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Antimicrobial Activity and Cytotoxic Effectof Cinnamon and Clove Mediated Gold Nanoparticles Based Mouthwash: An *in vitro* Study

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Abstract

Background: Nanotechnology is a new science that deals with the development of nanoparticles with various chemical compositions and sizes, as well as their application in health science for human benefit. We evaluated antimicrobial activity against oral pathogens and cytotoxicity using Brine Shrimp Lethality Assay of cinnamon and clove mediated gold nanoparticles based mouthwash.

Objective: The objective of the study is to evaluate antimicrobial activity and cytotoxic effect of cinnamon and clove mediated gold nanoparticles based mouthwash through an *in vitro* study.

Materials and methods: A total of 80 mL of 1 mm Gold chloride in distilled water were added to 20 ml of plant formulation. The active agent added was gold (III) chloride. In addition, a 0.3 gms of sodium lauryl sulfate, 0.3 gms of sucrose and 0.001 gms of sodium benzoate was added to formulate a mouthwash. Then this mouthwash was evaluated for its antimicrobial activity with estimating the zone of inhibition against oral pathogens and cytotoxic effect evaluated using Brine Shrimp Lethality assay (BSLA).

Results: Cinnamon and clove mediated gold nanoparticles were synthesized. Gold nanoparticles were characterized using Scanning Electron Microscope and were 525 nm in diameter. Brine Shrimp Lethality was done and the cytotoxicity of theses gold nanoparticles was found to be increasing with increasing concentration of the administered gold nanoparticles. The ZOI was noticed the highest with *streptococcus mutants* showing 42 mm of ZOI at 100 µl, while the lowest ZOI for *Candida albicans* and *Staphylococcus aureus* showing 36 mm at 100 µl. The nauplii alive at 5 µl were 7, which was the highest and the lowest was 1 nauplii alive at 80 µl.

Conclusion: There was dose based cytotoxicity with cinnamon and clove mediated gold nanoparticles, so the study concludes that assessing the safety levels is critical prior to administering nanoparticles for therapeutic and diagnostic purposes.

Keywords

Antimicrobial activity • Cytotoxic effect • Mouthwash • Brine shrimp Lethality assay

Introduction

In recent era, eco-friendly environmental decisions are a lot easier and also the current trend is to live a lifestyle that respects the earth by utilizing products that are eco-friendly. Interestingly, the people have started noticing about the increasing usage of synthetic-based antimicrobials that are being used in the consumer products [1]. Chemical preservatives such as benzoate, propionate, sorbate, nitrate, nitrite, and sulfites are widely antimicrobial agents [2], but these additives causes health ill effects on a longer time span such as liver damage, asthma, many allergic reactions and even malignant changes, which concerns increasing the use of natural antimicrobials [1]. Plants, animals, and microorganisms are considered to be the major sources of naturally available antimicrobial substances [3]. Most of the plant spices such as cinnamon, clove, turmeric, ginger, cardamom, etc. contains active compounds like 3-phenylprop-2-enal, 5-isopropyl-2-methylphenol, etc. that exhibits a great antimicrobial potential [4].

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Cinnamon is a native plant of Sri Lanka and atropical Asian spice obtained from the inner bark of several trees from the genus Cinnamomum [5]. Cinnamon includes a variety of species, all of which are common spices used in traditional and modern medicine around the world [6]. Cinnamon has been shown to have potent antimicrobial properties against a variety of microorganisms by Zaika earliest in 1988 [7]. Clove (*Syzygiumaromaticum*) on the other hand, is a plant widely cultivated in Spice Islands, Indonesia, Pemba and Zanzibar, though earlier production of the plant was in China [8]. Clove contains eugenol, oleic acids, and lipids, which is attributed to the antimicrobial properties. Also, the essential oil obtained from clove (*Syzygiumaromaticum*, L.) is known for its antimicrobial properties against a variety of pathogenic bacteria such as *S. aureus*, *E. coli*, *L. monocytogenes* and *S. typhimurium* [9].

Gold is one of the popular materials used in dentistry and the gold nanoparticles (AuNPs) are important components for biomedical applications. It is highly biocompatible and has antimicrobial properties that are well-known and as gold is converted to nanoparticles, its properties strengthen even further [10]. Since gold nanoparticles (AuNP) have a high surface conduction electron resonance and surface ratio, they can help in the movement of any biomolecules that come into contact with them [10]. Thus, gold nanoparticles (AuNP) are used to mediator in this study to fabricate a mouthwash using cinnamon and clove.

One of the critical aspects of staying healthy is to maintain a good oral hygiene and that is possible with various oral cleansing agents. Mouthwashes are one such agent but it's unaware that artificial mouthwashes can kill harmful bacteria while also killing your mouth's natural flora, exacerbating the problem and also alcohol in commercial mouthwashes will dry out your mouth [11]. So, this research is aimed to fabricate a mouthwash using natural products.

The objective of this research is to fabricate a mouthwash *in vitro* by analyzing the antimicrobial property of cinnamon and clove mediated by gold Nano particles.

Materials and Methods

Preparation of plant extract

The cinnamon and clove were collected, crushed and powdered. Accurately, 0.5 g of the powder of each was weighed and was added to 100 ml double distilled water, which was then boiled at 70°C for 30 min. This allows the phytochemicals present in the powder to get activated. The final formulation was filtered using Whattman filter paper 1, collected and stored for further use at low temperature.

Preparation of gold nanoparticles mouthwash

A total of 80 mL of 1 mM Gold chloride in distilled water were added to 20 ml of plant formulation. The active agent added was gold (III) chloride. In addition, a 0.3 g of sodium lauryl sulfate, 0.3 gms of sucrose and 0.001 gms of sodium benzoate was added. The prepared AuNP mouthwash was then kept in an orbital shaker for nanoparticle synthesis. The color change was observed and recorded periodically and photographs were taken.

Characterization of nanoparticles UV-visible spectrophotometric analysis of gold nanoparticles

The UV-visible spectroscopy was used to analyze the synthesized nanoparticles. The prepared AuNP mouthwash solution was taken in a cuvette and scanned in double beam UV visual spectrophotometer from 400-650 nm wavelengths. The results were recorded and graphical analysis was done.

Antimicrobial activity of mouthwash

Fresh bacterial cultures were prepared, in Hi-Veg broth medium, where 10 ul cultures of *Candida albicans*, *Streptococcus mutants*, *E. feacalis* and *Staphylococcus aureus* were inoculated, and incubated for 18 h. A nutrient agar medium was prepared and 5 mm wells were made, with different concentrations (25–100 µg/ml) of AuNPs added, along with the positive control ampicillin antibiotic disks. The plates were incubated for 18 h, at 37°C and the zones of inhibition were measured.

Brine Shrimp Lethality Assay (BSLA)

10 newly hatched Brine Shrimp (*Artemiasalina*) larvae (Nauplii) were taken and transferred into 6 wells. The synthesized AuNP mouthwash was introduced into each of the wells of varying concentrations of 5, 10, 20, 40 and 80 µL. One well with live nauplii was without the Gold nanoparticles and it served as the standard sample and were left undisturbed for 48 hours. The number of live nauplii after 48 hours was noted and the data was plotted in the form of a graph.

Results

UV-Visible spectrophotometric analysis

The cinnamon and clove mediated gold nanoparticles were synthesized and the color of the solution changed to blackish brown within 48 hours of reaction with the gold chloride solution, also the intensity of the color, increased with increase in incubation period (Figure 1). Spectrophotometer was used to measure the production of gold nanoparticles at a wavelength of 525 nm which was plotted in a graph.

Antimicrobial activity

The synthesized cinnamon and clove mediated gold nanoparticles were evaluated for its antimicrobial activity against oral pathogens; *Candida albicans*, *Streptococcus mutants*, *Staphylococcus aureus* and *Enterococcus faecalis*. The antimicrobial activity of AuNP mouthwashwas evaluated using the zone of inhibition (ZOI) against microbial cultures. The zone of inhibition increased with the concentration of the plantformulation. The ZOI was noticed the highest with *streptococcus mutants* showing 42 mm of ZOI at 100 µg/ml, while the lowest ZOI for *Candida albicans* and *Staphylococcus aureus* showing 36 mm at 100 µg/ml. The zone of inhibition against the evaluated pathogens are summarized in Table 1 and depicted in Figures 2 and 3.

Cytotoxicity using brine shrimp lethality assay

The nauplii were exposed to different concentrations (5, 10, 20, 40 and 80 µL) of AuNP mouthwash and left undisturbed for a time period of 48 hours. The number of live nauplii alive after 24 hours in each well inoculated with various AuNP mouthwash was noted for determining the level of cytotoxicity and a chart was formulated (Figure 4). The number of live nauplii was highest in 5 µL whereas the lowest was noticed in the well where a concentration of 80 µL was administered.

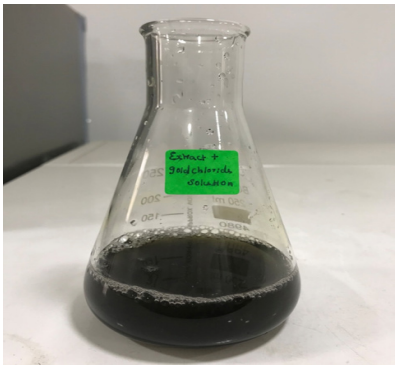

Figure 1. The cinnamon and clove mediated gold particles solution color changed to blackish brown within 48 hours of reaction with gold chloride solution.

Table 1. The zone of inhibition against the evaluated pathogens.

Concentration	Zone of inhibition (pathogens)			
	S.mutants	*S.aureus*	*C.albicans*	*E.faecalis*
25 µL	35	25	25	24
50 µL	40	32	27	35
100 µL	42	36	36	40
Control	30	12	25	18

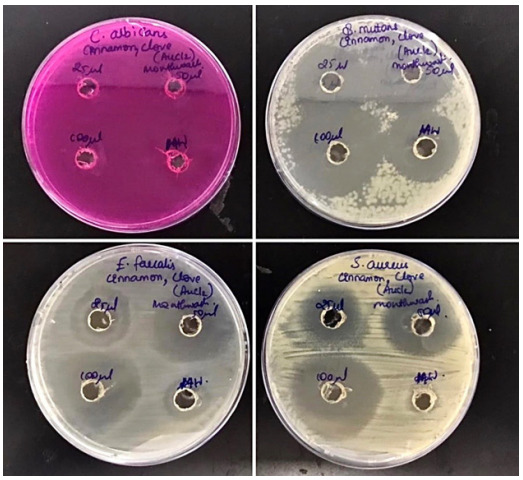

Figure 2. The AuNP mouthwash mediated zone of inhibition against the evaluated pathogens inoculated into 5 mm wells.

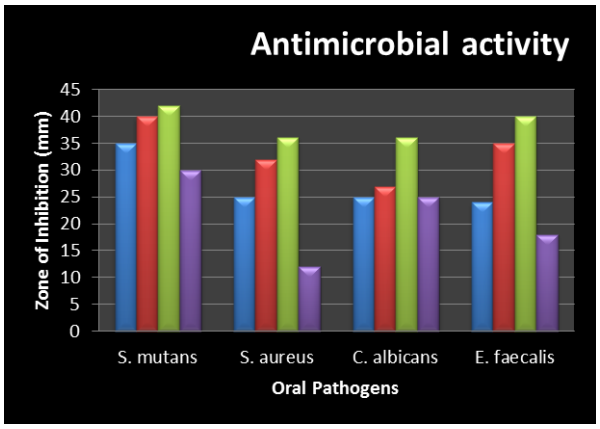

Figure 3. The graphical representation of AuNP mouthwash mediated zone of inhibition against the evaluated pathogens inoculated into 5 mm wells. **Note:** (■) 25 µL; (■) 50 µL; (■) 100 µL; (■) Mouthwash (Control).

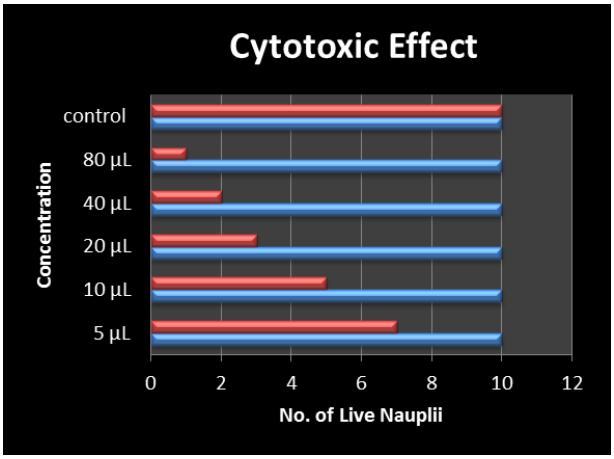

Figure 4. The graphical representation of AuNP mouthwash mediated cytotoxicity using Brine Shrimp Lethality Assay (BSLA). **Note:** (■) Day 2; (■) Day 1 .

Discussion

Metal ions, organic compounds, and biological molecules are all measured quantitatively using UV-visible spectroscopy. Color shifts and UV-visible spectrophotometer analysis at room temperature demonstrated the formation of gold nanoparticles. The resonance peak of gold nanoparticles was discovered in the spectra at 525 nm. Scanning Electron Microscope was used to examine the microstructure and scale of the biosynthesized gold nanoparticles. In a study conducted by Jochebed, et al. revealed that cinnamon oil mediated gold nanoparticles was found to possess maximum at a wavelength of 525 nm as viewed in a spectrophotometer that indicates that cinnamon in every form has similar mode of action [12].

The ZOI was noticed the highest with *streptococcus mutants* showing 42 mm of ZOI at 100 µg/ml, while the lowest ZOI for *Candida albicans* and *Staphylococcus aureus* showing 36 mm at 100 µg/ml but the lowest ZOI is still higher than the ampicillin antibiotic control used. A study conducted by Barma, et al. which shows 100 µl concentration a maximum zone of inhibition for *S. aureus*, *E. faecalis* of the silica nanoparticles [13]. But in this study, the mouthwash was formulated with clove oil alone along with silica nanoparticles. This could be the reason for change in the ZOI in different organisms. The antimicrobial activity of different compounds differs with different organisms because each compound has variations in their physical and chemical properties. Similar to silica, gold in the form of nanoparticles has found acceptance in the field of nanotechnology, as their efficacy is

primarily related to the fact that they reduce bacterial resistance. The gold nanoparticles are widely used in many applications like biomedical, food and environmental technology [14-20].

The nauplii alive at 5 µl were 7, which was the highest and the lowest was 1 nauplii alive at 80 µl. The brine shrimp lethality assay is a useful tool for determining the preliminary cytotoxicity of plant extracts based on their ability to kill laboratory cultured larvae (nauplii). It's a simple, low-cost method that only requires a small amount of test content. Artemiasalina is the organism most widely used in the Brine Shrimp Lethality Assay [14]. The nauplii do not obtain any food during the study period. The inoculated material of malnutrition may be causing the nauplii to die.

Conclusion

A control sample containing nauplii but no inoculation of the research material is used to ensure that the subject under study has a mortality impact. Since they still feed on their yolk sac, nauplii will live for up to 48 hours without food. Lethality of Brine Shrimp The number of live Shrimp larvae at higher concentrations was lower as compared to lower concentrations, and as the concentration of the Gold nanoparticles increased, so did the cytotoxicity, resulting in a lower number of shrimp larvae still alive after 48 hours.

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