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Green Synthesis of Silver Nanoparticles Using Bark Formulation of Symplocos Racemosa and Cinnamomum Verum and its Antimicrobial and Antioxidant Activity

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Abstract

Background and Aim: Noble metals nanoparticles are considered as promising metals due to their extraordinary physical properties. Among the noble metals, silver nanoparticles show a sharp and distinct absorption band in the visible region of the electromagnetic spectrum associated with localized surface Plasmon resonance.Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields. The use of nanoparticles of some metals is common and useful in several fields due to the good properties of these nanoparticles. In the present study,Symplocos racemosa (Lodhra) and Cinnamomum verum (cinnamon) bark extract was used as the reducing agent to synthesize silver nanoparticles using the method known as Turkevich method. Our aim is to produce silver oxide nanoparticles from lodhra and cinnamon extract and to find the activity of antimicrobial and antioxidant against oral pathogens

Materials and Methods: Preparation of plant extract is done by collection of plant extract in the form of powder. The prepared nanoparticles analysed for its antimicrobial activity against oral pathogens.

Results and Discussion: Normal value of zone of inhibition of bacteria for antibiotics was compared with zone of inhibition of each microorganism. The results from this study proved that the combination of Symplocos racemosa and Cinnamomum verum plant extract acts as an antimicrobial and antioxidant agent.

Conclusion: The research proves that silver nanoparticles of mixture of Symplocos racemosa and Cinnamomum verum have efficiently worked against Staphylococcus aureus which promotes the capability of this mixture of plants to be used as microbial remedies to treat bacterial pathogens.

Keywords: Silver Nanoparticles,Lodhra and Cinnamon,Cinnamon bark- turkevich method - Localized surface Plasmon resonance (LSPR).

Introduction

Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields. The use of nanoparticles of some metals is common and useful in several fields due to the good properties of these nanoparticles.Ag NP has high resistance to oxidation and antibacterial activity which make it desirable for a wide range of applications particularly in biomedical sciences. This led researchers to use materials with low toxicity to synthesize silver

nanoparticles which is termed green synthesis. In this method, a wide range of natural plant extract is used as the reducing agent instead of poisonous materials.

In the present era, green synthesis of silver nanoparticles is a new trend in nanoscience and nanotechnology. There is an increase in demand for biosynthesis of metal nanoparticles using microorganisms. In comparison with these organisms, plants seem to be the best candidate and they are suitable for large-scale biosynthesis of nanoparticles. Nanoparticles that are produced by plants are more stable and the rate of synthesis is faster than that of counterparts. Moreover, the nanoparticles comes in a varied shape and size in comparison with those produced by other organisms [1,2].

Researchers are interested in the use of silver nanoparticles in the fields of electronic, material science, and medicine. The use of silver nanoparticles in various fields is mainly because of their antimicrobial properties.[3,4].There are silver nanoparticles that show potential antimicrobial effects against infectious organism such as E coli, Pseudomonas aeruginosa and Staphylococcus aureus [5,6].According to previous research studies, the major processes underlying the antibacterial effects of NPs are disruption of the bacterial cell membrane, generation of ROS,penetration of the bacterial cell membrane and induction of intracellular antibacterial effects.

Moreover, these nanoparticles have been in the limelight to researchers because of their extensive applications in areas such as biomedical sciences, mechanics, optics, chemical industry, electronics, [7, 8], optoelectronic devices [9, 10], photo-electrochemical applications [11], and nonlinear optical devices.

Antioxidant action of Symplocos racemosa (Lodhra) and Cinnamomum verum (cinnamon) bark is mainly because of their high tendency to chelate and ligate with metals.These compounds comprises mainly of catechin, taxifolin, procyanidins of various chain lengths formed by catechin and epicatechin units, and phenolic acids. Lodhra compounds contain hydroxyl and carboxyl groups. These compounds may suppress the action of Fe ions by ligating and diminishing the superoxide-driven Fenton reaction, which is thought to be the most important source of reactive oxygen species. Hence, plants with high content of phenolic compounds like lodhra species are one of the best candidates for nanoparticle synthesis.Lodhra and cinnamon bark extract contains polymorphous and polyphenolic compounds that have considerable antioxidant activities. In this work, silver nanoparticles synthesized by a combination extract of Lodhra and Cinnamon plant barks were investigated.The objectives of this study were production of silver nanoparticles using combination of lodhra and cinnamon bark extract and optimization of the biosynthesis process [12,13].

Materials and Methods

Plant Material

Lodhra and Cinnamon bark specimens were collected from a botanical garden.The samples were collected in June month of 2022. The specimens were dried at room temperature, ground by using a conventional grinder, and stored at 4°C.

Plant Characteristics

Lodhra and Cinnamon is a medium-sized plant, reaching 3 feet high. The bark is brownish gray or light gray, not flaking, and head broad-topped. The leaves are stiff, 6–9?cm long, and green. The cones are pedunculate, solitary or in pairs, and light reddish brown. Scales irregularly rhombic, glossy, smooth, the whitish-gray apophysis concave: seeds blackish, 6-7?mm long, the reddish-brown wing 18–28?mm long.

Biosynthesis and Characterization of Silver Nanoparticles

Preparation of Lodhra and Cinnamon Extract

Fresh lodhra and cinnamon powder was collected from the market, Chennai .1g of freshly collected arrow root powder extract is mixed with 100ml of water and boiled for 3-5 mins in heating mantle then filtered using filter paper

Preparation of Ago Nanoparticles

0.861 g of AgO powder is added in 50ml of distilled water and to this arrow root extract has been added. The solution is kept in shaker and the reading were taken for every 2 hrs for analyzing the synthesis of nanoparticles

Evaluating Antimicrobial and Antioxidant Activity

11.2 of Lodhra in 300 ml of water and cinnamon in 100 ml of water are taken separately for antimicrobial and antioxidant activity respectively . These two flasks were Kept in a pressure cooker for 3 whistles and culture media was prepared.Turkevich method was used over here.

UV Spectrophotometry

It measures the attenuation of beam of light when it passes through a sample or after getting reflected from a sample. The color change of solution shows the formation of silver nanoparticles. The color change of the solution was observed using UV visible Spectrophotometer.

Test Pathogens

The antifungal activity of the green synthesized silver nanoparticles is tested against a variety of pathogenic microorganisms. The test pathogens were collected from the microbiology lab. Gram positive species such as Streptococcus aureus, Streptococcus mutans which is commonly found in the oral cavity, anaerobic Lactobacillus and gram-positive yeast such as Candida albicans were used for studying the antimicrobial activity.

Cultivation

Rose Bengal Agar (RBA) medium was prepared and kept for sterilization at 121 degree Celsius for 15 minutes. After sterilization, the medium was poured into petri-plates and was allowed for solidification. After solidification, the wells were made on the RBA medium using a well cutter. To that wells 50ul, 100ul, 150ul of Symplocos Ag NP pellet, solution was added. Fluconazole was used as an antibiotic (10ul). Then the plates were incubated at 37C for 48hrs. After incubation, the plates were observed and measured for zones of inhibition.

Assessment of antioxidant activity

DPPH Method

Antioxidant activity

DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of Symplocos racemosa and Cinnamomum verum bark extract interceded with zinc oxide 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. Ascorbic acid was used as standard. The percentage of inhibition was determined from the following equation,

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

Statistical Analysis

The diameter of the zone of inhibition for each pathogen was measured using a meter ruler. The mean values for each organism was also recorded and represented in millimeters.No spss analysis was used.

Results

Antimicrobial Activity

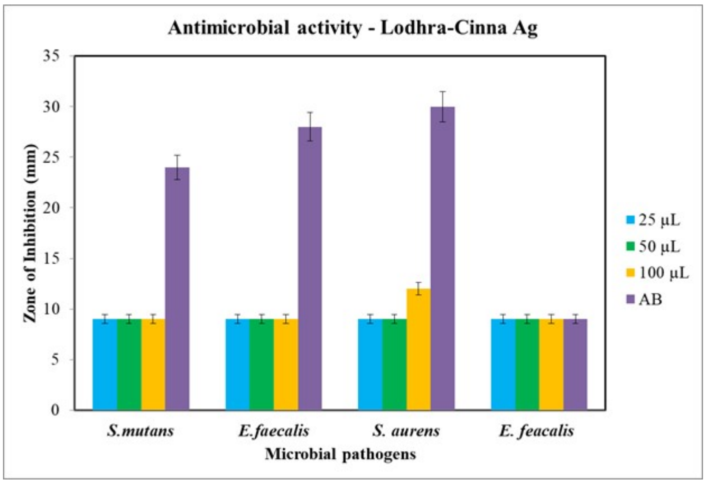

Figure 1: Graphical representation of percentage of zone of inhibition against the common microbial pathogens like Streptococcus mutans,Enterococcus faecalis,Staphylococcus aureus and Enterococcus faecalis.

The above graphs show the zone of inhibition and sensitivity of fungus towards Symplocos racemosa and Cinnamomum verum The amount of plant extract taken was 25µL, 50µL and 100 µL. Normal value of zone of inhibition of bacteria for antibiotics was compared with zone of inhibition of each microorganism

MICROBES	25µL	50µL	100 µL	150 µL
S.Mutans	9 mm	9 mm	9 mm	24 mm
S.Aureus	9 mm	9 mm	12 mm	34 mm
E.Faecalis	9 mm	9 mm	9 mm	30 mm
C Albicans	9 mm	9 mm	9 mm	9 mm

Table 1: The above Tabular column depicts the sensitivity and zone of inhibition value for each microorganism. The silver nanoparticles enter into the cell wall and kill the microbial growth.

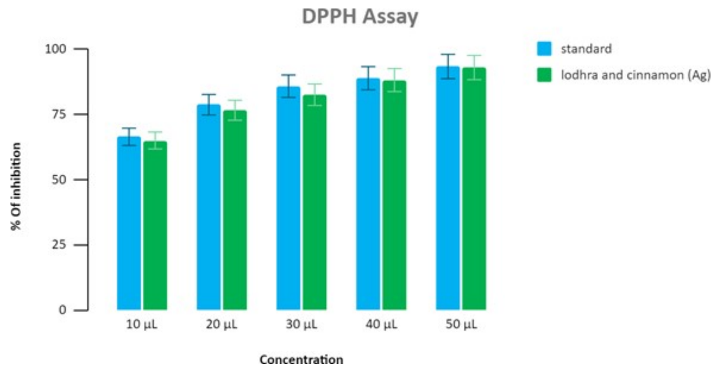

Figure 2: Graphical representation of percentage of inhibition against the concentration of common microbial pathogens like Streptococcus mutans,Enterococcus faecalis,Staphylococcus aureus and Enterococcus faecalis.The highest concentration of lodhra and cinnamon for its inhibitory activity is 50 microlitre for standard samples.

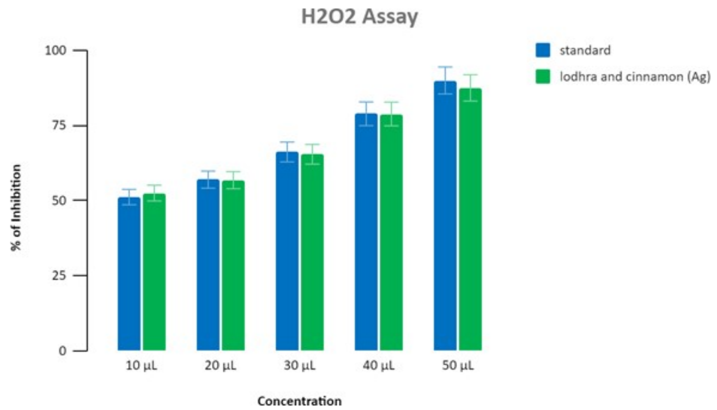

Figure 3: Graphical representation that shows the antioxidant activity with the percentage of inhibition against the concentration of common microbial pathogens like Streptococcus mutans,Enterococcus faecalis,Staphylococcus aureus and Enterococcus faecalis.

The above graphs shows that the maximum inhibitory concentration of lodhra and cinnamon (Standard) was found to be 50 microlitres for the standard samples.

In this study the antimicrobial and antioxidant activity of Lodhra and cinnamon bark mediated silver nanoparticles was assessed in five different concentrations of reaction mixture from 10µL, 20 µL,30 µL,40 µL and 50 µL.Antioxidant and Antimicrobial activity of different percentage of inhibition were 65%,70%,80%,80% and 100% respectively.Plant extract mediated by silver nanoparticle at 50µL of concentration exhibited a high antioxidant and antimicrobial of activity of 85%.This shows that Lodhra and cinnamon extract mediated nano particle has better antimicrobial and antioxidant activity even at 50µL which is more than

50%.

DPPH,Hydrogen peroxide, hydroxyl radicals, superoxide scavenging methods confirmed the silver nanoparticles has antioxidant, hydrogen peroxide, hydroxyl radicals and superoxide scavenging activities.These properties of silver nanoparticles occur due to the presence of functional groups on the surface of silver nanoparticles. The results of antimicrobial sensitivity confirmed the silver nanoparticles and silver nitrates have antibacterial activity. The AgNPs of this mixture have the maximum zone of inhibition against the Staphylococcus aureus (34 mm) at 150 µL and minimum against C.albicans (9 mm) fungi.

Discussion

Numerous studies have been conducted to analyze the antimicrobial and antioxidant effect of silver nanoparticles and its effects on humans .AgNPs have been widely used as antimicrobial materials in foods, medical products, and coatings for industrial products. Factors like size, surface chemistry, methods of exposure, and exposure times are critical to determining their pathways of tissue distribution.Its importance in antimicrobial and antioxidant activity is crucial.K.Ashwini et al (14) in their study has explained extensively about the benefits of the Lodhra and Cinnamon in the recent medicine field. They have also explains about the antimicrobial effects of silver nanoparticles in vitro study.

Cinnamon alone has high antimicrobial and antioxidant activity. AH.Saleim et al (15) in their study has explained about the biosynthesis of cinnamon in aqueous form taken from cinnamon zeylanicum bark. Aqueous cinnamon extract (ACE) proved to be more antimicrobial to bacterial pathogens at concentrations just above 24 mm (containing 50 µL cinnamaldehyde). At a critical concentration of 100 µL (containing 20 µLcinnamaldehyde), ACE treatment resulted in 85% growth inhibition of the majority of the microbial organisms, whereas at a similar concentration (10 µL) commercial cinnamaldehyde treatment resulted in 30% growth inhibition.

Lodhra though it has antimicrobial properties,it is less effective than cinnamon which is compensated by its synergistic effect. MA Ansari et al (16) in their study has explained the use of lodhra and its antifungal effect when incorporated into copper nanoparticles and its influence in various physiological systems.

From the above aspects we can find that our study has its own uniqueness of combination of Lodhra and cinnamon with silver nanoparticles.When compared with other other studies this is a new aspect where more than one compound is involved and the formulation is analysed for the synergistic effect of both antimicrobial and antioxidant activity.At the same time,the results were on par with the compared articles

Conclusion

The results from this study proved that the combination of Symplocos racemosa and Cinnamomum verum plant extract acts as an antimicrobial and antioxidant effect. The results of the study shows that silver nanoparticles of Symplocos racemosa & Cinnamomum veru, extract was effective in inhibiting the growth of Staph.aureus.There is an increased zone of inhibition is observed using Silver Oxide nanoparticles.The research proves that silver nanoparticles of Symplocos racemosa and Cinnamomum veruma has efficiently worked against Staphylococcus aureus.Thus,it promotes the capability of this extract of Symplocos racemosa and Cinnamomum verum plant combination to be used as herbal remedies to treat bacterial pathogens and its antioxidant effect on human cells..We can conclude that an increased zone of inhibition is observed using Silver oxide nanoparticles, especially its action against Streptococcus aureus (Antimicrobial growth) and a significant antioxidant effect on human cells.Hence,it can be used as a better drug of choice as a mixture of these two plant species.

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Conflict of Interest

The author declares that there was no conflict of interest in the present study.

Author Contribution

S.Ragul Prasath-Literature search,Data collection,Analysis and Manuscript writing. Anju Cecil- Data verification,Manuscript drafting.

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