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Synthesis and Characterization of Silver Nanoparticle from Citrus Limon Leaves and its Antibacterial Activity

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Abstract: ***The study is focused to biosynthesize silver nanoparticles using lime leaf extract to assess the antibacterial activity of silver nanoparticles against the selected microorganisms. 10g of dried leaf powder with 100ml of deionized water in 500ml of Erlenmeyer flask and boiled for 20mins. For the reduction of Ag+ ions, 10ml of leaf extract was mixed to 90 ml of 1mM of aqueous of AgNO3 and was heated at 60-80 for 20 mins. The bio reduction of Ag+ ions in solution was monitored using UV- vis spectrometer, TEM, analysis. The extract was tested against the microorganisms like Escherichia coli, Staphylococcus aureus, Shigella, Klebsiella pneumonia. The present research work emphasizes the use of lemon leaves for the effective synthesis of AgNPs and could be used against the microorganisms which are found to develop drug resistance towards board spectrum antibiotics.***

Keywords: lemon leaf, silver nanoparticles, microorganisms, UV-vis spectrometer

1. Introduction

The lemon *Citrus limon* (L.) osbeck is a species of small evergreen tree in the flowering plant family Rutaceae, native to asia. The trees ellipsoidal yellow fruit is used for culinary and non- culinary purposes throughout the world, primarily for its juice, which has both culinary and cleaning uses. The juice of the lemon is about 5% to 6% citric acid, with a pH of around 2.2, giving it a sour taste. The origin of the word ''lemon'' may be middle eastern. A genomic study of the lemon indicated it was a hybrid between bitter orange (sour orange) and citron. It was mainly used as an ornamental plant and for medicine. In the 19th century, lemons were increasingly planted in Florida and California. In 1747, James Lind's experiments on seamen suffering from scurvy involved adding lemon juice to their diets, Lemons are a rich source of vitamin C, Lemons contain numerous phyto chemicals, including polyphenols, terpenes and tannins. Lemon juice, rind, and peel are used in a wide variety of foods and drinks. Lemons were the primary commercial source of citric acid before the development of fermentation based processes.

Figure 1: *Citrus limon*

Collection of plant

Citrus limon leaves were collected from Bargur village in krishnagiri district, Tamil nadu, India .The plant was dried in shade at room temperature.

Identification of plant

The whole plant material *Citrus limon* is obtained from local area of krishnagiri district, Tamilnadu, India during the month of January, 2018.The fresh plant material was authenticated by a scientist from Botanical Survey of India, Coimbatore, Tamil Nadu, India.

Preparation of plant extract

The plant was dried in shade room temperature for 5 to 6 days .The leaves were powdered and used as raw material and stored in air tight container.

Solvent extraction

Plant leaf extract was prepared by mixing 10g of dried leaf powder with 100 ml deionized water in 500 ml of Erlenmeyer flask and boiled for 20 min. For the reduction of Ag + ions, 10 ml of leaf extract was mixed to 90 mL of 1 mM aqueous of AgNO3 and was heated at 60 - 80 for 20 min. A change from brown to reddish color was observed.

UV-VIS spectra analysis

UV-VIS spectra analysis the reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer UV-2450 (Shimadzu).

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) (HITACHI,H-7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra –thin specimen, interacting with the specimen as it passes through. Ag particle image was formed from the interaction of the electrons transmitted through the specimen; the image of Ag nanoparticles was magnified and focused onto an imaging device.

Antibacterial Activity

To perform antimicrobial activity using various bacterial and fungal species were selected viz., *Escherichia coli, staphylococcus aureus, pseudomonas species, bacillus species, klebsiella species; proteus species* are bacterial cultures.

Media and culture condition

Muller-Hinton Agar (MHA) and Nutrient Broth (NB) were used throughout the study for determining the antibacterial assay. The media was adjusted to the pH and autoclaved at 121°c for 15 minutes.

Preparation of the Bacterial Inoculum

Stock cultures were maintained at 4°c on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24 hours and at 37°c on shaking incubator and fungal cultures were incubated at 27°c for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours and fungal incubated at 27°c for 3-5 days. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27°c for 3-5 days.

Well Diffusion method

The antibacterial activity and antifungal activity of crude extract extracts was determined by Well Diffusion method (Bauer *et al*., 1996). MHA plates was prepared by pouring 20ml of molten media into sterile petriplates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile paper discs were dipped into required solvents then placed in agar plates. Then 10-15µl of plant extract was poured into the wells. After that, The plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. Well were put into the agar medium using sterile forceps. Plant extract were poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

2. Result

UV-VIS spectra analysis

Reduction of Ag ion into silver particles during exposure to the plant extracts could be followed by color change. Ag nanoparticle exhibit dark yellowish- brown color in aqueous solution due to the surface plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential plants for synthesizing the Ag nanoparticles. UV-VIS spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min has absorbance peak at 430 – 440 nm, broadening of peak indicated that the particles are polydispersed.

Figure 1: Synthesis of silver nanoparticles from citrus limon leaves

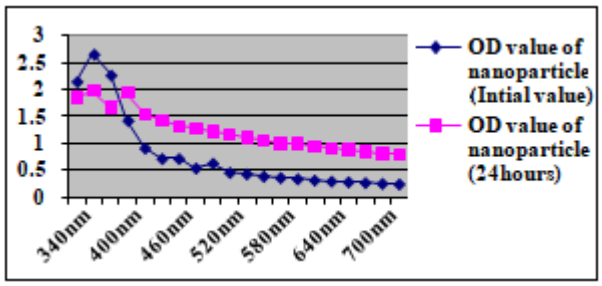
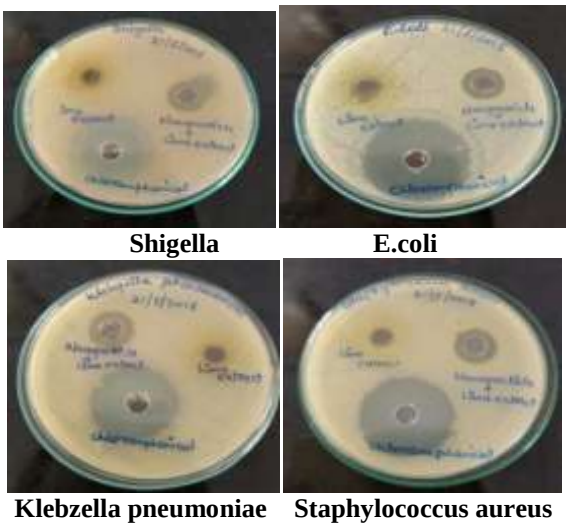


Figure 2: UV-VIS absorption spectra of silver nanoparticle synthesized from *citrus limon* leaves extract at 1mM silver nitrate

Antibacterial activity

Biosynthesis of Ag nanoparticles were studied for antibacterial activity against pathogenic microorganisms (clinical isolate) by using standard zone of inhibition(ZOI) microbiology assay with a well size of 5mm diameter and 30l was found to be Staphylococcus aureus (S. aureus) (2cm); Shigella (2cm); E.coli (1.7cm); Klebsiella pneumonia (K.pneumoniae) (1.8cm).



Shigella **E.coli**

Klebzella pneumoniae **Staphylococcus aureus**

Figure 3: Antimicrobial activity of silver nanoparticles synthesized from *citrus limon* leaf extract against pathogens.

3. Summary and Conclusion

Antibiotic resistance by the pathogenic has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents. In the present scenario, silver nanoparticles as antimicrobial agents have come up as a promising candidate in the medical field. There

are different physical and chemical methods for the synthesis of nanoparticles, but there is always a need for the development of eco-friendly route for the synthesis process. Hence, our current study proves to be an important step in this direction.

Formation and stability of silver nanoparticles in aqueous colloidal solution are confirmed using UV-Vis spectral analysis. As the *Citrus limon* leaf extract was mixed with aqueous solution of the silver nitrate, it started to change the color from watery to reddish brown due to reduction of silver ion, which indicated the formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape – controlled nanoparticles in aqueous suspensions. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 430-440 nm, which correspond to silver and broadening of peak indicated that the particles are polydispersed.

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