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SYNTHESIS OF SILVER PHYTO NANOPARTICLES AND THEIR ANTIBACTERIAL EFFICACY

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The synthesis of silver phyto nanoparticles and their antibiogram were included in this study. This is the first attempt of introducing silver herbal nanoparticle isolation and antimicrobial assessment. It also comprises about the Nanotechnology and their achievements in silver nanoparticles descriptions and production. The herbal leaves and their medicinal properties were already discussed in varieties of ayurvedic studies. The herbal leaves like *Ocimum sanctum* and *Vitex negundo* were included to analyze the productivity of nanoparticles. The synthesis of silver phyto nanoparticles were prepared by adding silver nitrate solution (10^{-3} M) and the silver phyto nanoparticles were isolated from these herbal leaves. The silver phyto nanoparticles were collected from each herbal plant and tested their antibacterial activity. The test cultures included in this study were *Staphylococcus aureus, Vibrio cholerae, Proteus vulgaris and Pseudomonas aeruginosa.* The pellets obtained were diluted at the concentration of 1:10 and were taken as 25µl, 50µl, 100µl and 150µl respectively are included in this study. The antibacterial activities of all the herbal nanoparticles obtained from *Ocimum sanctum* showed maximum inhibitory rate using 150µg of these plants extract compared with *Vitex negundo*. The silver nanoparticles from herbal leaves showed a good antibacterial activity than the plants used. Further studies needed to find out the efficacy, longevity and toxicity to improve the current investigation.

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1. Introduction

One of the important characteristics of biological system is their ability to change important properties in response to small environmental signals. Till recent times N Nanotechnology has been a domain of physics, chemistry, electrical engineering and material sciences; there has been a considerable progress in the visualization, construction and manipulation of materials at nanometer scale. Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology. This area of endeavor is an inter-phase between biology with engineering applications to recognize, organize and functionalize molecular materials which is applicable to medicine[1]. Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity. Oligodynamic silver have lethal effects spanned across all microbial domains (Viral, bacterial and fungal). The urinary tract related bacterial pathogens are known to be susceptible to oligodynamic silver[1,2]. In rats, silver was unevenly distributed in organs and tissues following, intravenous injection (wherein). The highest

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concentrations were found, in decreasing order, in the liver, pancreas, spleen and plasma[2-4]. It has been observed that intravenous silver administration will readily pass the so-called blood brain barrier, presumably allowing for interface and intervention with pathogens or prions associated with neuropathology.

Microorganisms that are exposed to pollutants in the environment such as metal ions have a remarkable ability to fight with metal stress. These metal-microbe interactions have already found an important role in Biotechnological applications. It is only recently that microorganisms have been explored as potential biofactories for synthesis of metallic nano particles[5]. Silver nano particles act as an antimicrobial, anti-bacterial, anti-viral, anti-biotic and anti-fungal agent when incorporated in coatings, nano fiber, first aid bandages, plastics soap and textiles, in self cleaning fabrics and as conductive filler. It is also used in nano wire and in certain catalyst applications. S-MITE HIV Inhibitor^{TM} is a proprietary form of silver nano powder that has been shown to deactivate HIV by inhibiting the virus from attaching to the host with undetectable levels of cytotoxicity[2,3]. Silver nano particles spectrally designed for selective coating for solar energy absorption and intercalation material for electrical batteries as optical receptors, polarizing filters and catalysts in chemical reaction. These particles also use as staining pigments for glasses and ceramics, transparent conducting coating, electronics, in surface-enhanced Raman Spectroscopy, bio-labeling and antimicrobial agents [2]. The use of anodic silver as preserving agents in cosmetics was also tested by a challenge test in a set of cosmetic dispersions with the addition of known preservative inhibitors or microorganism growth promoters such as humectants, hydro soluble collagen and vegetable extracts [5,6].

This is the first study of including the plants for synthesizing silver based nanoparticles. The present investigation revealed with the isolation and monitoring of silver nano particles from medicinally important plants like *Ocimum sanctum* and *Vitex negunda,* their antibacterial assessment was performed to produce novel drugs to overcome drug resistance and adverse reaction.

2. Materials and methods

Boiling /Collection of the extracts

Two herbs were included in this study (*Ocimum sanctu* and *Vitex negundo)* and were collected freshly from the market (stored leaves are not used in this study). Primarily the leaves were washed and the cleaned leaves were dried with water absorbent paper. Then it was cut into small pieces (Note: Do not grind), dispensed in 100ml of sterile distilled water and boiled for one hour at 80ºC. Then the leaf extracts were collected in separate conical flasks by standard filtration method.

Preparation of Silver nanoparticles

10^{-3} M Silver nitrate solution was prepared and stored in brown bottles. 5ml of leaf extracts was taken in BOD bottle separately and to this 100ml of $AgNo_3$ solution was added [7,9,10]. The same protocol was followed for other four leaf extracts. The color change of the leaf extracts from pale green to dark brown was checked periodically. Then the BOD bottles were incubated at room temperature for further incubation till 28 hours. The color change to brown indicated that the silver nano particles were synthesized from the leaves and centrifuged at 10000rpm for 25 minutes where pellets used for antibacterial activity.

Antibacterial Analysis

The Antibacterial activity of isolated plant-silver based nanoparticles pellets were tested by standard well cutting method. The test bacterial *Staphylococcus aureus, Vibrio cholerae, Proteus vulgaris* and *Pseudomonas aeruginosa* were included in this study to assess the susceptibility pattern of the compounds. Different concentrations (1:10 diluted) of the pellets like

25µl, 50µl, 100µl and 150µl of the diluted compounds were loaded on marked wells with the help of micropipette and plates were incubated at 37ºC for 24-48 hours for observing inhibition rate.

3. Results

Confirmation of metal-microbe interaction

It was found that aqueous silver ions when exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver hydrosol. The fungal biomass were pale green in color before addition of Ag^{+} ions and this changed to brownish color suggested the formation of silver nanoparticles. The bottles were observed periodically for change in color from green to different shades of brown (Table 1). The time duration of change in color varies from plant to plant where Ocimum *sanctum* obtained brown color within 2 hrs where as *Vitex negundo* obtained brown color within minimum of 4 hrs to change.

Table 1. Periodical colour change from green to brown of herbs with 10^{-3} M $Agno_3$

Time	Herbs	
	O. sanctum	***V. negundo***
0	-	-
10 mins	+	-
30 mins	+++	+
1 hr	+++	+
2 hr	++++	++
4 hr	+++++	+++
8 hr	+++++	++++
16 hr	++++++	+++++
24 hr	++++++	++++++
28 hr	++++++	++++++

- No colour change; + Dark Green; ++ Reddish greeen; +++ Red; ++++ Reddish brown; +++++ Tinge brown; ++++++ Brown threads

Antibacterial analysis

The inhibition rate of 25µl of this *Ocimum sanctum* extract nanoparticles against *S. aureus* was 4mm. The 50µl and 100µl concentrate were showed 9mm and 27mm where as the 150µl concentrate showed 26mm. The inhibition rate against *Pseudomonas aeruginosa* by Ocimum nanoparticles showed was 6mm, 12mm, 24mm and 36mm respectively. The maximum inhibitory rate was observed using 150µl of plant leaf nanoparticle showed 25 and 27mm in *Proteus vulgaris* and *Vibrio cholerae* respectively. The leaf extract nanoparticle of *Vitex negundo* showed minimum inhibition rate against bacterial battery included in this study and depicted in Table 2.

Table 2. Inhibition rate of various herbal nanoparticles against selective bacterial pathogens

Name of the test bacteria	Inhibition rate (in mm) in various concentration (µl) of herbal nanoparticles							
	Ocimum sanctum				*Vitex negundo*			
	25	*50*	*100*	*150*	*25*	*50*	*100*	*150*
Staphylococcus aureus	4	9	17	26	2	5	9	14
Proteus mirabilis	5	9	18	27	3	5	8	10
Vibrio cholerae	5	9	17	25	4	7	10	12
Pseudomonas aeruginosa	6	12	24	36	4	6	10	12

4. Discussion

The use of plant extracts has opened a new awareness for the control of plant disease, besides being safe and non-phytotoxic. It is found that the plant extracts are effective against various microorganisms including plant pathogens[8]. The search for antimicrobial agent has continued to be concentrated on lower plants, fungi and bacteria. Less research has focused on higher plants although identified plant compounds such as barberino, emetine, quinine and sanguinarine still find specialized uses[9].

The oil of *Ocimum sanctum* to possess significant antibacterial and insecticidal properties.it inhibits the in vitro growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes var. aureus*; in antitubercular activity, it has one tenth the potency of streptomycin and one fourth that isoniazid. Ether and alcohol extracts of leaves of *Ocimum sanctum* were also shown to posses significant activity against *Escherichia coli. O. sanctum* has been also extensively studied for therapeutic potentialities in various areas like immuno stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, anti-inflammatory, analgesic and antidiabetic[10].

A striking future of nanomedicine companies is their product diversity. The applications of these structures to a range of biotech challenges, including therapeutics, diagnostics, structural materials and electrical devices, are striking. Provided that environmental and safety concerns can be addressed in a meaningful and open fashion, there seems little doubt that this technology, after years of incubation, will finally accrue long sought-after benefit for its promoters[11]. The use of anodic silver ions as preserving agents in cosmetics was tested by a challenged test in a set of cosmetic dispersions with the addition of known preservative inhibitors or microorganisms growth promoters such as humectants, hydro soluble collagen and vegetable extracts. Silver's microbicidal efficacy, compared to that of imidazolidinyl urea or methyl p-hydroxybenzoate, showed a more efficient activity especially in the presence of proteinaceous material. This agent may represent a good and safe protection for finished products both in manufacture and during use[4,6,7,11].

One of the fundamental processes involved in the regulation of mineral deposition in biological systems is the organic matrix (proteins and /or other biological macromolecules) that controls the nucleation and growth inorganic structure. Inorganic binding proteins can be identified using either traditional or combinatorial approaches. Traditional approaches include isolation and identification of proteins that associate with inorganic structure in vivo using routine molecular biology techniques. The molecular basis for the biosynthesis of these silver crystals is not known, but it is speculated that the organic matrix contains silver binding proteins that provide amino acid moieties that serve as the nucleation sites. Based on this premise, we used a combinational approach to identify silver-binding peptides from a phage display library of random peptides instead of using the conventional molecular biology procedures for isolating the silver-binding proteins from bacteria. The silver-binding peptides were selected by incubating silver particles with the combinatorial phage peptide library. When the silver-binding clones were incubated in an aqueous solution of 0.1M silver nitrate for 24-48h at room temperature, the solution turned reddish in color, and a reddish colored precipitate was obtained when the solution was centrifuged. No change in the color or precipitate was observed with a non-specific phage. Silver nanoparticles are

known to exhibit a size-dependant characteristic surface plasmon resonance band that can be measured using ultra violet visible spectroscopy[12].

The efficiency of various silver based antimicrobial fillers (elementary silver and silver substituted materials) in polyamide (PA) toward their silver ion (Ag+) release characteristics in an aqueous medium was investigated and discussed. Anode Stripping Voltammeter (ASV) was used for the quantitative estimation of Ag+ release from these composites. The biocidal (Ag+) release from the composites was found to be dependant on the time of soaking in water and the nature of the filler. The long term Ag+ release capability of the elementary silver based PA/Ag composite is promising compared with the commercial counterparts. The silver ion release potential of polyamide composites where the silver filling was performed by using supercritical carbon dioxide ($scCO_2$) is also discussed. The composites release Ag at a concentration capable of rendering antimicrobial efficacy and proved to be active against the microbes[12,13].

5. Conclusion

The study included the synthesis of silver nanoparticles from the herbal leaves of *Ocimum sanctum* and *Vitex negundo* and their antimicrobial activity. From the study, it was concluded that the aqueous silver ions exposed to the herb were reduced and the nanoparticles were synthesized. The presence of nanoparticles was confirmed by the brown color formation. The brown color was observed by 2 hours in *Ocimum sanctum* whereas *Vitex negundo* developed brown color after 4 hours. The antimicrobial efficacy of *Ocimum sanctum* was more than *Vitex negundo* especially against *Proteus vulgaris* and *Vibrio cholerae*. Thus it was confirmed that the composite release of silver at a core is capable of rendering antimicrobial efficacy and proved to be active against the microbes.

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