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Study of antimicrobial activity of medicinal plants derived silver nano particles

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Abstract
The medicinal plants represents an enormous reservoir of potential antimicrobial compounds that could be useful as an alternative to synthetic microbicides and these are being used to develop drugs. In current study, the antimicrobial activity of various leaf extract were estimated by disc diffusion method. The results of antimicrobial activity of all the extracts showed good inhibitory activity against all the tested microbes.

Total four different medicinal plants namely tulsi, neem, castor & aloe vera leaf samples were collected from shree sai aurvedic research center, virag, district solapur to study synthesis of silver nanoparticles and antimicrobial activity from medicinal leaf extracts. Silver nano particles were synthesized by chemical reaction using methanol in 5% DMSO reagent. Lowest Optical Density measured by spectrophotometer at 300 nm which was 0.00 however highest Optical density recorded t 400nm by neem plant extract with silver nanoparticles which was 2.96 than other extracts. The silver nano particle synthesized by using medicinal plants extracts were assured for their antimicrobial activity against fungal and bacterial pathogen *viz., E.coli, Pseudomonas, Staphylococcus, Bacillus* and *Aspergillus niger* The primary screening of silver nano particles was done using disc diffusion method. In this view study was conducted to synthesize silver nano particles from medicinal plant extracts and its antimicrobial activity.

Keywords: antimicrobial activity, medicinal plants, silver nano

Introduction
In the recent years controllable synthesis of noble metal nanoparticles has attracted much attention due to their potential applications as antimicrobial, catalytic, and antifungal activity. The field of nanotechnology is one of the most active areas of research in modern material science. NPs produced by plants are more stable and the rate of synthesis is faster in the case of micro-organisms. Moreover, the NPs are more various in shape and size in comparison with those produced by other organisms.

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver nanoparticles is in medical industry and also these are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria.

Silver nanoparticles have unique optical, electrical, and thermal properties that play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering. So alternative methods are always searched on for the synthesis of silver nanoparticles without any toxic by-products. It is quite interesting that silver nanoparticles can also be synthesized from bacteria (Nanda and Saravanan, 2009), fungus and plants.

Materials and Methods
The present studies were carried out in Biochemistry and Molecular Biology Laboratory in the Lokmangal College of Agricultural Biotechnology, Wadala, and Solapur from Dec 2016-June 2017.

Plant sample collection
The plant sample of Tulsi, Neem, Castor and Aloe Vera leaves were collected from the local garden. The leaves were shed dried for 10 days, and then kept in the hot air oven at 60^0C for 24 to 48 hr. The leaves were ground to a fine powder by mixture grinder.

Solvent extraction
Ten grams of air dried powder was placed in 100 ml of organic solvent (90% methanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 180 to 200 rpm for 24 hr. After 24 hr, it was filtered through muslin cloth and centrifuged at 5000 × g for 10 min.

The supernatant was collected and the solvent was evaporated. The crude extract diluted with 5% of DMSO to make the final volume one-tenth of the original volume and stored at 4°C in air tight bottles for further studies.

Synthesis of silver nanoparticles

1mM silver nitrate was added to plant extract to make up a final solution of 200 ml and was centrifuged at 15,000 rpm for 30 min. The collected pellets were stored at 4°C. The supernatant was heated at 50 to 95°C. A change in the color of solution was observed during the heating process. Reduction of silver ion into silver particles during exposure to the plant extracts could be followed by color change.Silver nanoparticles exhibited dark yellowish – brown color in aqueous solution. The absorbance of silver nanoparticles was measured 400 to 430 nm.

UV- Vis spectroscopy

The reduction of pure Ag+ ions was monitored by measuring the UV-VIS spectrum of the reaction medium up to 1hour after diluting a small aliquot of the sample into distilled water. UV-VIS spectral analysis was done by using UV-VIS spectrophotometer.

Antifungal activity

The extracted sample of medicinal plant with silver nanoparticles were tested for their antifungal activity by the agar disc diffusion method. The plant pathogenic fungal strain *Aspergillus niger*. was used for this analysis. This fungal spore suspension was added on PDA plates by the pour plate technique. Prepared different concentration of 25µl, 50µl, 75µl, 100µl, of silver nanoparticle solution 0.5cm sterile disc loaded in PDA agar plates and then incubated at room temperature for 7 days. The formation of a clear zone (restricted fungal growth) around the cavity is an indication of antifungal activity.

Disc diffusion antibacterial method

The disc diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using nutrient agar media. The NA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. Prepared different concentration of 25µl, 50µl, 75µl, 100µl, of with the silver nanoparticle solution 0.5cm sterile disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Results and Discussion

Silver Nanoparticles synthesis was carried out by addition of silver nitrate solution to four prepared medicinal plant extracts. After 24 hr, as the solution turned into brown from yellow solution at room temperature suggesting formation of silver nanoparticles. The color became brown and turned into dark brown after 48 hrs. The reduction of Ag+ was confirmed from the UV–Vis spectrum of the solution. The nanoparticles were separated out from the mixture by centrifugation at 15000 rpm for 30min. The aim of this project was Tosyn the size silver nanoparticles from easily available and cost effective medicinal plant to check its antimicrobial property.

UV- absorbance study of medicinal plant extract

Prepared medicinal Plant extract was subjected to UV-absorbance study to confirm silver nanoparticles synthesis in plant extract. As soon as silver nitrate was added to each plant extracts colour change was observed from faint yellow to brown to reddish brown within 72 hr of incubation at room temperature. Similar, colour changes were observed in other studies which confirmed the reduction of silver ions to Ag NPs. Corresponding UV-visible absorbance spectrum of Ag Nps was recorded at the 60 minutes from the initiation of the reaction. The absorption band was observed at around 400nm for all four medicinal plants. From the results it was found that, the formation of Ag NPs occurred rapidly within 60 minutes representing rapid biosynthesis of silver nanoparticles and the absorption peak increased as the time increased.

Antibacterial activity of SNPs of medicinal Plant extract.

Different concentrations of silver nanoparticles synthesized plant extracts (25 µl, 50 µl, 75 µl, 100 µl) were used against three bacteria (*Staphylococcus sp., Pseudomonas sp., Bacillus sp.*) The significant zone of inhibition activity were recorded by all bacterial culture. The SNPs of neem extract showed highest antibacterial activity was recorded (3.25mm) against *E.coli.* Followed by SNPs of castor extract (3.12 mm) against *Staphylococcus sp.* Also, The SNPs of Aloevera and Tulsi extract were showed significant antibacterial activity against *Staphylococcus sp.* (2.87mm) and *E.coli sp.* (2.62mm) respectively. The lowest zone of inhibition was recorded by SNPs of neem (2.37mm) and castor (1.87mm) against *Bacillus sp.* and *E.coli sp*. respectively followed by Tulsi and Aloe vera against *E.coli sp.* and *Pseudomonas sp.* (1.75mm) respectively.

Similar findings with, Gebru *et al.* (2013) studied that, The antibacterial activity of synthesized nanoparticles against *E.coli* and *Staphylococcus aureus* was using paper disc diffusion technique. The magnitude of antimicrobial effect against *E.coli* and *Staphylococcus aureus* was determined on the basis on inhibition zone measurement. Hashoosh *et al.* (2014) [2] concluded that, the study was carried out to explain the role of *Aloe-vera*extract as a reducing agent for the production of silver nanoparticles. The UV-VIS spectrophotometer showed shift peak at 400nm. These nanoparticles gave significant effect on Gram negative bacteria *E. coil* and Gram positive bacteria *S. aureus* at concentration 3.5 mg\ml, but it did not have any antifungal effect on *Candida albican, Pencillium spp*. and *Aspergillus niger* Agarry *et al.* (2005) [1] revealed that, the antibacterial activity of aloevera gel extract was tested using agar well diffusion technique.

A**ntifungal activity of SNPs of medicinal Plant extract**

The significant antifungal activity observed by Aloe vera against *Aspergillus*

Niger (3.12mm) followed by Castor, Neem (2.87mm) and lowest zone of inhibition Observed by Tulsi (2.62mm).

Similar findings with, Medda *et al.* (2014) [3] revealed that, The synthesized AgNPs prepared from Aloe vera leaf extract showed antifungal activity against *Rhizopus sp.* and *Aspergillus sp.* Savithramma *et al.* (2011) [4] reported that the sensitivity testing of the plant extracts were determined by using disc diffusion method. 0.5 ml of standard.

Table 1: UV- Absorbance of Medicinal Plant extracts by using Spectrophotometer.

Sr. No	Wavelength (nm)	Tulsi	Neem	Castor	Aloevera
1.	300nm	00	00	00	00
2.	400nm	2.84	2.96	2.93	2.37
3.	500nm	2.48	2.22	2.84	1.94
4.	600nm	2.20	1.96	2.42	1.88
5.	700nm	1.80	1.62	1.94	1.74
6.	800nm	1.36	1.32	1.80	1.60

Table 2: Antibacterial activity from silver nanoparticles synthesized different medicinal plant extracts by disc diffusion method.

Sr. No	Microorganisms	Measurement of Zone of Inhibition in MM					
		25µl	50µl	75µl	100µl	Antibiotic	AVRAGE
A. Tulsi							
1	*E. Coli*	1	2.5	3	4	8	2.62
2	*Pseudomonas spp.*	1	1.5	2	2.5	5	1.75
3	*Staphylococcus aureus*	1	2	2.5	3	6	1.87
4	*Bacillus subtilis*	1	1.5	2	3	5	1.87
B. Neem							
1	*E. Coli*	2	3	3.5	4.5	8	3.25
2	*Pseudomonas spp.*	1	2	3.5	4	6	2.62
3	*Staphylococcus aureus*	2	2.5	3	4	7	2.87
4	*Bacillus subtilis*	1	1.5	3	4	5	2.37
C	**C. astor**						
1	*E. Coli*	2	1	2	3.5	6	1.87
2	*Pseudomonas spp.*	1	2	2.5	3	10	2.12
3	*Staphylococcus aureus*	1.5	2	4	5	8	3.12
4	*Bacillus subtilis*	1	1.5	2	3	5	1.87
D. Aloe vera							
1	*E. Coli*	1	1.5	2	2.5	6	1.75
2	*Pseudomonas spp.*	1	2	2.5	3	7	2.12
3	*Staphylococcus aureus*	1	2.5	3	5	8	2.87
4	*Bacillus subtilis*	1	2	3	3.5	4	2.37

Table 3: Antifungal activity from different medicinalplant extracts.

Sr. No	Fungal culture	Measurement of Zone of Inhibition in mm (at different concentrations)					
		25µl	50µl	75µl	100µl	Antibiotic	Average
A. Neem							
1	*Aspergillus niger.*	2	2.5	3	4	7	2.87
B. Tulsi							
1	*Aspergillus niger.*	1.5	2	3	4	6	2.62
C. Castor							
1	*Aspergillus niger.*	1	2	3.5	5	8	2.87
D. Aloe vera							
1	*Aspergillus niger.*	1	3	4	4.5	7	3.12

Conclusion

Silver nanoparticles have been successfully synthesized using a well-known medicinal plants like neem, castor, tulsi and aloe vera aqueous leaf extract. These study evaluated the antibacterial and antifungal efficiency of silver nanoparticles extract of 4 medicinal plants against 4 different bacteria and 1 fungus. The silver nano particles extract of 4 medicinal plants are found to be substantially active against microbe. In UV-absorbance study, the synthesized silver nanoparticles showed absorbance peak at 400nm wavelength. Results confirmed this protocol as simple, rapid, one step, ecofriendly, nontoxic and an conventional physical/ chemical methods. The synthesized silver nano particle using leaf extract exhibit good stability as well as good antimicrobial activity against *E-coli*, staphylococcus, Bacillus, Pseudomonas and *Aspergillus niger*.

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