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Research Article

SYNTHESIS OF SILVER NANOPARTICLES FROM PHYTOEXTRACTS AND TESTING THEIR POTENTIAL ANTICANCER ACTIVITY

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A B S T R A C T

Nanoparticles has gained importance in Nanotechnology for its unique properties and exhibiting medicinal activities. Size of these nanoparticles is one of the major factor for its applications in various fields. Synthesis of nanoparticles by chemical method becomes accountable for toxicity and hence Green synthesis is eco-friendly. In this study, Silver nanoparticles are synthesized from *Myristica malabarica, Vernonia amygdalina* and *Zanthoxylum ovalifolium*, an Indian flora found in Western Ghats ecosystem rich in secondary metabolites. The extracts of these medicinal rich plants when added to different concentration of silver nitrate yields Silver nanoparticles which upon visualization by UV-Visible Spectrophotometer had a characteristic peak between 410-440nm, the ideal wavelength range for Silver nanoparticles to exhibit maximum absorbance due to its Plasmon resonance frequency phenomenon. The characterization using Dynamic Light scattering, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscope techniques allowed its study against breast cancer cell lines to test their anticancer ability. This present study of Green synthesis aimed at less toxicity and eco-friendly environment.

INTRODUCTION

Nanotechnology deals with synthesis and manipulation of atoms and/or molecules having size between 1 to 100 nm. The nanoparticles in comparison with larger particles of bulk materials have unique properties due to its specific characteristics like size, morphology and distribution [4]. Nanoparticles synthesis by physical, chemical and biological approach are generally considered. The use of chemicals in chemical approach are toxic and hazardous to the environment, high radiation techniques are often not cost-effective approach for nanoparticle synthesis and hence green synthesis or the biological approach is often preferred as it is non-toxic and a cost-effective technique.

In this present study, synthesis from Phytoextracts were studied and characterized for its properties. The Silver nanoparticles were obtained from the phytoextracts of *Myristica malabarica, Zanthoxylum ovalifolium* and *Vernonia amygdalina*, the Indian flora considered to be rich in secondary metabolites, that help in nanoparticle synthesis by the process of reduction and behave as capping agents. The characterization of the synthesized nanoparticle by Dynamic Light Scattering, Scanning Electron Microscope, Fourier Transform Infrared Spectroscopy and UV-Visible Spectroscopy allowed the study of their anti-cancer potential of nanoparticles synthesized from plants.

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METHOD

Preparation of Phytoextracts

Fresh leaves of *Myristica malabarica, Vernonia amygdalina and Zanthoxylum ovalifolium* were collected from the Western Ghats ecosystem. The leaves were washed with tap water followed by washing with distilled water and shade dried overnight. Dried leaves were homogenized into powder using a grinder, 15g of the powder was added to boiling deionized water of about 200mL and the process of decoction was carried out for a period of 30 minutes [5]. The crude extract obtained was filtered using Whattman filter paper and the filtrate obtained as plant extract was used for synthesis of silver nanoparticles, the extract obtained was stored at 4˚C for further use.

Silver Nanoparticles synthesis

Silver nanoparticles were synthesized by taking 3 different concentration of silver nitrate (3mM, 5mM and 7mM). Equal amounts of about 30mL each of different plant extract and silver nitrate were mixed. The mixture was then incubated at 37˚C in dark overnight and the bioreduction reaction of silver from Ag^{+1} to Ag^{+0} was confirmed by measuring the UV-Visible spectra of the mixture [6].

$Ag^{+} NO^{3-}$ + plant extract → Ag^{0} NPs+ byproducts

Purification and Characterization of Silver Nanoparticles

The silver nanoparticles synthesized were centrifuged at 10000 rpm for 30 minutes at 4˚C.The supernatant obtained is discarded and the pellet being repeatedly washed with distilled water which helps reduce the impurities. After every wash the sample is centrifuged, and the supernatant is discarded [4]. The pellet is then weighed to determine the concentration of silver nanoparticles per mL of distilled water added and later the pellet is dried in hot air oven at a temperature of 60˚C overnight to obtain in the form of powder. The powder obtained is then used for characterization using SEM, FTIR and DLS.

UV-Visible spectral analysis: Silver nanoparticles synthesized using various silver nitrate concentration were confirmed from UV-Visible analysis (Eppendorf bio spectrometer) where the optical property is being measured between 350-500 nm wavelength using 2 mL quartz cuvette having 1 cm path length.

Scanning electron microscopy analysis: The purified samples of Silver nanoparticles were used to study the morphology using SEM (Hitachi TM-3000). The powdered samples were made into smear on a slide and coated with platinum to make the samples conductive so that there is sufficient amount of accelerating voltage provided usually about 20kV.

Dynamic light scattering analysis: The average particle size of silver nanoparticles was studied using DLS (Horiba scientific) where the synthesized nanoparticle samples were diluted several times with water and used for determination of the particle size, the size distribution and the poly dispersity index (PDI).

***Fourier Transmission Infrared Spectroscopy analysis*:** The synthesized nanoparticles were studied for its chemical composition from FTIR (Perkin-Elmer LS-55 luminescence spectrometer). The purified and dried powder were characterized within 4000-400 cm^{-1} of wavenumber by the process of KBr pellet technique.

Anticancer Activity

Test Solution: For studying cytotoxicity, 2.5mg/ml stocks were prepared using water and were diluted from 320µg/ml to 10µg/ml using DMEM plain media for treatment.

Culture Medium

The MCF-7 breast cancer cell lines were obtained, and stock cells grown in DMEM (10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml)) in a 5% CO_2 incubator at 37^oC [6].The cells dissociated using the solution that helps in cell dissociation (0.05 % glucose, 0.02 % EDTA, 0.2 % trypsin in PBS).The Cell viability were checked and centrifuged. Cells were added to 96 well microtiter plate and incubated at 37^oC in 5 % CO_2 incubator for 24 hrs.

Diluted cell suspension of about 100µL were added to 96 well microtiter plate. A partial monolayer formed is seen, and the supernatant is removed after 24 hrs incubation. Monolayer is washed with DMEM and different concentration of test drugs. Followed by incubation at 37^oC overnight in 5% CO_2 atmosphere. Then test solutions is removed and 100 µl of MTT (6 mg/10 ml of MTT in PBS) was added. Plates were incubated at 37^oC in 5% CO_2 atmosphere for a period of 4 hrs. Supernatant was discarded and DMSO was added of about 100µL and to solubilize the formazan formed. The sample was checked for its absorbance @590 nm. From the absorbance value IC_{50} value was calculated.

RESULTS

Silver nanoparticles in the solution are known to have dark brown or dark reddish color. The reason why silver nanoparticles exhibit this color is due to its Surface Plasmon Resonance Frequency phenomenon which helps silver exhibit characteristic peak between 410-445 nm when analyzed using UV-Visible spectrophotometer.

Table 1 UV-Visible spectral analysis

Phytoextracts	$AgNO_3$ concentration	Absorbance @peak (410-445 nm)
Myristica malabarica *Vernonia amygdalina* *Zanthoxylum ovalifolium*	3mM	0.513 (421 nm) 0.674 (418 nm) 0.448 (445 nm)
Myristica malabarica *Vernonia amygdalina* *Zanthoxylum ovalifolium*	5mM	0.934 (417 nm) 0.882 (415 nm) 0.793 (438 nm)
Myristica malabarica *Vernonia amygdalina* *Zanthoxylum ovalifolium*	7mM	1.284 (421 nm) 0.892 (423 nm) 0.880 (438 nm)

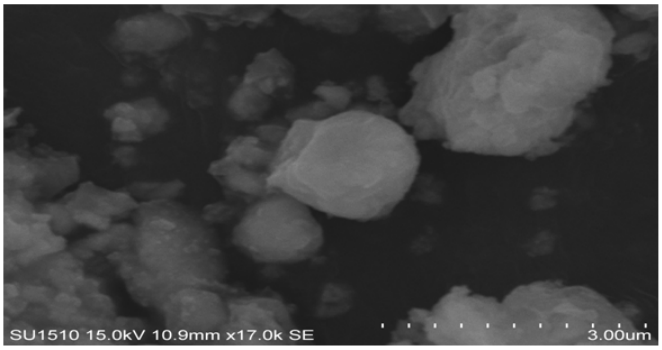
Also, higher the silver nitrate concentration better is the absorbance which implies that more silver nanoparticles are synthesized. Hence, Silver nanoparticles synthesized from higher concentration of silver nitrate was considered for characterization and anticancer studies.

Purification of Silver Nanoparticles

The purpose of silver nanoparticles to be purified by centrifugation at 10000 rpm and washing the pellet repeatedly is to remove the impurities present on the surface of silver nanoparticles like the phytochemicals. Also, the reason for centrifugation being carried out at higher speed is to prevent agglomeration of the nanoparticles, centrifugation carried out at higher speed initiates settling of larger particles first and since larger particles have lower surface area to volume ratio it prevents agglomeration and later the smaller particles settle down. In this manner the problem of nanoparticles getting agglomerated is prevented.

SEM Analysis

SEM analysis provides the morphology of nanoparticles. From ***Figure. 1*** the silver nanoparticles synthesized from *Myristica malabarica, Vernonia amygdalina* and *Zanthoxylum ovalifolium* are spherical in shape.



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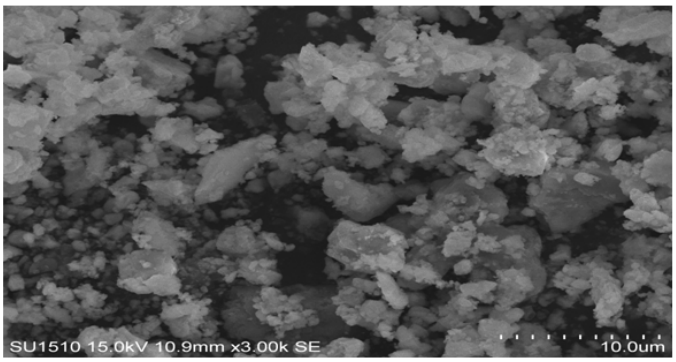

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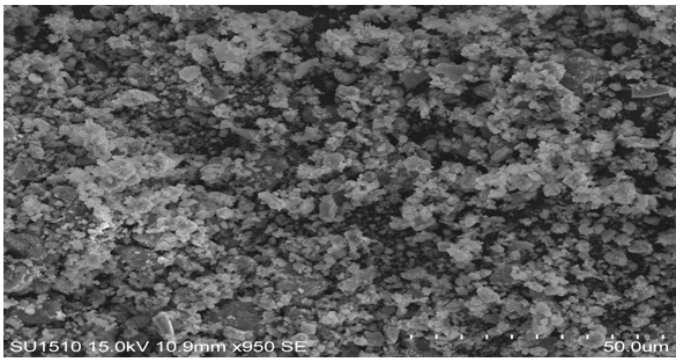
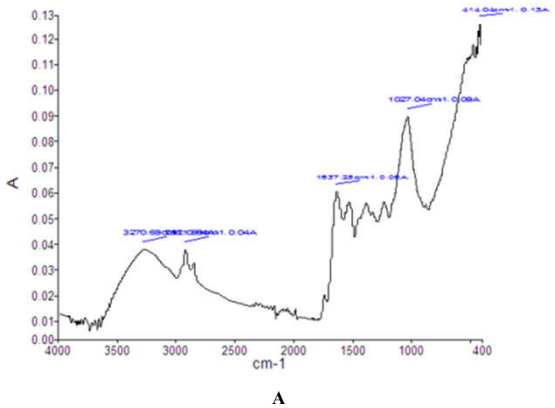


C

Figure 1 SEM images for Silver nanoparticles of a) Myristica malabarica, b) Vernonia amygdalina and c) Zanthoxylum ovalifolium

FTIR Analysis

Analysis of silver nanoparticles using FTIR helps identify the possible functional groups of phytochemicals that carries out bioreduction of $AgNO_3$ to Ag nanoparticles. FTIR shows how silver nanoparticles are biofabricated by the plants having phytochemicals.



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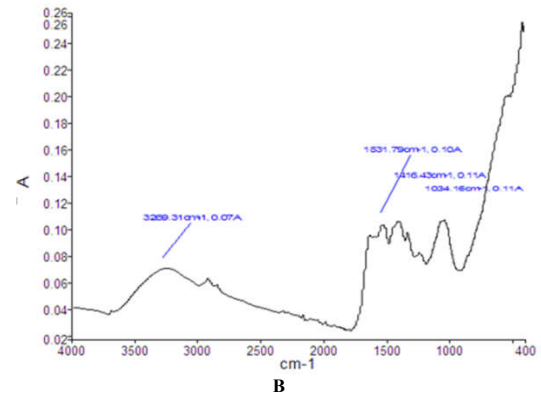

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Figure 2 FTIR spectra of Silver nanoparticles synthesized from a) Myristica malabarica andb) Zanthoxylum ovalifolium

Table 2 FTIR peaks in Silver nanoparticles synthesized from a) Myristica malabarica and b) Zanthoxylum ovalifolium

Peak number	X (cm^{-1})	Y (A)
1	3270.69	0.0382
2	2921.99	0.038
3	1637.25	0.0607
4	1027.04	0.0899
5	414.04	0.1262

Peak number	X (cm^{-1})	Y (A)
1	3269.31	0.0714
2	1531.79	0.1044
3	1416.43	0.1069
4	1034.16	0.1074

For Silver nanoparticles biofabricated from *Zanthoxylum ovalifolium* the peaks were identified at 3269.31 cm^{-1}, 1539.71 cm^{-1}, 1416.43 cm^{-1} and 1034.16 cm^{-1} indicating the presence of O-H absorption band (hydrogen bonded alcohols, phenols), C=C aromatic rings, C-H absorption band (alkanes) and C-O absorption band (alcohols, ethers, carboxylic acid, esters). From ***Table 2*** **(a)** the peaks were identified at 3270.69 cm^{-1}, 2921.99 cm^{-1}, 1637.25 cm^{-1}, 1027.04 cm^{-1} and 414.04 cm^{-1} respectively for silver nanoparticles biofabricated from *Myristica malabarica.* The peak therefore shows the presence of O-H absorption band (hydrogen bonded alcohols, phenols), C-H absorption band (alkanes), C=C absorption band (alkenes), C-O absorption band (alcohols, ethers, carboxylic acid, esters) and any peak less than 800 cm^{-1} is the silver nanoparticles. From this data it can be interpreted that the plant source used are rich in phenols, terpenoids, flavonoids and other compounds that are bound to the surface of silver nanoparticles and behave as capping agents and stabilize them.

DLS Analysis

Dynamic light scattering gives the size, the size distribution profile and poly dispersity index of the nanoparticles synthesized which is silver in this case present in colloidal suspension. ***Table 3.*** shows the size distribution profile of the silver nanoparticles obtained from *Myristica malabarica, Vernonia amygdalina* and *Zanthoxylum ovalifolium* using DLS also the table shows the poly dispersity index (PDI) which is the measure of silver nanoparticle distribution between 0.0-0.5.PDI value of less than 0.5 indicates that the nanoparticles are not aggregated/agglomerated and PDI value more than 0.5 is the indication of nanoparticles being aggregated/agglomerated.

Table 3: DLS data of Silver nanoparticles synthesized from the Phytoextracts

Phytoextracts	DLS (size)	PDI
Myristica malabarica	112.0 nm	0.373
Vernonia amygdalina	68.4 nm	0.524
Zanthoxylum ovalifolium	22.6 nm	0.296

Anticancer Activity

Table 4 shows that as the concentration is increased the color intensity of formazan decreases indicating that the number of viable cells are being reduced as depicted in %inhibition values. The %inhibition data helps determine the IC_{50} value which tells the significant concentration at which 50% of the viable cells are being killed.

Table 4: Concentration in µg/mL, OD at 590 nm and %inhibition for silver nanoparticles of a) Myristica malabarica, b) Vernonia amygdalina and c) Zanthoxylum ovalifolium

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition
Control	0	0.813	0.00
	10	0.741	8.80
	20	0.682	16.10
Myristica	40	0.610	24.92
nanoparticles	80	0.420	48.31
	160	0.201	75.30
	320	0.146	82.01

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition
Control	0	0.813	0.00
	10	0.750	8.40
	20	0.691	15.01
Vernonia	40	0.625	23.12
nanoparticles	80	0.410	49.57
	160	0.235	71.09
	320	0.160	80.32

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition
Control	0	0.813	0.00
	10	0.721	11.32
	20	0.622	23.49
Zanthoxylum	40	0.570	29.89
nanoparticles	80	0.405	50.18
	160	0.225	72.32
	320	0.150	81.55

Figure. 3 shows the anti-cancer activity for Silver nanoparticles of Phytoextracts after MTT assay where the viable cells having mitochondrial dehydrogenase enzyme converts MTT into formazan (purple colored). This gives the %inhibition data after measuring the resulting purple solution spectrophotometrically.

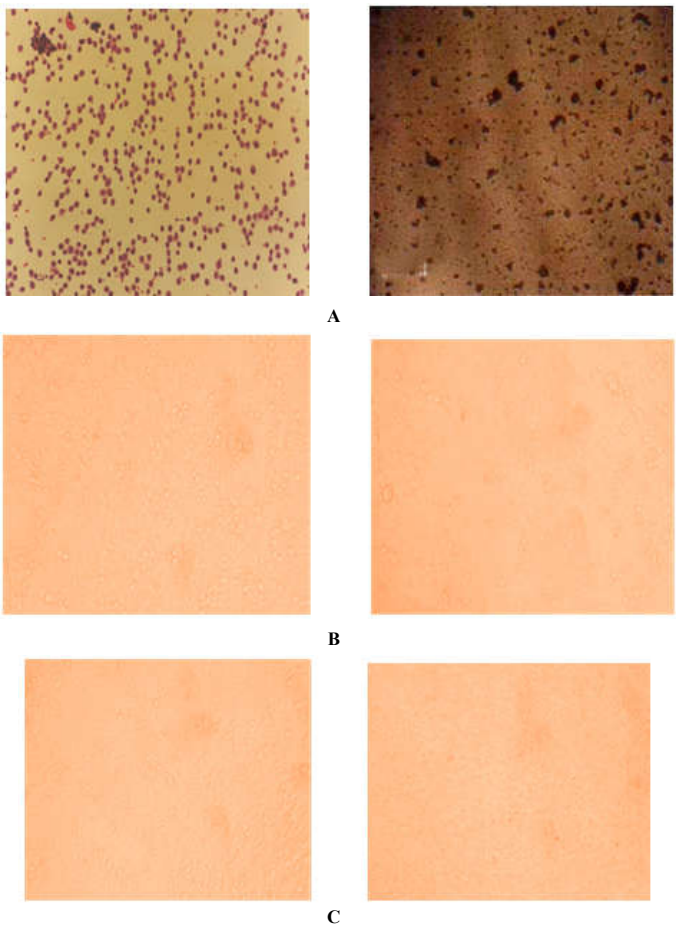

Figure 3 Control of MCF-7 breast cancer cell line and Silver nanoparticles of a) Myristica malabarica, b) Vernonia amygdalina and c) Zanthoxylum ovalifolium

CONCLUSIONS

The synthesis of Silver nanoparticles by green synthesis proved to be less time consuming, very effective, more potent and of much importance as the method employed was eco-friendly. The Silver nanoparticles synthesized from *Myristica malabarica, Vernonia amygdalina* and *Zanthoxylum ovalifolium* were spherical in shape when analyzed using SEM and the size was found to be within 120 nm from DLS analysis.

The PDI values from DLS showed that the nanoparticles synthesized were free from agglomeration and from FTIR spectra analysis the presence of certain functional groups like aldehydes, ketones, carboxylic acid, alcohols and phenols indicated the presence of phytochemicals that helped in stabilizing and capping of nanoparticles which are known to prevent agglomeration of the nanoparticles. Of the three plants used it was found that *Zanthoxylum ovalifolium* had smallest of silver nanoparticles synthesized of about 22.6 nm size.

The Silver nanoparticles synthesized from *Myristica malabarica, Vernonia amygdalina and Zanthoxylum ovalifolium* showed anticancer activity against MCF-7 breast cancer cell lines and the positive results against MCF-7 breast cancer lines has led to future studies against other cancer cell lines, also studying their antimicrobial activity.

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