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FACILE GREENY BIOLOGICAL NANOSYNTHESIS OF SILVER NANOPARTICLE USING PLANT LEAF ESSENTIAL OIL COMPOUND CITRONELLOL AGAINST HUMAN PATHOGENIC FUNGI

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ABSTRACT

The plant oil compound citronellol is enriched with bioactive compounds and displays multiple biological actions. Thus, the study planned to biosynthesize antifungal potent silver nanoparticles. The synthesized silver nanoparticles were confirmed by color transformation and Ultraviolet-visible (UV-visible) spectrophotometery at 480 nm. The size and morphology of the silver nanoparticles were characterized by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The presence of chemical compounds in silver nanoparticles was detected by Fourier Transform InfraRed spectroscopy (FTIR). The effect of synthesized silver nanoparticles with citronellol over pathogenic fungi such as *Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis* and *Candida kefyr* were examined which showed very high activity. The appearance of black color confirmed the synthesized silver nanoparticles. The silver nanoparticles showed large round shaped structure under scanning electron microscope and in transmission electron microscope the sizes were about 17 nm.

KEYWORDS: *Candida albicans*, Green synthesis, Ftir, fluroscence spectroscopy, silver nanoparticles.

1. INTRODUCTION

Synthesis of metal nanoparticles and their characterization has been an emerging field of nanotechnology since the past few decades because of their unique properties and potential application in the fields of physics, chemistry, biology and medicine (Vivek *et al*., 2011). Widely, nanoparticles are synthesized by different routes. However, the synthesis of nanoparticles by chemical methods is not environment friendly. Therefore, the synthesis of nanoparticles by biological route (plant extract and plant oil compounds) is the suggested alternative to the non eco-friendly methods (Song and kim,2009). The noble metals (Ag and Au) are widely used for the synthesis of nanoparticles (Hubenthal,2011) Among the noble metals, silver is the metal of choice because it is used as a health additive in traditional medicine (Dhanalakshmi and Rajendran, 2012) shows a strong toxicity over microorganisms. In addition, silver and its derivatives are widely used to treat many bacterial infections and burns (Radstig *et al*.,2008;Legendre et al., 2011; Samberg *et al*., 2011; Pettinari *et al*., 2011) However, the silver ions or salts have limited application as antimicrobial agents due to its inadequate release. The study hypothesize that the above limitation can be overcome by using silver nanoparticles, as these are highly reactive species due to their large surface area (Yacaman et al., 2001).

The bioreduction of Ag+ to silver nanoparticles involves plant extracts and plant oil compounds (Sanghi and Verma, 2009; Ghodake et al., 2010) It has been shown that variety of plant extracts served as green reactants in silver nanoparticles synthesis (Li *et al*., 2008; Vilchis-Nestor *et al*., 2008; Tavera-Davila *et al*.,2009;Loo *et al*., 2012) A recent study has demonstrated that the synthesized silver nanoparticles using plant oil compound citronellol displayed moderate anti-plasmodial activity (Pannerselvam *et al*., 2011).

The present review emphasizes reported plant leaf extract and many plant leaf oil compounds for the synthesis of different nanoparticles. Plants are known to possess various therapeutic compounds which are being exploited since ancient time as a traditional medicine. Due its huge diversity plants have been explored constantly for wide range of applications in the field of pharmaceutical, agricultural, industrial etc. Recent reports of plants towards production of nanoparticles is said to have advantages such as easily available, safe to handle and broad range of biomolecules such as alkaloids, terpenoids, phenols, flavanoids, tannins, quinines etc.are known to mediate synthesis of nanoparticles. With the advent of advance technologies

and improved scientific knowledge have paved a way for research and development in the field of herbal and medicinal plant biology towards intersection of nanotechnology.

2. MATERIALS AND METHODS

2.1 Plant leaf essential oil compounds

The plant leaf essential oils compounds were purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India). The silver nitrate were purchased from HiMedia (Mumbai, India).

2.2 *Biological green synthesis of silver nanoparticles*

About 5 ml of plant oil compound citronellol was mixed with 24 ml of 2 mM silver nitrate solution and kept in shaker at 37°C for 24 h and the color change was observed. The bio reduction of silver ions in the solution was monitored by sampling the aqueous component after incubation period and the absorption maxima was scanned at different wavelengths (420-500 nm) using a UV-Visible spectrophotometer.

2.4. Characterization of silver nanoparticles

After the incubation period, the silver nitrate treated with plant oil compound citronellol was centrifuged at 9,000 rpm for 15 min. The supernatant was taken for the analysis of size, shape and stability of the bioreduced silver nanoparticles using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Fourier Transform Infra Red Spectroscopy (FTIR).

2.5. *SEM analysis of silver nanoparticles*

Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. The excess solution was removed using a blotting paper. The film on the SEM grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using Scanning Electron Microscope.

2.6. *TEM analysis of silver nanoparticles*

Samples for TEM analysis were prepared by placing a drop of the silver colloidal solution on a TEM copper grid (200 meshes, carbon-coated, colloid on covered). The films on the TEM grids were dried and the excess solution woperated at an accelerating voltage of 80 KV. The size and shape of the bioreduced silver nanoparticles were obtained from TEM images. as removed using blotting paper. TEM measurements were performed on TECNAI 10 Philips;

the instrument was operated at an accelerating voltage of 80 KV. The size and shape of the bioreduced silver nanoparticles were obtained from TEM images.

2.7. FTIR analysis of silver nanoparticles

The AgNO3 treated with plant oil compound citronellol was centrifuged at 9,000 rpm for 25 min. The pellet was washed thrice with 20 ml of deionized water to get rid of free proteins/enzymes. The residue was dried and mixed with potassium bromide (KBr). The pellet was used for FTIR analysis in the range of 400-4000 cm-1 at a resolution of 4 cm-1.

2.8 XRD (X-ray diffraction)

The characterization of the purified IhAgNO3 was conducted with an XRD 6000 X-ray diffractometry (shimadzu, Japan) operated at voltage of 40 kV and current of 30 mA with cu k radiation in θ 20 configurations.

2.9 UV-Vis spectral analysis

The colour change in reaction mixture (silver ion solution + oil compound citronellol) was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots (0.5 *ml*) and subsequently measuring UV-Vis spectra of the solution. UV-Vis spectra of these aliquots was monitored as a function of time of reaction on UV-Vis spectrophotometer.

2.10 Fluroscence spectroscopy analysis

All spectrofluorimetric measurements were carried out by a Shimadzu RF-5301PC spectrometer. A xenon lamp source was used for excitation. The excitation and emission slit widths were 3 nm. The acquisition interval and the integration time were maintained at 2 nm and 0.02 s respectively. The oil samples were diluted in n-hexane (1% V/V) in a 10mm fused quartz cell. The low concentration of the samples was chosen to avoid spectral distortions, which may occur in more concentrated solutions (Sikorska *et al.*,2008) The synchronous fluorescence spectra were collected by simultaneously scanning the excitation the emission monochromators in the 220–900 nm range, with a constant wavelength difference $\Delta\lambda$ = 30, 40 and 50 nm between them.Minitab 16 was used for data processing and analysis.

Anti-fungal test

Agar well diffusion method

The antifungal analysis was done by the methods of (Ahmad *et al.*,1993) The synthesized silver nanoparticles of plant oil compound farnesol was taken upto 20µl for assay. Culture

suspension of 116 ul of the tested microorganisms 10^6 colony –forming unit (cfu)/ml of fungal cells (estimated absorbance at 600 nm) and 10^8 spores/ml of fungal strains were spread on potato dextrose agar medium. Then, bores(4mm depth, 6 mm diameter) were made using sterile cork-borer and were loaded with 25 µl of each sample Fluconazole and Amphoterecin-B were used as a positive reference. The fungal growth were noted after 48 hours and antifungal activity was evaluvated by measuring the diameter of the growth inhibition zones in millimeter. Then the values were tested through statistical analysis**.**

2. 10 Fungal cultures

The fungi used in this assay *Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis and Candida kefyr* was provided from Microlabs,Institute of research and technology, Arcot, Tamilnadu, India.

3. RESULTS

3.1Invitro antifungal test

The plant leaf essential oil compound citronellol showed moderate antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr* in (Table. 1).The plant essential oil compound citronellol showed no activity active against *Candida albicans* (0.00±0.00) and moderate activity against *Candida tropicalis* (4.08±0.34). Silver nitrate showed no activity against any fungal species. The silver nanoparticle citronellol was highly active against *Aspergillus niger* (5.26±0.57) and very highly active against *Aspergillus flavus* (11.58±0.59). All fungi were found to be sensitive to all test essential oil compound and synthesized silver nanoparticle farnesol and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent.

Table 1. Antifungal activity of synthesized silver nanoparticle oil compound citronellol.

Microorganisms	citronellol oil compound	Agno3	silver nanoparticle citronellol	Antifungal agents
Aspergillus niger Amphoterecin -B	0.00 ± 0.00[a]	0.00±0.00[a]	8.27±0.89[a]	8.08± 0.67[a]
Aspergillus flavus	2.05±0.47[b]	0.00± 0.00[b]	11.78 ±0.59[b]	14.26±0.56[b]
Amphoterecin-B *Candida albicans*	0.00±0.00[c]	0.00 ±0.00[c]	3.37±0.68[c]	26.48± 0.79[c]
Fluconazole *Candida tropicalis*	4.08 ±0.34[d]	0.00 ±0.00[d]	6.68±0.76[d]	24.48±0.17[d]
Fluconazole *Candida kefyr*	0.00± 0.00[e]	0.00±0.00[e]	2.58 ±0.25[e]	29.11±0.79[ce]

The values are represented as the Mean ± SD of plant leaf essential oil compound citronellol and synthesized silver nanoparticle citronellol. These plant leaf essential oil compound and synthesized silver nanoparticle citronellol have significant effect at 0.05 levels.

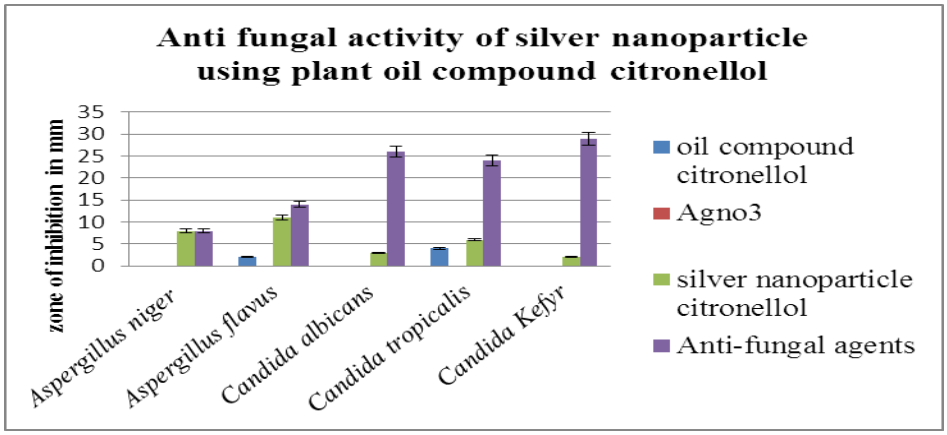
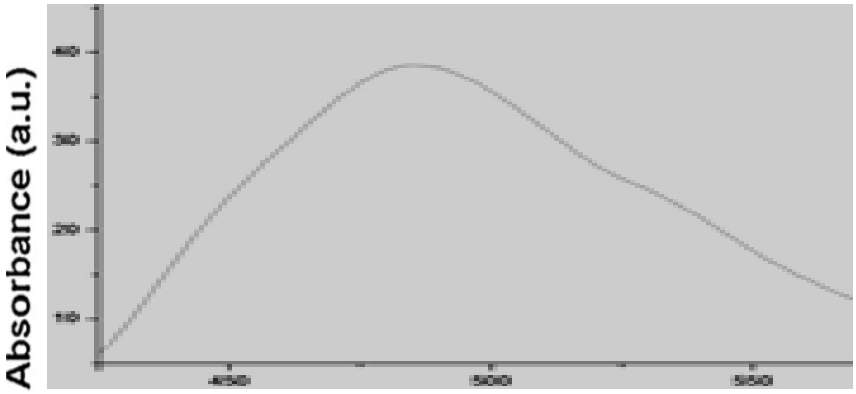


Fig 1. Inhibition of growth of selected fungi by synthesized silver nanoparticle from plant leaf essential oil compound Citronellol.

3.2 Greeny biological synthesis of silver nanoparticles

The 8 ml of plant oil compound citronellol was added to 90 ml of 2 mM Agno3 solution then after fifteen minutes time duration the colour changed into greyish colour after that the conical flask was kept for prolong incubation in a dark room for 78 hours of incubation which indicated the black colour formation showed the presence of silver nanoparticles. The UV–Vis spectra of silver nanoparticles synthesized by plant leaf oil compound farnesol are showed broad peak observed at 480 nm was seen in (Fig. 3).

Silver nitrate solution **Synthesised silver nanoparticles**



Wavelength n/m

3.3 Scanning electron microscope

The scanning electron microscope study showed the structure of synthesized silver nanoparticles was large round dispersed here and there was shown in.(Fig 3a).The analysis of energy dispersive spectroscopy (EDS) of the silver nanoparticles the presence of elemental silver signal was confirmed. (Fig 3b)

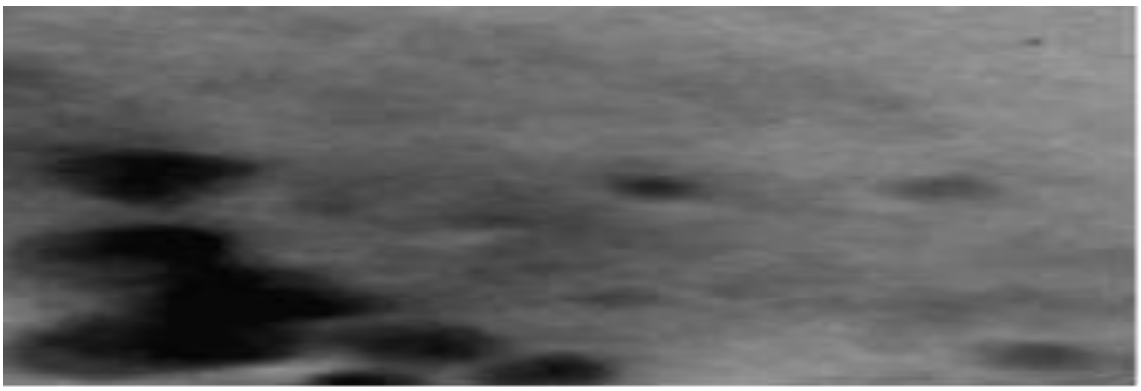


Fig (3a) Scanning electron microscope image of silver nanoparticle synthesized by plant leaf oil compound citronellol

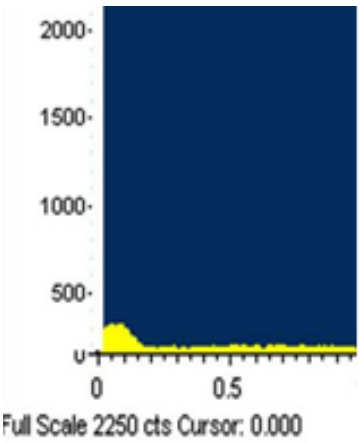


Fig (3b) Sem-EDS spectrum showed the presence of silver signal

3.4 Transmission electron microscope

The transmission electron microscope image of silver nanoparticle was showed in (Fig 4).The picture denotes that the very big round shaped silver nanoparticles are condensed with a diameter of 17 nm.

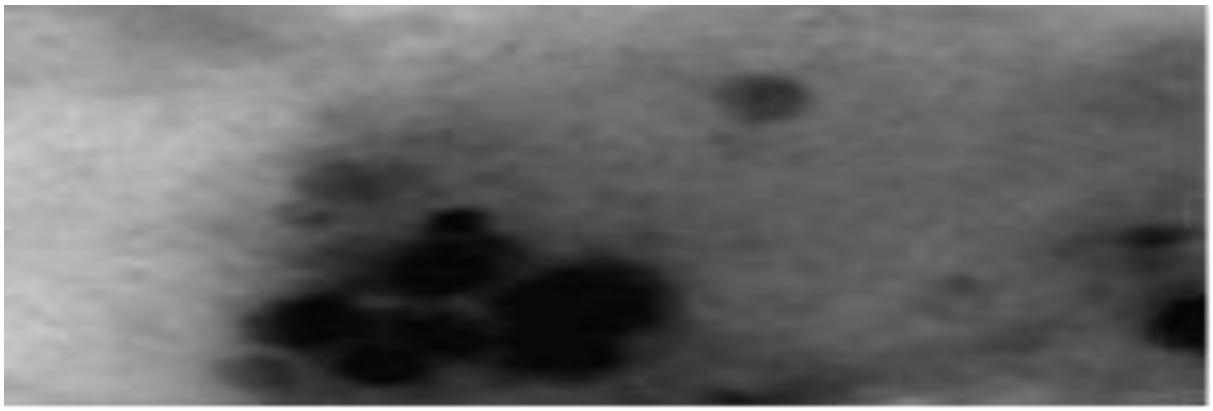
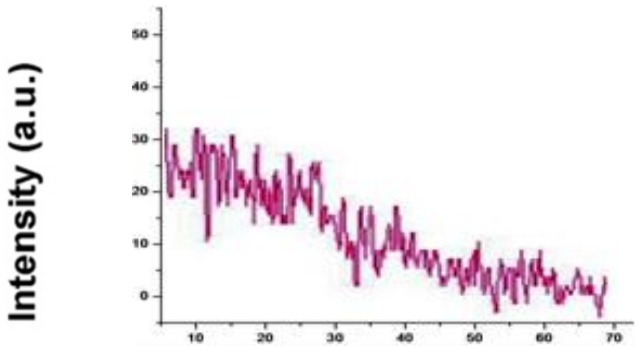


Fig (4) Transmission electron microscope image of silver nanoparticle synthesized by plant leaf oil compound citronellol

3.5 X-ray diffraction

The X-ray diffraction pattern of silver nanoparticle was shown in (Fig 5). The XRD pattern thus clearly illustrates that the silver nanoparticles present green synthesis method are powdery in nature.

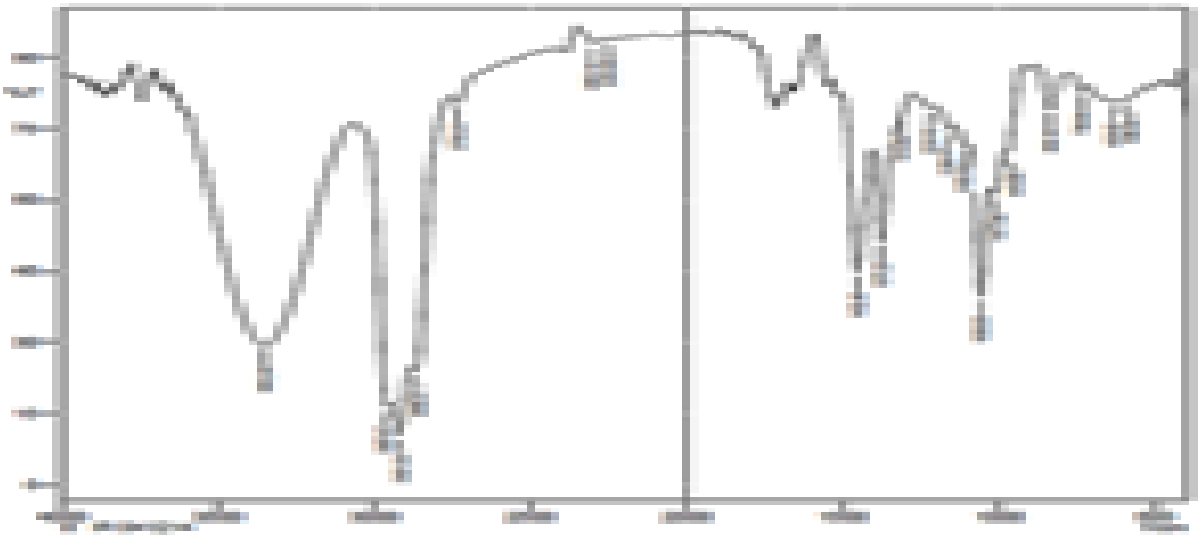


(Fig 5) XRD patterns of silver nanoparticles synthesized by plant leaf essential oil compound citronellol.

3.6 Fourier transform infrared spectroscopy

FTIR measurements were carried out to identify the potential biomolecules in plant oil compound citronellol responsible for reduction and capping of the bioreduced silver nanoparticles. The presence of four bands at about 3491.99 cm^{-1}, 2947.45 cm^{-1}, 2847.68 cm^{-1},

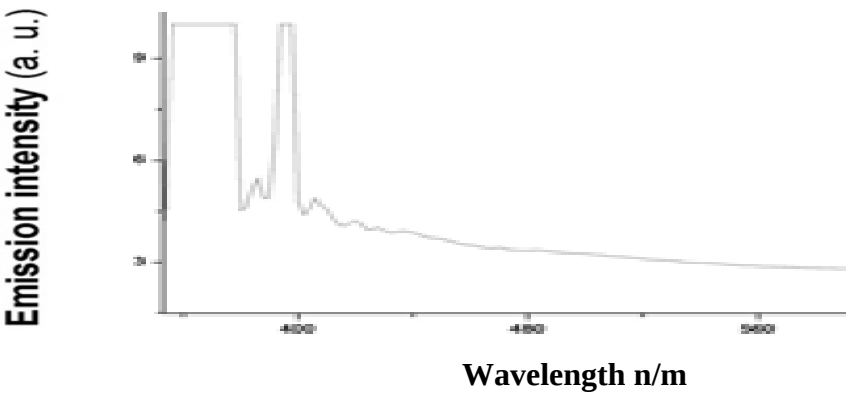
2643.78 cm^{-1}, 2543.24 cm^{-1}, 1403.56 cm^{-1}, 1302.89 cm^{-1}, 1079.99 cm^{-1}, 897.11 cm^{-1}. (Figure 6). The absorption band at 3491.99 cm−1 which is characteristic of the OH stretching of phenolic group. The absorption bands at 1403.56 and 1302 cm−1 correspond to carbonyl group present in the extract. The sharp band at 1079.99 cm−1 indicated C O group of ester and the band at 897.11 cm−1 is due to aromatic CH stretching vibrations.



(Fig 6). FTIR spectrum of vacuum dried powder of silver nanoparticles synthesized by plant leaf essential oil compound citronellol.

3.7 Fluroscence spectroscopy

The fluroscent spectra of synthesized gold nanoparticle were shown in (Fig 7). A broad emission band having prominent peak centered at ~420 nm is observed for the plant oil compound citronellol as it is excited at 400 nm. In this study emission intensity gradually increases with the decreasing concentration of Agno3. This decreasing intensity suggest that due to the close proximity of emissive species with nanoparticles, quenching of emission take takes place through energy transfer process.



DISCUSSION

In plant oil compound citronellol the carbohydrates, proteins, amino acids, tannins, Saponins, and steroids flavonoids, alkaloids and phenolics are present Ponarulselvam *et al.*,2012. The silver nanoparticles exhibited excellent antibacterial and antifungal activity with synthesized silver nanoparticle citronellol. The study suggests that the membrane interacting ability and its function modulating nature of silver nanoparticles synthesized by the plant oil compound citronellol could be the reasons for fungicidal activity of reduced metal nanoparticles (Dibrov *et al*.,2002) The fungicidal effect of silver nanoparticles is due to the inhibitory action of plant metabolite reduced silver and the suggested mechanism involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes (Cowan,1999) It has been suggested that polyol components are mainly responsible for the reduction of silver ions (Huang et al.,2007) Thus, the study suggest that water-soluble active constituents like polyphenols, flavonoids and terpenoids contents of extract might reduce Ag+ into Ag0.

It has been proven that the antifungicidal property of silver nanoparticles is size dependent. The silver nanoparticles <20 nm in size could attach to cell membrane and thereby disturb membrane functions and its interaction with biomolecules like proteins and DNA leading to more damage and also cardiac treatment in albino wistar rats. (Patil *et al*.,2012;Kevitek *et al*.,2008) The fungicidal effect of silver nanoparticles is due to the inhibitory action of plant oil compound reduced silver and the suggested mechanism involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes.

Silver nanosynthesis using plant essential oil compounds such as farnesol and citronellol may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, and medicine and water treatment. This increasing demand must be accompanied by ‘‘green’’ synthesis procedures. Nanosilver has many important applications. It is used as an antimicrobial agent. It is applied in textiles, home water purification systems, medical devices, cosmetics, electronics and household appliances.(Jabakumar and sethuraman, 2012)

The larvicidal property of silver nanoparticle synthesis using plant oil compound citronellol may be accounted for its effect on digestive tract enzymes, structural deformation in DNA, generation of reactive oxygen species (Feng *et al*.,2000;Li and Williams 1997; Patil *et*

al.,2012)Increased larvicidal spectrum may also be due to synergistic combination of AgNPs and proteins and other secondary metabolites adhering on AgNPs surface during reduction and stabilization and AgNPs. Different researchers (Naik *et al*.,2002;Kemp *et al*.,2009) reported similar reports of binding of protein and carbohydrate on silver and gold nanoparticles surface.

CONCLUSION

The biosynthesized silver nanoparticles using plant oil compounds displayed good antifungal activity over tested human pathogens. From a technological point of view, the obtained silver nanoparticles have potential applications in the medical field and this simple product has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications, as well as, large-scale commercial production. With the potential for commercial gain from silver nanoparticles antimicrobial fabrics comes a special responsibility to communicate openly and accurately about the strengths and weaknesses of the technologies. Biological approaches using plant oil compounds for metal nanoparticles synthesis have been suggested as valuable method to kill all the nosocomial infections causing pathogenic bacteria *Escherichia coli* and *Klebsiella pneumoniae*.

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