

**GREEN SYNTHESIS OF SILVER NANOPARTICLES AND  
CHARACTERIZATION USING PLANT LEAF ESSENTIAL OIL  
COMPOUND CITRAL AND THEIR ANTIFUNGAL ACTIVITY  
AGAINST HUMAN PATHOGENIC FUNGI**

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### **ABSTRACT**

The various plant materials used for the biosynthesis of nanoparticles is considered a green technology as it does not involve any harmful chemicals. In this paper we have reported the green synthesis of silver (AgNPs) nanoparticles by reduction of silver nitrate into silver using plant essential oil compound citral. In this research the synthesis of silver nanoparticles using citral has been investigated by UV-Spectroscopy showed the peak at 440 nm. The synthesized uniformly dispersed silver nanoparticles with spherical shape was showed in scanning electron microscope and with an average size of 45 nm was shown in transmission electron microscope. The x-ray diffraction technique showed the powdery nature of silver nanoparticle and in Ftir Spectroscopy the chemical groups such as amide and methylene of synthesized silver nanoparticle was noted. The antifungal activity

results showed that synthesized silver nanoparticle citral was highly active against clinically isolated human fungal pathogens, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr*.

**KEYWORDS:** *Candida albicans*, green synthesis, x-ray diffraction, silver nanoparticles, tem.

### **INTRODUCTION**

In the last decade, the biosynthesis of nano particles, as a representative intersection of nanotechnology and biotechnology has received increasing attention due to the growing need

to develop environmentally benign technologies in material syntheses. Although many synthetic technologies are well documented, the search for suitable biomaterials for the biosynthesis of nanoparticles continues among researchers worldwide. Early this decade, the potential of various microbes and plant biomasses for the synthesis of nano metals was explored. Sastry and co-workers examined the possibility of using microbes and plant materials as nano-factories (Mukherjee et al., 2001; Ahmad et al., 2002; Sastry et al 2003; Shankar et al., 2003; Shankar et al., 2004; Rai et al., 2006).<sup>[1,7,10,13,14]</sup> Since then, various microorganisms and plants have been employed for the synthesis of nanoparticles. In recent years, the biosynthetic method using plant extracts has received more attention than chemical and physical methods, and even than the use of microbes, for the nano-scale metal synthesis due to the absence of any requirement to maintain an aseptic environment. (Gardea-Torresdey et al., 2002; Gardea-Torresdey et al., 2003).<sup>[4,5]</sup> initially reported the possibility of using plant materials for the synthesis of nano-scale metals. Later, the bio reduction of various nano-sizes of various shapes, capable of meeting the requirements of diverse industrial applications, was extensively studied. Green processes with the use of economic, efficient and ecofriendly catalysis are gaining much importance due to the benefits associated with their use. The major advantage of green synthesis of nano materials is their important role in protecting the environment (Saifuddin et al., 2009).<sup>[11]</sup> All the green and clean technologies are expected to minimize things that contribute to environmental problems air and water emissions, greenhouse gases, non- renewable or toxic substances or materials. The utilization of products and materials from various plant species as well as several other biological materials for the synthesis of nano Ag particles are directly related to the mechanisms of nanotechnology and green chemistry. More and more research inputs are rendered for this and there has been an upsurge of interest in the biological synthesis of nano materials by using several plants, plant pure compounds, plant biochemical and microorganisms in the past few years (Song et al., 2008; Egorova et al., 2000; Mukherjee et al., 2002; Zhang et al; 2011).<sup>[3,8,21]</sup>

Biosynthetic methods for the production of nano particles have gained considerable attention in the past decade. This is mainly because there is no requirement of synthetic chemicals for the production protocols. The synthesis of nano particles through chemical routes has the drawback of the colloidal solutions being contaminated with various byproducts of chemical reactions. Considerable efforts have already been put to develop environment friendly protocols for the synthesis of nanoparticles. For instance, a great deal of work has been done

to exploit microorganisms for the production of metal nanoparticles. Bacterial species like *Lactobacillus* strains, *Pseudomonas stutzeri* AG259, *Lactobacillus* sp. A09, *Thermomonospora* sp., etc. have been demonstrated to successfully reduce metal ions to metal nanoparticles. Apart from bacteria (prokaryotic organisms), many fungal species (eukaryotic organisms) such as *Aspergillus flavus*, *Fusarium oxysporum*, *Torulopsis* sp., *Schizosaccharomyces pombe*, *Verticillium* sp., etc. have also been used to grow nanoparticles of different chemical compositions and sizes, mainly because of the ease in handling such biomass. Use of these microorganisms for the biological production of nanoparticles has been well reviewed (Thakkar *et al.*, 2010; Vaidyanathan *et al.*, 2009).<sup>[18,19]</sup>

In present study we synthesized silver nanoparticles from plant essential oil compound citral and characterization technique was completed. Then antifungal activity was performed to check the potential activity of synthesized silver nanoparticle citral against the deadly pathogens which causes many disease to human beings.

## 2. MATERIALS AND METHODS

### Plant leaf essential oils chemical compounds

The essential oils chemical compounds were purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India). The silver nitrate (AgNO<sub>3</sub>), were purchased from Hi Media (Mumbai, India).

### Antifungal assay

#### Agar well diffusion technique

Antifungal activity was performed based on the methods of (Ozcan and Boyarz, 2000; Wang *et al.*, 2005).<sup>[9,20]</sup> The effect of synthesized silver nanoparticle oil compounds was tested against yeast and mycelial growth of *Aspergillus Niger* and *Aspergillus fumigatus*. using potato dextrose agar *in vitro*. Medium (20 ml) was dispensed into each petri plate and 6 mm diameter plugs of each species were excised from the margin of diameter plugs of each species were excised from the margin of 14 day culture grown on potato dextrose agar. The plates were kept for incubation for 2 days. The colony diameter was measured at several points and the percentage was calculated through statistical analysis.

### Fungal cultures

The fungal isolates *Aspergillus Niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr* was used for antifungal activity. These cultures are received

from Christian medical college Vellore Tamil Nadu, India.

**UV–visible spectroscopic characterization of AgNPs** UV– visible spectra of these aqueous colloid samples of synthesized silver nanoparticle citral (1 mM) were measured on a Labomed Model UVD-2950 UV-VIS Double Beam PC Scanning spectrophotometer, operated at a resolution of 2 nm.

#### **FT-EDAX spectroscopic studies**

The functional groups present in the synthesized silver nanoparticle plant essential oil chemical compounds citral and their involvement in the synthesis of AgNPs was determined by the FT-IR studies. The dried aqueous extract and synthesized AgNPs were mixed with KBr to make pellet and the FT-IR analysis was carried out by JASCO FT-IR 400.

#### **XRD studies**

The synthesized silver nanoparticle plant essential oil chemical compounds geraniol solution was drop-coated onto a glass substrate and the XRD measurements were carried out using a Philips X' Pert Pro X-Ray diffractometer, with the following working conditions: CuK<sub>α</sub> Ni-filtered radiation; 40 kV, 30 mA; divergence slit 0.47°. A number of Bragg reflections with  $2\theta$  values of 38.03°, 46.18°, 63.43° and 77.18° correspond to the (111), (200), (220) and (311) sets of lattice planes are observed which may be indexed as the band for face centered cubic structure of silver. The XRD pattern thus clearly illustrates that the silver nanoparticles synthesized by the present green method are crystalline in nature.

#### **Transmission electron microscopy**

The synthesized silver nanoparticle of citral samples were analysed for high-resolution TEM analysis were prepared on carbon-coated copper grids. The films on the grids were allowed to dry in air prior to measurements on a JEOL model 3010 microscope operated at an accelerating voltage of 200 keV with wavelength ( $\lambda$ ) of 0.0251 Å. The size and morphology of nanoparticles were according to the transmission electron micrograph, the morphology of the synthesised silver nanoparticles from plant essential oil chemical compound citral was observed and approximately spherical. This reveals that the powder particles are slightly agglomerated but its size range of 90nm and the closed view of spherical nanoparticle has showed is a micrograph of the silver nanoparticles indicating that they are also spherical in shape in range of 60 nm. Above results suggested that the silver nanoparticles are synthesized due to the action of plant leaf essential oil citral, which act as good bio reluctant for

biosynthesis.

### Fourier transform infrared spectroscopy

In FTIR measurements. The synthesized silver nanoparticles citral samples were dried and analyzed on an Avatar330FTIR instrument in the diffuse reflectance mode operating in the range of  $-4000\text{Cm}_1$  and resolution of  $4\text{Cm}_1$

## 3. RESULTS

### *In vitro* antifungal activity

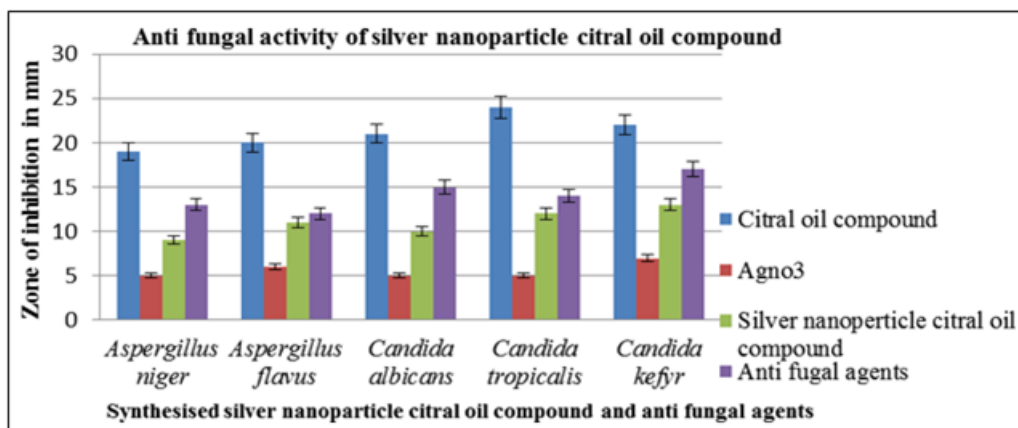
The plant essential oil compound citral showed notable antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Candida kefir* in (Table. 1). The essential oil compound citral was very highly active against *Candida tropicalis* ( $23.51 \pm 0.30$ ) and least against *Aspergillus niger* ( $17.50 \pm 0.28$ ). Silver nitrate solution was highly active against *Candida kefir* ( $10.23 \pm 0.33$ ) and least against *Aspergillus flavus* ( $5.09 \pm 0.29$ ). The silver nanoparticle citral was also highly active against *Candida kefir* ( $15.17 \pm 0.29$ ) and least against *Candida albicans* ( $9.87 \pm 0.28$ ). All fungi were found to be sensitive to all test essential oil compound citral and synthesized silver nanoparticle citral and mostly comparable to the standard reference antifungal drug Amphotericin B and ketoconazole to some extent.

**Table 1: Antifungal activity of synthesized silver nanoparticle citra.**

Microorganisms	Citral	Agno3 solution	Silver nanoparticle citral	Antifungal agents
<i>Aspergillus niger</i>	$17.50 \pm 0.28^a$	$6.56 \pm 0.29^a$	$11.33 \pm 0.14^a$	$12.54 \pm 0.06^a$
				(Amphoterecin –B)
<i>Aspergillus flavus</i>	$19.49 \pm 0.27^b$	$5.09 \pm 0.29^a$	$13.27 \pm 0.14^a$	$13.71 \pm 0.07^b$
				(Amphoterecin-B)
<i>Candida albicans</i>	$21.16 \pm 0.16^c$	$7.76 \pm 0.28^b$	$9.87 \pm 0.28^b$	$17.06 \pm 0.08^c$
				(Ketoconazole)
<i>Candida tropicalis</i>	$23.51 \pm 0.30^d$	$5.96 \pm 0.31^c$	$14.66 \pm 0.26^c$	$16.26 \pm 0.18^d$
				(Ketoconazole)
<i>Candida kefir</i> $22.64 \pm 0.35^e$		$10.23 \pm 0.33^d$	$15.17 \pm 0.29^d$	$20.85 \pm 0.09^e$
				(Ketoconazole)

The values are represented as the Mean  $\pm$  SD of essential oil compound citral and synthesized silver nanoparticle citral.

These essential oil compound citral and synthesized silver nanoparticle citral have significant effect at 0.05 levels.



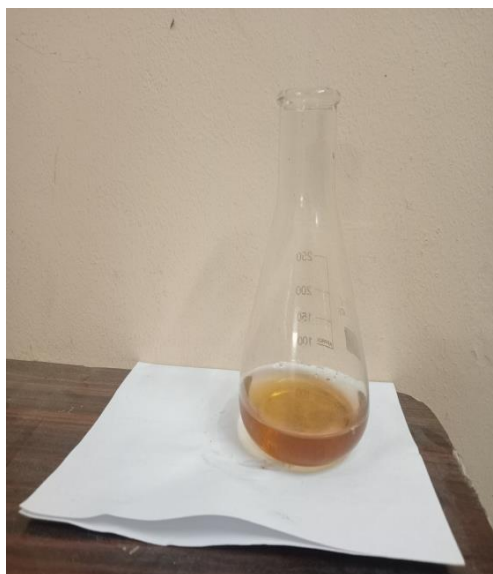
**Fig. 1: Inhibition of growth of selected fungi by plant leaf essential oil compound citral.**

### Biosynthesis of silver nanoparticles

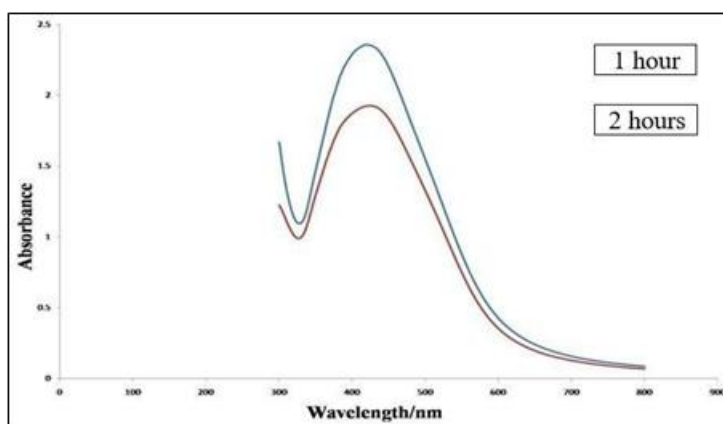
The silver nitrate solution was showed in (Fig 2a). The plant essential oil compound citral 9 ml was added to 90 ml of 1mM AgNO<sub>3</sub> solution in a conical flask and then magnetic stirrer is kept inside the conical flask and started to run vigorously on the hot plate the biosynthesis reaction started at 14 minutes and the color reaction was observed in which clear AgNO<sub>3</sub> solution changed into light brown color which indicates that formation of corresponding nanoparticles shown in (Fig. 2b). The UV–Vis spectra of silver nanoparticles synthesized by citral are showed the broad peak observed at 440 nm was seen in (Fig. 3).



**Fig. 2(a): Silver nitrate solution.**



**Fig. 2(b): Silver nanoparticle synthesized by citral.**

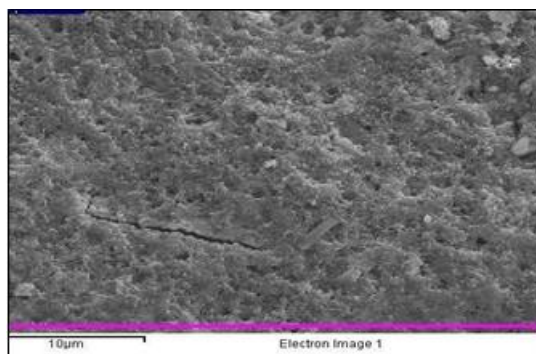


**Fig. 3: UV–Vis spectrum analysis of silver nanoparticle reduced by oil compound citral at 440 nm.**

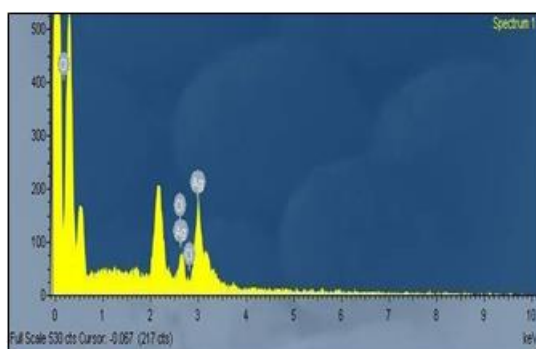
### Scanning electron microscopy study

In scanning electron microscopy the morphology of synthesized silver nanoparticle citral was noted and approximately spherical shaped in which the silver nanoparticles is in aggregated form (Fig 4a). This shows that the powder silver nanoparticles were agglomerated. The above results proved that the nanoparticles are synthesized due to the action of plant essential oil compound citral which act as good bio reductant for biosynthesis. The analysis of energy dispersive spectroscopy (EDS) of the silver nanoparticles the presence of elemental silver signal was confirmed. (Fig 4b).





**Fig (4a):** Scanning electron microscope image of silver nanoparticle synthesized by plant leaf oil compound citral.

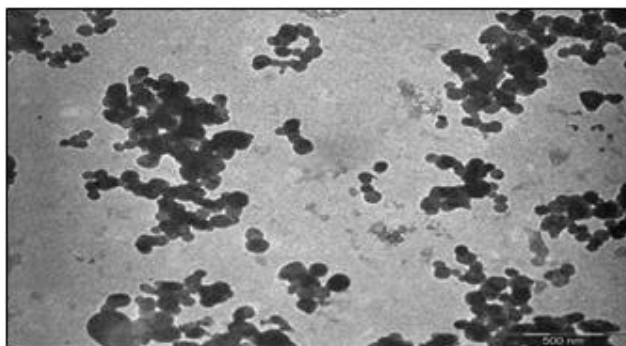


**Fig. (4b):** SEM-EDS spectrum showed the presence of silver signal.

### Transmission electron microscope study

The TEM image of silver nanoparticle synthesized by the plant oil compound citral was shown in (Fig 5).

The illustration suggests that the silver nanoparticles are spherical in shape with a diameter of 45 nm.

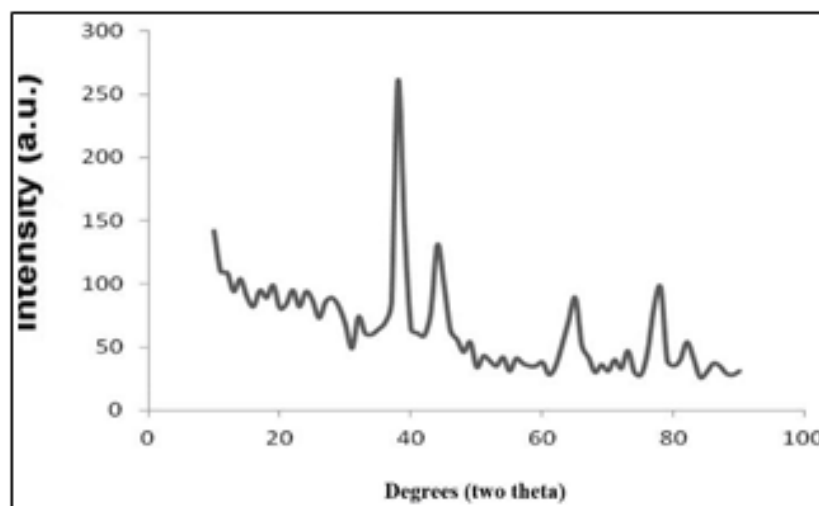


**Fig. 5:** Transmission electron microscope image of silver nanoparticle synthesized by plant leaf oil compound citral.



### X-Ray diffraction study

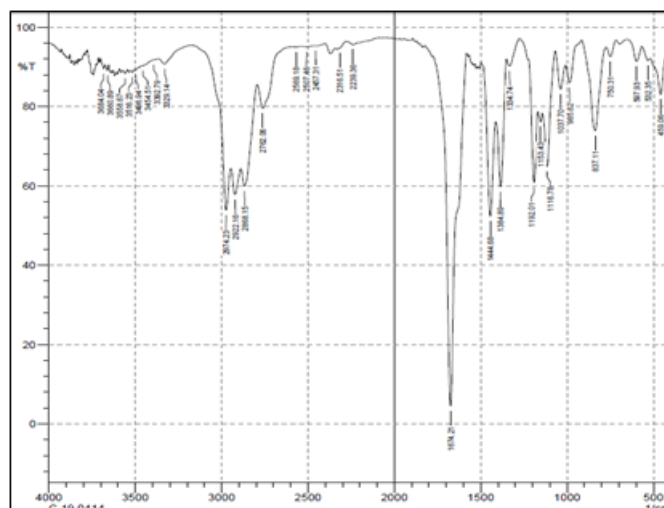
The X-ray diffraction pattern of silver nanoparticle synthesized by plant essential oil compound citral was showed in (Fig 6). The XRD pattern thus clearly illustrates that the silver nanoparticles present green synthesis method are powdery in nature.



**Fig. 6: XRD patterns of silver nanoparticles synthesized by plant leaf essential oil compound citral.**

### Fourier transform infrared spectroscopy study

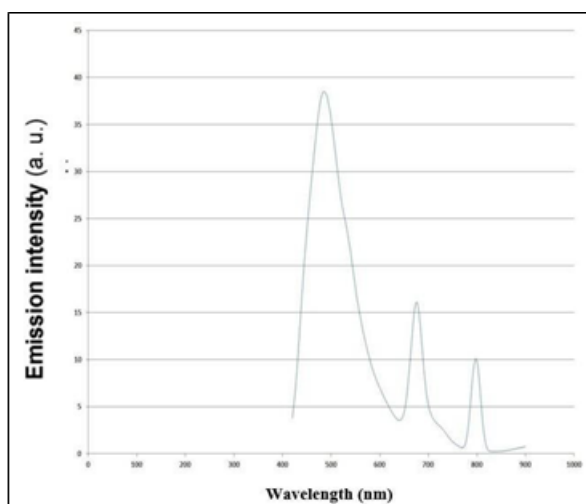
FTIR spectroscopy measurements was carried out to identify the biomolecules that bound specifically on the silver surface. (Fig. 7) shows the presence of three bands 1674, 1444, 1384 and 1192 $\text{cm}^{-1}$ . The strong absorption at 1674 $\text{cm}^{-1}$  is due to carbonyl stretching vibration of the acid groups of different fatty acids present in the synthesized plant oil compound citral. The bands at 1674 $\text{cm}^{-1}$  are characteristic of amide I and II band respectively. The amide band I is assigned to the stretch mode of the carbonyl group coupled to the amide linkage while the amide II band arises as a result of the N-H stretching modes of vibration in the amide linkage. The band at 1444 $\text{cm}^{-1}$  is assigned to the methylene scissoring vibrations from the proteins. It is well known that proteins can bind to silver nanoparticle through either free amine groups or cystein residues in the proteins and therefore stabilization of silver nanoparticles by the surface bound proteins is possible in the present green synthesis method.



**Fig. 7:** FTIR spectrum of vacuum dried powder of silver nanoparticles synthesized by plant leaf essential oil compound citral.

#### Fluorescence spectroscopy study

The fluorescent spectra of silver nanoparticle synthesized by the plant leaf essential oil compound citral were shown in (Fig 8). A broad emission band having prominent peak centered at ~500 nm is observed for the plant oil as it is excited at 420 nm. In this study emission intensity gradually increases with the decreasing concentration of AgNO<sub>3</sub>. 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.



**Fig. 8:** Fluorescence emission spectra (excitation at 420 nm) of plant leaf essential oil compound citral.

#### 4. DISCUSSION

Green chemistry approach towards the synthesis of nano particles has many advantages such

as, ease with which the process can be scaled up and economic viability. Based on the studies of synthesis of silver nanoparticles and its biomedical property was confirmed with various reports the appearance of intensity peak at 439nm corresponds to the absorption intensity steadily increased as a function of time reaction without any shift in the peak position (Shanker *et al.*, 2004; Kasturi *et al.*, 2009).<sup>[6,15]</sup> The potential ability of citral for the reduction of Ag<sup>+</sup> to silver nanoparticles was investigated in different condition. Characterization by UV-visible, TEM, SEM and EDS techniques confirmed the reduction of silver ions to silver nanoparticles. To the best of our knowledge, and based on a thorough literature surveys, this is the second report on the synthesis of silver nanoparticles using citral as a non- volatile compound from different plants leaf essential oils.

The enhanced antifungal effects of novel silver nanoparticles is characterized and also stated that once inside the cell, nanoparticles would interfere with the fungal growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrate critical for cell viability and division and the nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent [Shrivastava *et al.*, 2007, Duran *et al.*, 2005].<sup>[2,16]</sup> Also, in this investigation antifungal assay was used to check the effect of synthesised silver nanoparticle citral on human pathogenic fungi. The results showed high activity against all the clinical isolates of fungal pathogens. The synthesis described use environmentally safer materials (plant materials) compared to chemical synthesized nanoparticle, which often involve environmentally less acceptable or harmful chemicals. The evidence for the formation of a chemical bond between silver nanoparticles and the nitrogen of the amide group present in the amino acids. Biogenic synthesis is also favored due to its low toxicity to environment denoting its merit and prompt for preference in various industrial and medical applications.

## 5. CONCLUSION

To summarize, we succeeded in the biological reduction of silver nanoparticles by citral essential oil compound. Silver nanoparticles were synthesized in ambient conditions and characterization of synthesized nanoparticles was carried out by UV-Vis spectroscopy, FT IR, SEM and TEM. It is believed that phytochemicals present in the plant essential oil compound citral has reduced the silver nitrate into silver nanoparticles. The procedure for the biosynthesis of silver nanoparticles has several advantages such as cost- effectiveness, compatibility for biomedical and pharmaceutical applications as well as for large-scale

commercial production. In future, it would be significant to select such plants oil compounds, to understand the clear mechanism of biosynthesis and to technologically improve the nanoparticles in order to achieve better control over size, shape and absolute mono dispersivity to utilize the potential of herbal medicine in nanoscience for anti-fungal and biomedical applications. The synthesized silver nanoparticle exhibited a strong antifungal activity against both *Candida albicans* and *Aspergillus flavus*.

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