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PELARGONIUM GRAVEOLENS PLANT LEAF ESSENTIAL OIL MEDIATED GREEN SYNTHESIS OF SILVER NANO PARTICLES AND ITS ANTIFUNGAL ACTIVITY AGAINST HUMAN PATHOGENIC FUNGI

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ABSTRACT

The various plant leaf essential oils used for the biosynthesis of Nano particles is considered a green technology used to kill human pathogenic microorganisms. The present study reports the synthesis of silver (Ag) nanoparticles from silver precursor using plant leaf essential oil Pelargonium graveolens. Water-soluble organics present in the plant materials were mainly responsible for the reduction of silver ions to Nano-sized Ag particles. SEM, TEM, FTIR, XRD and fluroscence spectroscopy results confirmed the presence of nano-powedry Ag particles. The pH played a major role in size control of the particles. The plant essential oil produced more Ag nanoparticles which was attributed to the large availability of the reducing agents in the plant oil. Thus Pelargonium graveolens are a good bio-resource/biomaterial for the synthesis of Ag nanoparticles with antifungal activity. The antifungal activity results showed that synthesized silver nanoparticle from the plant oil was highly active against clinically isolated human fungal pathogens, Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis and Candida kefyr.

KEYWORDS: *Aspergillus flavus*, green synthesis, ftir, silver nanoparticles, tem.

1. INTRODUCTION

In the last decade, the biosynthesis of nanoparticles, 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).[33,1,43,44,38] 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).[13,14] initially reported the possibility of using plant materials for the synthesis of Nano-scale metals. Later, the bioreduction of various metals to Nano-sizes of various shapes, capable of meeting the requirements of diverse industrial applications, was extensively studied. Nanoparticles (NPs) are being viewed as fundamental building blocks of nanotechnology. Bio synthesised Ag NPs are used in antimicrobial (Duran et al., 2005),[7] anti-viral and anti-human immunodeficiency virus (anti-HIV) studies (Elechiguerra et al., 2005). Baiocco et al. (2011).[3] reported that Ag NPs showed the in vitro and in vivo inhibitory effect on Leishmania infantum. Using plants for NP synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale NP synthesis (Shankar et al., 2004).[45] Synthesis of NP using microorganisms or plants can potentially eliminate this problem by making the NPs more biocompatible. In addition, a large number of previous studies have dealt with the adverse effects of silver and silver NPs on microorganisms. Preparation and characterization of advanced nanomaterials of silver and gold using various chemical, physical, and biological methods has been the subject of large number of investigations from the last two decades.

[Marigner *et al*., 1985; Hanglein,1998; El-sayed, 2001; Jana *et al*., 2001; Pileni, 2003; Sharma *et al*., 2009; Nikoobakht and El- syed, 2003; Du *et al*., 2009].[31,16,19,37,46,36,9] Generally, a variety of chemicals such as polymers, surfactants, co- polymers, dendrimers, starch and lipids are essential to obtain fine and stable noble metal particles in the chemical and physical methods [Jain *et al*., 1989; chen *et al*., 2005; Bakshi *et al*., 2008; Bakshi and

Langmuir, 2009; Esumi., 2000].[18,4,12] These chemicals acted as stabilizing and/or capping agents. Biological approach provides advancement over other method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and chemicals [Xie *et al*., 2007; Shervani *et al*., 2011].[49,47] It has been established that extracts from algae, leafs, plants, and yeast acted both as reducing-, capping- and shape directing-agents in the green synthesis of Ag and Au-nanoparticles [Mehra *et al*., 1991; Duran *et al*., 2009; Sanghi and Verma, 2009; Rani and Rajashekarreddy, 2011].[8,32,40,39] Jose-Yacaman *et al*. reported a simple and fast biosynthetic method to the formation of gold and silver nanoparticles using living plants and/or its extract as reducing agents for the second time [Gardea-Torresday *et al*., 2002].[13]

The present study we synthesized silver nanoparticles from plant leaf essential oil *Pelargonium graveolens* and characterization technique was completed. Then antifungal activity was performed to know the potential activity of synthseised silver nanoparticle *Pelargonium graveolens* against the deadly pathogens which causes many disease to human beings.

2. Materials and methodsPlant leaf essential oils

The plant essential oil *Pelargonium graveolens* were purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India).The silver nitrate (AgNO3), were purchased from Hi Media (Mumbai, India).

Fungal cultures

The fungal isolates *Aspergillus niger, Aspergillus flavus, Canida 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 spectra analysis

The biosynthesis of Ag Nano particles was monitored periodically in a UV–visible spectrophotometer (Shimadzu, UV-2550, Japan). For the analysis, 0.2mL of the sample in a cuvette and was diluted to the 2mL with deionized water. The UV–visible spectra of the resulting diluents were monitored as a function of reaction time and biomaterial dosage at a resolution of 2 nm.

TEM observation of silver nanoparticles

Twenty minutes after the reaction, the biomass had settled at the bottom of the conical flasks and the suspension above the precipitate was sampled for transmission electron microscopy (TEM) observation. TEM samples of the aqueous suspension of Ag nanoparticles were prepared by placing a drop of the suspension on carbon-coated copper grids and the films on the TEM grids were allowed to stand for 2 min, after which the extra solution was removed using a blotting paper and the grid was allowed to dry prior to measurement. TEM observations were performed on a HITACHI-JP/H7600 instrument (Japan) operated at an accelerating voltage of 200 kV. The size distribution of the resulting nanoparticles was estimated on the basis of TEM micrographs with the assistance of Sigma Scan Pro software (SPSS Inc., Version 4.01.003). Energy dispersive X-ray (EDX) analyses were performed on a JEOL JSM-6400 microscope (Japan) fitted with Oxford-6506 (England) EDX analyser.

2. 5XRD Analysis

The redispersed silver nanoparticles were kept in an oven in order to obtain the powdered form with high purity. The dry powders of the synthesized silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 10 ° to 80 ° at 2 theta angles. XRD analysis was performed using an X’Pert Pro X-ray diffract meter operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation.

FTIR analysis

The plant essential oil *Pelargonium graveolens* when reacted with silver nitrate solution it produced a synthesized silver nanoparticles, the residual solution of 200 ml after reaction was centrifuged at 20,000 rpm for 20 min. This was followed by re dispersion of the pellet of Ag-NPs into 2 ml of deionised water. 20μl of the sample of the formed silver nanoparticles from the plant essential oil was subjected to FTIR analysis using a Paragon 600, Perkin Elmer-RX2 spectrophotometer in the diffuse reflectance mode at a resolution of 2cm−1 in KBr pellets.

SEM-EDX analysis

To determine the morphology of the synthesized silver nanoparticles using seed powder extract, the sample was analysed with Zeiss 600 Scanning electron microscope (SEM).

The redispersed nanoparticles were dried in an oven to obtain a powdered form. Then, 20 mg of the sample was redispersed in ethanol and the sample was prepared in thin films on carbon coated copper grid. EDX was used for elemental analysis in the sample.

Antimicrobial activity

The biological activity against yeasts was determined by employing the standard discs diffusion technique (Malabadi, 2005; Malabadi and Vijayakumar, 2007).[25,26] Antifungal activity was assessed on the yeasts *Candida albicans*, *Candida tropicalis* and *Candida kefyr* and molds *Aspergillus flavus*, *Aspergillus niger*. The effect of synthesized silver nanoparticle oil compounds was tested against yeast and mycelial growth of *Candida albicans* 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 15 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.

Results antifungal assay

The plant essential oil *Pelargonium graveolens* showed notable antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr* in (Table. 1). The essential oil *Pelargonium graveolens* was very highly active against *Candida tropicalis* (16.52±0.22) and least against *Aspergillus flavus* (5.46±0.11). Silver nitrate solution was highly active against *Aspergillus niger* (4.24±0.35) and least against *Candida albicans* (0.00±0.30). The silver nanoparticle *Pelargonium graveolens* was also highly active against *Candida tropicalis* (13.89±0.38) and least against *Candida albicans* (6.12±0.45). All fungi were found to be sensitive to all test essential oil *Pelargonium graveolens* and synthesized silver nanoparticle *Pelargonium graveolens* and mostly comparable to the standard reference antifungal drug Amphotericin B and ketoconazole to some extent.

Table 1: Antifungal activity of plant *Pelargonium graveolens* synthesized silver nanoparticle.

Microorganisms	P. graveolens	Agno3 solution	silver nanoparticle	P. graveolens Antifungal agents
Aspergillus niger	6.45± 0..18[a]	4.24±0.35[a]	11.44±0.55[a]	2.35±0.02[a]
(Amphoterecin –B)				
Aspergillus flavus	5.46± 0.11[b]	3.17± 0.36[a]	9.06±0.73[a]	2.66± 0.05[b]
(Amphoterecin-B)				
Candida albicans	9.79± 0.14[c]	0.00±0.30[b]	6.12±0.45[b]	16..31±0.07[c]
(Ketoconazole)				
Candida tropicalis	16.52±0.22[d]	1.36± 0.44[c]	13.89±0.38[c]	14.37± 0.15[d]
(Ketoconazole)				
Candida kefyr	14.77±0.39[e]	0.00±0.31[d]	11.22±0.88[d]	17.69±0.24[e]
(Ketoconazole)				

The values are represented as the Mean ± SD of plant essential oil *Pelargonium graveolens* and with the synthesized silver nanoparticle. These plant essential oil *Pelargonium graveolens* and with the synthesized silver nanoparticle have significant effect at 0.05 levels.

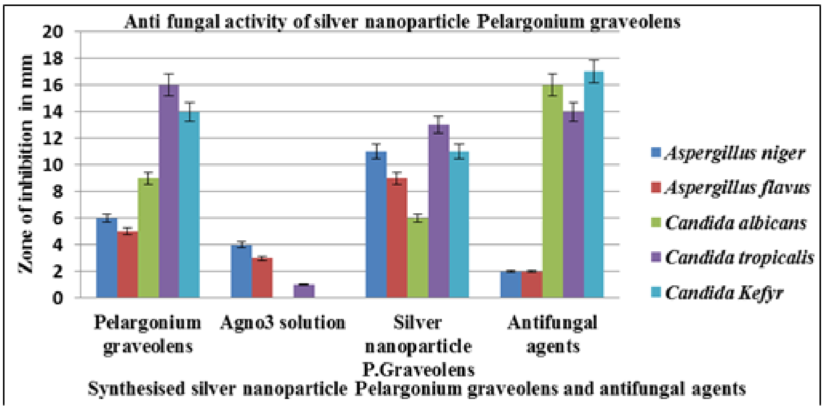

Fig. 1: Inhibition of growth of selected fungi by plant leaf essential oil *Pelargonium graveolens.*

Green synthesis of silver nanoparticles

The silver nitrate solution was showed in (Fig 2a). The plant essential oil *Pelargonium graveolens* 6 ml was added to 80 ml of 2mM AgNO3 solution in a conical flask and the magnetic stirrer is kept inside the conical flask and started to run vigorously on the hot plate the biosynthesis reaction started within 15 minutes and the color reaction was observed in which clear AgNO3 solution changed into dark brown color which indicates that formation of corresponding silver nanoparticles shown in (Fig. 2b). The UV–Vis spectra of silver nanoparticles synthesized by plant leaf essential oil *Pelargonium graveolens* are showed the broad peak observed at 420 nm was seen in (Fig. 3).

Fig. 2: (a) Silver nitrate solution.

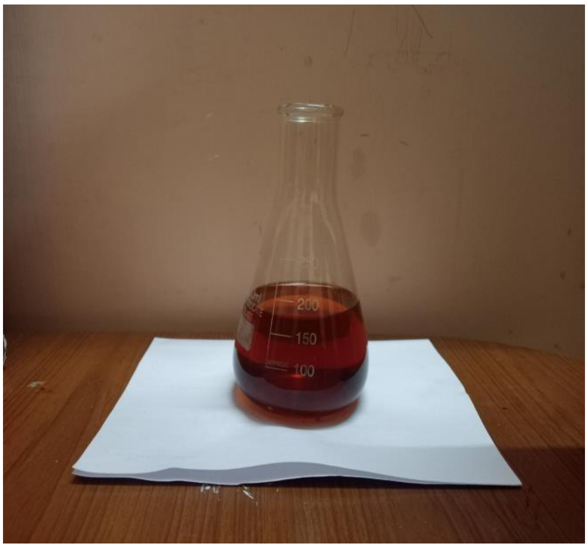

Fig. 2(b): Silver nanoparticle *Pelargonium graveolens* oil.

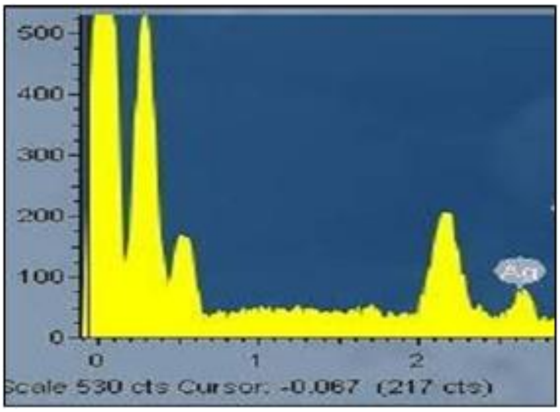

Fig. (4b): Sem-EDS spectrum showed the presence of silver signal.

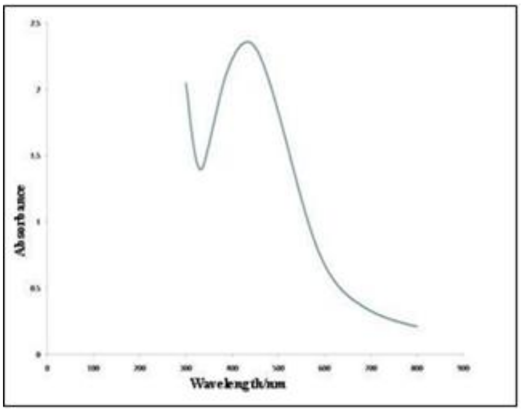

Fig. 3: UV–Vis spectrum analysis of silver nanoparticle reduced by plant leaf oil *Pelargonium graveolens* at 420 nm.

Scanning electron microscopy

The scanning electron microscopy the structure of synthesized silver nanoparticle *Pelargonium graveolens* was noted in which the silver nanoparticles is in agglomrated form (Fig 4a). The results shown below proved that the nanoparticles are synthesized due to the action of plant essential oil *Pelargonium graveolens* which act as good biomedical properties to kill pathogenic microorganisms. The analysis of energy dispersive spectroscopy (EDS) of the silver nanoparticles thepresence of elemental silver signal was confirmed. (Fig 4b).

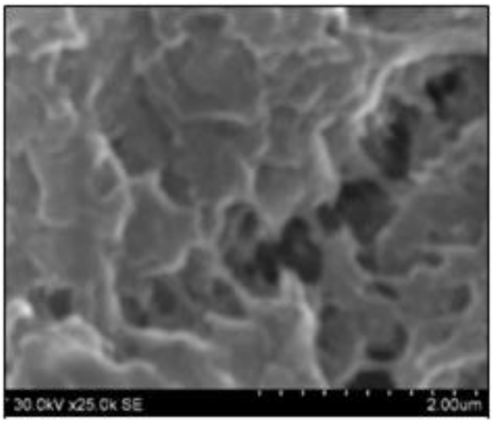

Fig (4a): Scanning electron microscope image of silver nanoparticle synthesized by plant leaf essential oil *Pelargonium graveolens.*

Transmission electron microscope

The TEM picture of silver nanoparticle synthesized by the plant essential oil *Pelargonium graveolens* was shown in (Fig 5). The microphotography image shows that the silver nanoparticles are with a diameter of 164 nm and 122 nm.

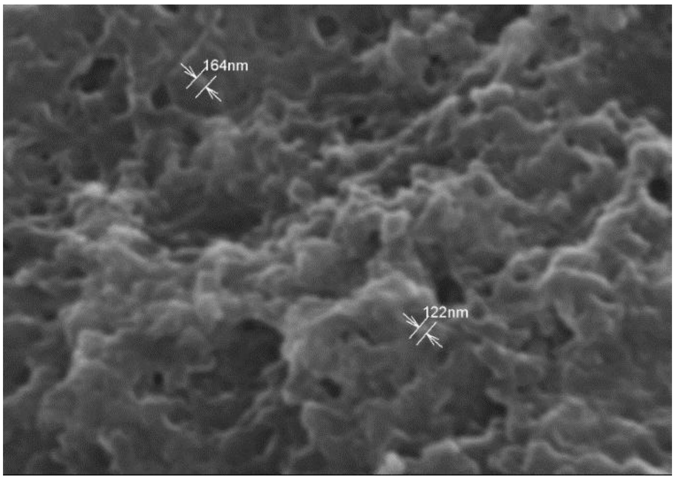

Fig. (5): Transmission electron microscope image of silver nanoparticle synthesized by plant leaf essential oil *Pelargoniumgraveolens.*

X-Ray diffraction study

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

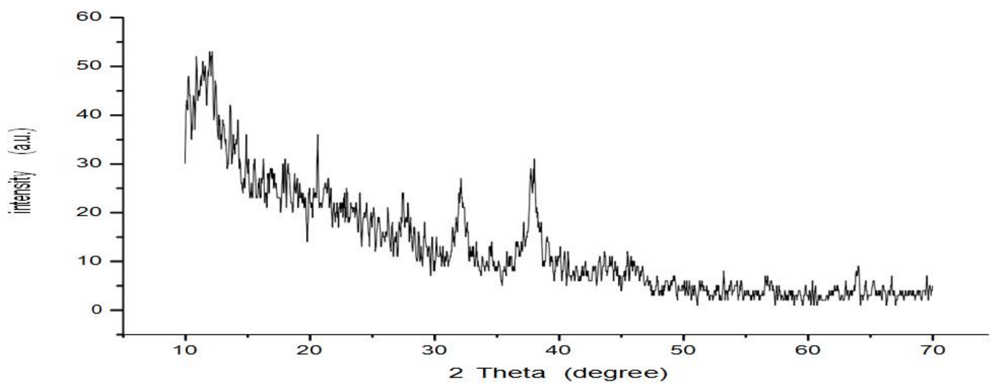

Fig. 6: XRD patterns of silver nanoparticles synthesized by plant leaf essential oil *Pelargonium graveolens.*

Fourier transform infrared spectroscopy

The possible potential biomolecules responsible for the reduction of silver ions to silver nanoparticles was identified using FT─IR analysis. Figure 7 shows the FT─IR spectrum of plant essential oil *Pelargonium graveolens* assisted silver nanoparticles. The band at 3434.3 cm-1 represents N─H stretching groups of amides. The band at 1726.2 cm-1 corresponds to N─H groups of primary amines. The peak at 1379.1 cm-1 corresponding to amide II and amide III of aromatic rings either may by poly phenols associated with synthesized silver nanoparticles which is segregated by plant essential oil *Pelargonium graveolens*. The band at

1,056.5 cm-1 shows that C─O stretching vibrations of alcohols, carboxylic and C─N stretching of aliphatic amines. The intense peak of 836.3, 742.1, 534.2, and 436.9 cm-1 shows that C─Cl, C─Br and C─I group of alkyl halides.

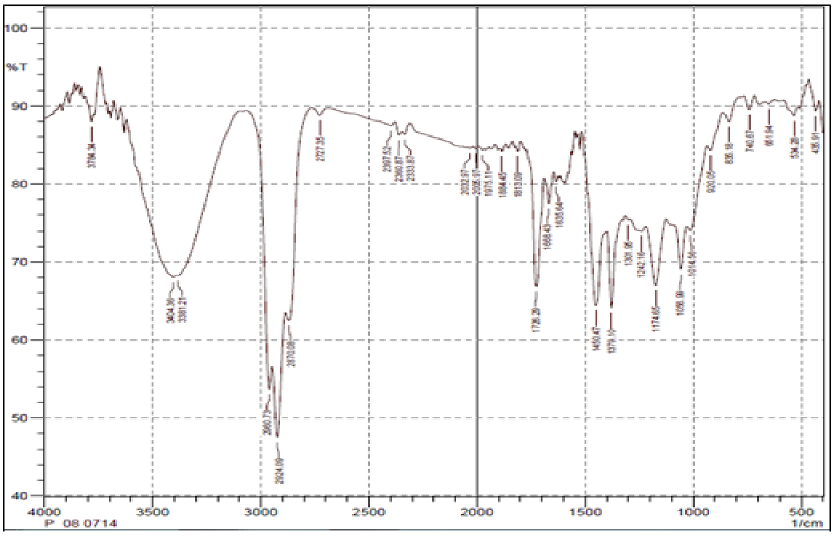

Fig. 7: FTIR spectrum of vacuum dried powder of silver nanoparticles synthesized by plant leaf essential oil *Pelargonium graveolens*.

Fluroscence spectroscopy

The fluroscent spectra of silver nanoparticle synthesized by the plant essential oil *Pelargonium graveolens* were shown in (Fig 8).A broad emission band having prominent peak centered at ~200 nm and 400 nm was observed for the plant oil *Pelargonium graveolens*. In this study emission intensity gradually increases with the decreasing concentration ofAgNO3.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.

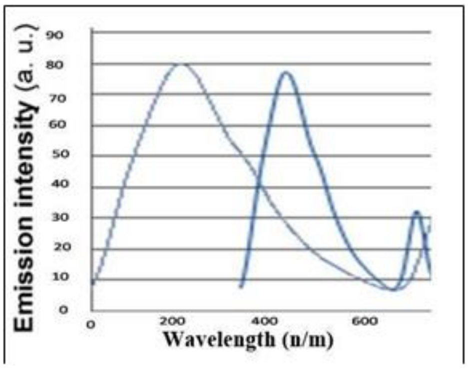

Fig. 8: Fluorescence emission spectra showed prominent peak at 200 nm and 400nm of plant leaf essential oil *Pelargonium graveolens.*

DISCUSSION

Plants or their extracts can be efficiently used in the synthesis of gold and Ag NPs as a greener route. Control over the shape and size of nanoparticles seems to be very easy with the use of plants. Such nanoparticles produced using plants have been used in various applications for human benefit. Elucidation of the mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research (Hettiarachchi and Wickramarachchi, 2011).[17] The potential uses and benefits of nanotechnology are enormous.

The antifungal activity of silver nanoparticles from plant leaves showed enhancement in activity due to synergistic effect of silver and essential oil (Vankar and Shukla, 2012).[48] Silver is inherently anti-microbial and antibacterial substance. Silver has been widely utilized for thousands of years in human history. Among the silvers many applications, its disinfectant property is being exploited for hygienic and medicinal purposes, such as treatment of mental illness, nicotine addiction and infectious diseases like syphilis and gonorrhea (Mukunthan *et al.* 2011).[37] It has been observed that nanoparticles loaded with plant essential oil are efficacious against *Aspergillus flavus* (Ankamwar *et al*. 2005).[2] The results of the present study show that synthesized Ag NPs from plant essential oil kills all human pathogenic bacteria and fungi. Nanoparticle size also determines the colour of the solution, the smaller the size of Ag NPs and the greater the colours shift towards red (Ghosh *et al*. 2012).[12] Some other interesting findings were reported by different scientists on plants. In our present study, plant leaf essential oil killed all pathogenic microorganisms due to the presence of dimeric alkaloids, vinblastine and vincristine in plant essential oil of *Pelargonium graveolens* which are indispensable drugs for a number of anticancer chemotherapies (Kulkarni *et al.* 1999; Dutta *et al*. 2005; Malabadi *et al.* 2008).[22,10,27] On the other hand, roots accumulate the antihypertensive alkaloids, ajmalicine and serpentine (Dutta *et al*. 2005; Malabadi *et al*. 2008).[10,27] The antimicrobial activity in terms of inhibition zone significantly varied with test microbes and the type of the extracts. This differential antimicrobial activity of silver nanoparticles can be attributed to their differential sizes and shape: the antimicrobial activity increases size of the silver nanoparticles (Nabikhan *et al.* 2010; Malabadi *et al.* 2012a, 2012b, 2012c).[35,28,29,30] The results of this study also clearly indicated that silver nanoparticles synthesized from leaf, root has many pharmaceutical applications for the control of deadly pathogens (Malabadi *et al.* 2012).[28] The highest antifungal activity of plant essential oil *Pelargonium graveolens* are probably due to the presence of mono-terpenoid indole alkaloids (MIA’s) in different organ (Jordan *et al*. 1991;

Laflamme *et al*. 2001; Mahroug *et al.* 2006; Malabadi *et al.* 2008).[21,23,24,27] The plant essential oil *Pelargonium graveolens* reacted with silver nitrate solution confirmed silver nanoparticle synthesis through the steady change of greenish colour to reddish brown (Satyavani *et al.* 2011).[42] They also confirmed that plant based silver nanoparticles possess considerable anticancer effect compared with commercial nanosilver (Satyavani *et al.* 2011).[42] The reduction of the metal ions through the plant essential oil leading to the formation of silver nanoparticles of fairly well defined dimensions. Use of the synthesized silver nanoparticle from plant extract should emerge as one of the novel approaches in cancer therapy and when the molecular mechanism of targeting is better understood, the applications of silver nanoparticles are likely to expand further (Satyavani *et al.* 2011).[42]

CONCLUSION

In this present study, we have proposed a green approach for synthesizing silver nanoparticles using plant leaf essential oil *Pelargonium graveolens* as reducing mediator. We investigated the nanoparticles formation using UV-Vis spectrophotometer, the powedry nature of formed nanoparticles was confirmed by using X-Ray Diffraction patterns, SEM micrograph demonstrated that the shape of the nanoparticles and the TEM showed the size of the silver nanoparticle was about 164 nm. The possible biomolecules amide and polyphenol groups may responsible for the reduction of silver nitrate to silver nanoparticles are identified by FT-IR. The formed silver nanoparticle was found to have high antifungal activity in *Aspergillus Niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr*. We trust that the silver nanoparticles have great promising for application in biological based nan medicine, biosensors and food industries. In the present study we found that plant leaves were good source for the synthesis of silver nanoparticles has advantages such as, ease with which the process can be scaled up, economic viability and to obtain smaller particle size. This study demonstrated the possibility of use of biologically synthesized silver nanoparticles and their incorporation in materials, providing them sterile properties. The plant extract and plant essential oil incorporated with these silver nanoparticles exhibited very high antimicrobial activity against the common deadly human pathogens. Prepared nanoparticles can be used as bactericidal and fungicidal and in wound healing and also in the field of medicine.

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