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Research Article

RAPID GREEN SYNTHESIS OF GOLD NANOPARTICLE USING PLANT LEAF ESSENTIAL OIL *CYMBOPOGON MARTINII* AND THEIR ANTIFUNGAL ACTIVITY AGAINST DEADLY HUMAN PATHOGENIC FUNGI

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ABSTRACT

A new method for the synthesis of gold nanoparticles is reported. The plant leaf essential oils extracted from the fresh leaves of Cymbopogon martinii are used for the reduction of chloroauric acid to Au nanoparticles (NPs). The formation and morphology of synthesized AUNPs are investigated with the help of UV–visible, TEM and FTIR spectroscopy. The AUNPs synthesized at room temperature are mono-dispersed and big round in shape with an average size of 47 nm while those prepared at higher temperature are composed of a mixture of anisotropic particles. The UV–visible absorption spectra of these anisotropic AUNPs show asymmetry in the longer wavelength side. The quantity of leaf oil is an important criteria on modulating the shape of AUNPs. Possible chemical compounds of AUNPs are studied using FTIR spectroscopy. The antifungal activity results showed that synthesized gold nanoparticle from the plant oil Cymbopogon martinii was highly active against clinically isolated human fungal pathogens, Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis and Candida kefyr.

KEYWORDS: *Candida tropicalis, Green synthesis, Fluroscence spectroscopy, Gold nanoparticles, Tem.*

INTRODUCTION

The properties of noble metal nanoparticles gold have previously been changed with many stabilizing and capping agents for various applications. The biological means of synthesizing nanoparticles provides an edge over chemical means as it is cost-effective, does not involve physical barriers for lessening agents. There are several plants that have been identified to synthesize nanoparticles and the rate of synthesis of nanoparticles by plant leaf essential oil is comparable is a faster green synthesis technique.(Castro *et al*., 2010; Cruz *et al*., 2010) Similarly, AUGNPs have been considered as an important area of research because of their unique and their applications in biomedical science including drug delivery, tissue or tumor imaging, photo thermal therapy, and immune chromatographic identification of pathogens in clinical specimens.(Khalil *et al*., 2012) The AUGNPs are used for developing biosensors, DNA labeling.

Gold has been used in the form of metallic gold, chloroauric acid to treat burns, wounds, and severe bacterial infections. (Dar *et al*., 2013; Khan *et al*., 2013) The synthesized silver ions have been used in many kinds of formulations. Recently, it was shown that hybrids of AUNPs with amphiphilic hyper branched macromolecules display effective antimicrobial surface coatings. The most important applications of AUNPs are in the medical industry, such as topical ointments to prevent infection in burns and open wounds (Dar *et al*., 2013).

In this study, we show that an plant leaf essential oil *Cymbopogon martinii*, placed in 2mM aqueous solution $HAuCl_4$, resulted in reduction of gold ions to form AUGNPs. These green-synthesized AUGNPs of were examined by ultraviolet-visible (UV-vis) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analysis for size and shape.

Gold in nanoscale display novel properties and have diverse activities that make it appropriate for therapeutic use and broad applications in nanobiotechnology (Kim *et al*., 2004; Sperling *et al*., 2008) Phytochemical constituents in the plants and spices extract like essential oils (terpenes, eugenols, etc), polyphenols and carbohydrates these compounds contain active functional groups, such as hydroxyl, aldehyde and carboxyl units which may play important role for reduction of $HAuCl_4$ to AuNPs. Gold nanoparticles produced by using phytochemicals or other extract components remain stable for certain time (Singh *et al*., 2010; Chandra *et al*., 2011) Further plants and spices mediated stabilized or capped AuNPs may cross the barrier of cytotoxicity which is a prior requirement for biomedical application of AuNPs (Das *et al*., 2011) The antibacterial and antioxidant properties of bio molecules present in the plants and spices extract have facilitated excellent stability of the nanoparticles (Ankamwar, 2005). Green gold nanoparticles derived from phyto chemicals can be show excellent biocompatibility, such biogenic gold nanoparticles with high biocompatibility may be clinically useful as contrast enhancement molecular imaging agents for cancer diagnosis (Chandra *et al*., 2011).

This present study the gold nanoparticles were synthesized from plant leaf essential oil *Cymbopogon martinii* and these nanoparticles was characterized. Antifungal activity test was done to know the biological activity of synthesized gold nanoparticles against the most pathogenic fungi.

MATERIALS AND METHODS

1. Plant leaf essential oils:

The plant leaf essential oils were purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India). The Chloroauric acid ($HAuCl_4$) was purchased from Hi Media (Mumbai, India).

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2. UV-Vis absorbance spectroscopy analysis:

The bioreduction (by $HAuCl_4$) of nanoparticles was recorded periodically using a UV-Vis 3000+ double beam spectrophotometer (Lab India, Maharashtra, India), which had slit widths of 0.5, 1.0, 2.0, and 5.0 nm. The samples were diluted with 2 mL of deionized water and measured by UV-Vis spectrum at regular time intervals. The deionized water was used as a blank for background correction of all UV-VIS spectra. All samples were loaded into a 1 cm path length quartz cuvette for sampling. The UV-Vis spectrometric readings were scanned from 200 to 800 nm and recorded at a scanning speed of 0.5 nm interval. The UV-VIS spectra were fit with Gaussian curves correcting for a cubic background for full-width at half maximum (FWHM) and wavelength of maximum absorbance measurements. The Gaussian fits to the UV-VIS spectra all had goodness of fit values ($\chi 2 \sim 1$), showing accurate curve analysis.

3. SEM analysis of AUGNPs:

The prepared AUGNPs were characterized using high resolution SEM analysis (JSM-5600 LV; JEOL, Tokyo, Japan). The samples were prepared by a simple drop coating of suspended gold solution on to an electrically heated clean glass and allowing the solvent (water) to evaporate. The samples were left to dry at room temperature.

4 FTIR spectroscopy analysis of dried biomass after bioreduction:

To identify the biomolecules present in the plant leaf essential oil and the biomolecules within the SNPs and GNPs after synthesis, a carefully weighed quantity of the synthesized nanoparticles were subjected to FTIR analysis (PerkinElmer RX1; PerkinElmer, Waltham, MA, USA). The bioreduced chlorauric and silver solutions were centrifuged at 10,000 rpm for 15 minutes, and the pellets were washed three times with 20 mL of deionized water. The resulting purified suspensions were dried and ground with KBr pellets and analyzed by FTIR. The FTIR were recorded in the range of 400–4000 cm−1. To obtain a good signal and noise ratio, 512 scans were recorded [26].

5. X-ray diffraction studies:

The formation and structure of AuNPs were checked by X-ray diffraction (XRD) spectrum. For XRD, a drop coated films of the bio-reduced HAucl4 solution was prepared on glass substrates. The powedry nature of the films were analyzed by using an X-ray diffractometer (Make: Seifert, Model 003 T/T) with CuKá radiations operated at 40kV and30mA.

6. Green synthesis of AuNPs:

Locally available medicinal plant leaves oil *Cymbopogon martinii* were used for bio-synthesis of AuNPs. The plant leaf oil were mixed with 6 ml in 100 ml of deionized water in an Erlenmeyer flask, and kept for 20minutes and then the gold ion complex formed, it started to change colour (after 20 minutes) to a ruby red colour.

7. Fluroscence spectroscopy:

Both conventional fluorescence and TFS emission spectra were recorded on a Hitachi Model F-4500 fluorimeter. The working parameters for conventional spectra were an excitation wavelength of 337 nm and an emission range from 350-650 nm (5 nm intervals) with 5.0-nm excitation and 2.5-nmemission slits with a scan rate of 60nm/s. For TFS, the excitation range was 230–600 nm (5-nm intervals) and the emission range 300–750nm (5-nm intervals) with 5.0-nm slits and a scan rate of 1200 nm/min. For SFS, a PerkinElmer Model LS50 fluorimeter was employed with excitation from 250–650 nm, 15-nmexcitation and 10 nm emission slits and a scan rate of 500nm/min. An Edinburgh Instruments Model LP900 laser flash photolysis spectrometer operated in the fluorescence mode was utilized for the TRFS measurements. Samples were excited with 5-ns pulses of the third harmonic (355 nm; power of 40mJ/s) of a pulsed Nd-YAG laser.

8. TEM analysis of gold nanoparticles:

Sample is dispersed in double distilled water. A drop of thin dispersion is placed on a "staining mat". Carbon coated copper grid is inserted into the drop with the coated side upwards. After about twenty minutes, the grid is removed and air dried. Then screened in JEOL JEM 100SX Transmission Electron Microscope at an accelerating voltage of 80kv.

Antifungal test:

9. Agar well diffusion method:

The antifungal assay was performed done on the methods of (Berghe and Vlietinck, 1991). The synthesized gold nanoparticles of *Cymbopogon martinii* oil were taken upto 29 µl for assay. Culture suspension of 150 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 borer and were loaded with 11 µl of each sample Fluconazole and Amphoterecin-B were used as a positive reference. The fungal growth was noted after 2 days and antifungal activity was evaluvated by measuring the diameter of the growth inhibition zones in millimeter. Then the values were tested through statistical analysis.

10 Fungal Strains:

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

RESULTS

1. Invitro antifungal assay:

The plant essential oil *Cymbopogon martinii* showed notable antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans, Candida tropicalis* and *Candida kefyr* in (Table. 1). The essential oil *Cymbopogon martinii* was very highly active against *Candida kefyr* (13.77±0.34) and least against *Aspergillus niger* (3.67±0.17). Chloroauric acid showed no activity against any fungal species. The gold nanoparticle *Cymbopogon martinii* was also highly active against *Candida tropicalis* (27.48±0.35) and least against *Aspergillus niger* (5.08±0.78). All fungi were found to be sensitive to all test essential oil *Cymbopogon martinii* and synthesized gold nanoparticle *Cymbopogon martinii* and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent.

2. Biological green synthesis of gold nanoparticles:

The plant leaf essential oil *Cymbopogon martinii* 11 ml was added to 60 ml of 2 mM chloroauric acid solution and the magnetic is kept into the conical flask solution and then the magnetic stirrer was started to run vigorously on the hot plate. The magnetic stirrer is continuously stirred for 24 minutes finally the ruby red colour formation indicated the presence of gold nanoparticles shown in Fig (2b). The UV–Vis spectra of gold nanoparticles synthesized by plant leaf oil *Cymbopogon martinii* are showed broad peak observed at 540 nm was seen in (Fig. 3).

3. The SEM:

The sem study showed the structure of synthesized gold nanoparticles was agglomerated here and there was shown in (Fig 3a). The analysis of energy dispersive spectroscopy (EDS) of the gold nanoparticles the presence of elemental gold signal was confirmed (Fig 3b).

4. The TEM:

The transmission electron microscope image of gold nanoparticle was showed in (Fig 4). The picture denotes that the small and big round shaped gold nanoparticles are distributed widely with a diameter of 47 nm.

5. The XRD:

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

6. The FTIR:

The size distribution and characterization of the AUGNPs was further corroborated by FTIR, as shown in Figure 6.The interaction of gold nanoparticles with plant leaf essential oil *Cymbopogon martinii* showed intensive peaks at 1708, 2904, 1467, 1074, and 1215 cm−1. Relative shifts in position and intensity distribution were confirmed with FTIR. This clearly shows that the oxidized polyphenols capped the surface of the nanoparticles and kept them stable for longer periods.

7. The Fluroscence Spectroscopy:

The fluroscent spectra of synthesized gold nanoparticle were shown in (Fig 7). A broad emission band having prominent peak centered at ~535 nm is observed for the plant oil as it is excited at 525 nm. In this study emission intensity gradually increases with the decreasing concentration of $HAucl_4$.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.

Table No. 1: Antifungal activity of synthesized gold nanoparticle *Cymbopogon martinii* oil

Microorganisms	*Cymbopogon martinii* oil	$HAucl_4$	Gold Nanoparticle *C.martinii*	Antifungal agents
Aspergillus niger	3.67± 0.17[a]	0.00±0.00[a]	5.08±0.78[a]	2.03± 0.56[a] Amphoterecin -B
Aspergillus flavus	4.39±0.17[b]	0.00± 0.00[b]	7.57±0.69[b]	3.08±0.89[b] Amphoterecin -B
Candida albicans	9.47±0.38[c]	0.00±0.00[c]	9.24±0.16[c]	25.37± 0.58[c] Fluconazole
Candida tropicalis	12.36 ±0.48d	0.00±0.00[d]	27.48±0.35[d]	24.02±0.47[d] Fluconazole
Candida kefyr	5.89± 0.59e	0.00±0.00[e]	23.46±0.88[e]	25.04±0.57[e] Fluconazole

The values are represented as the Mean ± SD of plant leaf essential oil *Cymbopogon martinii* and synthesized gold nanoparticle *Cymbopogon martinii*. These plant leaf essential oil *Cymbopogon martinii* and synthesized gold nanoparticle *Cymbopogon martinii* have significant effect at 0.05 levels.

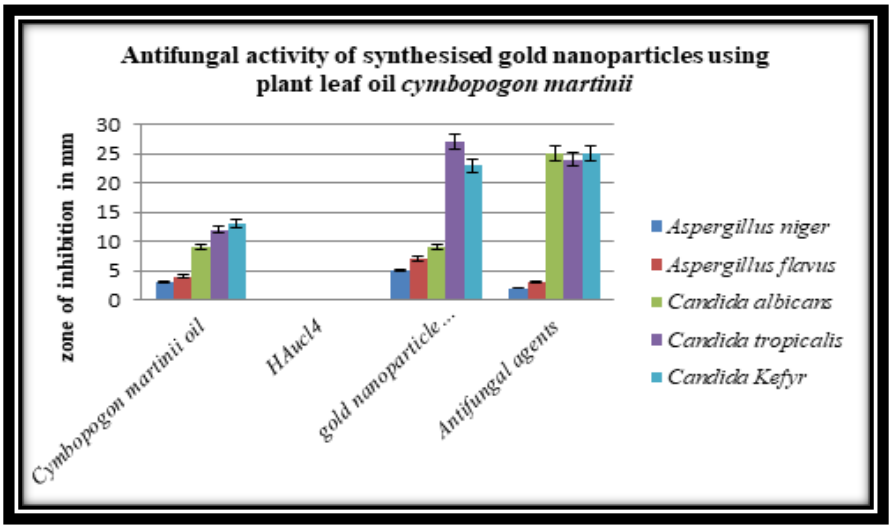

Fig. 1: Inhibition of growth of selected fungi by synthesized gold nanoparticle from plant leaf essential oil *Cymbopogon martini*

(a)

(b)

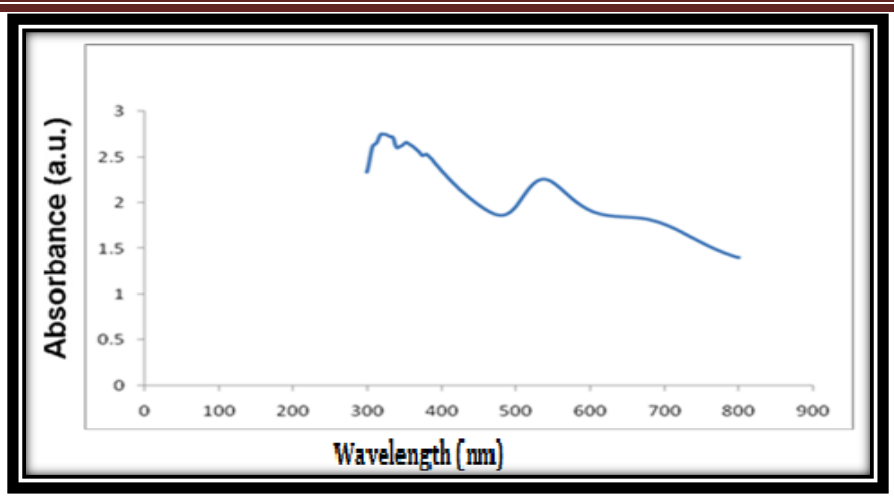

Fig. 3: (a) Chloroauric acid; (b) Synthesised gold nanoparticles;
(c) UV–Vis spectrum analysis of gold nanoparticle reduced by plant leaf oil *Cymbopogon martinii* at 540 nm

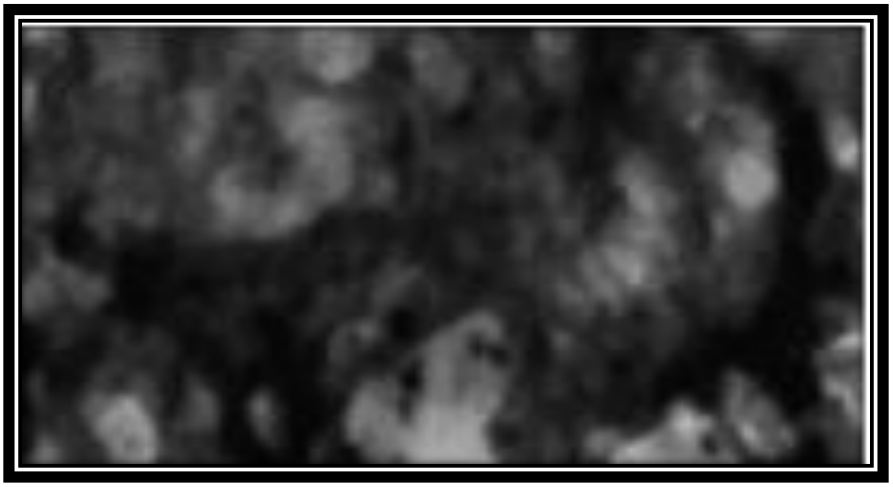

Fig. 3a: Scanning electron microscope image of gold nanoparticle synthesized by plant leaf oil *Cymbopogon martini*

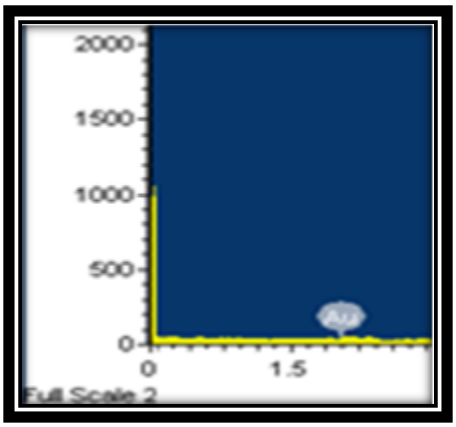

Fig. 3b: Sem-EDS spectrum showed the presence of gold signal

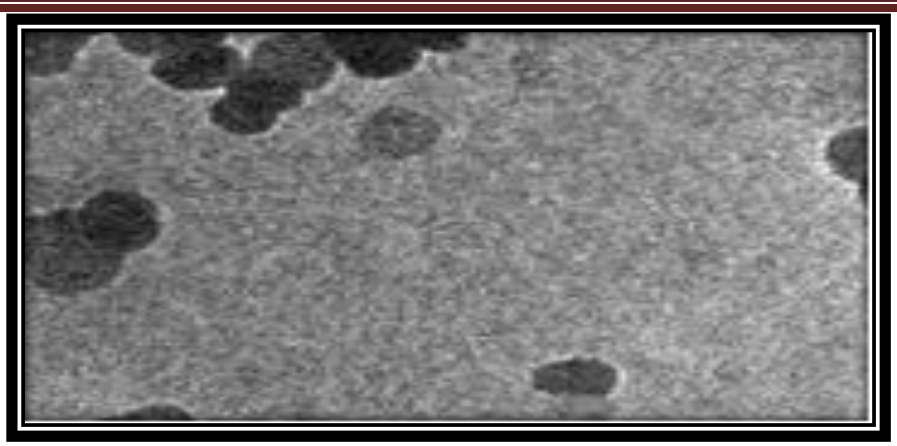

Fig. 4: Transmission electron microscope image of gold nanoparticle synthesized by plant leaf oil *Cymbopogon martinii* oil

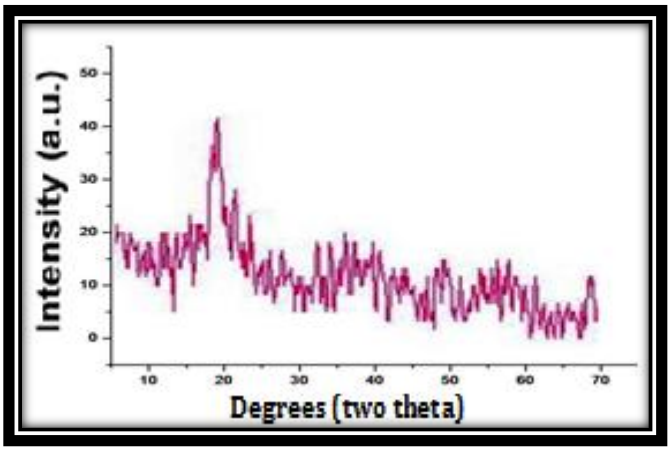

Fig. 5: XRD patterns of gold nanoparticles synthesized by plant leaf essential oil *Cymbopogon martinii*

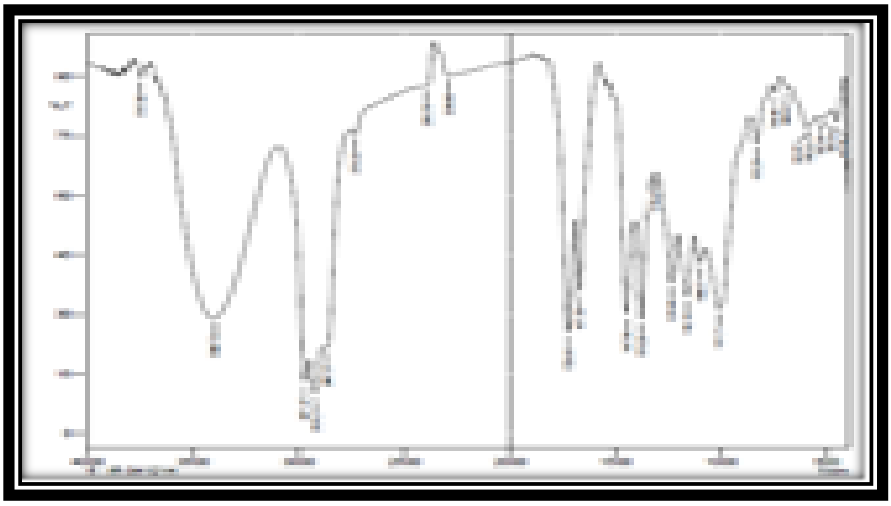

Fig. 6: FTIR spectrum of vacuum dried powder of gold nanoparticles synthesized by plant leaf essential oil *Cymbopogon martinii*

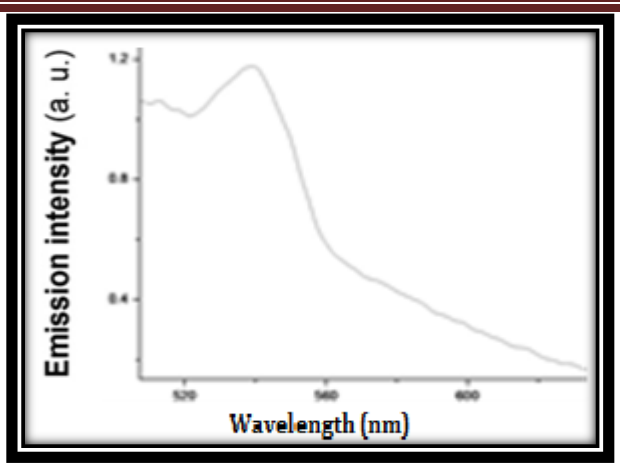

Fig. 7: Fluorescence emission spectra (excitation at 535 nm) of synthesized gold nanoparticles from plant leaf essential oil *Cymbopogon martinii*

DISCUSSION

Gold nanoparticles synthesized with *cymbopogon martinii* plant leaf oil an effective germ fighter are widely recognized as being expecially effective because of their enormously high surface area. Gold nanoparticles do not remain 'nanosize' when they come in contact with normal environmental samples such as water but they agglomerate to form much larger, much effective. There is no possibility that gold nanoparticles can ever form gold ions, except in the presence of strong oxidizing substances. With all the surface area and the energy that exists, the nanoparticles need to be held together.

Plants leaf essential oils can be efficiently used in the synthesis of gold 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 leaf oil mediated synthesis of nanoparticles is a very promising area of research (Kumar and Yadav, 2009).The potential uses and benefits of nanotechnology are enormous. These include agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nano capsules for herbicide delivery, vector and pest management and nano sensors for pest detection (Scrinis and Lyons, 2007).

In terms of green synthesis technology using *Cymbopogon martinii* oil-in-water micro emulsions as a nano-pesticide delivery system to replace the traditional emulsifiable concentrates (oil), in order to reduce the use of organic solvent and increase the dispersity, wet ability and penetration properties of the droplets is being developed. The advantages of using oil-in-water micro emulsions for improving the biological efficacy and treatment for cancer diseases would be a useful strategy in green nanotechnology. Synthesis of metallic nanoparticles using green resources like *Cymbopogon martinii* plant leaf oil is a challenging biological synthesis, since this novel green synthesis is pollutant free and eco-friendly synthetic route for gold nanoparticles. Characteristics of gold nanoparticles such as shape and size are important not only for augmenting the antimicrobial activity, but also for reducing tissue and eukaryotic cell toxicities. Anti-toxicity studies of *Cymbopogon martinii* oil with gold nanoparticles on human pathogen and on cancer cell opens a door for a new range of anticancer agents (Chang and Cheng, 2002) .

Biosynthesized AUGNPs with plant leaf oil *Cymbopogon martinii* oil can interact with and alter the function of certain mammalian tissues. For example, metallic NPs can interfere with the antioxidant defense mechanism, leading to accumulation of reactive oxygen species, destruction of mitochondria and cell apoptosis (Valodkar *et al*., 2011) The metallic AUGNPs can be used for both drug delivery and cancer cell targeting .The targeted delivery of AUGNPs themselves is therapeutic or the AUGNPs act as carriers for some other agent, biosynthesized AUGNPs are becoming increasingly important in nano medicine.

CONCLUSION

The above results described a simple and eco-friendly time-dependent method to biosynthesize green powedry AUNPs and GNPs in metal solution using medicinal plant leaf essential oils which does not need special physical conditions. The plant leaf essential oils that could be worthwhile are drug delivery, gene delivery, and biosensor applications where there is a direct contact of these nano particles with blood serum. This eco-friendly method for AUGNPs and GNP biosynthesis does not use any chemicals and thus has the potential to be exploited in biomedical applications and will play an important role in future optoelectronic and biomedical applications. In our recent studies, we have conferred the ability of the gold nano particles for preventing biofilm in urinary catheters. Which makes it possible to combine the advantages of using these plant leaf essential oils in the form of nano fibrous mats to serve as skin graft substitutes or as nano fibrous wound dressings for the treatment of burns and wounds.

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