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Green Synthesis of Gold Nanoparticles Characterization by using Plant Essential Oil *Menthapiperita* and their Antifungal Activity against Human Pathogenic Fungi

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Abstract

The present study reports about green method for the synthesis of gold nanoparticles using plant essential oil *menthapiperita*. The effects of gold concentration, extract concentration and extract quantity were identified through nanoparticles synthesis. Gold nanoparticles of synthesized essential oil *menthapiperita* were characterized with different techniques such as UV–Vis spectroscopy, SEM-EDX, X-ray diffraction, transmission electron microscopy and fourier transform infrared spectroscopy. Transmission electron microscopy showed that these nanoparticles are 60 nm. The SEM image showed that theses gold nanoparticles are small dot round shaped structure. X-ray diffraction pattern showed these gold nanoparticles are powdery in nature. Clinical isolates in the group of molds such as *Aspergillusniger*, *Aspergillusflavus,* and yeast like fungi such as *Candida tropicalis, Candida albicans and Candida kefyr,* were used for antifungal assay. The synthesised gold nanoparticle essential oil *menthapiperita* showed high activity against these deadly pathogenic fungi.

Keywords: *Menthapiperita*; Green synthesis; *Aspergillusflavus*; Gold nanoparticles; TEM

Introduction

Science of the nanotechnology is supposed to have started by the lecture of Richard Feyman on "There is Plenty of Room at the Bottom" at the annual meeting of the American Physical Society at the California Institute of Technology in 1959. Due to the optical, magnetic and electrical properties [1,2], nanomaterials have a long list of applicability in improving the human life and its environment. The first relation between human life and nanoscale was developed naturally in ayurveda, which is 5000-year-old Indian system of medicine. It had some knowledge of nanoscience and technology before the term 'nano' was even formed, which modern science has just started exploring in the 21st century [3]. Several physical and chemical processes [4-6] for synthesis of metal nanoparticles were developed considering the real life application of nanoparticles in the area of medicine [7], catalysis [8], detection [9], etc. Recently the studies started under green chemistry for the search of benign methods for the development nanoparticles and searching antibacterial, antioxidant, and antitumor activity of natural products. Biosynthetic processes have received much attention as a viable alternative for the development of gold nanoparticles where plant extract is used for the synthesis of nanoparticles without any chemical ingredients [10-14]. Leaf extracts of neem, geranium, hibiscus, cinnamon, tamarind and coriander have also found suitable for the biosynthesis of gold nanoparticles [15-20]. Among various nanoparticles, AuNPs have several effective applications as antibacterial, sensors and detectors besides their biomedical applications [21-25].

Synthesis of gold nanoparticles has gained great significance during the last few years due to biological properties. Chemical methods are among the most important approaches in metallic nanoparticles synthesis. However, these methods generally use expensive and toxic reagents as reducing and stabilizing agents, and it is very likely that trace amounts of unreacted reagents remain in the solution. Therefore, the environment is polluted. Moreover, these nanoparticles may have adverse effects in biomedical applications [26]. For this reason, one of the most essential needs in nanotechnology is to develop environmentally friendly and green approaches in nanoparticles synthesis. The biosynthesis of nanoparticles has been at the center of attention as a green and benign method in recent years. In biological methods, nanoparticles are synthesized using microorganisms (bacteria, fungi, algae) and plant extracts. In microorganism-mediated methods, the synthesis reaction takes a long time (24–124 h) [14] and the process of maintaining cell cultures is time-consuming [13,27] whereas in plant-mediated methods, reaction time is greatly saved.

Among microbes, fungi are considered as potent nanofactories for the synthesis of extracellular gold nanoparticles because of its high metal tolerance, efficient secretion of soluble proteins and other reducing components, easy scale up, economic viability and easy handling. Extracellular synthesis of gold nanoparticles is reported using fungi such as *F. oxysporum* [28], *Colletotrichum sp*. [16], *Trichothecium* sp. [18], *Trichodermakoningii* [29] and *Penicillium sp.* [24]. Filamentous fungi are commonly used as industrial producers of various primary and secondary metabolites and enzymes. The sclerotia of certain fungi produced during stress conditions contains the hard compact mass of fungal mycelia, which can be used as inoculum in adverse conditions [30]. *Sclerotiumrolfsii*is a soil-borne filamentous fungi belonging to Basidiomycota, which secretes a wide range of enzymes extracellularly for the degradation of cellulose and hemicellulose. It also secretes enzymes for the degradation of polymers like mannanase,

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polygalacturonase and glycosidases [31]. Normally, under optimized conditions, fungus secretes more than 30 g/L of protein in the extracellular medium [32]. The overproduction of NADPH/NADH-dependent enzymes by genetic manipulation of strain can facilitate the commercial production of nanostructures extracellularly. Screening of new isolates of fungi paved the way to unravels such novel enzymes/components. In the present investigation, for the first time, we report the facile synthesis of anisotrophic and isotrophic gold nanoparticles using the cell free filtrate of fungi *S.rolfsii*.

Although gold nanoparticles synthesis using plant extracts has already been reported in various plants such as geranium [16], neem [26], Aloe vera [33], *Cinnamomumcamphora* [34], mushroom [17], *Magnolia kobus* [13], pear fruit [30] and *Mangiferaindica* [17], there is still a lot of attention paid to this field because of the diversity and the high potential of plants in producing nanoparticles with different shapes. Among these nanoparticles, gold nanoparticles (GNPs) have attracted the attention of many researchers interested in the field due to their biological applications like cancer therapy and imaging [35-36].

This present study the gold nanoparticles were synthsized from plant essential oil *menthapiperita* 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

Essential oils chemical compounds

The essential oils chemical compounds were purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India). The Chloroauric acid (HAuCl4) were purchased from HiMedia (Mumbai, India).

Determination of antifungal activity

Agar well diffusion method: Antifungal activity was performed according to the method described by [37]. The synthesized gold nanoparticles of menthapiperita oil was taken upto 20 µl for assay.

Culture suspension of 200 µl 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 (they measured by Malassez blade) were spread on potato dextrose agar medium. Then, bores (4 mm depth, 6 mm diameter) were made using sterile borer and were loaded with 20 µl of each sample Fluconazole and Amphoterecin-B were used as a positive reference. The petric dish were kept for 2 hours at 4°C and then incubated at 37°C for overnight. The fungal growth was noted after 48 hours and antifungal activity was evaluated by measuring the diameter of the growth inhibition zones in millimeter. Then the values were tested through statistical analysis.

Fungal strains: The fungi used in this assay *Aspergillusniger, Aspergillusflavus, Candida albicans, Candida tropicalis and Candida kefyr* was provided from Microlabs Institute of Research and Technology.

Green Synthesis of HAucl4

For the biosynthesis gold nanoparticles, 1.5 ml of plant essential oil is mixed with 34 ml of HAucl4 solution (1 mM/ml) and incubated at 29°C for 24 h. Small aliquot of solution is used for the UV–Vis spectroscopy is performed to the plant essential oil which was exposed before and after addition to the chloroauric acid solution. The reactions mixture is centrifuged at 6000 rpm for 11 minutes and the pellet was suspended in small amount of sterilized double distilled water and then small amount of suspension was sprayed on glass slide to make thin film. The thin film was kept in hot air oven to dry and then the thin film was used for characterization studies.

UV–Visible spectral analysis

The colour change was observed in the chloroauric acid solution incubated with cell-free culture filtrate. The bioreduction of HAuCl4– ions in solution was monitored by periodic sampling of aliquots (0.1 mL) of aqueous component and measuring the UV–Vis spectra of the solution in 10-mm-optical-path-length quartz cuvettes with an Amerhsam pro 600 UV–Vis spectrophotometer at a resolution of 1 nm between 200 and 1100 nm with a scanning speed of 1856 nm/min. The nanoparticle solution was diluted to 20 times with deionized water to avoid errors due to high optical density of the solution

X-ray diffraction (XRD) studies

The bioreduced chloroauric acid solution was drop-coated onto glass substrate and powder X-ray diffraction measurements were carried out on a PAN alytical X'pert PRO X-ray diffractometer (Netherlands). The pattern was recorded by Cu K_1 radiation of 1.5406˚A and nickel monochromator filtering the wave at tube voltage of 40 kV and tube current of 30 mA. The scanning was done in the region from 30°C to 80°C at 0.02°/min and the time constant was 2 sec. The size of the nanoparticles was calculated through the Debye–Scherrer's formula D= 0.94/˚1/2 cos, where D is the average crystal size, "_" is the X-ray wavelength (_= 1.5406A˚), full width at half-maximum (FWHM) in radians.

Fourier transform infra-red (FTIR) spectroscopy

For FTIR measurements, the bioreduced chloroauric acid solution was centrifuged at 10,000 rpm for 15 min and the pellet was washed with deionized water to get rid of the free proteins/enzymes that were not capping the gold nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Thermo Nicolet model 6700 spectrum one instrument in the diffuse transmittance mode operating at a resolution of 4 cm^{-1} over 4000–500 cm^{-1}. In order to obtain a good signal/noise ratio, 512 scans were recorded.

Transmission electron microscopy (TEM)

Samples 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 (_) of 0.0251A˚. The size and morphology of nanoparticles were examined shows typical TEM images of Ag NPs synthesized using *Menthapiperita* plant oil at different exposure time periods. At the lower exposure periods (5 min), the obtained AUNPs were mostly irregular and agglomerated with a larger size (Mean ± Standard Deviation) of 120 ± 13 nm. The reason for these large sized particles is due to the aggregation of two or more NPs together which in turn result due to the presence of excess amounts of reducing moieties (at the lower exposure times the consumption of reducing molecules for the reduction process is very low) and the interactions between the stabilizing molecules bound to the surface of particles and secondary reduction process on the surface of the performed nuclei. When the light exposure period was increased to 10, 20, 40 and 80 min, the particle sizes (Mean ± Standard Deviation) were decreased to 28 ± 11, 27 ± 15, 18 ± 8 and 17 ± 12 nm, respectively, and the number of spherical shaped Ag NPs was also increased the TEM micrographs of the AuNPs clearly indicating the presence of coatings surrounding the AuNPs showed it was gold nanoparticles (Figure 1).

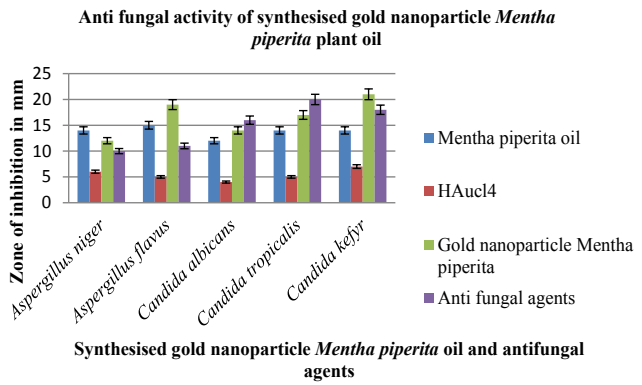

Figure 1: Inhibition of growth of selected fungi by synthesized gold nanoparticle from plant essential oil *Menthapiperita*.

Microorganisms	Menthapiperita oil	HAucl4	gold nanoparticle *M.Piperita*	Antifungal agents
Aspergillusniger	11.49 ± 0.28a	5.31 ± 0.18a	9.51 ± 0.27a	8.02 ± 0.57a
				Amphoterecin-B
Aspergillusflavus	12.47 ± 0.27b	6.26 ± 0.15b	18.49 ± 0.29b	9.03 ± 0.58b
				Amphoterecin-B
Candida albicans	10.44 ± 0.25c	4.02 ± 0.04c	9.24 ± 0.15c	14.14 ± 0.67c
				Fluconazole
Candida tropicalis	9.46 ± 0.15d	4.16 ± 0.08d	14.47 ± 0.30d	17.02 ± 0.59d
				Fluconazole
Candida kefyr	11.85 ± 0.29e	5.17 ± 0.11e	20.32 ± 0.18e	15.04 ± 0.60ce
				Fluconazole

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

Table 1: Antifungal activity of synthesized gold nanoparticle *Menthapiperita* oil.

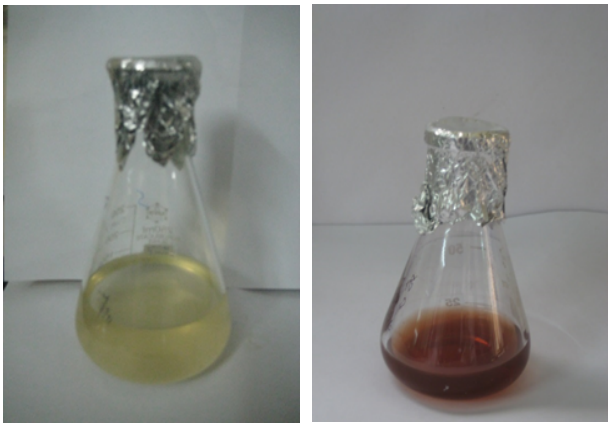

Figure 2: a) Chloroauric acid solution; b) Gold nanoparticle *Menthapiperita* oil.

Results

Invitro antifungal assay

The plant essential oilv *Menthapiperita* showed notable antifungal activity against *Aspergillusniger*, *Aspergillusflavus*, *Candida albicans*, *Candida tropicalis*and *Candida kefyr* (Table 1). The essential oil *Menthapiperita* was very highly active against *Aspergillus flavus* (12.47 ± 0.27) and least against *Candida tropicalis* (9.46 ± 0.15). Chloroauric acid solutionwas highly active against *Aspergillus flavus* (6.26 ± 0.15) and least against *Candida albicans* (4.02 ± 0.04). The gold nanoparticle *Menthapiperita* was also highly active against *Aspergillus flavus* (18.49 ± 0.29) and least against *Candida albicans* (9.24 ± 0.15). All fungi were found to be sensitive to all test essential oil *Menthapiperita* and synthesized gold nanoparticle *Menthapiperita* and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent (Figures 2a and 2b).

Biosynthesis of gold nanoparticles

The plant essential oil *Menthapiperita* of 10 ml was added to 90 ml of 2 mMHAucl4 solution, the biosynthesis reaction started within few minutes and the color reaction was observed in which clear HAucl4 solution changed into vivid ruby red color which indicates that formation of corresponding nanoparticles shown in Figure 3. The UV–Vis spectra of gold nanoparticles synthesized by *Menthapiperita* oil are showed narrow peak observed at 530 nm was seen in Figure 4.

SEM study

In this scanning electron microscope study the structure of synthesized gold nanoparticles of *Menthapiperita* oil was observed and it is round shaped in which the gold nanoparticle is in condensed form (Figure 4a). The analysis of energy dispersive spectroscopy (EDS) of the gold nanoparticles the presence of elemental gold signal was confirmed (Figure 4b).

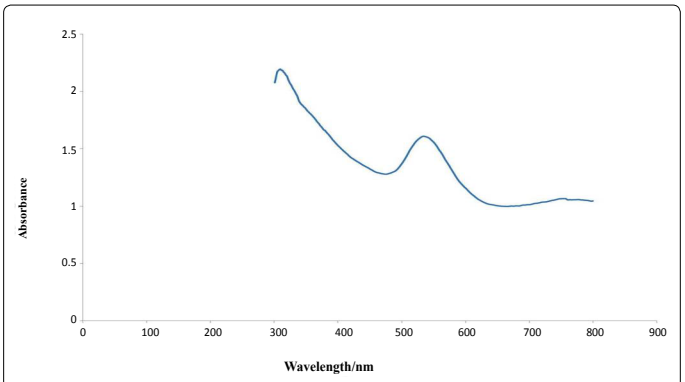

Figure 3: UV–Vis spectrum analysis of gold nanoparticle reduced by *Menthapiperita* oil at 530 nm.

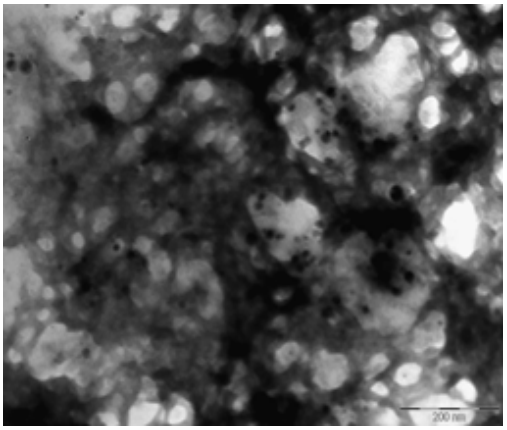

Figure 4a: Scanning electron microscope image of gold nanoparticle synthesized by plant oil *Menthapiperita*.

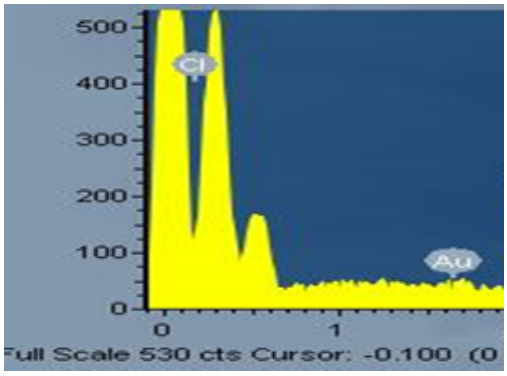

Figure 4b: EDS spectrum showed the presence of gold signal.

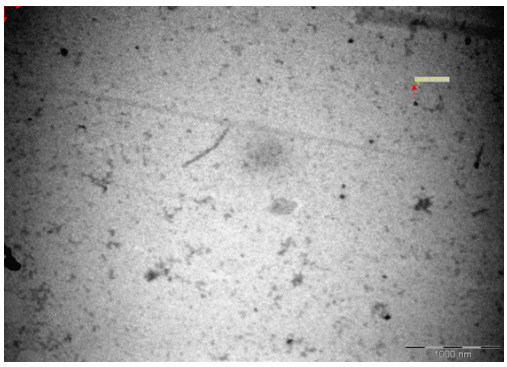

Figure 5: Transmission electron microscope image of gold nanoparticle synthesized by plant oil *Menthapiperita*.

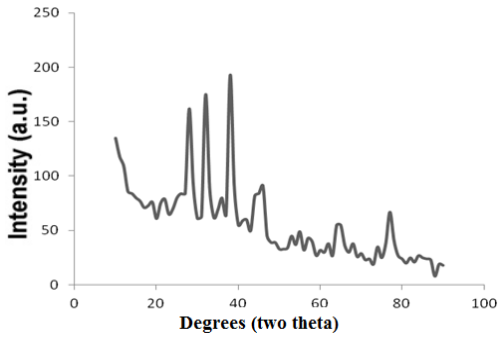

Figure 6: XRD patterns of gold nanoparticles synthesized by plant essential oil *Menthapiperita*.

TEM study

The transmission electron microscope image of gold nanoparticle synthesized by the plant oil *Menthapiperita* was noted in Figure 5. The picture suggests that the small dot round shaped gold nanoparticles are distributed widely with a diameter of 60 nm.

XRD study

The X-ray diffraction pattern of gold nanoparticle synthesized by plant essential oil *Menthapiperita* was shown in Figure 6. The XRD pattern thus clearly illustrates that the gold nanoparticles present green synthesis method are powdery in nature.

FTIR study

FTIR spectroscopic studies were carried out to investigate to find possible bioreducing agents present in the plant oil. The spectra of plant oil were recorded before and after adding the chloroauric acid solution (Figure 7). The interferrogram exhibit a broad at 3412 cm^{-1} is assigned to the N–H group from peptide linkage present in the plant oil. Formation of C-C bonds is energetically favored over S C bonds, as the latter will impose severe geometrical constraints on the molecule more specific in thiol group and less in acidic as compared to alcohols and that makes elimination of hydrogen attached to sulfur group. There is a decrease in the concentration of the amide linkage in the aqueous solution after the formation of gold nanoparticle.

Fluroscence spectroscopy study

The fluroscent spectra of gold nanoparticle synthesized by the plant essential oil *Menthapiperita* were shown in Figure 8. A broad emission band having prominent peak centered at ~520 nm is observed for the plant oil as it is excited at 400 nm. In this study emission intensity gradually increases with the decreasing concentration ofHAucl4. 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.

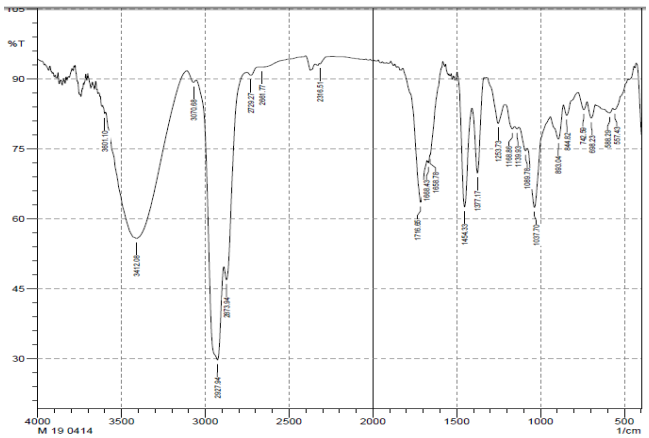

Figure 7: FTIR spectrum of vacuum dried powder of gold nanoparticles synthesized by plant essential *Menthapiperita*.

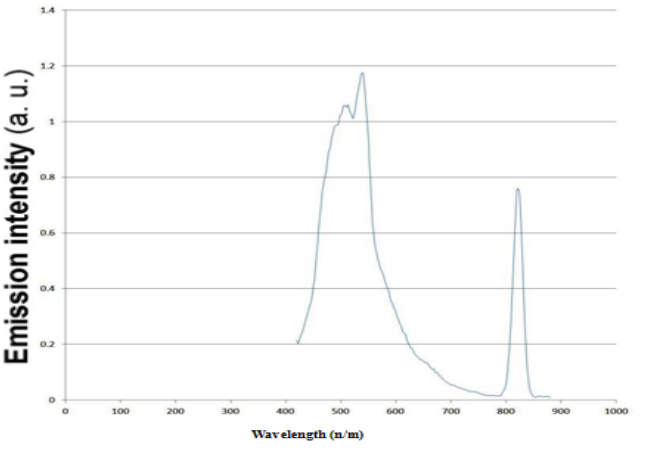

Figure 8: Fluorescence emission spectra (excitation at 400 nm) of plant essential oil *Menthapiperita*.

Discussion

Colloidal Au nanoparticles possess a lot of interesting properties that make them useful for biological applications. So far there is no indication of Au particle corrosion, and Au particles are inert, which make them relatively biocompatible [23]. Their unique features such as tunable core size, monodispersity, large surface to volume ratio, and easy functionalization with virtually any molecule or biomolecule allow targeting, transport, and tuning of delivery processes [38] reviewed the use of colloidal gold nanoparticles for fabrication of anisotropic and multicomponent nanoparticles [39]. Developed gold nanoparticles functionalized with a valine-derived formamide as catalysts for the reduction of ketimine 1 with trichlorosilane in toluene [40] deposited gold nanoparticles onto the surface of indium–tin oxide electrode surface used for the amperometric sensing of glucose at alkaline and neutral solutions [41]. Sharma et al. denoted the assembly of nanoparticles into three-dimensional (3D) architectures which allow greater control of the interactions between gold nanoparticles with biomolecules [42]. Wanga et al. reported that biogenic gold nanoparticles could facilitate the electron transfer between p-nitrophenol and glassy carbon electrode (GCE) by immobilizing on the electrode [43]. Huang et al. proved the versatility of AuNP applications in the direct or competitive surface plasmon resonance kinetic assay of the interaction between small molecule inhibitors and their target proteins with a high sensitivity [34] used gold nanorods with different localized surface plasmon resonance based quenching process of quantum dot (QD) emission to be efficient in DNA detection [44] achieved the multimodal delivery of antibody-conjugated PEGylated gold nanoparticles enhancing the contrast in in vivo optical coherence tomography images of oral dysplasia in a hamster model [45] reported about the microbiological intelligence of gold nanoparticles when conjugated with poly paraphenyleneethynylene to identify two different strains of E. coli in minutes [46].

It has been stated that either through free amino groups or cysteine residues, the protein can bind to gold nanoparticles that lead to the stabilization of gold nanoparticles by surface bound protein [28]. Various phytochemicals like alkaloids, flavones, steroids, polysaccharides, amino acids, oximes and proteins in general and menthol in particular. A biological route of gold nanoparticles to promote the anisotropic growth of different crystal planes under ambient condition. Shanker have reported that the synthesis of a high percentage of thin, flat, and single-crystalline gold nanotriangles by using the lemongrass plant. Very recently, Rai et al. have demonstrated that the presence of halide ions and modulation of temperature can control the morphology of biologically synthesized gold triangles using lemongrass leaf extract [47]. It has also been shown that the size of nanoparticles using this technique can be controlled through pH adjustment, exposure time, and temperature reaction.

The capability of *menthapiperita* oil for the reduction of 'Au' to gold nanoparticles was monitered in many conditions. Characterization by UV-spectrophotometer, XRD and FT-EDX techniques confirmed the reduction of gold ions to gold nanoparticles. To the best of our knowledge, and based on a thorough literature surveys, this is the second report on the synthesis of gold nanoparticles using *menthapiperita* oil as a volatile compound. The antifungal activity was done to check the effect of synthesized gold nanoparticle *menthapiperita* oil on human pathogenic fungi. The results showed high activity against all the clinical isolates of fungal pathogens.

Conclusion

Menthapiperita plant essential oil was successfully used for the biosynthesis of gold nanoparticles in the size range of 60 nm. Gold particles were observed from UV-spectrophotometer, TEM imaging, XRD technique and FTIR analysis. In this article we presented a new method for the synthesis of gold nanoparticles. The use of natural extracts, distilled water and practically nontoxic reagents allows the synthesis pathways presented to be considered as "green" and so permitting the synthesized AUNPs to be used in sensitive areas such as biomedicine. The size of the silver nanoparticles was estimated as 42 nm. Crystallinity of the AUNPs was confirmed from XRD pattern. The zeta potential of AUNPs was found to be 55.0 mv, this high negative value confirms the repulsion among the particles and thereby increase in stability of the formulation. The antifungal assay results of synthesized gold nanoparticles of *menthapiperita* showed notable activity against all pathogenic human fungi. The study results support the environmental friendliness and promising potential of nanoparticle tailoring by bio-inspired or biological routes. To the best of our knowledge, this is the rapid synthesis of gold nanoparticles using a microbial component

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