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APIUM GRAVEOLENS LEAF EXTRACT-MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND ITS ACTIVITY ON PATHOGENIC FUNGI

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Green synthesis of metallic nanoparticles is gaining momentum in the field of nano-research because it is a single-step, fast, low cost and eco-friendly alternative to well known physical and chemical procedures that involve costly instruments or hazardous by products at the end. Here, we report a single-step, novel and cost-effective biosynthesis of silver nanoparticles from silver salt using leaf extract of celery (*Apium graveolens*). Silver nanoparticle formation was studied at regular intervals by UV-Vis spectroscopy. The crystallinity and phases were characterized by X-ray diffraction (XRD) analysis. Transmission electron microscopy (TEM) was performed to determine the size, shape and morphology of the nanoparticles. Fourier transform infra-red (FTIR) spectroscopy was done to detect the organic functional molecules that reduced the silver ions and capped the particles during interaction. Antifungal activity of these biogenic silver nanoparticles was studied against two pathogenic fungi- *Aspergillus niger* and *Aspergillus wentii* and these nanoparticles showed effective fungicidal property against the tested fungal strains.

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Keywords: Silver nanoparticles, celery (*Apium graveolens*) leaf extract, UV-Vis Spectroscopy, XRD, TEM, Antifungal activity

1. Introduction

Nano-research initiates with the primary step of nanoparticle synthesis through a simple, low cost and eco-friendly method. Synthesis methods like chemical reduction [1], electrochemical reaction [2], microwave-assisted [3] or photocatalytic reaction [4] are widely used in nanolabs to prepare metallic nanoparticles from bulk materials. But these complicated methods are time-consuming and involve costly equipments for preparation of metallic nanoparticles [5]. In addition, the hazardous by-products of these procedures cause serious damage to the environment as described in previous reports [6,7]. In last few years, green synthesis routes ushered in this field of nanoparticle synthesis and eliminated the major disadvantages of the conventional methods. Biological route for nanoparticles synthesis has been reported to be simple, fast and environment friendly as they involve plant extracts that produce nanometals in a single step [8]. Bacteria [9,10], algae [11] and fungi species [12,13] have been used for biosynthesis of metal nanoparticles earlier but use of plant extract is a better choice as they are easy to prepare and safe to handle unlike microorganisms [14]. Leaf extracts of *Aloe vera* [15], parsley [16], lemon grass [17], gooseberry [18] etc. which are potential source of ascorbic acid were so far used for the synthesis of metal nanoparticles. Celery (*Apium graveolens*) leaves was chosen here as they contain a few functional organic molecules (including ascorbic acid) that have ability to reduce silver ions and can stabilize the particles in the medium [19].

Aspergillus niger and *Aspergillus wentii* are two pathogenic fungal species of the genus *Aspergillus* that cause Aspergillosis, a pulmonary disease common in humans with symptoms like

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fever, chest pain, cough and difficulty in breathing [20]. Antifungal resistance is always a serious concern in medical practice and silver nanoparticles was found to have fungicidal property against these fungi as reported by a few [21,22].

In this paper, we report for the first time, the green synthesis of silver nanoparticles from silver salt by using leaf extract of celery (*Apium graveolens*) and the antifungal assay was carried out to test the antifungal activity of these biogenic Ag nanoparticles against two pathogenic fungi- *Aspergillus niger* and *Aspergillus wentii*.

2. Materials and Methods

2.1. Materials- Fresh celery (*Apium graveolens*) leaves (shown in Figure 1) were collected from the local market for preparing extract. Silver nitrate ($AgNO_3$) required for this work was purchased from Merck India Ltd. The fungal strains were procured from National Chemical Laboratory, Pune (India) and the potato dextrose agar used for antifungal assay was purchased from Himedia, India.

Fig. 1: Fresh celery (Apium graveolens) leaves

2.2 Methods

2.2.1 Preparation of silver nanoparticles

To prepare the leaf extract, fresh celery (*Apium graveolens*) leaves (weight around 100 g) were cleaned, chopped and crushed inside a grinder with a small amount of de-ionized (DI) water. It was later filtered and centrifuged at 4000 rpm for 10 minutes to obtain clear soup of celery leaf extract. 20 mM stock solution of silver nitrate was prepared by dissolving around 0.34 g of $AgNO_3$ in 100 ml DI water. To reduce the silver cations, equal amount (100 ml) of celery leaf extract was added gradually to the $AgNO_3$ solution resulting into a half diluted (10 mM) mixture of the silver nitrate. The reacting mixture was then kept at room temperature and after half an hour of incubation, the color of the mixture started to change from colorless to light brown (shown in Figure 2). To obtain the nanoparticles after completion of reaction, the reacting mixture was centrifuged at 10000 rpm for 20 minutes to separate other components from the particles. The supernatant was decanted and the precipitate formed at the bottom of the centrifuge was re-dispersed into small amount of DI water. The dispersion was further centrifuged at 5000 rpm for 10 minutes to remove the organic biomass attached to the nanoparticles. At the end, the pellet of nano-silver was collected from the bottom of the centrifuge tube carefully and dried inside a desiccator to obtain dry powder of biogenic Ag nanoparticles.

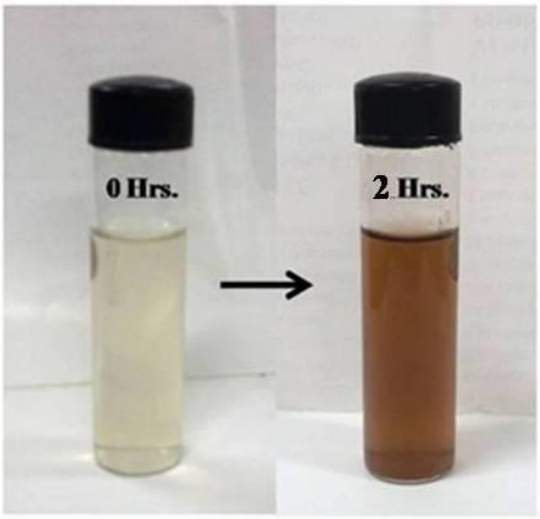

Fig. 2: Change of mixture color with reaction time

2.2.2 Characterization of Silver nanoparticles

Absorption spectra of the reacting solution were recorded at regular intervals by using Perkin Elmer UV-Vis spectrometer (USA). XRD of the dry silver nanoparticles was done by Rigaku Ultima-III x-ray diffractometer (λ=0.154, Japan). FTIR spectroscopy of the nanoparticles was performed by IR-Prestige FTIR spectrometer (Shimadzu, Japan). To prepare the sample for TEM analysis, the dry Ag nanoparticles were suspended in DI water maintaining standard concentration (50µg/ml). A few drops of this suspension was then placed on the carbon coated copper grid and dried in vacuum before scanning under high resolution TEM (JEOL-2010, USA; operating voltage is 200 kV).

2.2.3 Study of Antifungal activity

Antifungal activity of these biosynthesized nanoparticles was studied against two pathogenic fungi- *Aspergillus niger* (strain no.-NCIM 1213) and *Aspergillus wentii* (strain no.-NCIM 667) following agar disc diffusion procedure. The fungal strains were maintained on favourable medium prepared from potato dextrose agar. Pure leaf extract of celery (*Apium graveolens*) was taken as control to compare the results of antifungal assay. The pure leaf extract and two different suspensions of nanoparticles having two different concentrations i.e. 25µg/ml and 50µg/ml were added separately into three different wells created on each disc seeded with fungus. Each disc was made in triplicates to get more accurate results considering statistical variance. The prepared discs were then placed inside an incubator (at 30ºC) for 48 hours to allow the fungi to grow. The fungicidal activity of nano-silver was evaluated by measuring the diameter of inhibition zone formed around the wells after 48 hours incubation period.

3. Results and Discussions

3.1 UV-Vis Spectroscopy

Celery (*Apium graveolens*) leaves are rich source of functional organic molecules (including ascorbic acid) that can reduce silver ions present in the silver nitrate solution. After half an hour of extract addition to the $AgNO_3$ solution, the mixture color began to change from colorless to light brown indicating formation of nanoparticles in the medium (shown in Figure 2). The color became darker with reaction time and turned dark brown after 4 hours of incubation.

The change in the coloration of reacting mixture may correspond to the surface plasmon resonance of silver nanoparticles [23]. Formation of nanoparticles in the mixture was investigated by scanning the mixture under Perkin Elmer UV-Vis spectroscope at regular intervals (every hour). The peak absorbance observed near 450 nm further confirmed the production Ag nanoparticles in the mixture. The UV-Vis spectra recorded at various time intervals are shown in Figure 3(a). Figure 3(b) shows the variation of peak absorbance with reaction time. It may be observed from Figure 3(b) that the peak absorbance increased almost linearly with reaction time till 2 hours of incubation. This may be due to the production of more number of nanoparticles in the medium. After 4 hours of incubation, the formation rate saturated denoting the completion of reaction.

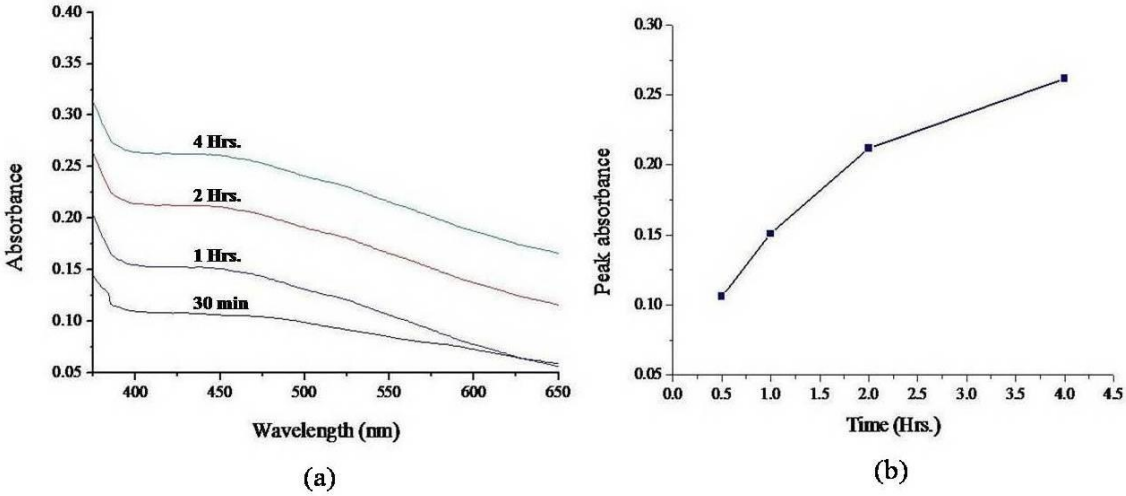

Fig. 3(a): UV-Vis spectra of the reacting solution at different time intervals
(b): The graph indicates variation of peak absorbance with reaction time

3.2 XRD Analysis

The XRD graph of dry Ag nanoparticles (shown in Figure 4) shows peaks of silver at 2θ = 27.8˚, 32.1˚, 46.1˚, 55.1˚, 56.9˚ and 77.8˚ that may correspond to (220), (122), (231), (331), (241) and (311) planes of fcc structure of silver (correlated to JCPDS card: File no. 4–783). Absence of any other peak except the characteristic peaks of silver indicates high level of purity of these biogenic silver nanoparticles.

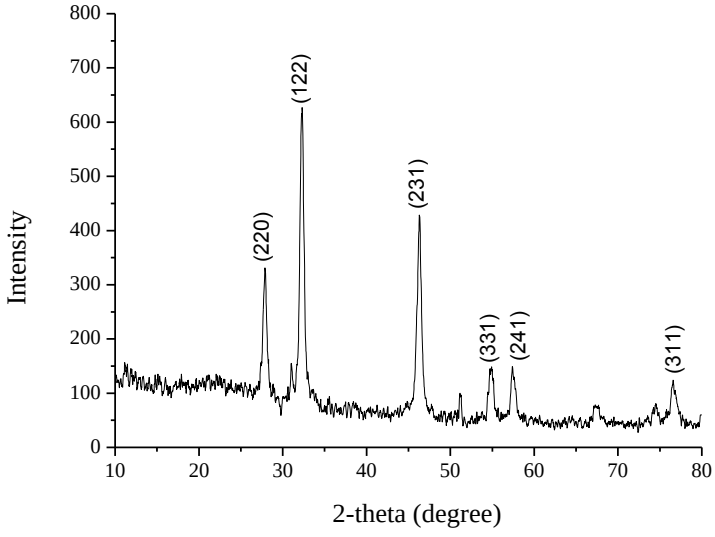

Fig. 4: X-ray diffraction curve of biogenic silver nanoparticles

3.3 TEM Study

Transmission electron microscopy was performed to obtain detail information about the microstructure and particle dimensions of the silver nanoparticles prepared using celery leaf extract. The high resolution TEM images (shown in Figure 5) show that the silver nanoparticles are almost spherical in shape with an average diameter closely 25 nm. The inter-planar spacing, as observed from the images, can be manipulated to be around 0.282 nm that may correspond to (122) planes of nanosilver.

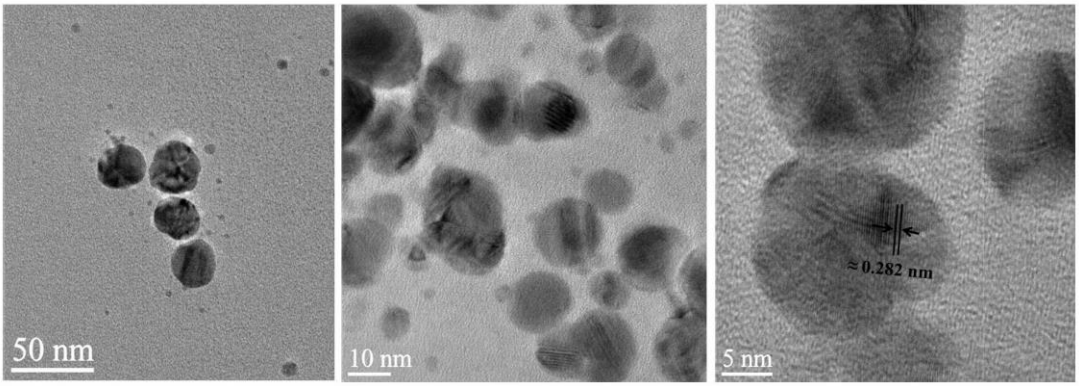

Fig. 5: High resolution TEM images of Ag nanoparticles

3.4 FTIR Spectroscopy

Fig. 6 shows the FTIR spectra of both the leaf extract and biogenic silver nanoparticles in absorbance mode. The spectrum of leaf extract consists of eight noticeable peaks throughout the entire range. The bands at 1658 $cm^{-1}$and 3292 cm^{-1} indicate C=O stretching of tertiary amides and O-H stretching of benzene ring compounds (phenol, ascorbic acid etc.) respectively [24]. A band at 1078 cm^{-1} indicates the C–N stretching vibration of amines whereas a distinct band at 1595 cm^{-1} denotes the bending of C–H bonds present in hydrocarbons [25]. The bands noticed at 1406 cm^{-1} and 648 cm^{-1} may be attributed to C-H bending of alkanes and stretching vibrations of halo-alkanes respectively [26]. The remaining two bands found at 729 cm^{-1} and 883 cm^{-1} may correspond to the stretching vibration N-H bonds present in amines [27]. The FTIR spectrum of biogenic nanoparticles reveals no distinct peak in the observation range suggesting purity of the particles prepared in this method. From this analysis, it is clear that the functional organic molecules like amines, phenol, ascorbic acid etc. present in the celery (*Apium graveolens*) leaf extract can play important role for reducing silver ions and stabilizing the colloidal particles in the medium.

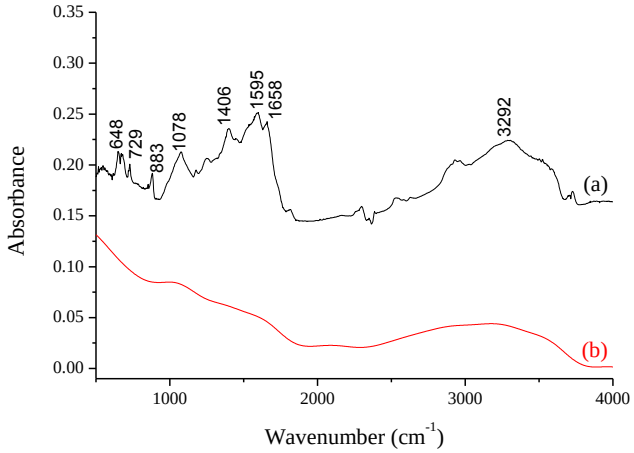

Fig. 6: FTIR spectra of (a) pure leaf extract and (b) biosynthesized silver nanoparticles

3.5 Antifungal Activity

The antifungal activity of these biosynthesized silver nanoparticles was examined against two pathogenic fungi- *Aspergillus niger* and *Aspergillus wentii* and the obtained result is shown in Table 1. After applying the control and two suspensions (conc. 25µg/ml and 50µg/ml) of Ag nanoparticles separately into the wells created on each plate, the discs were incubated and after 48 hours, distinct inhibition zones were noticed around the wells where suspensions were added. The control i.e. pure leaf extract of celery (*Apium graveolens*) was unable to curb the growth of these fungi as no inhibition zone was observed around the cups where it was applied. From the result of antifungal study, it is clear that the suspension of nanoparticles prevented the growth of the fungi to various levels. Better antifungal activity was reported against *Aspergillus wentii* (12.01 mm) followed by *Aspergillus niger* (11.59 mm). It was further observed that the size of the inhibition zone increased with the increase of concentration of nanoparticles in the applied suspension as depicted in Figure 7.

Table 1: Result of Antifungal study

Fungus tested	**Concentration of Ag nanoparticles in suspension (µg/ml)**	**Diameter of inhibition zone (mean of triplicates) (mm)**
Aspergillus niger	Control	0
	25	5.56
	50	11.59
Aspergillus wentii	Control	0
	25	5.79
	50	12.01

Reports on the antibacterial activity of Ag nanoparticles are redundant in the literature but reports on the effect of nanosilver on fungal species are scarce. Though the antifungal mechanism of silver nanoparticles is not completely understood yet, but a few reports provide better understanding of the acting mode of biogenic silver nanoparticles. Nasrollahi et al. reported that when the nanoparticles come in contact with the fungal cells, conformity of the cell membrane gradually diminishes and cells fail to survive for long [28]. Vazquez-Munoz et al. showed that the Ag nanoparticles induce changes in the morphology of fungal cell membrane during interaction that leads to the formation of “pits” on cell surface [29]. As a result, the cells rupture gradually

causing leakage of the cytoplasmic component and eventually succumb to death as described by Chen et al [30].

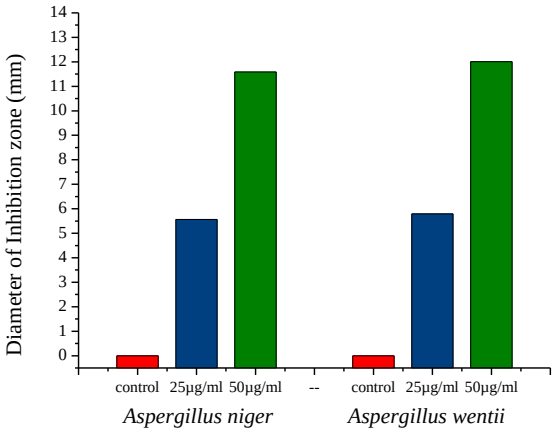

Fig. 7: Bar graph shows the result of concentration dependent antifungal study

4. Conclusion

Plant extract mediated procedures for nanoparticles synthesis is gaining popularity among the researchers as it is a single step, low cost and eco-benign alternative to widely known chemical and physical methods. In this study, we prepared silver nanoparticles from silver nitrate solution using leaf extract of celery (*Apium graveolens*). The obtained nanoparticles were found to be closely spherical in shape with average diameter 25 nm. Crystalline nature of the particles was verified by XRD whereas the reducing and capping organic molecules present in the extract were identified by FTIR spectroscopy. The antifungal assay reported that these biosynthesized Ag nanoparticles have effective fungicidal property to curb the growth of two pathogenic fungi- *Aspergillus niger* and *Aspergillus wentii*. Hence, it can be explored as a promising option to reduce the infective potential of the tested pathogenic fungi.

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