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RESEARCH ARTICLE

Preparation of Silver Nanoparticles from *Bael* (*Aegle marmelos*) Leaves Extract and their Doping with Multiwalled Carbon Nanotubes for Antibacterial Utility

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ABSTRACT:

A green methodology was adopted for the preparation of silver nanoparticles. The leaves of Bael Tree (*Aegle Marmelos*) were possessed from the area of Redhills, Chennai, Tamil Nadu, India. From the clean and dried leaves, the Bael tree leave extract was obtained by a simple chemical process. This extract was further employed for the green synthesis of silver nanoparticles. When the extract was added into the colourless silver nitrate solution and stirred, a periodic colour change was observed indicates the formation of silver nanoparticles. Besides, Multiwalled Carbon Nanotubes (MWCNTs) were synthesized by a simple chemical approach using potassium persulphate as the solid oxidant. The synthesis of MWCNTs involves both the Conc. Sulphuric acid and Conc. Nitric acid were added to 99.9% pure graphite powder slowly with stirring for a period of 30 min. The solid oxidizing agent potassium persulphate was then added with stirring and the whole reaction mixture was refluxed to get the MWCNTs. The target Silver-doped multiwalled carbon nanotubes were produced by thermal treatment of the reaction mixture containing silver nanoparticles and MWCNTs. The antibacterial utility of the synthesized silver-doped MWCNTs was tested on different bacterial culture using Well diffusion method. The bacterial cultures employed for the anti-bacterial study are *Enterococcus faecalis* (MTCC 2729) and *Pseudomonas aeruginosa* (MTCC 424). The antibacterial investigation furnished that the Silver-doped carbon nanotubes exhibit good antibacterial activity. A higher antibacterial behavior was noticed on *Pseudomonas aeruginosa* than *Enterococcus faecalis* upon acted by silver-doped MWCNTs.

KEYWORDS: *Aegle Marmelos*, Silver Nanoparticles, Multiwalled Carbon Nanotubes, Silver-doped Multiwalled Carbon Nanotubes, Antibacterial study.

INTRODUCTION:

Nanoscience and technology is one of the rapidly growing fields of science, which experiences enormous interest for researchers since the early 90s of the last century to the present day scientists. This exciting field of research still provides an abundant opportunities, and applications to explore.

In order to fulfill the chemical, biological, biochemical or pharmacological requirements of human being, this area of research has become an inherent part of modern day science and technology[1-3]. Nanoparticles and Nanomaterials reveal an unexpected and unparalleled properties, and thus attracts an innumerable applications including as antibacterial agents, industrial catalysts, healthcare-related products, medical device coatings,

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optical sensors, diagnostics, orthopedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs[4-6]. More specifically nanomaterials in the form of nanoparticles (NPs) warrant implication as these NPs possess novel and size-related physico-chemical properties differing significantly from larger matter[7]. Their remarkable advances in the field of biotechnology have opened manifold opportunities for molecular diagnostics and therapy[8]. The magnificent properties of NPs have been capitalized in a wide range of prospective applications including medicine, cosmetics, renewable energies, environmental remediation and biomedical devices[9,10]. The fascinating nano-size nature of these particles enable diverse interactions with biomolecules on the cell surfaces and within the cells in a number of ways that can be decoded and designated to various biological, biochemical and physiochemical properties of these cells[11].

Among a diverse nanoparticles, silver nanoparticles (AgNPs) are progressively utilized in numerous fields including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. The specific properties exhibited by nanoparticles comprised of optical, electrical, and thermal, high electrical conductivity, and biological properties[12-14]. Moreover, the silver nanoparticles are also used in the enhancement of decimation of tumors[15-17]. Silver-based nanoparticles are the leading therapeutic agents in medicine for a number of diseases and surgical infections and it was reported that the benefits of silver are more than the risk factors[18]. When silver nanoparticles are doped with multiwalled carbon nanotubes (MWCNTs), the catalytic effects are enhanced[19]. Herein, we report a green synthesis of silver nanoparticles, using the extract of *Aegle marmelos* leaves.

Aegle Marmelos is a species of tree native to India and it belongs to the family Rutaceae[20-21]. It is commonly known as Bael, Golden apple, and Bengal quince which is seen throughout South East Asia. The fragrant leaves and fruit of the plant have medicinal value and were used to treat dyspepsia and sinusitis. Leaves of the *A. Marmelos* contain alkaloids of which aegeline (N-[2-hydroxy-2(4-methoxyphenyl)ethyl]-3-phenyl-2-propenamide) is a known constituent and is consumed as a dietary supplement.

Fig. 1: Leaves of *Aegle marmelos*

The leaves of the Bael tree used for the preparation of silver nanoparticles are shown in Figure **1**. MWCNTs were synthesized by our simple chemical approach[22]. The synthesized silver nanoparticles are then doped on MWCNTs. The resultant silver-doped MWCNTs are employed for antibacterial application.

EXPERIMENTAL:

Materials and Methods:

For the synthesis of silver nanoparticles, Bael Tree (*Aegle Marmelos*) leaves were collected from the area of Redhills, Chennai, Tamil Nadu, India. The leaves were cleaned with fresh water thrice and dried in air under room temperature for 24 hrs. The dried clean leaves are then stored in metal containers and ready for usage. Silver nitrate, Graphite powder, 99.9% pure, Conc. H_2SO_4, Conc. HNO_3, Potassium persulphate were purchased from Sigma-Aldrich (India) Limited, Chennai.

All the chemicals used were of analytical grade and used as such without any further purification. Millipore water was purchased from Lab Tech Chemicals, Chennai, India. UV-Visible absorption spectra were recorded on a Shimadzu UV-visible spectrophotometer (UV-1800, Japan). The morphology of the sample was observed with Field Emission Scanning Electron Microscopy (FE-SEM) Hitachi Ltd (SU-6600). The X-ray Diffraction (XRD) pattern was taken with a Philips instrument (JSO Debye Flex 2002 Seifert) in the angular range 10^o to 80^o. The bacterial standards of *Enterococcus faecalis* (MTCC 2729) and *Pseudomonas aeruginosa* (MTCC 424) were procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

Preparation of *Aegle Marmelos* Leaves Extract:

The extract was prepared by using 10g of fresh leaves of *Aegle Marmelos*. Fresh leaves from Bael Tree were washed thoroughly (three times) with distilled water, dried in air at room temperature for about 24 h. The dried and cleaned leaves were cut into fine pieces and transferred into a 500ml Erlenmeyer flask, followed by the addition of 100ml of distilled water into it and boiled

for 15 min. The orange coloured extract formed was cooled to room temperature and filtered and used immediately for the synthesis of silver nanoparticles.

Synthesis of Silver Nanoparticles:

Silver nitrate ($AgNO_3$) was used as a precursor for the synthesis of silver nanoparticles[23]. 0.1mM aqueous solution of silver nitrate was used for nanoparticles preparation. Silver nanoparticles were obtained by adding 25ml of plant extract to 75ml of silver nitrate solution in a clean beaker at room temperature. A distinct color change was observed during the course of the reaction. The initial colourless solution of silver nitrate turned to dark yellow after 2 min, suggesting the formation of silver nanoparticles. The colour became darker and turned into dark brown after 5 min. The reaction mixture was stirred further for about 15 min to complete the reaction. The reduction of silver ions was confirmed by the UV–Vis spectrum of the solution. The synthesized nanoparticles were separated out from the solution by centrifugation at 10,000rpm for 20 min. Centrifugation process was repeated for 3 to 4 times by dispersing the pellet in distilled water for removing organic matters of leaf extract. The pellet was collected carefully from the bottom of the centrifuge tube in a watch glass and dried in hot air oven at 60ºC.

Synthesis of Multiwalled Carbon Nanotubes:

We have followed a similar procedure employed in our previous paper (Rani et al. 2018) for the preparation of MWCNTs. In a clean 500ml beaker, a mixture of 30ml of concentrated nitric acid and 60 ml of concentrated sulphuric acid were taken. To this acid mixture about 5g (0.4166mol) of 99.9% graphite was added slowly with stirring for about 30 min. Once the addition of graphite is done, the solid-oxidant potassium persulphate [128g (0.473mol)] was added steadily for about 50 min with constant stirring. As this reaction is an exothermic one, a lot of heat was produced while adding potassium persulphate into the mixture. Therefore, special care must be taken during this step. Following this, the resultant mixture was heated for 3 h at 110°C and kept as such for 24 h at room temperature. After 24 h, the entire content was then added into 1 litre of Millipore water and stirred well with the help of magnetic stirrer fastly for one and half hour. The top floating layer (of MWCNTs) was collected separately with extreme care, washed with water repeatedly, filtered and dried. The CNTs prepared in the reaction mixture is given in the Figure **2**.

Fig. 2: View of MWCNTs prepared in our lab

Synthesis of Silver-doped MWCNTs:

Silver nanoparticles prepared as above by the green synthesis (15mg) were added into Multiwalled carbon nanotubes (100mg) taken in a 100ml RB flask. About 50ml of distilled water was added into the reaction mixture and heated to 70°C and the mixture was continued to be kept at the above temperature for further 3 h with constant stirring. The product formed (Figure **3**) was cooled to room temperature and filtered washed with distilled water for multiple times and dried.

Fig. 3: Silver-doped MWCNTs as suspension in water

RESULT AND DISCUSSION:

Firstly the silver nanoparticles were synthesized by a green route using the leaves extract of *Aegle Marmelos*. Subsequently, the silver nanoparticles were doped with the synthesized MWCNTs to prepare silver-doped MWCNTs (Figure **3**). The XRD data of the obtained multiwalled carbon nanotubes was similar to that of the reported pattern of MWCNTs prepared by our research group [22] as well as by Das *et al.*[24]. The XRD analysis point out that graphite was converted into MWCNTs and the possibility of amorphous carbon materials was very less. The formation of MWCNTs was further evidenced by the SEM image which exhibits the tubular form of CNTs (Figure **4**).

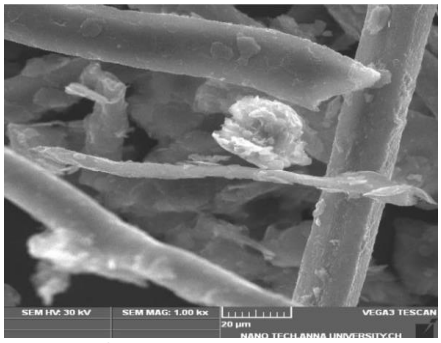

Fig. 4: SEM image of MWCNTs

Antibacterial activity of Silver-doped MWCNTs:

The stock cultures of *Enterococcus faecalis* (MTCC 2729) and *Pseudomonas aeruginosa* (MTCC 424) were maintained on slants of nutrient agar in 4˚C. Active cultures for screening their susceptibility were prepared by transferring loop full of cells from stock cultures to test tubes containing Mueller Hilton Broth and were incubated at 37˚C for 24 h. Well diffusion method was used to screen the antibacterial activity. For assay, Mueller Hinton Agar (Beef Extract - 2g; Acid Hydrolysate of Casein - 17.5g; Starch - 1.5g; Agar - 17g. was used and Final pH was maintained at 7.3±0.1) (MHA) was used. Onto the sterile MHA plates 0.1mL of the saline suspension of individual cultures were swabbed uniformly. The well of 8mm diameter was bored on the MHA plates. Different concentrations of the extracts (15mg, 20mg and 25 mg) were loaded onto the well. The plates were incubated at 37˚C for 24 hours to assess the biological effect. After incubation period, the diameter of inhibition zones formed around the well was measured in millimeter. These studies were performed in duplicates for all the bacterial samples and the results are given in Table **1** and the zone of inhibition against *Enterococcus faecalis* and *Pseudomonas aeruginosa* are shown in Figure **5**.

The silver-doped MWCNTs were evaluated for their antibacterial activity by the Well diffusion method. The occurrence of a clear zone (cramped bacterial growth) around the cavity is an indication of antibacterial activity. The result of our antibacterial study shows that silver-doped MWCNTs displayed very good antibacterial action against both the bacterial cultures. Moreover, it was also experienced that the silver-doped MWCNTs induce a very high activity against *Pseudomonas aeruginosa* than *Enterococcus faecalis*. Besides, silver-doped MWCNTs exhibit a towering antibacterial activity at a lower concentration similar to reported result[13].

A B

Fig. 5: Silver-doped MWCNTs show the zone of inhibition against (A) *Enterococcus faecalis,* and (B) *Pseudomonas aeruginosa*

Table:1 Antibacterial activity of silver-doped MWCNTs showing the values for zone of inhibition

Sample	Species	Zone of inhibition (in mm)			
		15 mg	**20 mg**	**25 mg**	**Control (Streptomycin -10 mg)**
AgCNT	*Enterococcus gaecalis*	12	14	22	22
		14	14	22	22
		13	14	22	22
	Pseudomonas aeruginosa	6	8	8	28
		6	8	8	28
		6	8	8	28

CONCLUSION:

Silver nanoparticles were prepared by implementing a green methodology using the extract of Bael Tree leaves. MWCNTs were also obtained by employing a simple chemical approach by utilizing potassium persulphate as the solid-oxidant. The earmarked silver-doped MWCNTs were synthesized and their antibacterial behavior was evaluated against the bacterial cultures of *Enterococcus faecalis* (MTCC 2729) and *Pseudomonas aeruginosa* (MTCC 424). The capability towards antibacterial action was a remarkable one and it was discerned that silver-doped MWCNTs showed a higher activity over *Pseudomonas aeruginosa* than the *Enterococcus faecalis*. Furthermore, a larger antibacterial capability towards both the bacterial cultures was noticed at a lower concentration.

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