RESEARCH ARTICLE

Synthesis of Ag-Fe_2O_3 nanoparticles with Anti-bacterial and anti-fungal impacts on dental microbia

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ABSTRACT

The arrangement of silver doped iron oxide nanoparticles (Ag-Fe_2O_3 NPs) through the exploitation of *Prosopis fracta* leaf extract was successfully performed in this work, which was proceeded by configuring the produced NPs by the outcomes of Powder X-ray Diffraction (PXRD), Field Energy Scanning Electron Microscopy (FESEM), Energy-Dispersive X-ray (EDX), Raman, Fourier Transform Infrared (FT-IR), and Vibrating-Sample Magnetometer (VSM) procedures. Apparently, the observed spherical particulates contained a size of 50-60 nm, while exhibiting antimicrobial performances towards *Streptococcus mutans bacteria* and *Candida albicans fungi*. They also seemed to possess potential antibacterial and anti-fungi properties at the volumes of 10 and 75 µg/mL, respectively, and thus, a viable proposal can be made regarding our synthesized product as a suitable option for dental and oral implementations. In addition, this biosynthesis method can be a suitable option for the synthesis of nanoparticles due to advantages such as cost-effectiveness, environmental friendliness, and no use of chemical solvents.

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INTRODUCTION

The presented substantial platform by nanotechnology for altering the physicochemical features of countless materials, under the aim of generating effectual antimicrobials, Nanomaterials (NM), can offer strategical benefits in the form of active antibacterial groups with exceedingly large surface areas in proportion to their sizes. The capability of nano sized particles in causing high rates of activity despite the usage of small doses has been well proved, which confirms the capacity of Nanomaterials in supplying an alternative for antibiotics in the course of controlling bacterial infections [1]. Metal oxides such as ZnO, TiO_2, Fe_2O_3 and CuO nanoparticles are included among the metal oxides with a strong potential for acting as antibacterial products. For instance, many researchers invested their interest in Fe_2O_3 nanoparticles that contain a band gap of ~2.2 eV for bacterial inhibition, which is attributable to its visible light absorption qualities (~564 nm), distinctive magnetic features, and biocompatibility [2-5] . The interaction of released iron oxide nanoparticle into the environment with air, water, and soil usually induces alterations in the surface quantities of particles and leads to particle accumulation or modifications in particle charge and other surface features. Different surface adjustments were attempted for discovering the right designs of these non-biodegradable nanoparticles with superior biocompatibility[6-8] . The approach of surface modification is performed under the objective of enhancing the surface characteristics of iron oxide nanoparticles. This technic can inhibit the occurrence of aggregation, as well as upgrade the compatibility of iron oxide nanoparticles with biological surroundings and their stability in suspensions [8-10] . The fundamental stance of silver nanoparticles in the progress of novel antimicrobial substances against certain pathogenic microorganisms is undeniable. Additionally, the smaller size of these nanoparticles is quite effectual in enhancing the

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antibacterial performance through the lysis of bacterial cell wall[2, 11-14]. Apparently, although not many papers were published on the synthesis of Ag/Fe_2O_3 nanoparticles [15]. there are reports on the synthesis of various heteromers of Ag/Fe_2O_3 nanoparticles and their bactericidal performances [16], the synthesis of Ag/Fe_2O_3 nanoparticles by glucose, and most recently [17] on the synthesis of Ag/Fe_2O_3 nanoparticles through the exertion of green *Adathoda vasica* leaf extract to achieve antibacterial, antifungal and anticancer features.

Numerous research results confirmed the upgraded antimicrobial functionality of Ag/Fe_2O_3 nanoparticles in contrast to the solitary case of Fe_2O_3 nanoparticles [15, 17] . Their observations pointed out the significance of released Ag^+ ions from Ag/Fe_2O_3 nanoparticles in the process of antibacterial activities [12, 15, 17]. There is no doubt in the superior effectiveness of Ag/Fe_2O_3 nanoparticles than that of solitary Ag NP in the cases of pathogens similar to bacteria, as well as fungi as multi drug resistant pathogens, which is attributable to the manageable release of Ag^+ ions [11]. Our work is based on the synthesis of Fe_2O_3 nanoparticles through a Hydrothermal approach and proceeded by the arrangement of Ag doping Fe_2O_3 through a photodeposition route [18]. We also assayed the constructional configurations and antibacterial performances of Fe_2O_3 and Ag/Fe_2O_3 nanoparticles towards *shigella dysenteriae* bacteria.

Performing the biosynthesis of materials through plant extracts proved to be uncomplicated, methodical, cost-efficient, and pertinent, which can serve as fitting substitutes for conventional preparation routes of extending the manufacturing of nanoparticles [19]. The biomolecules of plants, similar to carbohydrates, proteins, and coenzymes, can efficiently facilitate the reduction of metal salts into nanoparticles while preserving their stabilization [19] . Considering these facts, our work proceeded under the objective of synthesizing Ag-Fe_2O_3 NPs through the exploitation of leaf extract of *Prosopis farcta*, which was continued by assessing the antimicrobial performance of our product towards *Streptococcus mutans* bacteria and *Candida albicans* fungi.

MATERIALS AND METHOD

Synthesis of Ag-Fe_2O_3 NPs

As the first step, the impure substances of *Prosopis farcta* leaves were dispatched by being washed with DI water to be dried in the course of 2 days at ambient temperature. Subsequent to weighing out 10 g of the leaves and appending 100 mL of DI water, the mixture was heated for 30 min at 90 °C, which was then filtered to cleanse out the broth and collect the resultant extract for the upcoming experiments. The synthesizing process of Ag-Fe_2O_3 required the consistent insertion of a fitting aqueous solution of extracted Prosopis farcta to the mixture of 0.1 M of $FeCl_3$ and 0.025 M of Ag (NO_3) within an Erlenmeyer flask under stirring conditions by a magnetic stirrer at 75 ◦C for 2 h. Succeeding to tuning the pH of solution through the exertion of NaOH (1M, Merck, pH=11), the prepared mixture was heated in oven at 90 °C for 12 h. The prepared precipitate by drying the resultant was calcinated in a furnace at 400 °C for 2 h to fabricate the brown powder of Ag-Fe_2O_3 NPs.

Characterization

The characteristics of Ag-Fe_2O_3 NPs was determined through X-ray powder diffractometer (XRD) and scanning electron microscope (SEM). The type of exerted XRD was PANalyticalX'Pert PRO MPD system X-Ray Diffractometer, which implicated the 2θ values in a scale of 20° to 80° along with the Cu Kα radiation source (λ=1.54060) that functioned at 40 kV and 25 mA. Furthermore, the employed type of SEM was MIRA3 TESCAN.

Assessment of the Inhibitory Activity of Ag-Fe_2O_3 NPs

In the following, we performed a well-diffusion trial to collect data on the inhibitory performance of Ag-Fe_2O_3 NPs towards *Streptococcus mutans* bacteria. For this purpose, the streaking technic was exerted to inoculate the experimental bacteria into the sterile Petri plates that were composed of 20 mL of Mueller–Hinton. As the wells were made ready by the employment of sterile corn borer (6 mm), the appending of 200 μL of NPs at the volumes of 1, 4, 8, 10 mg/mL was required to continue the process. We stored the inoculated plates in a refrigerator for 45 min to complete the adequate diffusion of NPs and thus, performed the bacteria assay through an incubation procedure at 37 °C for 24 h. Lastly, the surrounding inhibition zone of every well was determined.

Assessment of Antifungal Activity of Ag-Fe_2O_3 NPs

The antifungal functionality of our product was configured through the outcomes of agar well diffusion technic. Under this objective, we exerted week-old fungal cultures grown on potato

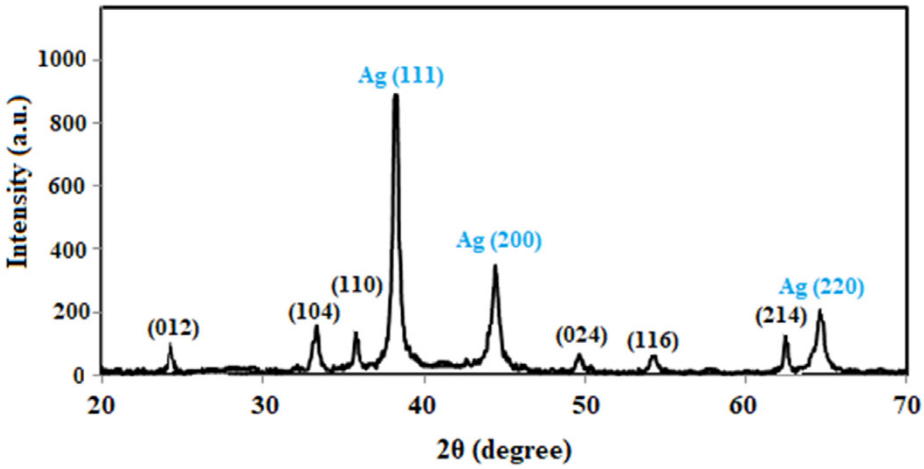

Fig. 1. PXRD spectrum of Ag-Fe_2O_3 NPs

dextrose medium to assay the antifungal potential of designated NPs. The inoculation of aliquots of 0.02 mL inocula from every fungal pathogen was done within 20 mL of molten Sabouraud dextrose agar medium in culture tubes, which were subsequently homogenized among hands to be inserted into 90 mm Petri plates. As the following step, we provided the solidification of culture plates in laminar airflow chamber to prepare the wells on the agar plate by a 5 mm standard corn borer. The exerted culture tubes, culture plates, and laminar airflow were procured from Thermo Fischer Scientific, New Delhi, India. A diverse range of NPs concentration (25, 50, and 75 µL of 0.10 mg/mL NPs) was appended to respective wells, while Hexahit 0.1 mg/mL (20 µL/disc) was implicated as the standard (positive control). We examined the impact of our product on fungal pathogens and conducted a comparison between its results and that of the standard. Thereafter, we sealed the plates to perform an incubation at 25 ± 2 °C for the duration of 2 days and ultimately, the antifungal functionality was required to be determined through the measurement of inhibition zone through the standard scale.

RESULTS AND DISCUSSION

PXRD analysis

Fig. 1 exhibits the powder x-ray diffraction spectrum of Ag-Fe_2O_3 NPs, which displays the diffraction pattern of Fe_2O_3 with peaks in the 2θ positions of 25.21, 34.12, 35.58, 49.51, 53.71, 58.86, and 61.28° that were associated with the (021), (104), (110), (024), (116), (018), and (214) planes. In coordination to this figure, certain other peaks were discerned throughout the 2θ position of 38.1, 43.2, 65.4, and 72.1° as the signs of silver atoms existence in the crystalline construction of doped nanoparticles. In consonance with the diffraction peak at 2θ°=35.58°, we determined the crystalline particle size of Fe_2O_3 through the Debye-Scherer's equation to be 41.59 nm for Ag-Fe_2O_3 NPs.

FESEM and EDX analysis

The data of SEM images (Fig. 2A) aided the configuring process of synthesized Ag-Fe_2O_3 NPs in term of morphology and size of particles, which exhibited spherical doped nanoparticles in the size of 50-60 nm. Similar to PXRD outcomes, Fig. 2A proves the doping impacts of silver into the iron oxide structure on causing a decrease in particle size. The EDX data in Fig. 2B provides an evident proof for the appearance of doped silver throughout the nanoparticles.

VSM analysis

The analytical data of VSM helped in configuring the magnetic features of synthesized Ag-Fe_2O_3 NPs (Fig. 3) and as it is observable, the value of saturation magnetization was 0.16 emu/g, while the same parameter (M_s) for the Fe_2O_3 of our previous study was reported to be 1.8 emu/g [20]. These outcomes imply the effect of existing silver in nanoparticles construction on reducing the rate of saturation magnetization in samples, which is probably attributable to the nature of paramagnetic substances as a hard magnetic material. A direct connection among the Ms content of these NPs

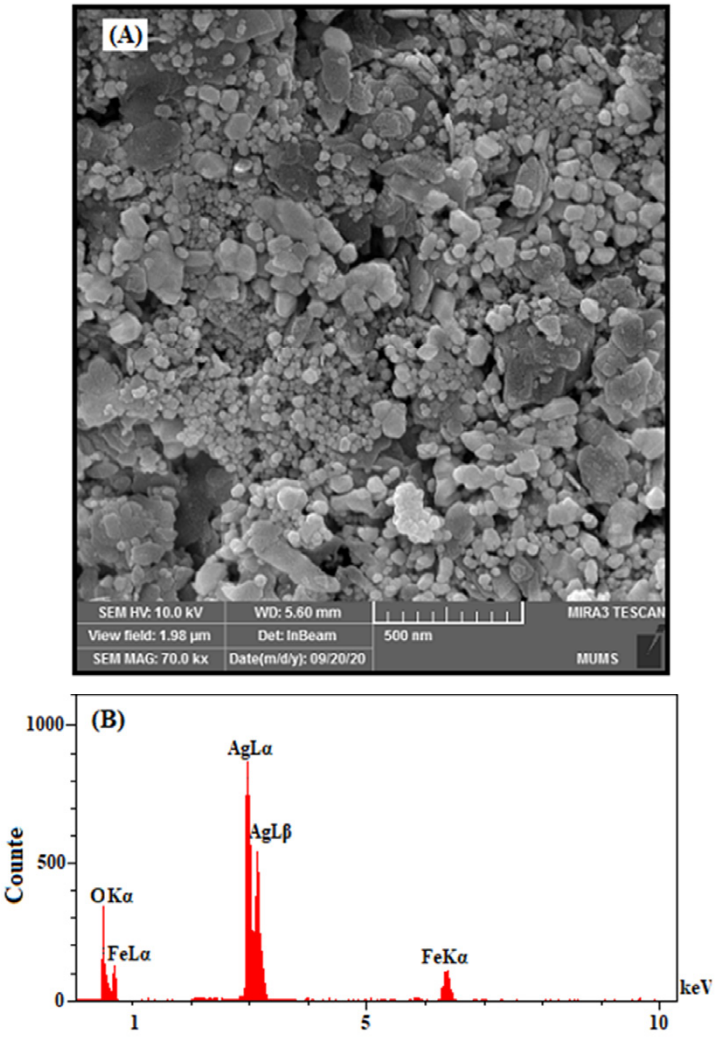

Fig. 2. (A) FESEM and (B) EDX spectra of Ag-Fe_2O_3 NPs

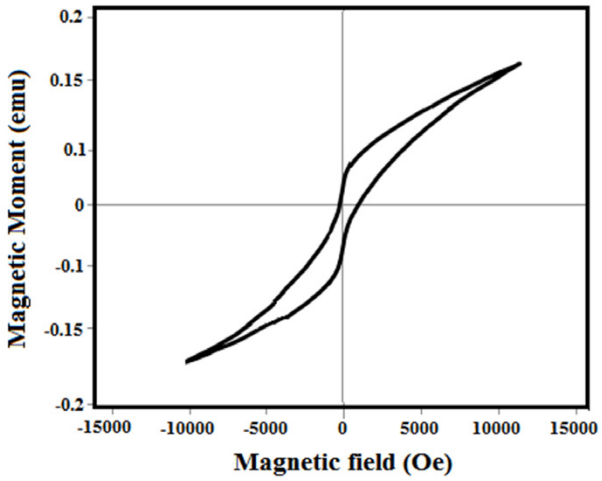

Fig. 3. VSM spectra of Ag-Fe_2O_3 NPs

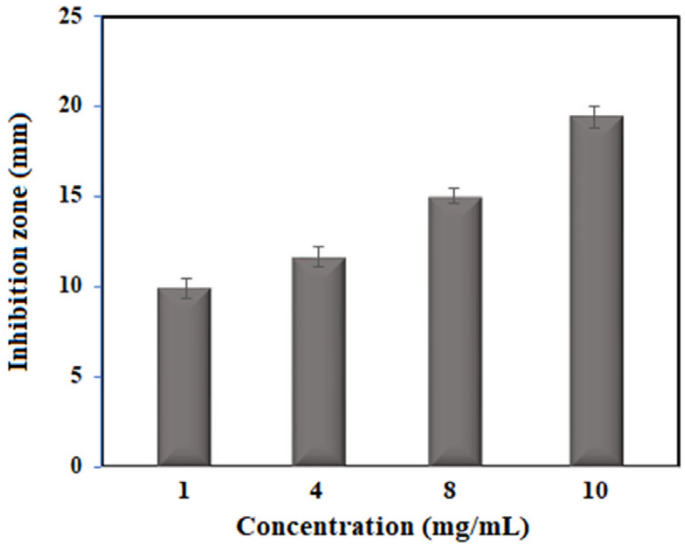

Fig. 4. Antimicrobial activity of the synthesized Ag-Fe_2O_3 NPs

Table 1. Antifungal activity of Ag-Fe_2O_3 NPs

	Activity against the fungi (mm)			
	Control (20 µL)	Ag-Fe_2O_3 (25 µL)	Ag-Fe_2O_3 (50 µL)	Ag-Fe_2O_3 (75 µL)
Candida albicans	25	10	12	16

and their particle size and shape anisotropy were discovered by the survey of Bepari and his coworkers on the magnetic features of Fe_2O_3 NPs [20].

Anti-bacterial assay

Fig. 4 displays the data of well diffusion procedure, which was exerted to assess the antibacterial efficacy of Ag-Fe_2O_3 NPs towards *Streptococcus mutans*. The maximal dosage of Ag/Fe_2O_3 (10 g/mL) exhibited a high level of inhibitory performance on *Streptococcus mutans* along with the inhibition zones of 19 ± 0.45 mm (Fig. 4). A related assessment proved the superior antimicrobial impacts of silver particles on hazardous Gram-positive or Gram-negative bacteria [21]. Similarly, some recent researchers studied the antibacterial performance of Ag-Fe_2O_3 and approved its suitability on Gram-negative and Gram-positive bacteria as well [22-24]. The antibacterial functionality of Ag-Fe_2O_3 towards bacteria is attributable to its capabilities in damaging the cell walls, disrupting constructional proteins, inactivating enzymes, preventing electron transport chains, injuring nucleic acids (DNA), and facilitating the induction of oxidative stress through reactive oxygen species (ROS) [10, 25-27]. In this regard, the emergence of Ag-Fe_2O_3 as a practicable antibacterial treatment choice can be quite advantageous in medicinal fields as well.

Anti-fungi assay

As a role model sample, *Candida albicans* fungi was exerted to assess the antifungal performance of Ag-Fe_2O_3 NPs. It is considerable that a very limited number of reports are available on the application of iron oxide NPs in the form of antifungal materials towards these two fungi. The work of Nazanin implicated the exploitation of spherical iron oxide NPs in the size of 30–40 nm for the case of fungal infection by Candida species, which resulted in exhibiting the antifungal capacity of NP against pathogenic *Candida* spp. [28]. Moreover, Nehra et al. [29]reported their successful results on illustrating the antifungal functionality of chitosan-coated iron oxide NPs in opposition to *A. niger* and four different organisms. In a close correspondence to our discoveries, the mean diameter of inhibition

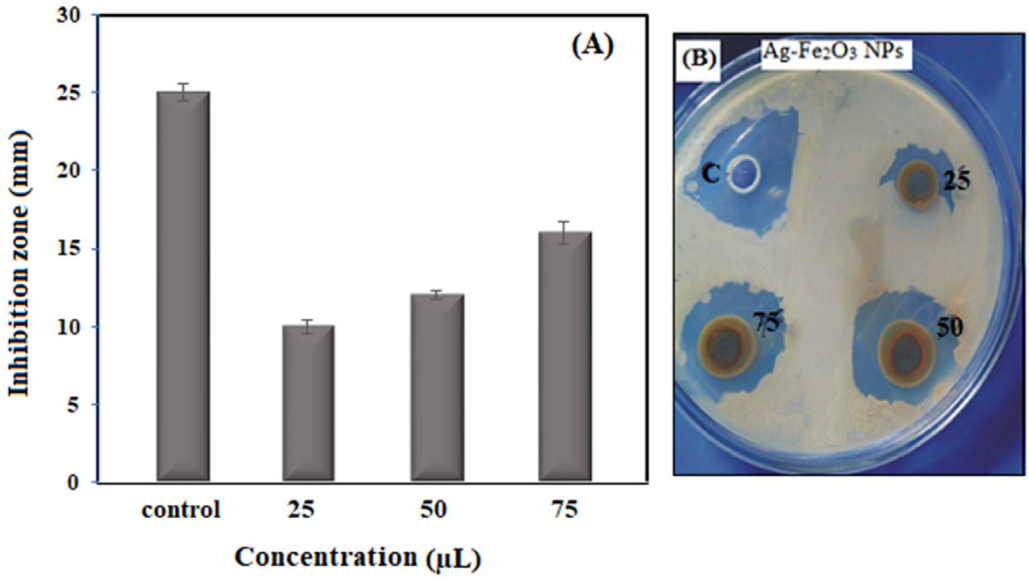

Fig. 5. Antifungal activity of Ag-Fe_2O_3 NPs against *Candida albicans*

zone of the prepared chitosan-coated NPs contained a scale of 14.5–18.5 mm.

The activities of Ag-Fe_2O_3 NPs are outlined in Table 1 and rendered in Fig. 5. The large surface-to-volume ratio of NP can facilitate their strong adherence to the cell surfaces of fungus, while their small sizes can provide a direct penetration into the cells and result in injuring the cell walls. Ag-Fe_2O_3 NPs are abled to inactivate fungus by utilizing the head on interactions of NPs with cell surfaces, which influences the permeability of membranes upon the entry of NPs to induce oxidative stress in fungus cells. This process ultimately prevents the growth of cells and gradually results in cell annihilation [30] .

In conformity to literature, there are the probabilities of membrane injury through the straight or electrostatic interaction of Ag-Fe_2O_3 NPs with cell surfaces, NPs cellular internalization, and the generation of active oxygen species similar to H_2O_2 within cells by metal oxides [31]. Furthermore, Fig. 5B exhibits the noticeable antifungal performance of Ag-Fe_2O_3 NPs towards *Candida albicans*. There is a firm belief among the authors on the robust interaction of Ag-Fe_2O_3 NPs with the cell surface of *Candida albicans*.

CONCLUSIONS

This paper was set out to exploit the extracted product of *Prosopis farcta* leaf to conduct an uncomplicated, quick, and effective synthesizing technic to fabricate Ag-Fe_2O_3 NPs. The configuration of our products qualities required the outcomes of certain analyses similar to PXRD, Raman, and FESEM and accordingly, the particle sizes were reported to be 50-60 nm for Ag-Fe_2O_3 NPs whilst observed in a spherical form. The data of hysteresis loop appointed the saturation magnetization (Ms) of Ag-Fe_2O_3 NPs to be 0.17 emu/g. We also assayed the capability of our product in exhibiting anti-bacterial effects on *Streptococcus mutans* and anti-fungi in opposition to *Candida albicans* and the attainted data approved our proposal of these synthesized NPs with potential applicability throughout the fields of oral and dental health.

CONFLICT OF INTEREST

There is no conflict of interest

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