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A COMPARATIVE IN VITRO ANTIFUNGAL STUDY OF CAMELLIA SINENSIS MEDIATED IRON NANOPARTICLES

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ABSTRACT

Nanotechnology in medicine is going to have a wide impact on the survival of the human race. The objective of the nanotechnology is to create the eco-friendly designs with nanotechnology and use it to reduce health and environmental hazards by seeking methods to replace present applications with green nanotechnology products. In the present study, we report a simple green method for the synthesis of iron nanoparticles using *Camellia sinensis* leaf extract. The synthesized iron nanoparticles were characterized by visual inspection UV, XRD, EDS and SEM. The antifungal activity of iron nanoparticles was evaluated using agar-well diffusion method. The maximum zone of inhibition for *Camellia sinensis* mediated iron nanoparticles obtained against *Fusarium oxysporum* (37.33 ± 1.15 mm) followed by *Aspergillus fumigatus* (34.33 ± 1.15 mm), *Fusarium solani* (29 ± 0.57 mm) and *Aspergillus flavus* (24.33 ± 1.15 mm). Results obtained that the *Camellia sinensis* mediated iron nanoparticles showed excellent antifungal activity on selected pathogens than the *Camellia sinensis* plant extract alone.

KEYWORDS: Nanotechnology, *Camellia sinensis,* Iron nanoparticle, Antifungal activity, Zone of Inhibition.

INTRODUCTION

In India, infectious diseases accounts for high proportion of health problems. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created.[1] Over the years, antibiotics have been used to control infections resulting from both community and hospital environments[2]. Current advances in the field of nanobiotechnology, particularly the ability to prepare metal oxide nanomaterials of specific size and shape, are likely to lead to the development of new antibacterial agents. The key aspects of green technology involves nano products that provide solutions to environmental challenges and producing nano materials with a view towards minimizing harm to human health and the environment.[3] Zero valent iron nanoparticles are relatively inexpensive, non-toxic, and high energy effectiveness.[4] The iron nanoparticles are gaining importance for their uses in environmental remediation technologies. Zero valent iron nanoparticles exhibited a stronger antimicrobial activity than other iron-based nanoparticles.[5] A very wide range of biological resources of bacteria, yeast, fungi, algae, viruses and plants can be used for nanoparticle synthesis.[6] Plant extracts reduce the metal ions in a shorter time as compared to microbes. Several recent studies have reported on the antimicrobial activity of zero valent iron nanoparticle.[7] *Camellia sinensis* possesses antibacterial, antitoxin, antiviral and antifungal activities.[8] The phytochemical screening of *Camellia sinensis* revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols. Iron nanoparticles rich with iron oxide can be readily prepared using green tea extract.[9] The iron nanoparticles were synthesized by green route using extracts of *Camellia sinensis.* *Camellia sinensis*, a member of *Theaceae* family is an evergreen shrub that attains a height of 10 - 15 m in the wild. The leaves are light green, short stalked, alternate, lanceolate, serrate margin, glabrous or pubescent beneath, varying in length from 5 - 30 cm and about 4 cm width.[10]

MATERIALS AND METHODS

Collection and identification of plant

Healthy, disease free, mature leaves of *Camellia sinensis* were collected from the region of Pandalur, Wayanad district, Kerala. The collected plant material was identified and authenticated by Dr. G.V.S. Murthy (Botanist), Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Preparation of plant extract

120 g of freshly collected *Camellia sinensis* leaves were washed thoroughly with running tap water. It was again washed with sterile distilled water to remove dirt and air dried. The dried leaves were crushed nicely using a mortar and pestle. Distilled water was added in 2:1 ratio[11] with crushed leaves. The mixture is then filtered out using Whatmann's No.1 filter paper to get plant extract.

Green synthesis of iron nanoparticles using *Camellia sinensis*

A solution of 0.1 M $FeCl_3$ was prepared. For the green synthesis of nanoparticles, the *Camellia sinensis* extract was introduced to the ferric chloride solution at a 1:2 volume ratio. Mixing was carried out at room temperature applying a vigorous agitation up to 30 minutes and the formation of *Camellia sinensis* mediated iron nanoparticles was marked by appearance of intense black precipitate. The resulting iron nanoparticles were separated from the reaction mixture by centrifugation at 4000 r/min for 5 minutes. Then the particles were washed thrice with distilled water and one times with 99% absolute ethanol.[11] Finally, the green synthesized iron nanoparticles were dried overnight at room temperature. Then the resulting powder was used for further analysis.

Characterization of green mediated iron nanoparticles

Visual inspection

The reduction of metal ions was roughly monitored by visual inspection of the reaction solution by color change and formation of precipitate.

UV-Visible Absorption Spectroscopy

UV-Vis spectroscopy of iron nanoparticles was performed on Shimadzu dual beam spectrophotometer (model UV-2000 Shimadzu) in the range of 300-800nm operated at a resolution of 1nm.

X-Ray Diffraction (XRD)

The crystallographic analysis of the samples was performed by powder X-ray diffraction. The X-ray diffraction patterns were recorded in the scanning mode on an X'pert PROPAN analytical instrument operated at 40 kV and a current of 30 mA with Cu α radiation (λ=1.54060 Å). The diffraction intensities were recorded from 10.02^o to 79.92^o in 2θ angles. The diffraction intensities were compared with the standard JCPDS files. The software gave

the information about the crystal structure of the particle and the average size of the particles can be estimated using the Debye-Scherer equation,

$D = k\lambda / \beta \cos\theta$

Where D is the thickness of the nano crystal, 'k' constant, 'λ' wavelength of X-rays, 'β' width at half-height of the reflection after correction for the instrumental broadening at Bragg's angle 2θ, 'θ' Bragg's angle.

Scanning Electron Microscopy (SEM)

Morphology of the synthesized iron nanoparticles was observed with the scanning electron microscope (JSM 35 CF JEOL) operated at a resolution of 60 Å at 15 kV. The solid samples were sprinkled on the adhesive carbon tape which is supported on a metallic disk. The sample surface images were taken at different magnifications. The magnification of the microscope was 5.0 kV. The scale was about 8.4 mm to 5 μm.

Energy Dispersive Spectroscopy (EDS)

EDS analysis provides qualitative as well as quantitative details of elements that may be involved in the formation of nanoparticles. It was used for the determination of elemental composition and purity of the sample by atomic percentage of metal. In order to get the EDS micrograph, samples were prepared on a carbon coated copper grid and kept under vacuum desiccation for 3 hours before loading them onto a specimen holder. Elemental analysis of iron nanoparticles was carried out using EDS instrument (JSM 35 CF JEOL) in a resolution of 60 Å, operated at 15.0kV with a magnification of about 5K.

Antifungal activity of green mediated iron nanoparticles

Fungal culture

The fungal pathogens namely, *Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum,* and *Fusarium solani* were cultured. Fungal cultures were grown on nutrient PDA agar plates and maintained in the slants at 4^0C. Culture in the nutrient broth was used for the present experimental study.

Assay to evaluate antifungal activity of green mediated iron nanoparticles

The antifungal activity of iron nanoparticles was evaluated using agar-well diffusion method.[12] The fungicidal effect of the iron nanoparticles can be assessed by the inhibition of mycelial growth of the fungus and is observed as a zone of inhibition near the wells. Potato dextrose agar medium was used to evaluate the antifungal activity. Potato Dextrose Agar

medium was prepared and poured on to the petriplates. The fungal pathogens were spread over the PDA agar plates. Wells of 6 mm diameter were made on PDA agar plates using sterile cork borer and 50 µl of leaf extracts and iron nanoparticles were poured into each well. The plates were incubated at 37^0C for 72 hours. The antifungal effect was seen as crescent shaped zones of inhibition.[13]

RESULTS AND DISCUSSION

Characterization of green mediated iron nanoparticles

Visual inspection

The appearance of black colour in the reaction mixture is the indication of formation of iron nanoparticles (Fig.1a). The formation of colour in the reaction solution is due to the surface plasmon excitation of the iron nanoparticles in the reaction mixture. Fig. 1b represents the powder form of iron nanoparticles. The present results coincide with earlier finding.[14]

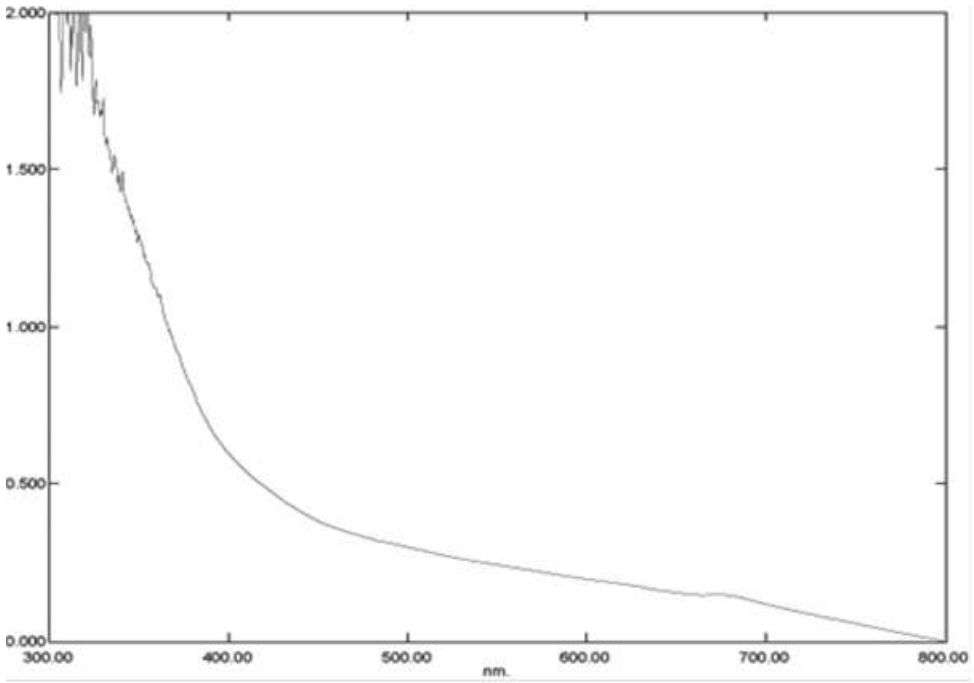
Figure 1a. Formation of iron nanoparticle reaction mixture

Figure 1b. Powder form of *Camellia sinensis* mediated iron nanoparticles

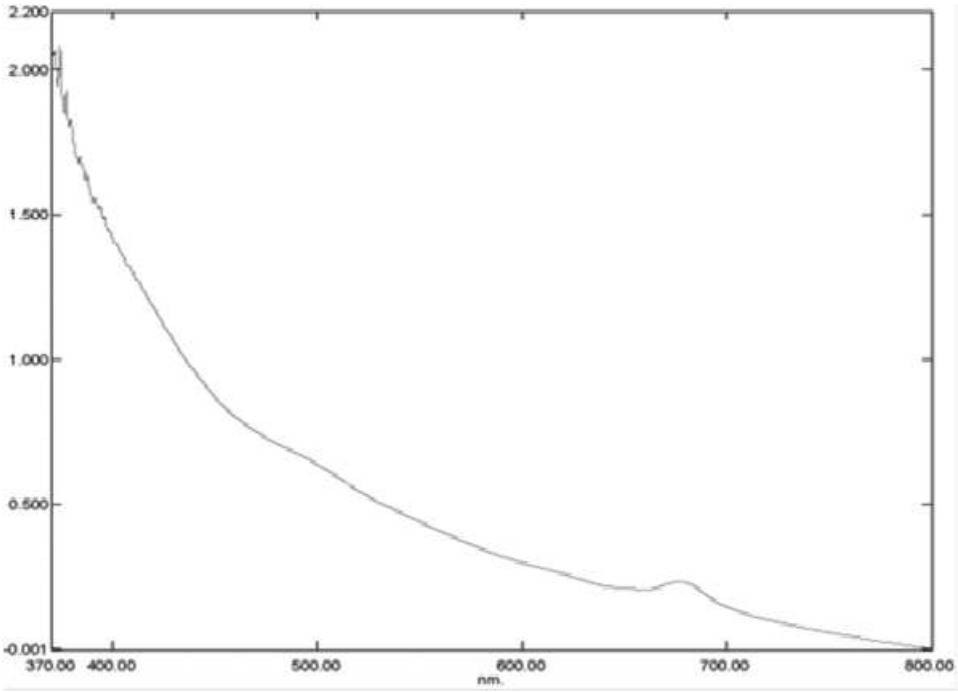
UV-Visible Absorption Spectroscopy

The UV-Vis spectra of *Camellia sinensis* plant extract and colloidal solution of *Camellia sinensis* mediated iron nanoparticles showed strong absorbance peaks at 660 nm and 680 nm respectively (Fig. 2a and Fig. 2b). A strong absorbance peak was observed 680 nm in the nanoparticle solution indicated the formation of iron nanoparticles. Yadav and Mendulkar, 2016[15] reported the absorption peak of *Camellia sinensis* mediated iron nanoparticles at 270 nm. There is a slight difference in the observed peak of current study with the previous

studies.[16,17] The difference is may be due to the mode of synthesis adopted for the fabrication of the iron nanoparticle.



(a)



(b)

Figure 2: UV-Vis absorption spectra of *Camellia sinensis* plant extract (a) and *Camellia sinensis* plant mediated iron nanoparticle (b).

X-Ray Diffraction (XRD)

The X-ray diffraction pattern of *Camellia sinensis* mediated iron nanoparticles were shown in Fig. 3. The pattern shows that the particles are in amorphous stage and they are in tetragonal shape. The intensive diffraction peaks were observed at 2θ value of 44.85^{o} unequivocally indicated that the particles are made of pure iron. The earlier studies observed that the characteristic main diffraction peak at 2θ = 44.7^{o} confirmed crystallization of nanoparticles.[18] In this diffraction spectrum five more additional bands were observed at14.09^{o}, 27.10^{o}, 36.39^{o}, 45.00^{o} and 65.21^{o}. The size of the synthesized *Camellia sinensis* mediated nanoparticles was found to be 30.1 nm.

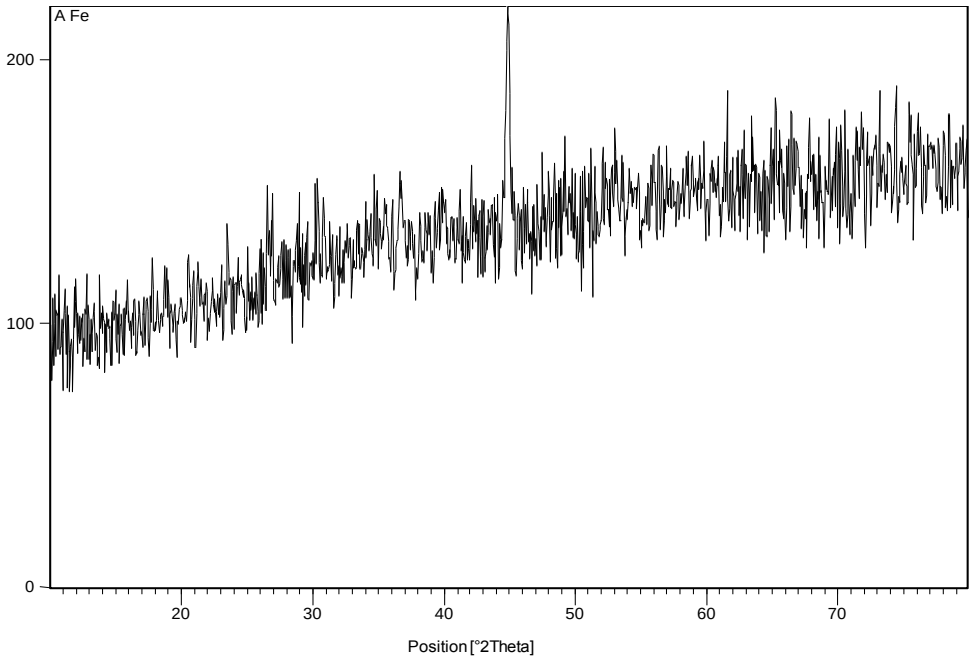


Figure 3: X-ray diffractogram of *Camellia sinensis* plant mediated iron nanoparticles

Scanning Electron Microscopy (SEM).

SEM micrograph of *Camellia sinensis* mediated iron nanoparticles revealed that the particles were more or less spherical and tetragonal shape in nature (Fig. 4). The synthesized *Camellia sinensis* mediated iron nanoparticles showed uniform arrangement. The particles get agglomeration due to intermolecular interaction and Vander walls force. Pattanayak and Nayak[19] reported similar kind of morphology of iron nanoparticle with diameter of around 100 nm. The surface of iron nanoparticles was rough and aggregated round shape.[20] P. Prema *et al.*, 2011[21] depicted that iron nanoparticles are present as nano spheres and hexagonal in nature.

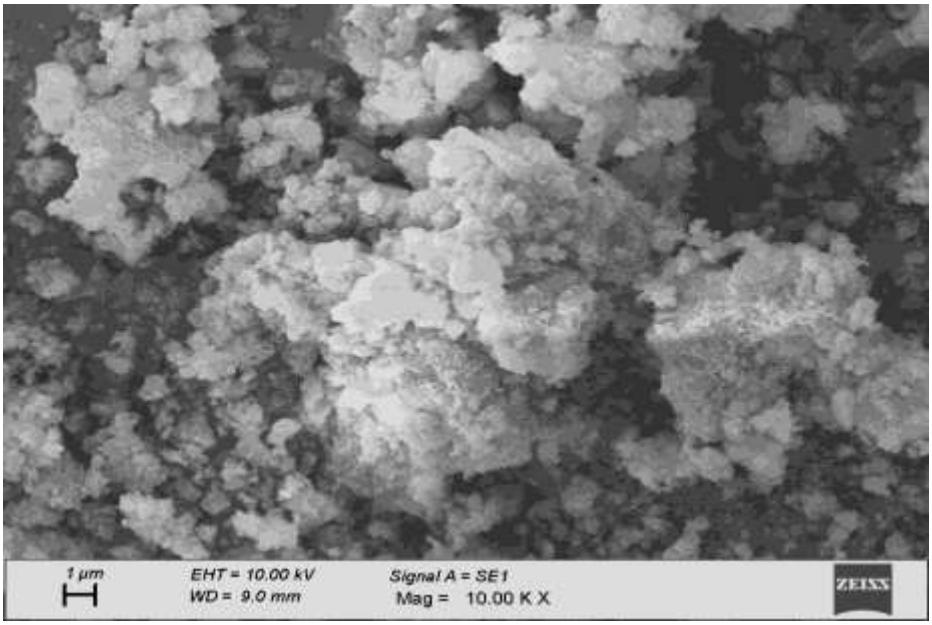


Figure 4: Scanning Electron Micrograph of *Camellia sinensis* plant mediated iron nanoparticles.

Energy Dispersive Spectroscopy (EDS)

The EDS analysis of *Camellia sinensis* mediated iron nanoparticles showed a green signal at 14.140 KeV in the iron region and thus confirmed the presence of iron nanoparticles in the prepared sample (Fig. 5). The micrograph showed that iron was present followed by Cl, C and O. The appearance of additional peaks for C, Cl, O and Au can be attributed due to the chemicals used for the synthesis of nanoparticles.

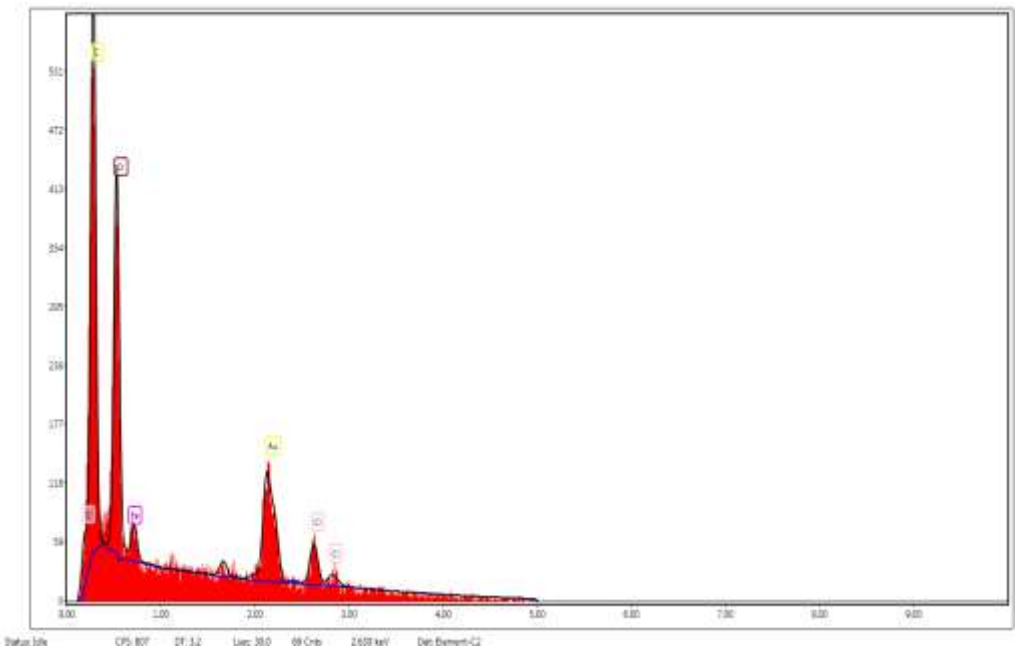


Figure 5: EDS of *Camellia sinensis* plant mediated iron nanoparticles.

Antifungal studies of green mediated iron nanoparticles

The antifungal activity of *Camellia sinensis* mediated iron nanoparticles were investigated against fungi such as *Aspergillus flavus, Aspergillus fumigatus, Fusarium solani* and *Fusarium oxysporum* and are depicted in Fig. 6. The plant extract of *Camellia sinensis* and *Camellia sinensis* mediated iron nanoparticles showed varying degrees of antifungal activities against the selected pathogens.

The antifungal activity of plant extract of *Camellia sinensis* against tested pathogens showed maximum zone of inhibition (29.33 ± 1.15 mm) against *Fusarium oxysporum* followed by *Aspergillus fumigatus* (28.33 ± 2.88 mm), *Fusarium solani* (24.66 ± 0.57 mm) and *Aspergillus flavus* (19.33 ± 1.15 mm). The maximum zone of inhibition for *Camellia sinensis* mediated iron nanoparticles obtained against *Fusarium oxysporum* (37.33 ± 1.15 mm) followed by *Aspergillus fumigatus* (34.33 ± 1.15 mm), *Fusarium solani* (29 ± 0.57 mm) and *Aspergillus flavus* (24.33 ± 1.15 mm). The presence of inhibition zone clearly indicates the mechanism of the biocidal action of nanoparticles involve disrupting the membrane. Extend of inhibition depends on the concentration of nanoparticles as well as on the initial fungal concentration. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction.[22] Padmavathy and Vijayaraghavan (2008)[23] reported that the size of the inhibition zone increased significantly with decreasing size of the nanoparticles. The *Camellia sinensis* mediated iron nanoparticles showed greatest antifungal activity on selected pathogens than the *Camellia sinensis* plant extract alone. The previous study reported that the zinc nanoparticles synthesized using *Camellia sinensis* have antifungal activity towards *A.flavus* and *A.fumigatus*.[24]

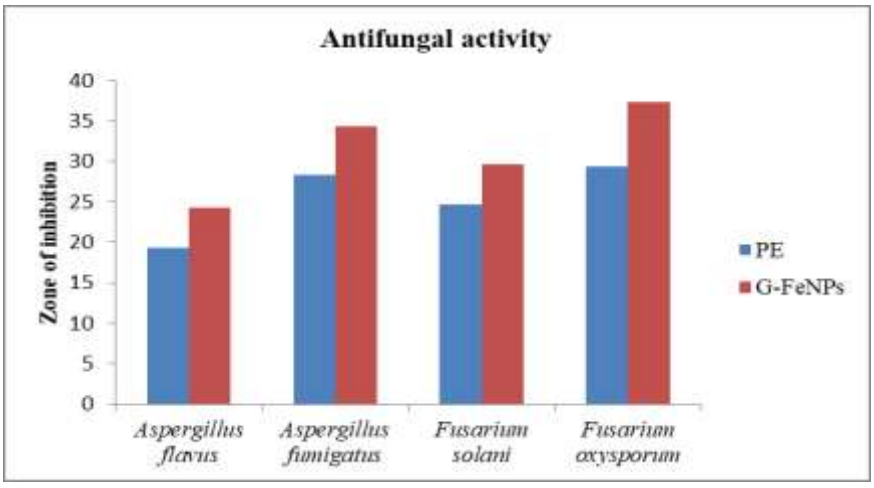


Figure 6: Antifungal activity of leaf extracts of *Camellia sinensis* (PE) and *Camellia sinensis* mediated iron nanoparticles (G-FeNPs) against selected fungal pathogens.

CONCLUSION

Plant extracts are plant derived compounds are likely to produce a valuable source of new medicinal agents and the urgent need for alternative treatment has led to screen natural products for therapeutic use. Results on fungicidal effect revealed that plant extract of *Camellia* sinensis and *Camellia sinensis* mediated iron nanoparticles showed varying degrees of antifungal property against the selected pathogens in the present study. In conclusion, the present findings clearly indicated that plant extract of *Camellia sinensis* and *Camellia sinensis* mediated iron nanoparticles possess the capabilities of being a good candidate in the search for a natural antifungal agent against diseases caused by fungi.

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