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FACILE SYNTHESIS OF ZN-AG DOPED FUNCTIONAL NANOPARTICLES BY CO-PRECIIPITATION TECHNIQUE USING TAMARINDAS INDICA LEAVES & THEIR ANTIMICROBIAL APPLICATIONS

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ABSTRACT: The synthesis of metal oxide nanoparticles has emerged as a burgeoning field of research due to its promising applications in advancing technological frontiers. Particularly, the utilization of biologically derived nanomaterials has gained significant traction within the realm of nanotechnology. *Tamarindus indica* plant extract has demonstrated efficacy as a viable precursor for the synthesis of silver nanoparticles. *Tamarindus indica*, being a locally abundant plant, boasts a rich composition of essential amino acids and vitamins. This investigation presents the biosynthesis of metal oxide nanoparticles using an aqueous extract derived from *Tamarindus indica*. The aqueous extract and resultant nanoparticles underwent comprehensive characterization techniques such as XRD, DLS, UV, SEM, and EDS. Additionally, this study delves into the antimicrobial properties of the synthesized compound. By minimum inhibitory concentration (MIC) and Agar diffusion method of the extract against various test organisms was conducted, with results indicating antimicrobial activity against both gram-positive and gram-negative bacteria as well as fungi. The efficacy of the antimicrobial effect was assessed by measuring the diameter of the inhibition zone. The Zinc doped silver nanoparticles showed spherical shaped nanocrystals ranging from 200nm in SEM and exhibited potent inhibitor effect against *Salmonella typhi* and *A. nigrospora*.

INTRODUCTION: The nanotechnology is an emerging technology, which has extended to a novel revolution in all field of science. This technology is used with affiliation to optics, electronics, bio medical and materials sciences ¹. Nanotechnology deals with nanoparticles that are characterized by size varying between 1 nm to 100 nm ².

Its subsidiary field Nanochemistry is one of best field to identify the chemical activities at atomic, molecular and super molecular level (size 1-100nm) ³. It has wide applications in various fields like medicinal, agriculture, electrical and others ⁴. Nano medicines are getting more popular because getting wide variety application that can be used for Nano drug delivery ⁵.

In recent years, metal and metal oxide nanoparticles have been intensively studied ⁶, as they have immense applications in numerous fields ranging from water treatment, cosmetics, medicine and engineering to name a few ⁷. The Nanosized materials have been an important subject in the field of basic and applied sciences ⁸.

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Nanomaterials have different size and shapes so, its appealed considerable attention because of their peculiar physicochemical properties compared to the bulk materials⁹. Nano sized aluminum containing particles are used in industrial, medical products and in energetic systems (composite propellants) to replace lead primers in artillery, etc. For example, aluminum nanoparticles are used in explosive combinations and titanium dioxide nanoparticles are mostly used as photo catalysts and adsorbents in consumer products like in sunscreens and as catalysts in sterilization and chemical engineering¹⁰. Various groups have studied the antibacterial activity of these metal oxides including ZnO and AgO. Ag NPs are one of the multifunctional inorganic nanoparticles that have many features like chemical and physical stability, effective antibacterial activity as well as intensive ultraviolet and infrared adsorption with broad range of applications¹¹.

The use of nanoparticles of silver and zinc oxide has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles¹². The behavior of dissolved metals in natural bodies of water is strongly influenced by particulate inorganic and organic material. Hydrous metal oxides, clays, humic substances and biota are all capable of binding metallic ions from solution¹³. The international awareness of medicinal plants has enormously increased during the last few years. The World Health Organization (WHO) estimated that 80% of the population in developing countries rely on traditional medicine, mostly plant-based drugs, for primary health care¹⁴. The rising incidence in multidrug resistance among pathogenic microbes has further necessitated the need to search for newer antibiotic sources. *Tamarindus indica* is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhea and jaundice and as skin cleanser¹⁵. *Tamarindus indica* is a good source of zinc and used to make dawwa (porridge) commonly consumed during pregnancy¹⁶. The extracts of *Tamarindus indica* have broad spectrum antibacterial activity against both gram positive and gram-negative bacteria¹⁷. Include activities of *T. indicus* in different extracts.

MATERIALS AND METHODS: The plant material was collected in Botanical Garden,

Mysore and authenticated at DOS in Botany, University of Mysore. Zn (NO₃) (99%, Sigma chemicals, India) and AgNO₃ (99.9% Sigma Aldrich, India), Media for culture used in bacteriological studies were obtained from HIMEDIA laboratories.

Preparations of Plant Extract using *Tamarindus indica*: 20g of leaf washed several time with distilled water and cut into small pieces then placed in RB flask containing 200mL deionized water and was heated at 60° C for 1 Hrs, then cooled at room temperature. The extract was filtered through Whatmann no.1 filter paper; a brownish aqueous extract was obtained and stored in 4°C for further use to synthesize the nanoparticles.

Synthesis of Pure and Ag Doped Zinc Oxide Nanoparticle by Co Precipitation Method: 30 mL of the aqueous plant extract was taken and stirred until it reaches 60 to 70°C for 30 min. 0.1ml of 3M zinc sulphate was added. The above solution was stirred continuously and 8ml of NaOH was added, the pH 10 was maintained. At this point white color precipitate was formed which is considered as source for pure compound (undoped ZnNPs), this white precipitate was collected and centrifuged, the supernatant was removed, and the precipitate was washed using water followed by ethanol for removing impurities, the resulting sample was dried for 12 hrs at 60°C in hot air oven. The doped nanoparticle was prepared using same method with minor modification. Briefly, 30mL of plant extract was taken and stirred until it reaches to 60-70°C. 3M of Zinc sulfate was added followed by 2mL of 0.05 M AgNO₃ and stirred continuously and the pH was maintained by addition of NaOH. The color change to brown was observed. The precipitate which was formed was allowed stand at room temperature. The supernatant was removed, and the precipitate was washed several times with deionized water followed by ethanol, the sample was dried at 60°C at 12 hrs using hot air oven.

Characterization: Synthesized pure ZnO and ZnO-Ag doped nanomaterials were characterized at Centre for Material Science and Technology, University of Mysore. The PXRD studies were performed using Rigaku smart Lab-II, 2θ = 10°–80° using Cu Kα radiation at λ = 0.154 nm with step size of 0.0001 deg. External morphology and

elemental composition was determined using Hitachi S-3400 N Scanning Electron Microscope with attached EDS. A Perkin Elmer infrared spectrophotometer was used for the determination of the surface functional groups. Particle size of synthesized nanoparticles was obtained by DLS using Microtrac Zeta analyzer. The diffuse reflectance UV-Visible spectra (DRS) of the nano sized materials were recorded using a UV-visible spectrophotometer.

Antibacterial Activity of Pure ZnO and Ag Doped ZnO of *Tamarindus indica* Nanoparticles:

The antibacterial activity of pure and Ag doped Zn nanoparticles were determined using antibiotic disc

diffusion assay¹⁸. The pure microbial culture was obtained from microbial type cell culture and Gene banking (MTCC). The bacteria used in this study were, *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 433), *Pseudomonas aeruginosa* (MTCC 1934), *Salmonella typhi* (MTCC 98), *Staphylococcus aureus* (MTCC 3160)¹⁹.

RESULTS AND DISCUSSION:

Powder X-ray Diffraction Studies: The XRD was analyzed in University of Mysore. The main application of this XRD is to identify the intensity, average size, helps to determine the lattice parameters, determine the grain size in polycrystalline blocks.

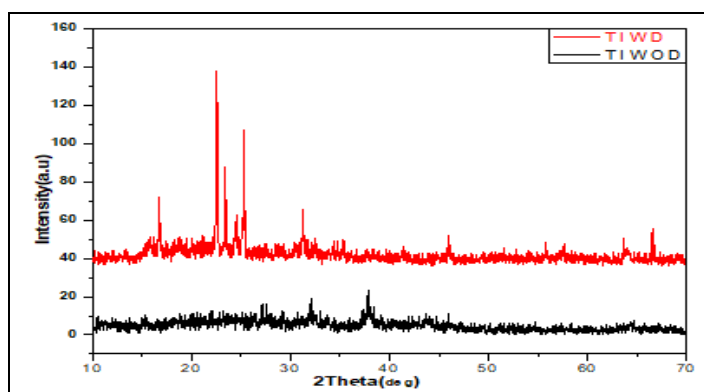


FIG. 1: CHARACTERISTIC SPECTRUM OF XRD PATTERN ANALYSIS OF TAMARINDUS INDICA PLANT WHICH CONTAINS BOTH DOPED AND UN-DOPED METAL OXIDE

The above graph was analyzed by using Bragg's law. XRD pattern of synthesized metal oxide from leaf extract of *Tamarindus indica* is shown. The AgO NPs diffraction peaks clearly indicate it is highly crystalline in nature. The sharp and narrow diffraction peaks appearing at about 2θ starts from 10 and ends at 70 and the step size of this is 0.02

and the speed is 5.00 sec. The XRD graph shows diffraction peaks for Pur Zn and Ag doped nanoparticle shows the crystalline nature, the peak showed for pure Zn nanoparticle at 2θ angles of 15.39° , 27.64° , 32.2° , 32.23° , 37.95° , 45.7° the above peak shows well resolved six diffraction peaks²⁰.

Particle size Determination using DLS:

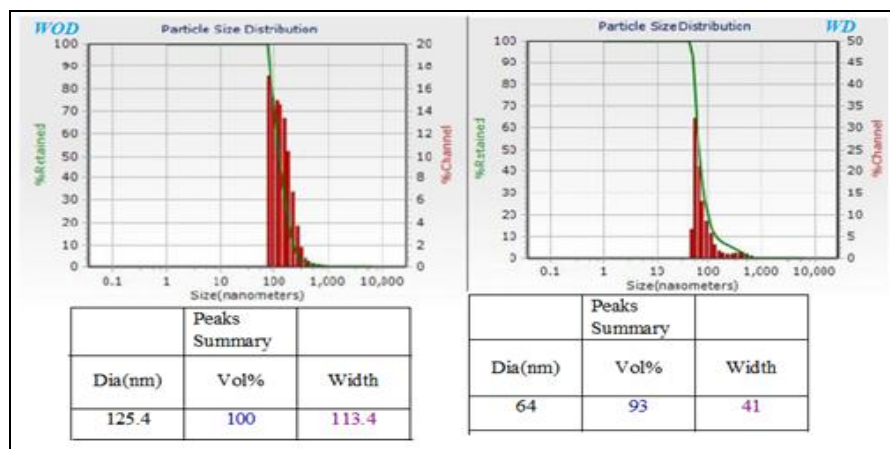


FIG. 2: CHARACTERISTIC SPECTRUM OF DYNAMIC LIGHT SCATTERING PATTERN OF TAMARINDUS INDICA

Scanning Electron Microscope:

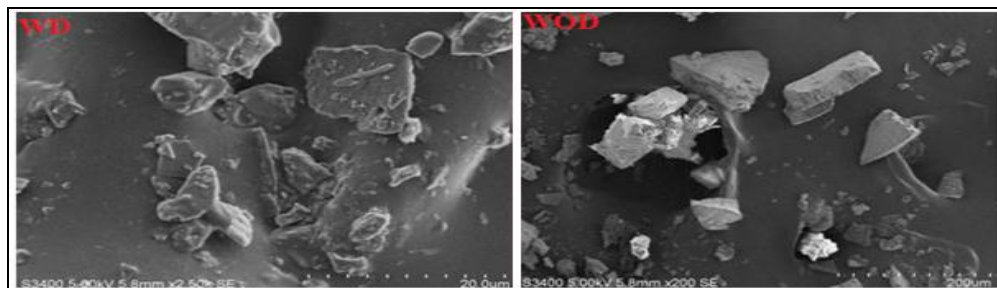


FIG. 3: CHARACTERISTIC SPECTRUMS OF SCANNING ELECTRON MICROSCOPE WERE WE INVESTIGATE THE ACHIEVABLE RESOLUTION OF BOTH DOPED UN-DOPED METAL OXIDE OF *TAMARINDUS INDICA*

The size and topology of the AgNPs were studied using SEM analysis. SEM analysis revealed fairly well dimensioned spherical shaped nanocrystals ranging from 200µm. The ZnO and Ag Doped ZnO

nanoparticle morphology shows the shape like rod shaped ZnO and spherical shaped particle present above the Zn nanoparticle.

Energy Dispersive X-ray Studies:

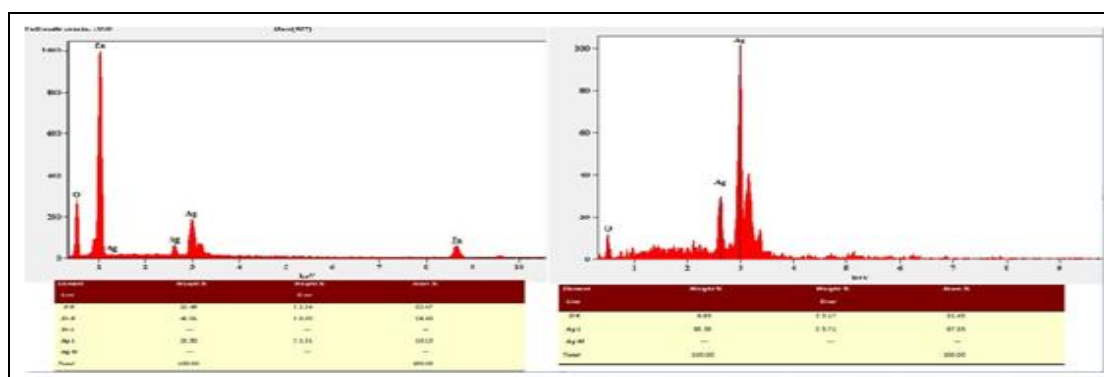


FIG. 4: CHARACTERISTIC SPECTRUM OF AN ENERGY DISPENSIVE X-RAY SPECTROSCOPY ANALYSIS OF *TAMARINDUS INDICA* PLANT EXTRACT. THIS CONTAINS BOTH DOPED AND UN-DOPED METAL OXIDE

Antibacterial Activity Disc Diffusion Method:

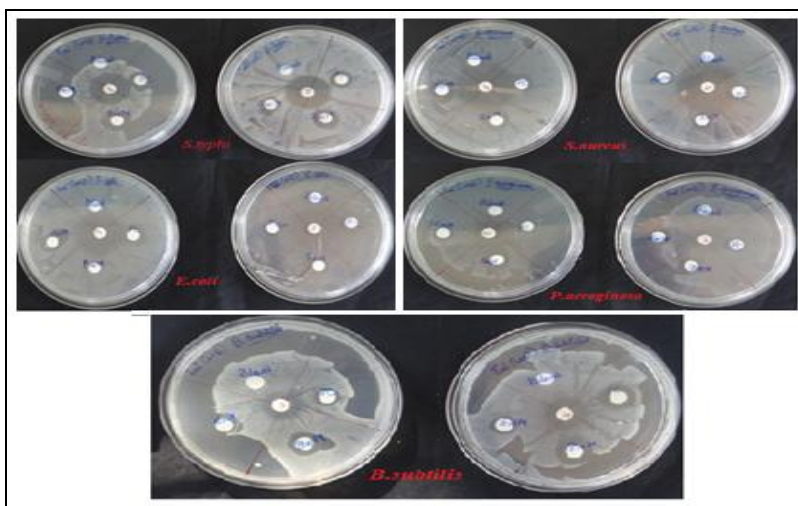


FIG. 5: ANTIBACTERIAL ACTIVITY OF DOPED AND UN-DOPED METAL OXIDE OF *TAMARINDUS INDICA*. ZOI AS OBSERVED IN A. *SALMONELLA* TYPHI, B. *STAPHYLOCOCCUS AUREUS*, C. *ESCHERICHIA COLI*, D. *PSEUDOMONAS AERUGINOSA* AND E. *BACILLUS SUBTILIS*

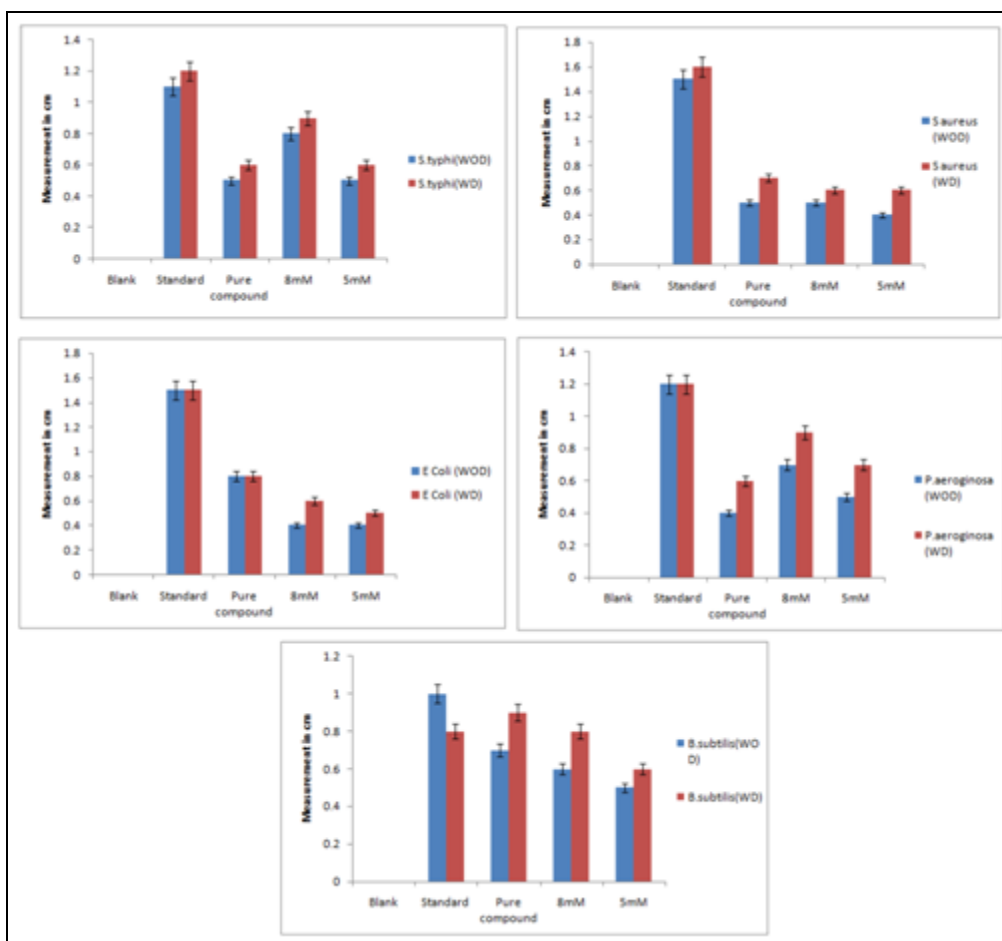


FIG. 6: EFFECT OF DOPED AND UN-DOPED METAL OXIDE OF TAMARINDUS INDICA ON SURVIVAL OF A. *SALMONELLA TYPHI*, B *STAPHYLOCOCCUS AUREUS*, C. *ESCHERICHIA COLI*, D. *PSEUDOMONAS AERUGINOSA* AND E. *BACILLUS SUBTILIS*.

The antibacterial potential of the doped and un-doped metal oxide of *Tamarindus indica* is tested against *S. typhi*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* and shown in Fig. 6.

The both doped and un-doped metal oxides showed antibacterial effect on all the tested bacterial strains. The bactericidal effects of doped metal oxides were found to be higher than that of un-doped metal oxides.

Doped metal oxides of *Tamarindus indica* showed a significant zone of inhibition for *Salmonella typhi* (9mm), *Staphylococcus aureus* (6mm), *Escherichia coli* (6mm), *Pseudomonas aeruginosa* (8mm) and *Bacillus subtilis* (10mm) whereas the zone of inhibition was observed less for *Salmonella typhi* (6mm), *Staphylococcus aureus* (4mm), *Escherichia coli* (6mm), *Pseudomonas aeruginosa* (8mm) and *Bacillus subtilis* (10mm)⁵.

Antifungal Activity:

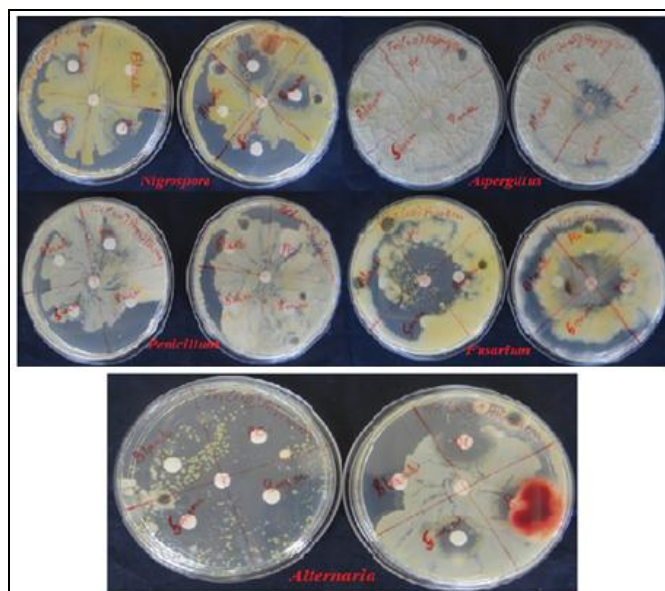


FIG. 7: ANTIFUNGAL ACTIVITY OF DOPED AND UN-DOPED METAL OXIDE OF TAMARINDUS INDICA. ZOI AS OBSERVED IN A. *NIGROSPORA*, B. *ASPERGILLUS*, C. *PENICILLIUM*, D *FUSARIUM* AND E. *ALTERNARIA* SPECIES

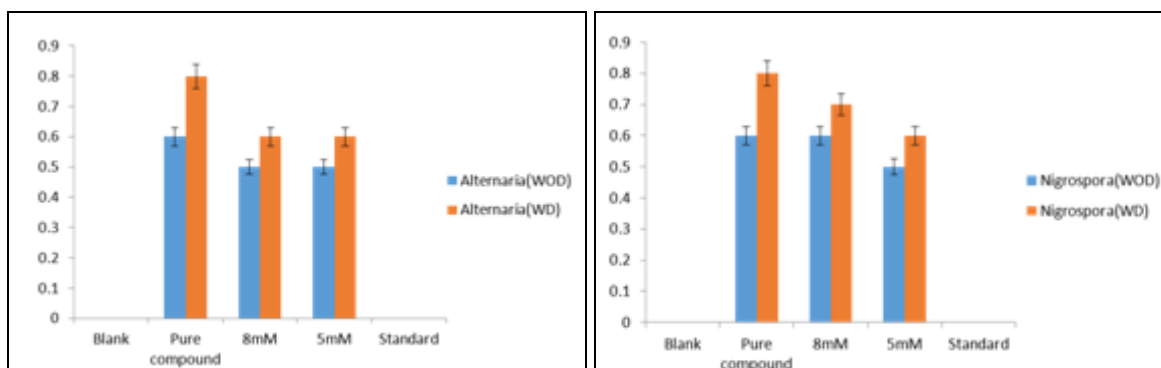


FIG. 8: GRAPHICAL REPRESENTATION OF THE EFFECT OF DOPED AND UN-DOPED METAL OXIDE OF TAMARINDUS INDICA. ZOI AS OBSERVED IN A. NIGROSPORA, B. ASPERGILLUS, C. PENICILLIUM, D FUSARIUM AND E. ALTERNARIA SPECIES

The antifungal potential of the doped and un-doped metal oxide of *Tamarindus indica* is tested against *Nigrospora*, *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* Species and shown in **Fig. 8**. The both doped and un-doped metal oxides showed antifungal effect only on *Nigrospora* and *Alternaria* Species. Both Doped metal oxides have better antifungal activity when compared to un-doped.

CONCLUSION: In the current study, Zn doped Ag nanoparticle were synthesized by using the green approach by *T. indica* co-precipitation method. The size of synthesized NPs with Zn doped was measured to be in the range of 64–70 nm whereas non doped NPs showed 120-124nm. Further XRD, FTIR, SEM, TEM, EDX, AFM, and DLS techniques were used for the characterization of synthesized NPs. The synthesized ZnO NPs have shown antibacterial efficacy against Gram-positive and Gram-negative pathogens. The antibacterial activity of Ag doped and undoped ZnO nanoparticle showed inhibitory effect bacterial strains like *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* by Doped metal oxide's synthesized with *Tamarindus indica* show more antibacterial activity than un-doped metal oxide developed with *Tamarindus indica* growth was similar to that obtained with earlier methods, However, both doped and un-doped metal oxides showed antifungal effect only on *Nigrospora*, and *Alternaria* strains but had no effect on other species competing against commercial products even better than colloidal silver. Doped metal oxide's synthesized with *Tamarindus indica* show more antimicrobial activity than un-doped metal oxide

developed with *Tamarindus indica*. It would be interesting to try the synthesis of AgNPs with purified flavonoids and compare the biocidal activity of the obtained nanoparticles.

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CONFLICT OF INTEREST: All the authors declare No conflict of interest to publish the paper.

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