Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 12, Issue 7 July 2021: 12818 – 12829

Research Article

Bactericidal And Radical Scavenging Activity Of Natural Wood And Chitosan Based Novel Nanocomposite

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Abstract

Wood is a flexible material appreciated extremely for its cost effective, great quantity, and biocompatibility. In addition, the wood materials possess prominent antibacterial properties and it can withstand efficiently when compared to other materials like glass, steel and plastics. The present study aimed to fabricate chitosan, silver nanoparticles incorporated *Borassus flabelifer* trichome and *Prosopis juliflora* wood-based biomaterial, to evaluate its antibacterial activity by broth dilution method and agar diffusion method against *S. aureus, E. coli, S. typhi* and *B. cereus* which cause digestive problems. Furthermore, *in vitro* free radical scavenging was performed to evaluate its ability to scavenge radicals of DPPH. The TC-Ch-AgNPs-W biocomposite showed minimum percentage of scavenging activity. It was compared with standard ascorbic acid and TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Chand TC-Ch-AgNPs. The efficiency of antibacterial activity of selected microorganisms and were observed around 85 % which reached up to maximum enormity. The increased antibacterial activity was significantly observed in *E. coli and it* was exhibited by TC-Ch-AgNPs-W.

Keywords: Chitosan; Silver Nanoparticle; Antibacterial activity; *Prosopis juliflora;* Antioxidant activity

1. Introduction

Wood is a wide natural reusable resource which gives an impact in different industries makes the researcher to work on it. The naturally existing wood porous acts as a good carrier to build functional composites are still rarely used. Nanotechnology is a promising area to enhance the efficiency and effectiveness towards the heavy metals polluted water purification process. Its improved reactivity and surface area to volume ratio employed great impact which compared with those bulk materials[1,2]. This latest material possess to incredible durability, biodegradability, biocompatibility, nontoxicity and improved adsorption applications in pharmaceuticals, agriculture, forestry, skin care products, food and water treatment. In India, palmyra tree is the official tree of Tamil Nadu state. In tradition it is called as karpaha, celestial tree, nungu and extremely cherished by the people and compared as god[3]. The immeasurable and vital constituents of *Borassus flabeliferare* gums, fats, albuminoids, carbohydrate like sucrose, spirostane type steroids like borassosides, steroidal glycosides and dioscin[4].

Silver nanoparticles are considered to have strong therapeutic ability and show enhanced antimicrobial properties[5,6]. In addition, they have a combination of antiviral, antifungal and antioxidant functions as well as high efficiency at very low concentrations to possess strong potential to treat a variety of diseases. Silver nanoparticles (AgNPs) incorporation plays great impact on water purification process due to its stability, chemical catalytic ability, low toxicity to humans and effective antimicrobial property[7-9].

The investigation was aimed to evaluate antioxidant activity of TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Ch, TC-Ch-AgNPs and TC-Ch-AgNPs-Wand to determine the antibacterial activity and against two gram negative and gram positive bacteria (*S.typhi, S.aureus, E.coli* and *B.cereus)* by broth dilution method and agar well diffusion method.

2. Methodology

2.1 Chemicals and Samples

From Sigma–Aldrich Chemical Company, USA, the required fine chemicals like silver nitrate was purchased and all other requirements are first class analytical grade.

2.2 Preparation of chitosan solution (Ch)

The purified chitosan was significantly obtained by performing alkaline deacetylation of chitin from crab shells. The mixture was obtained by dissolving 1 gram of chitosan in 100 ml of 0.25 N Hydrochloric acid[10].

2.3 Preparation of *Borassus flabelifer* trichome –chitosan biocomposite (TC–Ch)

In 50 ml double distilled water 1 g of *Borassus flabelifer* trichome and 1% chitosan (Ch) was mixed to this solution. The pH of the solutions was adjusted to 7.0 and the resultant materials were used for further use. It was denoted as TC-Ch.

2.4 Synthesis of silver nanoparticles solution (AgNPs)

Silver nanoparticles were effectively prepared by using modified method of Turkevich[11]. In brief in 30 ml of 1mM silver nitrate solution was boiled and kept in magnetic stirrer. Once it starts to boil add 3ml of 10mM trisodium citrate in the ratio of one drop per second and wait till it changed into brown color. Finally denoted as AgNPs.

2.5 Preparation of Wood Sample (W)

The wood pieces were collected from *Prosopis juliflora* and hard wood of the plant was finely powdered. The obtained finely powdered wood sample was collected and it denoted as W.

2.6 Incorporation of AgNPs and Wood on to the TC–Ch biocomposites (TC–Ch–AgNPs-W)

The prepared TC-Ch biocomposite was soaked in solution which having 0.01% of AgNPs and wood sample for about 24 hrs. Silver nanoparticles (AgNPs) and wood integrated TC-Ch was taken and dried at 37°C. The obtained material was expressed as TC–Ch–Ag-W.

2.7Determination of Antibacterial activity

The bacterial cultures *S. typhi*, *S. aureus*, *E. coli* and *B. cereus* were purchased from microbiology laboratory, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, and India.

2.7.1 Determination of Minimum Inhibitory Concentration by Broth Dilution Method

The Broth dilution method offered by Ericsson and Sherris[4] was effectively used to determine Minimum Inhibitory Concentration (MIC) for TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Ch, TC-Ch-AgNPs and TC-Ch-AgNPs-W against four selected Gram negative and positive bacteria *S.aureus*, *S.typhi*, *E.coli* and *B.cereus*. The samples were significantly diluted into different concentrations *viz*., 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml in a sterilized nutrient broth. At 37 °C for 24 hours the inoculated tubes were incubated and thereafter growth or turbidity was observed. The turbidity was measured at 580 nm by using UV Visible Spectrophotometer. The experiments were allowed for triplicates.

2.7.2 Determination of Percentage growth inhibition

The Percentage bacterial growth inhibition of bacteria was determined by using the formula:

% Growth Inhibition	=	OD of preliminary growth (Nutrient broth + Bacteria) – OD of Inhibited growth of Bacterial (after addition of different samples) / OD of Initial Bacterial growth × 100.

2.8 Antibacterial Activity (Agar well diffusion method)

Mueller Hinton Agar plates were equipped and inoculated using sample bacterial isolates by dispersal of the bacterial inoculates. Exactly 6 mm in diameter well was punched and loaded 50 µl of TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Ch, TC-Ch-AgNPs and TC-Ch-AgNPs-W were directly placed in the culture medium. In this DMSO and Gentamicin used as negative and positive control. The prepared plates were kept for incubation for 24 hours at 37°C in a sterile environment. The effective antibacterial activity was evaluated by observing the zone of inhibition in all three replicates.

2.9 DPPH radical scavenging assay

With slight modification of available literature[11,12], the prepared reaction mixture containing 50ml methanol with 1mM DPPH (Diphenyl-2-picryl radical). In the solution TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Ch, TC-Ch-AgNPs and TC-Ch-AgNPs-W added with concentrations ranging from 20 - 100µl. Methanol DPPH solution was used as a positive control (ascorbic acid) and blank methanol alone. When DPPH reacts in the sample with antioxidant, color changes from deep purple to light yellow. Calorimetrically estimated at 518 nm.

3. Result and Discussion

From the present investigation, TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Ch, TC-Ch-AgNPs and TC-Ch-AgNPs-Wcomposite have been prepared by modified method of available literature and it was systematically evaluated the antibacterial activity towards four different bacteria (*S.aureus*, *S.typhi*, *E.coli* and *B.cereus)*was performed and represented in the figure 2 to 5. In addition, the antioxidant activity was performed and expressed in figure6 to 8. All the above results were interpreted with available literature[13-15].

Minimum Inhibitory Concentration (MIC)

The sensitivity towards the selected gram positive and negative bacterial strains against different percentage of inhibition of TC, Ch, AgNPs and W are shown in figure 2.

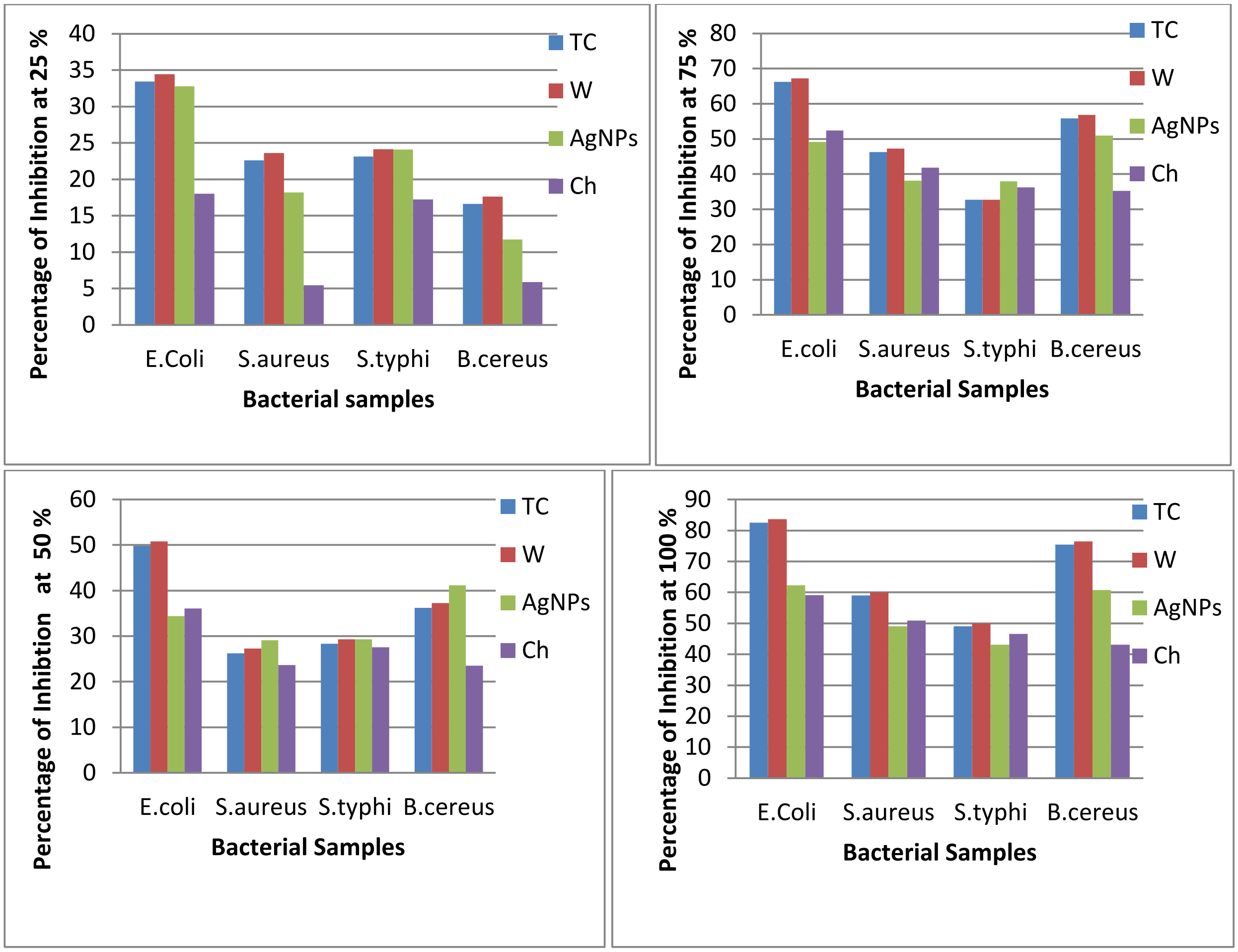

Figure 1: Minimum inhibitory concentration of different concentration of TC, W, AgNPs and Ch against four different bacteria

The minimum inhibitory concentration investigation was conducted by using different concentration of TC, AgNPs, Ch and W viz, 25, 50, 75, 100µl/ml. From the above concentration the rate of inhibition was observed in all bacterial strains in increasing the growth of inhibition. From the figure it represents that, among all four different samples the maximum activity shown by wood and most similar results were observed for TC against *E. coli*.

Followed by the proportion of TC-Ch, TC-AgNPs and TC-W are allowed to interact with different selected microbes and the results were expressed in figure 2.

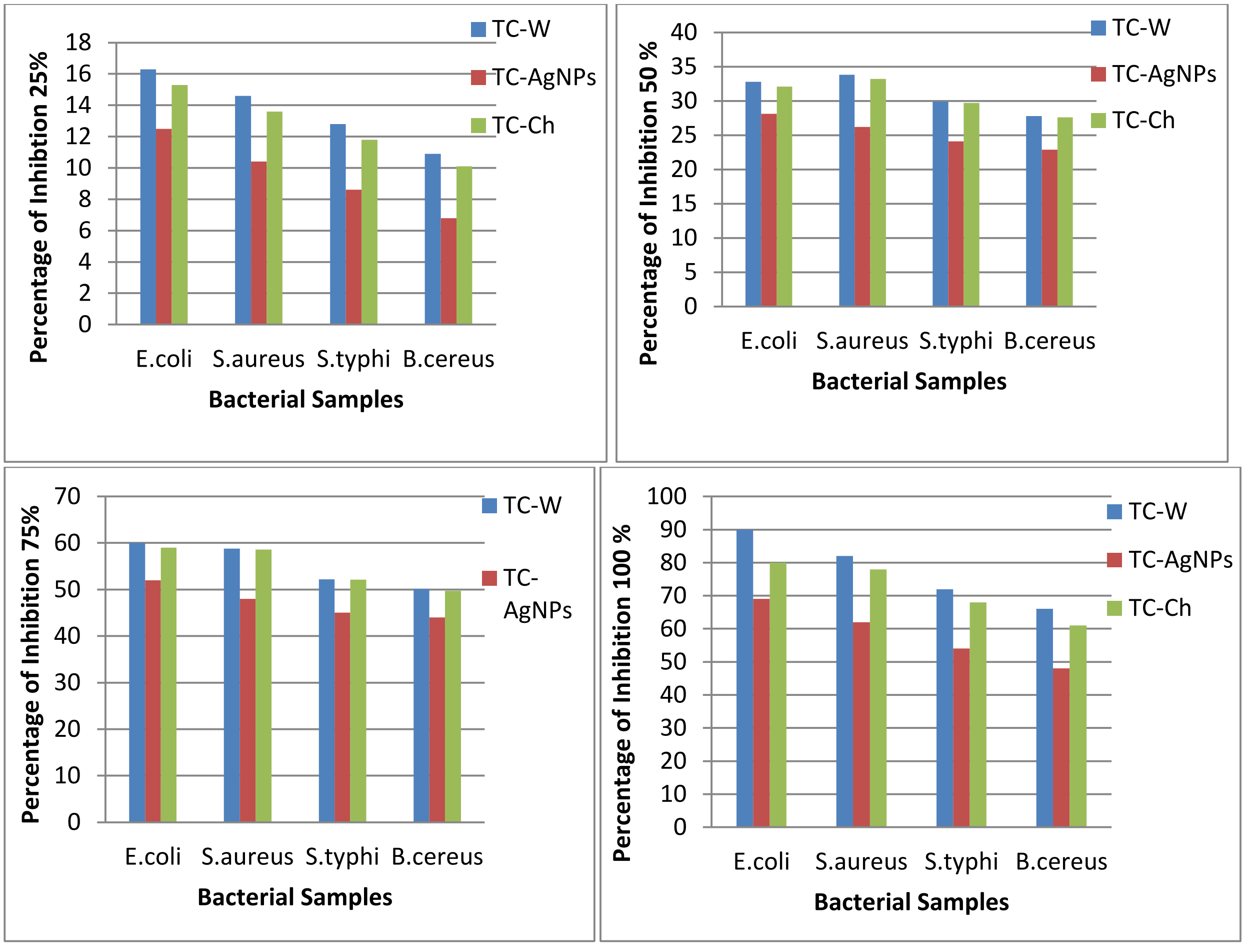

Figure 2: Minimum inhibitory concentration of different concentration of TC-W, TC-AgNPs and TC-Ch against four different bacteria

The results show TC-W exhibits maximum zone of inhibition against *E. coli* and most similar zone was observed in *S. aureus* when compared to TC-Ch and TC-AgNPs. The percentage of inhibition was gradually increased with concentration in different bacterial strains. When compared to TC-Ch and TC-AgNPs, TC-Ch shows great growth inhibition against *S. aureus*.

From the above results, fabricated biomaterial with the proportion of TC-Ch-AgNPs and TC-Ch-AgNPs-W were allowed to interact with it desired concentration against four different bacterial strains to determine the efficiency of growth inhibition shown in figure 3.

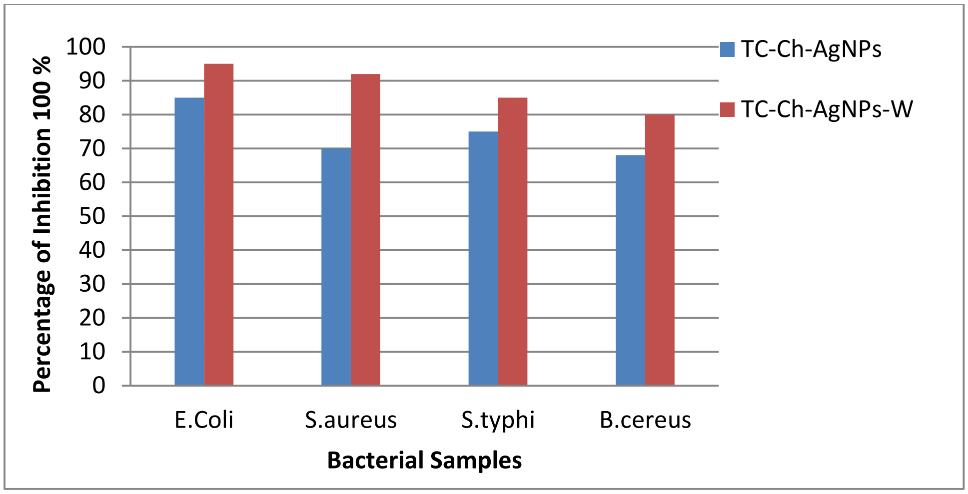

Figure 3: Minimum inhibitory concentration of TC-Ch-AgNPs and TC-Ch-AgNPs-W against four different bacteria

The results show, among the fabricated nanomaterials TC-Ch-AgNPs-W acquired enhanced inhibitory activity against all four bacteria. The inhibition percentage is comparatively increased with single (TC, W, AgNPs and Ch) and double proportions (TC-W, TC-AgNPs and TC-Ch). The great growth inhibition was observed *E. coli* and *S. aureus* shows slightly different. All the obtained results were compared with available literatures[16-18].

The MIC for targeted samples was tested using a twofold broth dilution method at the concentration ranging from 0.2-1 µg/l. All these samples showed great antibacterial activity against selected bacterial isolates. However, the selected samples expressed remarkable antibacterial activity among all four different bacteria (*S. aureus, B. cereus, S. typhi* and *E. coli)*. It concludes the selected bacterial strains showsTC-Ch-AgNPs-W > TC-Ch-AgNPs > TC-W > TC-Ch > TC-AgNPs >W >TC > Ch> AgNPs order of growth inhibition. In The results were agreed with available literates and shows significant compatibility was confirmed by broth dilution method[19,20].

Antibacterial Activity (Agar well diffusion method)

The antibacterial activity was significantly performed by agar well diffusion method with prescribed concentration of sample by MIC against selected bacterial samples like *B. cereus, S. aureus, S. typhi* and *E. coli*. The activity was expressed in figure 4-6. DMSO acts as negative control.

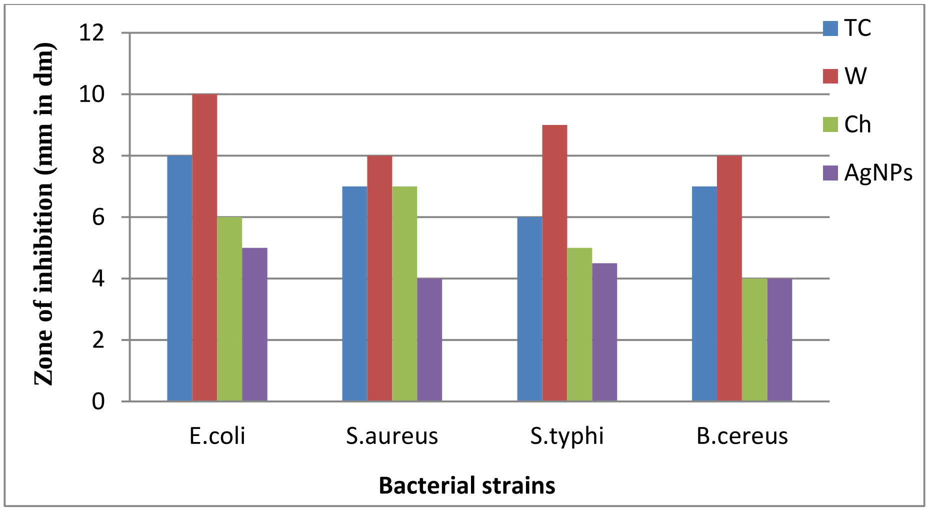

Figure 4: Antibacterial activity of TC, W, AgNPs and Ch against four different bacteria

From the figure 4, the antibacterial activity was performed for TC, W, AgNPs and Ch and all the samples express its desirable zone of inhibition. The increasing zone of inhibition was observed for *E. coli* exhibited by W.

Followed by the proportion of TC-Ch, TC-AgNPs and TC-W are allowed to interact with selected microbes was performed and the zone of inhibition was expressed in figure 5.

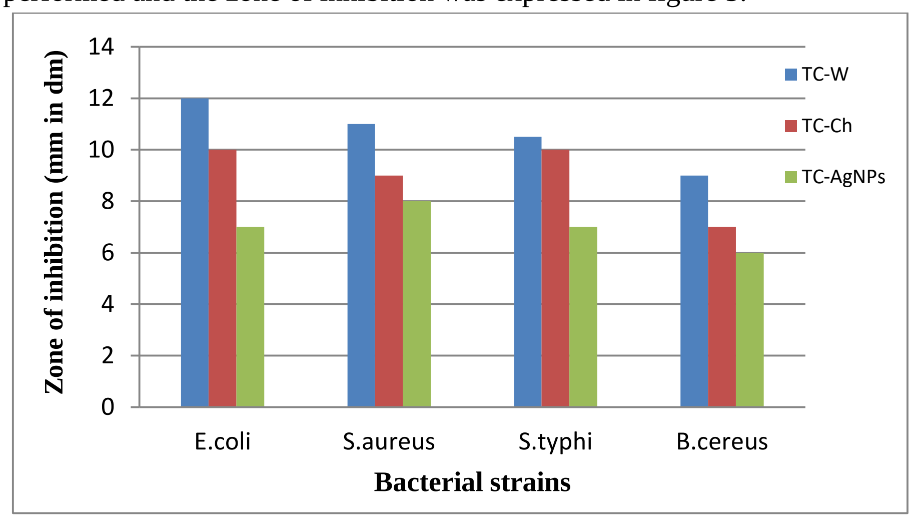

Figure 5: Antibacterial activity of TC-W, TC-AgNPs and TC-Ch against four different bacteria

The results show TC-W exhibits maximum zone of inhibition against *E. coli an*d most similar zone was observed in *S. aureus* when compared to TC-Ch and TC-AgNPs. When compared to TC-Ch and TC-AgNPs, TC-Ch shows great growth inhibition against *S. typhi and E. coli.*

From the above outcome, fabricated nanomaterial with the combination of TC-Ch-AgNPs and TC-Ch-AgNPs-W were allowed to interact with its desired concentration against four different bacterial strains to determine the efficiency of zone of inhibition shown in figure 6.

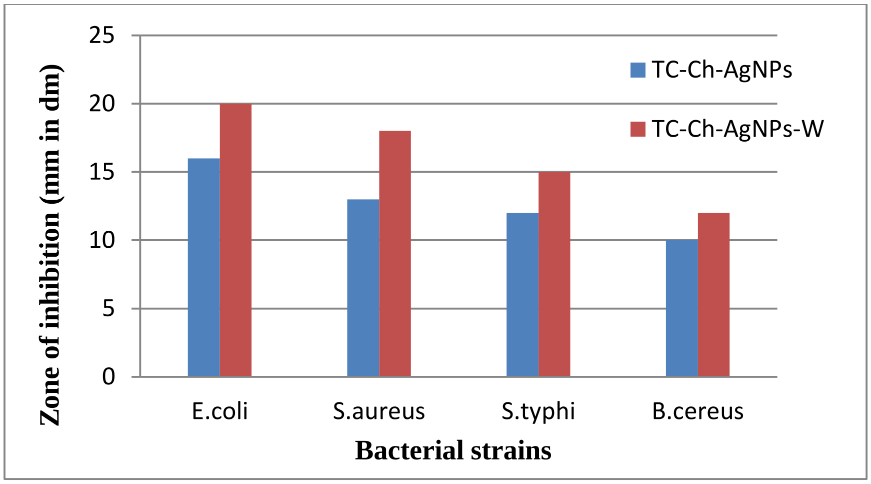

Figure 5: Antibacterial activity of TC-Ch-AgNPs and TC-Ch-AgNPs-W against four different bacteria

The results show among the two fabricated nanomaterials, TC-Ch-AgNPs-W acquired enhanced inhibitory property against selected bacteria. The increased zone of inhibition was observed *E. coli* comparatively increased with single (TC, W, AgNPs and Ch) and double proportions (TC-W, TC-AgNPs and TC-Ch). The great growth inhibition was observed E. coli and S. aureus with slightly different. The results were agreed with available literates[21-23] and shows significant compatibility was confirmed by agar diffusion method.

Antioxidant Activity

The results confirmed the TC, Ch, AgNPs and W possess the efficient antioxidant activity. The TC has 42 %, Ch possesses 33 %, AgNPs has 28 % and W possesses 38 % of antioxidant activity at the concentration of 100 µg/ml. The figure 6, it expressed that antioxidant activity increases which increases the concentration. The results were in agreement with available literature[24,25].

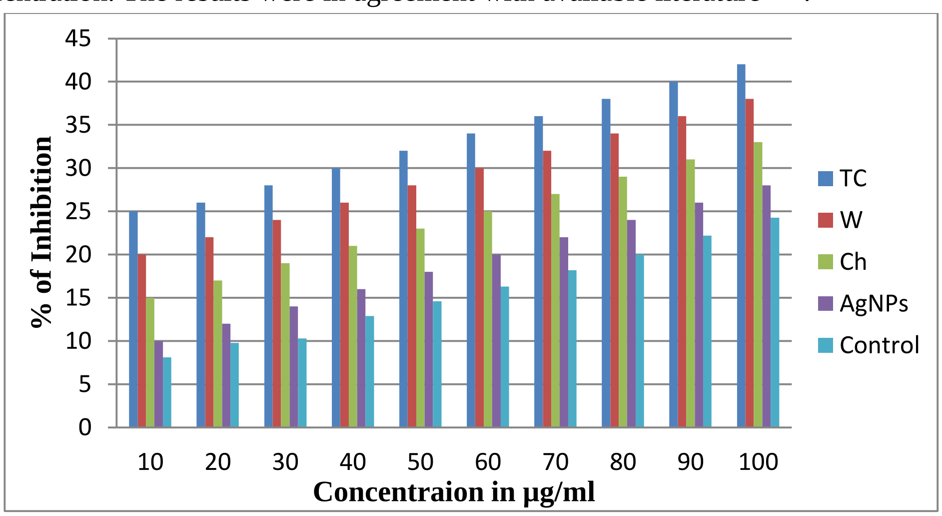

Figure 6: Antioxidant activity of TC, W, AgNPs and Ch

From the figure 7 the results were confirmed that TC-W, TC-Ch and TC-AgNPs possess the increased antioxidant activity compared with single proportion. The percentage of inhibition was observed for TC-W, TC-Ch and TC-AgNPs around 72.89, 69.12 and 65.28 %. The concentration of samples increases with increase the percentage of scavenging activity. The results were in agreement with available literature[26].

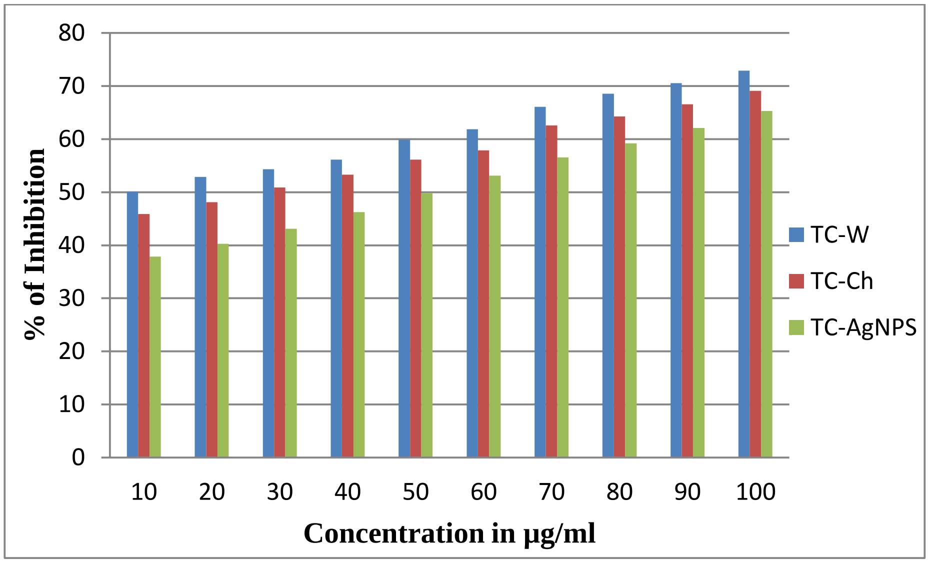

Figure 7: Antioxidant activity of TC-W, TC-AgNPs and TC-Ch

From the figure 8 the results were confirmed the TC- Ch-AgNPs –W and TC-Ch-AgNPs possess the improved antioxidant activity compared with Figure 6 and 7. The percentage of inhibition was observed for TC- Ch-AgNPs –W and TC-Ch-AgNPs around 98.79 and 88.55 percent of scavenging activity at the concentration of 100µg/ml. The concentration of samples increases with increase the percentage of scavenging activity. The results were in agreement with available literature[27].

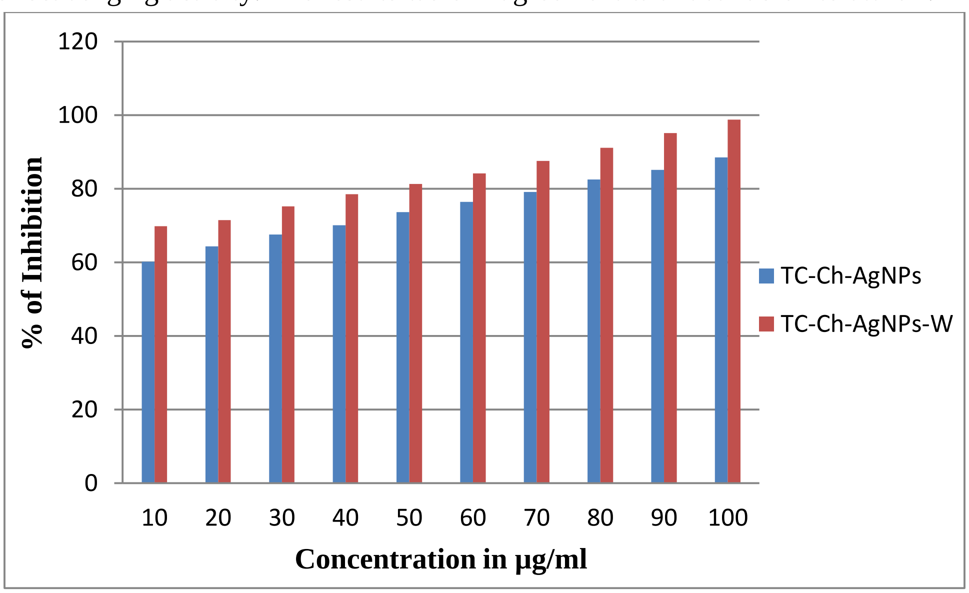

Figure 8: Antioxidant activity of TC-Ch-AgNPs and TC-Ch-AgNPs-W

4. Conclusion

From the present investigation TC, AgNPs, Ch, W, TC-AgNPs, TC-W, TC-Ch, TC-Ch-AgNPs and TC-Ch-AgNPs-W were allowed to evaluate the antibacterial activity was performed by broth dilution method and agar well diffusion method against *E.coli*, *B.cereus*, *S.typhi* and *S.aureus*. For enhanced activity was observed byTC-Ch-AgNPs-W > TC-Ch-AgNPs > TC-W > TC-Ch > TC-AgNPs >W >TC > Ch > AgNPs order. The increased activity was observed against *E. coli* by TC-Ch-AgNPs-W. In addition, the antioxidant activity was performed and compared with all other samples. TheTC-Ch-AgNPs-Wpossess increasing percentage of inhibition which was confirmedand compared with standard ascorbic acid. From the proposed research the prepared materials possess great impact on the microorganisms and antioxidant activity. In addition, it also may offer different bioremediation processes with ecofriendly nature.

ACKNOWLEDGEMENTS

The first author would like to thank the management of Sacred Heart College Tirupattur for providing all the facilities to do the research activities in the form of Fr. Carreno Research Grant (2021–2022). All the authors would like to thank their respective management of their institutes for rendering academic liberty for the successful completion of the research project.

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