Antibacterial Evaluation of *Nerium Oleander* Extract Enhanced by Titanium Oxide Nanoparticles

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Abstract

The antimicrobial activities of ethanolic extract of the *Nerium oleander* have been screened by anti-bacterial action against *Staphylococcus aureus, Klebsiella pneumonie and Escherichia coli* utilizing the disc diffusion test. An endeavor was additionally made to enhanced the antimicrobial activity of the ethanolic extract of *Nerium oleander* by blending of TiO_2 nanoparticles with *Nerium oleander* extract. In view of the general results, the ethanolic extract of *Nerium oleander* enhanced by TiO_2 Nps demonstrated the most activities against a wide range of tested bacteria, yet the ethanolic extract alone was observed to be dynamic against the *S. aureus* and *E. coli*, while a powerless antibacterial activity was observed against the ethanolic extract demonstrate a *K. pneumonie*. Inhibition efficiency increased with increasing concentration of the ethanolic extract of and also with enhanced by TiO_2 Nps. The inhibition zone at the concentration 300 mg/mL in presence and absence of TiO_2 NPs were 21 mm and 24 mm *against Staphylococcus aureus* and against *Klebsiella pneumonie* were 12 mm and 25 mm, finally for *Escherichia coli* were 23 mm and 29 mm respectively. [DOI: 10.22401/JNUS.20.2.04]

Keywords**:** antimicrobial activities, *Nerium oleander*, TiO_2 Nps, *Staphylococcus.*

Introduction

The investigation of plant extracts as minimal effort and ecofriendly antibacterial inhibitors is of incredible benefit of an ecological viewpoint and is enticing a noteworthy field of interest. Green anti-microbial inhibitors possess a hopeful outlook for the feature of the ecological since they don't possess heavy minerals or other poisonous compounds. What's more, they are bio-degradable with renewable origin of minerals [1]. As of now, over a 100 new items are in clinical advancement, especially as anti-cancer agents and against infectives [2,3,4]. This has affected a large number of pharmacological organizations to manufacture novel anti-microbial formulas that extracts of plant. The bio-active molecule come in plants as auxiliary metabolites and as impedance systems, mechanisms versus herbivores, fungal attack, microbial invasion and viral-infection. Amid the previous decade, strong agents have gotten to be accessible against viral diseases. In this manner, plants extracts with phyto-chemical have been obtaine more critical as potential source for viral inhibitors amid the late decade. Broad studies have demonstrated that therapeutic plants of a few parts of the world contain compounds dynamic against infections that bring about human diseases [5,6,7]. In the conventional medication system, parts of this plant are utilized for the treatment of different human ailments. The leaf is utilized as a cardiotonic, diuretic, anti-bacterial in cutaneous eruptions, and is likewise powerful against snake-nibbles; the root is utilized for curing distinctive sorts of cancers, ulcers with leprosy. The root-bark is utilized particularly against ring worm and the leaves extracts, branches, roots and flowers are harmful to specific insects [8]. A few phytochemicals have been distinguished in different parts of the plant and they incorporate principally cardiotonic glycosides, terpenoids and steroids [9]. The oxide of metal NPs, like, tinoxide, ironoxide, zincoxide and titanium are extremely attract and assume a noteworthy part in different manufactured uses [10,11]. Generally they were utilized as a part of restorative domain, electronic, sensor, optic, catalyst, photo-electrochemical devices, drug delivery, bio-sensors, bio-imaging, anti-microbial exercises, nourishment preservation with photonics. The best of semiconductors are TiO2 that are generally utilized as a part of dye-sensitized solar cell (DSSC). A DSSC is a device for the change of obvious light power

into power in view of the sharpening of wide band hole semiconductors, for example, TiO_2 [12]. Till now, TiO_2 is the foundation semiconductor for dyesensitized nanostructured terminals for DSSC. Because of the non-lethal, effectively accessible and minimal effort attributes, TiO_2 has been the generally favored semiconductor for the photoelectrode [13]. Despite the expansive quantities of plant concentrates researched and tried as antimicrobial, writing is insufficient on the inhibitive properties of plant concentrate supplemented by TiO_2 Nps for the eliminating microscopic organisms. Along these lines the target of our study is to examine the inhibitive properties of ethanol extract of Nerium oleander as antibacterial and improvement of this extract by TiO_2 Nps.

Materials and Methods

Titanium oxide nanoparticles all the chemicals used in our work were obtained from Merck and Sigma-Aldrich Chemical Company and all were used without further purification.

Medicinal plant collection

The *Nerium oleander* were collected, sealed in plastic-bags, transported to laboratory and deposited for further investigations.

Extraction and isolation

The air-dried *Nerium oleander*, (250 g) was powdered and extracted had been done utilizing the continuous extraction strategy utilizing the Soxhlet mechanical assembly with ethanol (1000 mL). The extraction was completed for five hours. After the extraction procedure, the crude was filtrate and the ethanol in the filtrate was evacuated via air-drying. The crude was weight and dissolved in dimethyl sulfoxide to yield the working stock solution. The percentage of crude extraction was computed by utilizing the equation (1).

$$Crude\ Extaction\% = \frac{M_b - M_a}{M_b} \times 100$$

where M_b and M_a are the mass in grams of *Nerium oleander* samples before after extraction respectively.

Antimicrobial activity assay

Disc diffusion method:

The antimicrobial activity of *Nerium oleander* with and without TiO_2 Nps (1.0 mg with each extracted test) were assessed by rules of the National/ Committee/ for/ Clinical/ Laboratory/Standards (1997-NCCLS) utilizing the agar disc diffusion strategy [14]. Quickly, culture of tried gram positive microscopic organisms to be specific *S. aureus*, and gram negative microorganisms in particular *K. pneumonie* and *E. coli* were blended with sterile saline (0.85%). Petri plates having 20 mL of Agar (Mueller Hinton) had been utilized for every one of the microscopic organisms tried. Inoculums had been spread on hardened media surface of the Whatman (No. one filter-paper) disc (6 mm in diameter) impregnate and the *Nerium oleander* with and without TiO_2 Nps set on the plates. Ciprofloxacin utilized as positive control for bacteria. A paper disc impregnate and DMSO (dimethylsulfoxide) had been was utilized as negative control. Plates inoculated with the microorganisms were incubated for one day at 37ºC. The inhibition zone distances across had been measured in mille-meters. All investigated had been done in triplicate moreover the normal was taken for the final test.

Statistical analysis

Statistical analysis of variance (ANOVA) wasused for analyzing the data obtained. Data were expressed as mean±standard deviation.

Results and Discussion

Anti-bacterial activity

A essential neutralize of studies had been published on the impact for photo-catalytic of TiO2 NPs on micro-organisms [15-18]. These investigations display that the photo-catalytic operation in water is efficient versus the wide domain of organisms, like alga, virus and microbs. It could be noted that the various investigations had been carried out in aqueous slurry or with aqueous inoculum, confirm the major function of water in the micro-organism photo-killing operation [19]. The irradiation of TiO2 with ultraviolet light, whose wavelength is less than 385 nm, leads to generation of an electron-hole pair on the TiO2 surface. The electrons and holes react on the surface and convert water and oxygen in to reactive oxygen species like hydrox-radical, superoxide and hydrogen peroxide [20]. The primary

oxidizing agents are short-living hydroxy-radical that may bonded to the surface. Those radicals are significantly short life span (10^{-9}), that prevent from diffusing to a long distance. Consequently, only microbial cells that adhere to the surface of the TiO2 catalyst may react with the hydroxyl radical that usually causes the loss of membrane integrity [21]. In turn, superoxide ions are long-lived, although due to the negative charge, they cannot penetrate into the cell membrane. The penetration is possible by hydrogen peroxide [22]. Also, free TiO2 particles can attack intracellular components directly if it is enhanced with UV irradiation. This may lead to physical and chemical damages of DNA and RNA, causing the conversion of the pyrimidine and purine bases to carbon dioxide, ammonia and nitrate ions [23,24]. Although there are at least three hypotheses of the antimicrobial effect of TiO2 photocatalytic reaction, that mechanism is still to be proved.

The results of anti-bacterial were assessed of all the tested samples are demonstrated in Figs. (1, 2 and 3). Samples that have TiO_2 Nps demonstrated moderate to good activity in DMSO. Particularly, Ciprofloxacin demonstrated higher activities (zone of inhibition up to 19, 24 and 27 mm at concentration of 300 mg/mL against, *S. aureus*, *K. pneumonie* and *E. coli* respectively as synergistic effect with TiO_2 Nps that were higher than Ciprofloxacin in absence of TiO_2 Nps. Ciprofloxacin in presece of TiO_2 Nps did not show any deference agains *K. pneumonie* while *Nerium oleander extract* synergistic with TiO_2 Nps. Show much higher than without the synergistic effect. Inhibition efficiency for the tested plant extract increased with increasing concentration of the ethanolic extract of and also with enhanced by TiO_2 Nps. From Fig.(1), the inhibition zone at the concentration 50, 150 and 300 mg/mL in absence of TiO_2 NPs were 9, 13 and 21 mm but in presence of TiO_2 NPs were 13, 16 and 24 mm *against S. aureus* more over for the Ciprofloxacin without TiO_2 NPs 18, 22 and 26 but in presence of TiO_2 NPs were 20, 23 and 28 respectively against *S. aureus*.

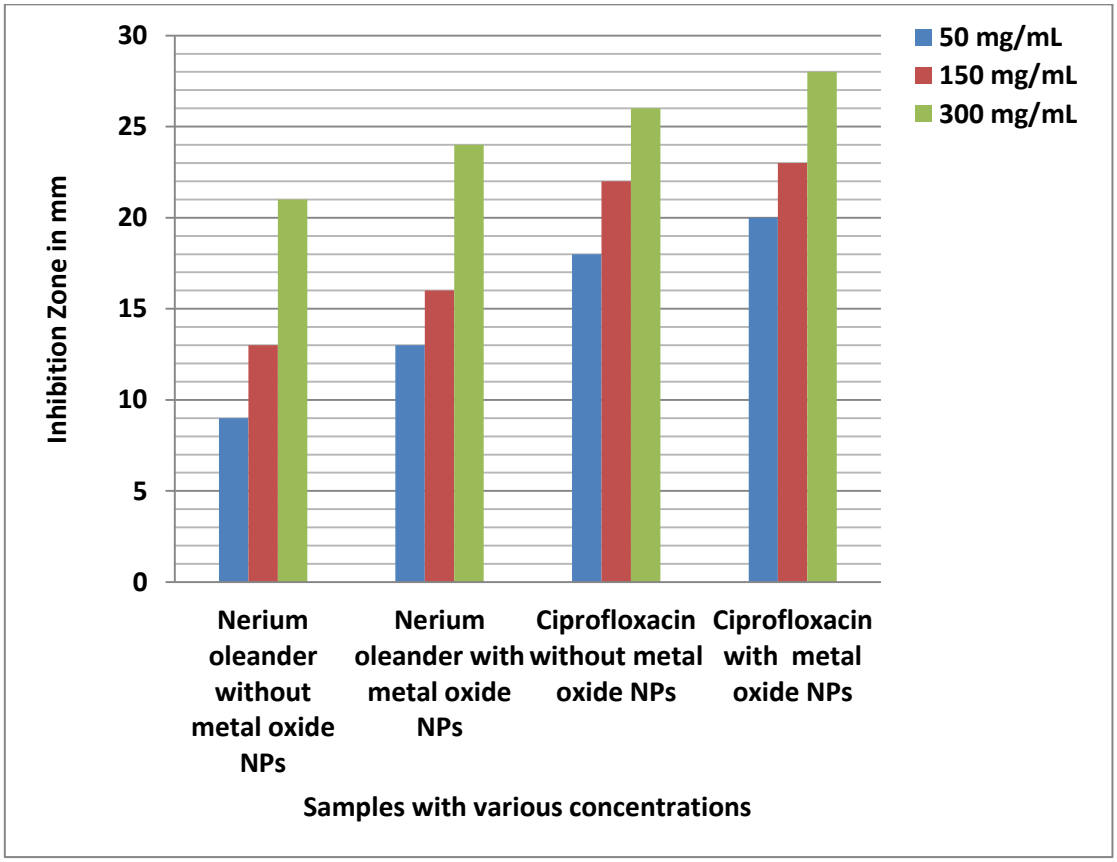

Fig.(1): Inhibition zone (mm in diameter) (Mean±SD) (n=3) for tested samples against Staphylococcus aureus.

From Fig.(2), the inhibition zone at the concentration 50, 150 and 300 mg/mL in absence of TiO_2 NPs were 5, 10 and 12 mm but in presence of TiO_2 NPs were 11, 17 and 25 mm *against K. pneumonie* more over for the Ciprofloxacin without TiO_2 NPs 11, 15 and 21 but in presence of TiO_2 NPs were 19, 22 and 24 respectively against *K. pneumonie*.

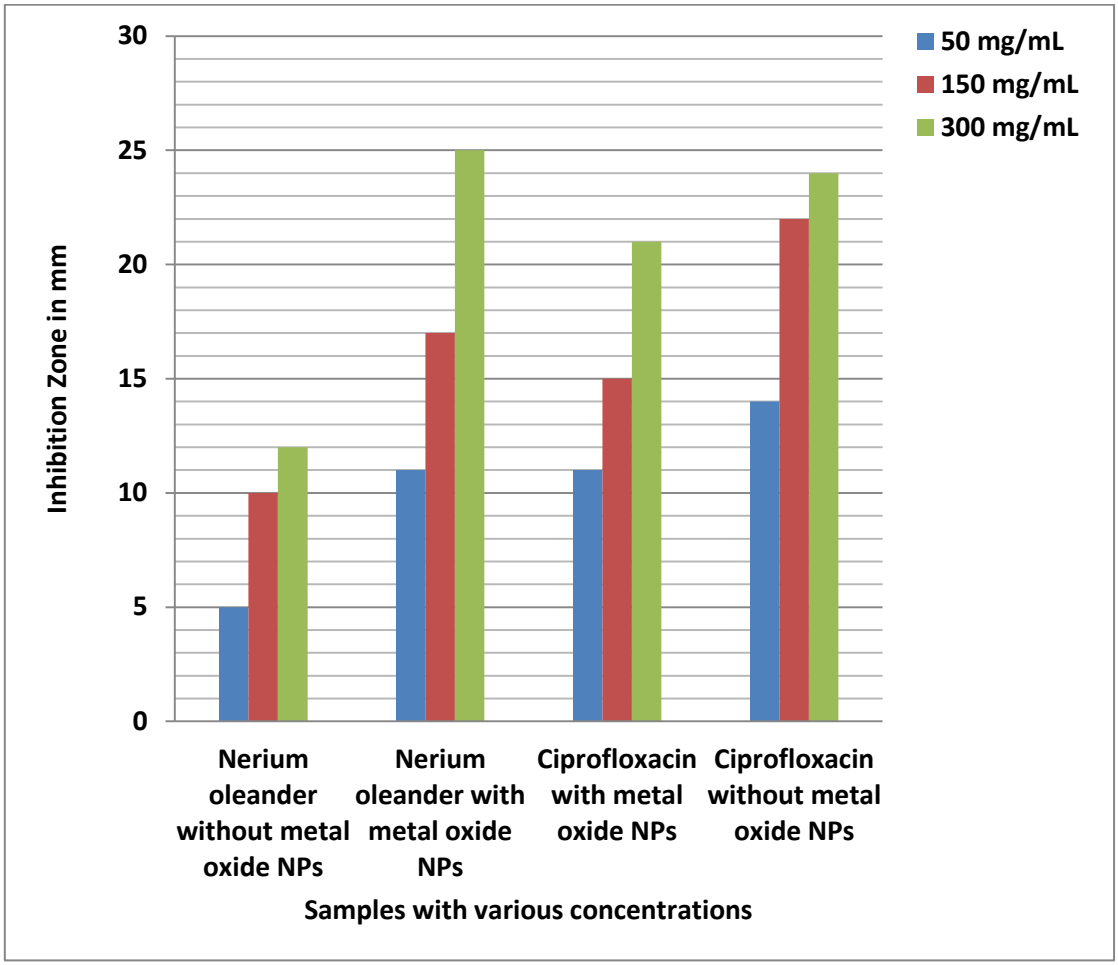

Fig.(2): Inhibition zone (mm in diameter) (Mean±SD) (n=3) for tested samples against K. pneumonie.

From Fig.(3), the inhibition zone at the concentration 50, 150 and 300 mg/mL in absence of TiO_2 NPs were 13, 17 and 23 mm but in presence of TiO_2 NPs were 14, 25 and 29 mm *against Escherichia coli* more over for the Ciprofloxacin without TiO_2 NPs 11, 15 and 24 but in presence of TiO_2 NPs were 12, 19 and 24 respectively against *Escherichia coli*.

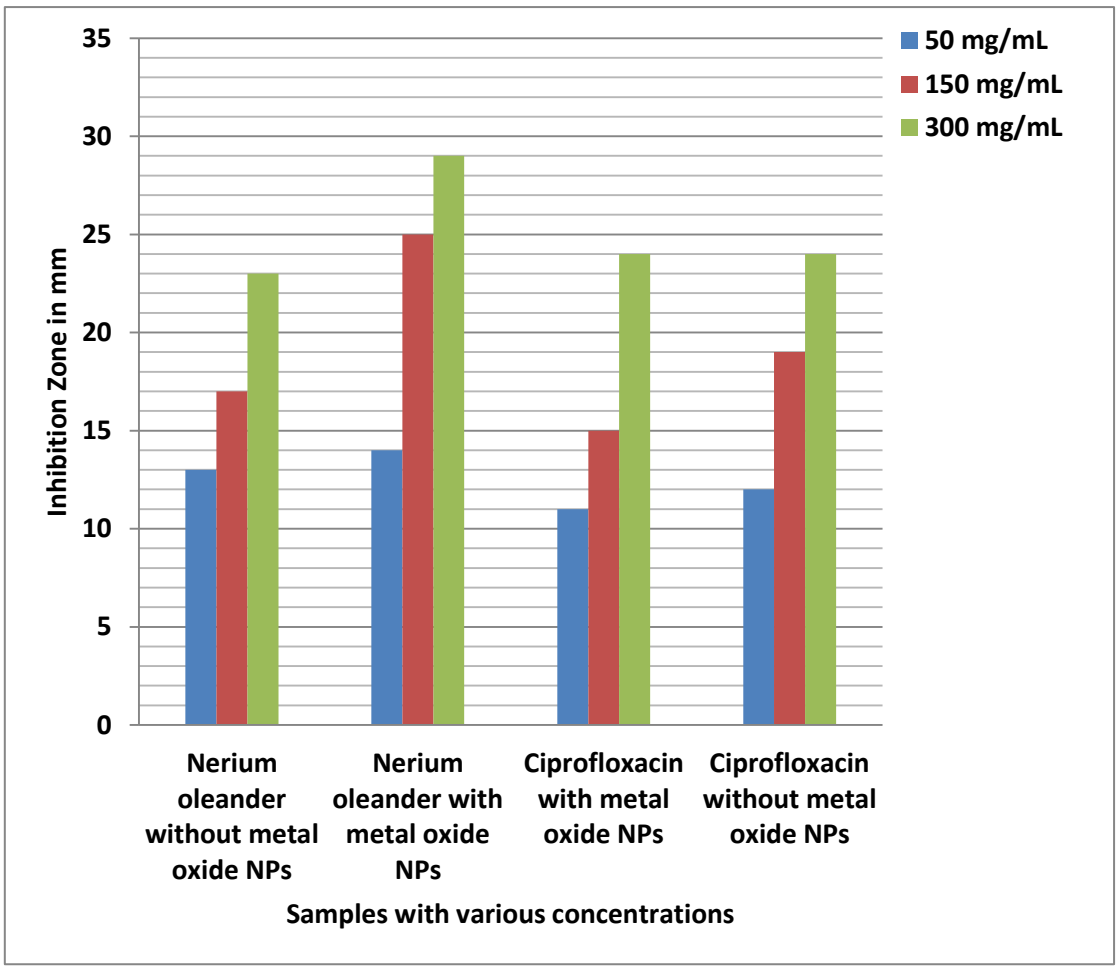

Fig.(3): Inhibition zone (mm in diameter) (Mean±SD) (n=3) for tested samples against Escherichia coli.

Conclusions

Nerium oleander extracts were highly inhibition against the Gram positive bacteria *S. aureus*, and gram negative bacteria *E. coli*, but low activity against *K. pneumonie*. Antibacteria activities were enhanced and become much higher by synergistic effect of TiO_2 Nps. It can be conclude that enhancing of *Nerium oleander extract* by TiO_2 Nps will raise the inhibition activities against all types of selected bacteria specially against *K. pneumonie*.

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