International Journal of Scientific Research in Chemical Sciences
Vol.**11**, Issue.**2**, pp.**01-06**, April **2024**
E-ISSN: 2455-3174
Available online at: www.isroset.org

Research Article

Pharmacological Potential of TiO_2 Nanoparticles synthesized by Chemical and Green method – A Comparative study

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Received: 05/Feb/2024; **Accepted**: 08/Mar/2024; **Published**: 30/Apr/2024

Abstract— In modern times, nanoparticles have materialized as indispensable things in contemporary medicine, with variety of uses in clinical, drug and gene conveyance. In the current study, TiO_2 nanoparticles prepared from the leaf extracts of *Mollugo oppositifolia* and *Trianthema portulacastrum* were compared with the chemical TiO_2–nanoparticles for antibacterial and antioxidant activities. The synthesized TiO_2-NPs are assessed for antimicrobial and antioxidant activities. Green TiO_2-NPs has an antibacterial effect against Pseudomonas aeruginosa (17 + 0.56 mm) and Staphylococcus aureus (16 + 0.24 mm) at concentrations as low as 100 µl. TiO_2-NPs Green showed high inhibition of DPPH I radical (50 µg/m) at 95.17 ± 21. Therefore, TiO_2-NPs represent eco-friendly properties that aid in the degradation of dyes due to their antioxidant activity.

Keywords— Nanoparticles, *Mollugo oppositifolia, Trianthema portulacastrum*, Antioxidants, Phyto-active compounds, TiO_2, antibacterial

1. Introduction

Phytochemicals are considered as the secondary plant matter which are always active in human biology and often has health benefits [1]. Many types of compounds found in plants are biochemically important classes, comprising the alkaloids, glycosides, polyphenols, terpenes etc. In recent years, numerous chemical compounds obtained from floras are used in the defense mechanism against many diseases. These are often useful as drugs, and possess many pharmacological activities [2]. Phyto-active chemicals kindle the insusceptible system that is the body's resistance against viruses, bacteria and other pathogens. Most of the phytochemicals are antioxidants and they (i) block the probable carcinogenic substances produced in the body, (ii) Reduces oxidation, the destruction of cells that occurs with aging and exposure to toxic wastes, (iii) Check DNA damage and help with DNA restoration mechanisms and (iv) Help in regulating the hormones such as estrogen and insulin. High levels of these hormones increase the risk of breast and colon cancer [3].

Nanotechnology is the most dynamic and constantly evolving field of science and technology for producing new nanoscale materials in an economical and environmentally friendly way. Apart from that, nanotechnology deals with the possibility of producing nanoparticles of preferred shapes, sizes, etc. according to the requirements of different applications. TiO_2-NPs are produced largely in recent years because of their wide usage in various fields due to high stability, increased catalytic activity, etc. This increased catalytic activity and high surface area are the reasons for which TiO_2-NPs are being used in numerous applications including waste water treatment from industries [4]. In medical field, TiO_2-NPs are under examination for their valuable outfits in imaging and nano-therapeutics [5]. Distinctive properties of TiO_2-NPs make them as an ideal choice in the composition of several skin care products. TiO_2-NPs preparations are presently observed as unique treatments for acne vulgaris, recurrent condyloma accuminata, atopic dermatitis, hyper pigmented skin diseases, and new non-dermatologic diseases [6]. TiO_2-NPs also possess antimicrobial activity under Ultra Violet light radiation [7]. Nanoparticles of Ag, Au, MgO, CuO, Al and TiO_2 have been reported by many authors for their antibacterial activity and are effective against various drug-resistant bacteria, viruses and fungal strains [8]. Hence, a nanoparticle has ability to serve as an alternate to antibiotics and to limit microbial infections caused by Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae and Salmonella typhi [9][10]. Biologically active phytochemicals like alkaloids, glycosides, polyphenols and terpenes can be used as reducing agents to reduce metal oxide ions [11]. The synthesis of TiO_2-NPs by employing extracts of various plants by green synthesis method was reported by several researchers [12],[13], [14], [15].

The main objective of this work is to ascertain the advantages of the TiO2-NPs produced by biosynthesis process from the

extracts of *Mollugo oppositifolia,* and *Trianthema portulacastrum* plants over the chemically synthesized TiO_2-NPs in terms of their properties and pharmacological applications [16], [17].

2. Related Work

This work is intended to present the advantages of the nanoparticles synthesized from the extracts of various parts of the plants over the chemically synthesized nanoparticles. Biosynthesized nanoparticles possess better antioxidant and pharmacological potential than chemically synthesized. [18] [19] [20] [21].

3. Theory

Collection of material
The leaves of Mollugo oppositifolia and Trianthema portulacastrum were collected from Mayiladuthurai, Tamil Nadu, India. The leaves free from microbial infection were collected and cleaned in Tap water followed by distilled water to dry a period of 5 days in a shadow. An electric blender was used to pulverize the dried leaves and stored in an anaerobic container.

Preparation of Extract
About 50 gm of finely powdered form of leaves was soaked for 72 hrs in ethanol and the subsequent extract was then filtered and stored at 10oC for further use.

Synthesis of TiO2-Nanoparticles
Chemical Synthesis of TiO2-NPs
Solution gel method was adopted to prepare TiO2-NPs. In a characteristic production, 0.1 M solution of titanium nitrate was prepared with deionized water and the mixture was stirred constantly to get a blue colour solution with homogeneous nature. Then 0.2 M NaOH solution was mixed with the already prepared solution and heated for 2 hrs at 80°C with ultrasonic stirring until obtaining a black precipitate. The precipitate was cooled to room temperature. The resultant precipitate was filtered and washed with the surplus quantity of methanol to eliminate the initial constituents. Finally, muffle furnace was used to calcinate the powder for 4 hrs at 400°C for more depiction.

Biosynthesis of TiO2-NPs
0.1 M concentration of Titanium nitrate was prepared by dissolving in 100ml of deionized water by constantly rousing at room temperature. The alcoholic extract solution prepared with water was added to the Ti (NO3)4 solution with uninterrupted rousing at 85°C for 2 hr to acquire colloids. The change of the precipitate colour from blue to dark black confirmed the formation of nanoparticles. Later, with the help of muffle furnace the precipitate was calcinated at 400 °C for 4 hrs and the further study was carried out with the obtained powder.

DPPH radical scavenging assay
The basis of this analytical method is the reduction of DPPH free radical. A maximum absorption at 517nm (purple colour) provided by the odd electron present in the free radical DPPH•. Then the free radical DPPH• reacts with antioxidant, forming a stable free radical it is paired in the presence of a hydrogen donor and is DPPH•, leading to a color change (yellow) related to the number of electrons trapped(Fig. 12 (a)). The greater the colour changes, the better the reducing power. This test is the maximum broadly used version for comparing the loose radical scavenging interest of latest drugs. [22] [23] [24].

Mixing a solution of DPPH• with a substance capable of donating a hydrogen atom yields the reduced state (dipheny1 picry1 hydrazy1: non radical) by losing its violet color (It is expected that the pale yellow of the picry1 group still remains). Reports exposed that the plant may be used as source of antioxidants for the possible potential antioxidant by DPPH• scavenging method [25] [26].

Assay of Antibacterial Activity
Gram positive and Gram-negative organisms like Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli were used to find out the antibacterial activity of the biosynthesized nanoparticles utilizing sterile media like Mueller-Hinton Agar etc. by disc diffusion method. The zone inhibited by the synthesized compounds was noted and it was compared with ciprofloxacin - a standard drug.

Biosynthesized nanoparticles used to possess antibacterial activity against microorganisms of certain family.[27] All the biosynthesized nanoparticles were tested for their anti-bacterial activity against bacterial and fungal strains by paper disc diffusion method [28]. Dimethyl sulfoxide (DMSO) was used as solvent to prepare the solutions with following concentrations viz. 5,10,20,40,80 and 100µg-mL. DMSO has been chosen for antibacterial studies because of its co effect on the bacterial strains chosen [29]. To sterilize the media, the bribed medium was autoclaved at 50 pa for 20 mins and kept at 120º C for 10 mins. The media was then poured in to petri dishes slowly in laminar flow environment allowed to solidify and kept 30º C for 24 hrs. [30] using paper disc (8mm) in nutrient agar culture medium. 50 and 100 µg-mL of the newly synthesized nanoparticles were loaded through bacteria free micro pipettes. The antibacterial activity was determined by measuring the zone of inhibition in millimeters and compared with standard drug ciprofloxacin for bacteria and the findings of antibacterial evaluations.

Culture medium
Nutrient broth was used for the preparation of inoculum of the bacteria and nutrient agar was used for the screening method.
Composition of Nutrient agar medium

Peptone	5.0 gm
Sodium chloride	5.0 gm
Beef extracts	1.5 gm
Yeast extracts	1.5 gm
Agar	15.0 gm
Distilled water	1000 ml
pH	7.4 ± 0.2

4. Experimental Method

The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37ºC ± 1˚ C for 18 hrs, were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121˚ C for 15 min. The petri plates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160˚C, for an hour. Into each sterilized petri plate (20 cm diameter) was poured about 125 ml of molten nutrient agar medium which was already inoculated with the respective strain of bacteria (5 ml of inoculum to 250 ml of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification. After solidification, the piper discs containing the derivatives were placed at different areas on the surfaces of each plate and labeled accordingly.

Each test compound (5 mg) was dissolved in dimethyl sulfoxide 5 ml (Analar grade) to give a concentration of 1000 µg/ml. ciprofloxacin solution was also prepared to give a concentration of 1000 µg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using conc. HCl .All the compounds were tested at dose levels of 1000 µg and DMSO used as a control. The solutions of cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1˚ C for 24 hours. After incubation the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

Nitric oxide generation and assay of Nitric oxide scavenging method:
Nitric oxide (NO) was produced from sodium nitroprusside (SNP) and measured with a Griess reagent. SNP in aqueous solution at physiological pH spontaneously produces NO[11], which can be estimated with the Griess reagent / NO scavengers compete with oxygen, reducing NO production. SNP (3 ml) in phosphate-buffered saline (PBS) was mixed with drug dissolved in different concentrations (1.5-1000 µg/ml) in respective solvents and incubated at 25 °C for 150 min. The mentioned samples were reacted with Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dichloride, and 3% phosphoric acid) by diazotizing the nitrite with sulfanilamide and subsequent coupling with sulfanichloronamine, followed by reading the absorbance of dilam chloride dilamido dichloride. At 546 nm and showed the absorbance of quercetin used as a positive control, similarly treated with Griess reagent. Experiments were repeated in triplicate. The percentage removal of nitric oxide radical activity was calculated using the formula below.

Nitric oxide Inhibition % = [(A control – A test/ A Control)]. × 100
Where A control = Absorbance of control reaction, A test = Absorbance in the presence of the sample of extracts.

Ic50 values were determined as the concentration of the test mixture that reduced absorbance by 50% compared to the blank control. Experiments were repeated in triplicate. Quercitin has been used as a conventional positive antioxidant.

5. Results and Discussion

Antibacterial activity
The synthesized TiO_2-NPs in the concentration of 50µl and 100µl, and are used to determine the antibacterial effect by the agar dispersion method. The synthesized nanoparticles exhibited antimicrobial activity when TiO_2-NPs were tested against of gram positive and gram negative pathogens. It was found that antibacterial activity is dependent on concentration, while antimicrobial activity is augmented with the increasing in concentration of TiO_2-NPs (Fig. 1 and 2). The zone of minimum resistance coefficient in the range of 25 mm in 100µl was measured. As a result it is clear that the cells were highly sensitive to all concentrations of TiO_2-NP, which was confirmed by the level of the inhibitory zone [31]. Biologically synthesized TiO_2-NP, gram-negative bacteria, (Table 4) exhibited a good antibacterial activity. The effect of antibacterial activity was minimal when the inhibitory concentration was 50µl. This result is possible due to the difference in the reactive concentration of TiO_2-NPs in the gram negative cell wall and it contains peptidoglycan as thin layer.TiO_2- NPs infiltrates the membrane of bacteria and divides the cell wall, resulting in the killing of bacteria. It is clear from the Fig. 7 and 8 that the biologically synthesized TiO_2 nanoparticles have a powerful bactericidal activity [32].

Antioxidant activity
3.6.1. DPPH radical scavenging assay
As represented in Fig. 9.a,b, the biosynthesized TiO_2NPs indicated less free radical scavenging activity at lower concentration but at higher concentration it exhibited as like the standard. The free radical scavenging was 6–69% for chemically synthesized TiO_2-NPs, 12-87% for Bio(M.P), and 15– 91% Bio (T.P)TiO_2-NPs respectively and when it is compared to 21–96% effect with standard ascorbic acid at 25–500 µg/mL concentrations. The obtained results strongly supports that the concentration of the extract determines the free radical scavenging activity [32]. The size and morphology of titanium dioxide nanoparticles decides the radical scavenging activity. Therefore, TiO_2-NPs produced from alcoholic extract of *T. portulacastrum* exhibited the maximum radical scavenging in whole range of the concentrations owing to its fine particle dimension of 25 nm with the large size of 100 nm. TiO_2-NPs synthesized from the alcoholic extract of *T. portulacastrum* was found to possess maximum scavenging ability of 91% at 500 µg/mL concentration. Similarly, Biosynthesized TiO_2-NPs from the alcoholic extract of *M. oppositifolia* found to have 87% of radical scavenging at 500 µg/mL concentration with 30 nm particle size. The better radical scavenging ability of metal nanoparticles can be correlated with the existence of phytoactive components in alcoholic extracts. *T. portulacastrum* extracts have been reported to contain higher amounts of phenolic compounds compared to *M. oppositifolia* extracts. This might be the

reason that *T. portulacastrum* extract mediated TiO_2-NPs owns better radical scavenging potential than *M. oppositifolia* extract mediated TiO_2-NPs.

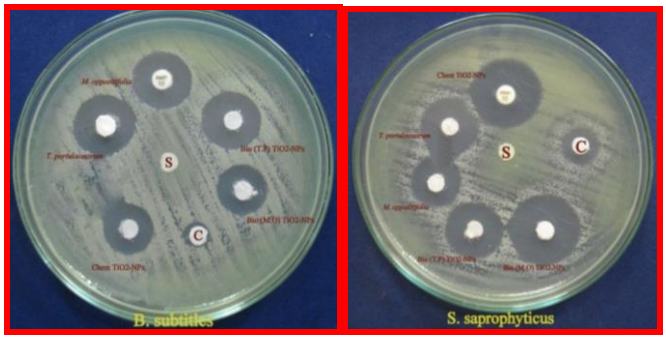
Radical scavenging activity of alcoholic leaf extract of *T. portulacastrum* plant were measured by 2,2 dipheny2 2 picry1 hydrazyl [DPPH•]. DPPH• in ethanol was prepared with the concentration of 0.1 mM solution. The newly prepared solution [1µ1] was added to various extracts in ethanol and of varying concentration [5, 10, 15, 20, 25, and 30]. The various concentration of the extracts were prepared by dilution method by using the ethanol soluble portion. After shaking the mixture vigorously, it was leave at room temperature for 30 minutes. Spectrophotometer [UVIS] Shimadzu Corporation was used to measure the absorbance at 517 nm. Experiments were performed to calculate the sample required to inhibit 50% of DPPH free radicals using ascorbic acid as a reference standard. A lower absorbance of the reaction mixture indicated a higher radical activity of the extract [24], [25]. The calculation of the percentage of DPPH• scavenging effects was done with the aid of an equation (1).

Nitric oxide scavenging activity

The nanoparticles synthesized by green synthesis, at intermediate concentrations, it has a significant scavenging activity against nitric oxide(NO)-induced free radical release 25 from 500 µg/mL are shown in Fig. 3 (b).

The ratio of nitric oxide scavenging action of chemosynthetic and biosynthesized TiO_2NPs at 25–500µg/mL is 6%–78%, 4%–62%, and 5%–63% respectively. Alcoholic extracts of plant leaves showed the lowest radical scavenging action with the maximum IC_{50}µg/ml (177.25and 173.56). However, the chemically synthesized TiO_2 NPs showed 155.79 and the biosynthesized TiO_2-NPs exhibited moderate scavenging action with IC_{50}µg/mL (130.41 and 125.56). A low IC_{50}µg/mL value confers superior antioxidant capacity to the samples.

Greater antioxidant action was perceived in the *T. portulacastrum* facilitated TiO_2 NPs than the other two NPs produced chemically and biosynthetically in this study using *M. oppositifolia* alcoholic extract. Polyphenols act as protective caps for TiO_2- NPs as mentioned earlier, the size-dependent activity of TiO_2 NPs may be the cause for the explanation given in this test. However, drawing firm inferences requires a detailed analysis to reveal the likely tools underlying the toxic nature of chemically synthesized TiO_2-NPs.



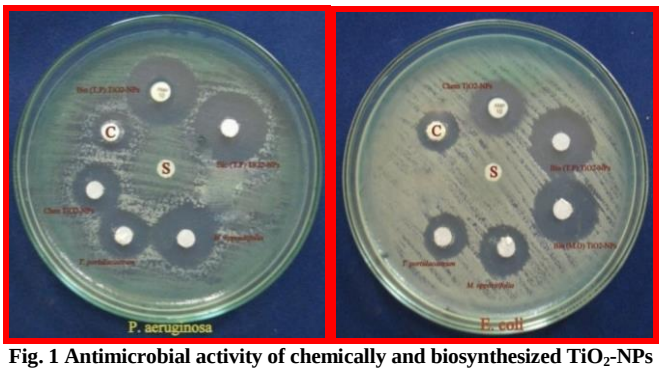


Fig. 1 Antimicrobial activity of chemically and biosynthesized TiO_2-NPs using *M. oppositifolia* (M.P)

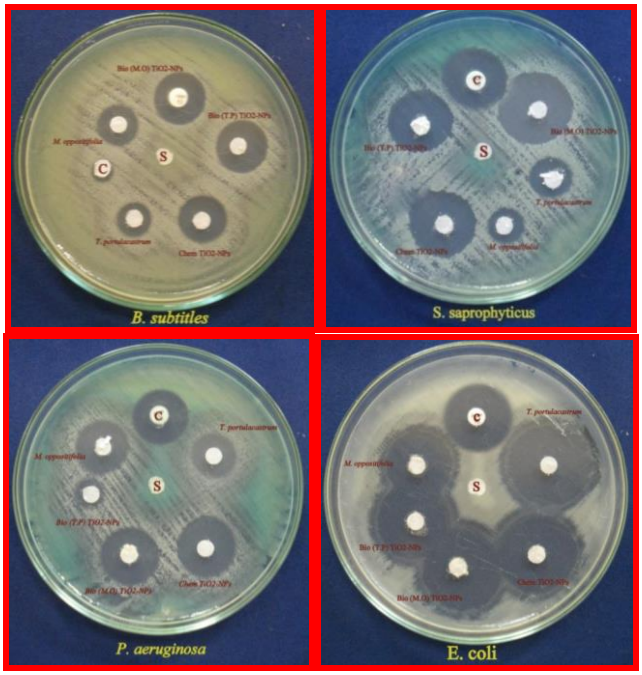


Fig. 2 Antimicrobial activity of chemically and biosynthesized TiO_2-NPs using *T. portulacastrum* (T.P)

Table 1. Antimicrobial activity of TiO_2-NPs synthesized by chemically and biosynthesis methods

Bacteria	Inhibition Zone											
	50 µl						100 µl					
	M.P	T.P	Chem.	Bio1	Bio2	St	M.P	T.P	Chem.	Bio1	Bio2	St
Bacillus subtitles	2	3	2	7	9	15	7	8	7	18	21	25
Staphylococcus saprophyticus	2	2	4	7	10	15	7	7	8	18	19	25
Pseudomonas aeruginosa	3	2	5	9	10	16	8	8	15	20	22	25
Escherichia coli	4	3	2	11	12	18	10	11	17	19	22	26

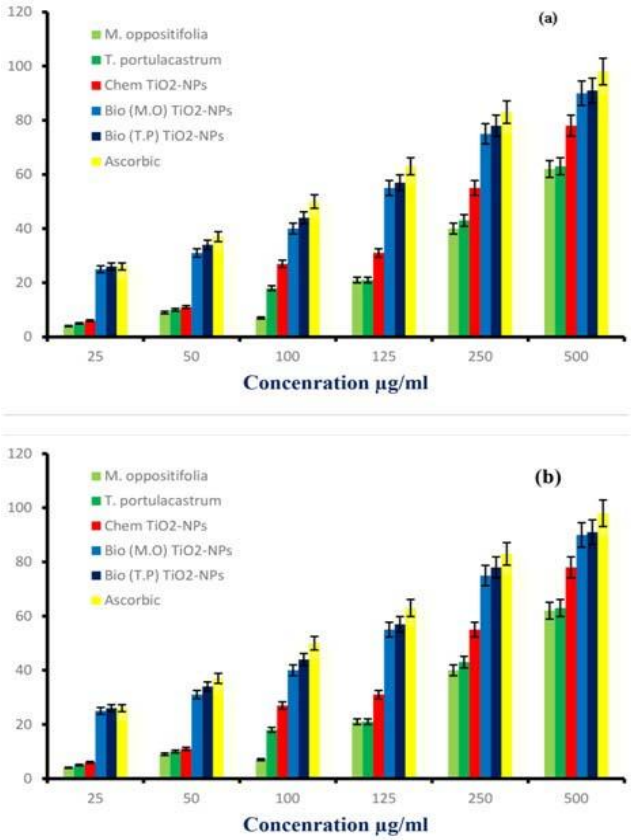


Fig. 3.(a). DPPH˙ assay for free radicals; b) Nitric oxide scavenging activity of chemically and biosynthetically produced TiO_2-NPs.

Table 2. DPPH radical scavenging activity of chemically and biosynthetically produced TiO_2 NPs.

Concentration (µg/mL)	M. oppositifolia Extract	*T. portulacastrum* Extract	Chem. TiO_2-NPs.	Bio TiO_2-NPs. (M.P)	Bio TiO_2-NPs. (T.P)	Standard
500	50 ± 0.2	54±0.4	69±0.7	87±0.4	91±0.2	96±0.5
250	27 ±0.4	28±0.8	51±0.1	69±0.1	75±0.7	81±0.2
125	18 ±0.8	18±0.1	31±0.5	49±0.1	51±0.3	63±0.6
100	11 ±0.1	12±0.2	17±0.4	31±0.3	32±0.4	43±0.9
50	5±0.4	5±0.7	12±0.5	22±0.9	22±0.9	31±0.7
25	3±0.5	4±0.9	6±0.7	12±0.5	15±0.2	21±0.5
IC_{50}	173.06	170.54	154.30	127.83	125.04	116.42

Equation/Formula

Scavenging effect (%) or Percentage of Inhibition = $[A_0A_1 /A_0] X 100$. --- (1)

A_0 & A_1, are the absorbance of control and test solution or standard sample solution respectively.

6. Conclusion and Future Scope

Titanium dioxide nanoparticles were efficaciously synthesized by chemical and biogenic means employing extracts of *T. portulacastrum and M. oppositifolia*. Biosynthesized TiO_2-NPs confirmed higher antibacterial interest in opposition to Gram-terrible microorganism than Gram-high-quality microorganism and also better antioxidant activity against scavenging free radicals.

A metal oxide nanoparticle synthesized by green synthesis has numerous exclusive advantages over the chemically synthesized. The mode of green synthesis is easy to execute, quick, economical and environmentally friendly. Biosynthesized metal oxide nanoparticles have shown significant antibacterial activity as compared to chemical synthesis. Biosynthesized Zinc oxide nanoparticles have greater sensitivity against microorganisms like Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis. In general, biosynthesized metal oxide nanoparticles have moderate antioxidant property as compared to standard ascorbic acid and chemically synthesized metal oxide nanoparticles have shown a negligible antioxidant property. Finally transition metal oxide nanoparticles produced by green method showed good possession of antibacterial and antioxidant activity as compared to the rare earth metal oxide nanoparticles.

Conflict of Interest

We declare that we do not have any conflict of interest.

Funding Source

None

Authors' Contributions

Author-1 reviewed the literature and compiled the study. Author-2 involved in collecting samples and carried out experiments.

Acknowledgements

Authors acknowledge the support of their respective managements for their support.

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