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RESEARCH ARTICLE

Anti-Bacterial Effect of Titanium-Oxide Nanoparticles and their Application as Alternative to Antibiotics

Afsheen Mansoor[1,2], Mazhar Mehmood[3], Syed Mujtaba Ul Hassan[3], Muhammad Ishtiaq Ali[1], Malik Badshah[1] and Asif Jamal[1]*

[1]Department of Microbiology, Quaid-i-Azam University, Islamabad, 45320, Pakistan; [2]Department of Dental Material Sciences, School of Dentistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad,44080 Pakistan.
[3]Department of Metallurgy and Materials Engineering, Pakistan Institute of Engineering and Applied Sciences, Islamabad, Pakistan.
*Corresponding author: asifjamal@qau.edu.pk

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ABSTRACT

Bacterial resistance towards antibiotics has been increasing globally posing a serious challenge for agriculture, animal-human health and public health policy makers. The development of plant derived innovative materials for agriculture, medical and dental applications is growing rapidly. The metal oxide nanoparticles are considered as the viable option for the treatment of microbial infections in both humans and animals. In present work, *Mentha spicata* leaves were used to synthesize the titanium-oxide Np's and their characterization was carried out by atomic force microscope (AFM), fourier transform infrared spectroscope (FTIR), scanning electron microscope (SEM), energy dispersive x-ray spectroscopy (EDX), x-ray diffraction pattern spectroscopy (XRD), dynamic light scattering (DLS), and uv/vis diffuse reflectance spectroscopy (DRS). The antimicrobial sensitivity was done by disc diffusion test against pathogenic bacteria of animal origin such as *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis,* and *Pseudomonas aeruginosa*. Our results indicated that titanium-oxide Np's were round, smooth with particle size of 39nm. These Np's were having anatase phase which were pure in their composition and functional compounds. The antimicrobial sensitivity testing revealed zones of 24, 23, 17 and 24mm against *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. On the basis of results, it can be concluded that titanium-oxide Np's prepared through *Mentha spicata* were safe, sustainable and having potent antimicrobial activity against bacterial strains which can be used as an alternative antimicrobial agent for medical and dental applications.

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INTRODUCTION

The raising antibiotic resistance and depletion of existing spectrum of antimicrobial drugs have been posing great challenge for public health and safety. The multidrug resistant (MDR) bacteria have zoonotic potential and circulate among animal-human-environment triad. Animal food chain and non-judicious use of antibiotics in animal production and in humans led to emergence of antimicrobial resistance (AMR). Microorganisms are continued to adopt innovative mechanisms to resist the antimicrobial drugs and thus putting great burden on the existing health care system and agriculture. Considering ongoing quest of antibiotic resistance, various new drugs are being developed globally. Among various contemporary techniques, use of nanomaterial for the treatment of bacterial infections is growing tremendously. These materials can be developed by using various agricultural resources and plant extracts (Mansoor *et al.*, 2022). The Np's display excellent properties and physiochemical characteristics as compared to the synthetic medicines (Khalil *et al.*, 2017). There are various factors responsible for the versatility of Np's which include temperature, pH, pressure, reducing, capping and stabilizing agents. The synthesis protocols are considered as the most important factor affecting the biological and physico-chemical properties of the nanoparticles. There are two classes of Np's depending on their routes of synthesis such as conventional and sustainable Np's. The conventional class of Np's incorporates the physical and chemical methods, which includes spray pyrolysis, laser ablation, sol gel, electrophoretic deposition, oxidation, hydrolysis, microwave assisted, sonochemical, template based, solvothermal, hydrothermal, and electrochemical anodization etc. These synthesis methods are categorized in the conventional regime because of using toxic chemicals, combustible pressure, high temperature, strong reducing and stabilizing agents, resulting in the toxic byproduct formation. Furthermore, these Np's are drastically expensive and more energy dependent (Singh *et al*., 2019). Thus, conventional methods are considered hazardous for the human-animal health and environmental safety. Therefore, the alternative class of sustainable nanotechnology is the need of this modern era (Dobrucka and Długaszewska, 2016). The sustainable class of Np's recruits the biogenic and green methods such as microorganisms, plants and their derivatives. These regimes utilize the natural biomolecules which serve the purpose of reducing, oxidizing and stabilizing the Np's without any toxic byproduct formation. These Np's are cost-effective and show more safe, stable and sustainable behavior (Singh *et al*., 2019). Nanoparticles have gained success worldwide in various other significant fields such as cosmetics, sensors, agriculture, waste management, water plants, electronics, solar cells, electric batteries, energy production and drug delivery systems (Mansoor *et al*., 2022). The utilization of Np's in aforementioned fields have enhanced the quest of researcher to fabricate Np's through novel green routes in order to utilize them in medical and dental applications more tremendously without the fear of non-biocompatibility (Kirthi *et al*., 2011). The current literature has reported that metal-oxide Np's synthesized through green routes are useful, safe and eco-friendly in their nature (Edmundson *et al*., 2014), which might impose beneficial effects in different aspects.

Recently, antimicrobial resistance (AMR) to bacteria, virus, parasite and fungi has become the major cause of death among humans and animals. Moreover, AMR has become a threat for food security, heath care systems and livelihood. The estimated deaths globally due to AMR have been found to be 4.50 million where leading resistance was observed against the pathogens such as *P. aeruginosa, S. pneumoniae, S. aureus, E. coli, K. pneumoniae*, and *A. baumanniii* (Murray *et al.*, 2022). The World health organization has declared these six bacteria as the most lethal AMR pathogens (WHO, 2021). Since then, various additional bacterial, parasitic and fungal infections in humans and animals became more complicated due to the enhanced resistance developed against them with every passing day that, needs to be encountered properly (Dobrucka *et al*., 2017). Certain methods and techniques have been employed to eliminate the contagious bacterial, parasitic and fungal infections in humans and animals, but it was difficult to eradicate them because of their extra strength (Chatterjee *et al*., 2017). The metal-oxide Np's have gained success in displaying the potent antimicrobial sensitivity against variety of microorganisms (Kaur *et al*., 2019). The titanium-oxide Np's have emerged as a new class of antimicrobial agent whose inhibitory activity against microorganisms needs further investigation in order to incorporate them in agriculture, medical and dental applications on the larger scale. The *mentha spicata plant* has potent advantages because of which, it has been used safely in flavors, foods, industry and pharmacy (Salehi *et al*., 2018). The *mentha spicata plant* is mainly composed of various chemotypes such as carvacrol, piperitone -oxide, carvone, p-cymene, pulegone-1, 1-8cine´ole, camphene, transecarveol, linalool, menthone-s, germacrenes D, limonenee, p_cymene, *B*-car yophyllene, dihydrocarvone, and α-pinene. The carvone is the main chemotype present in *mentha spicata* but its quantity varies when grown in agricultural land of various countries (Zekri *et al*., 2019). Pakistan is well known for the growth of *mentha spicata plant* containing about 50% of carvone chemotype along with other countries including Iran, Hungary, Bangladesh and Serbia, (Farahbakhsh *et al*., 2020). The carvone in *mentha spicata plant* might be capable of generating potent beneficial effect in agriculture, and health care fields. Previously a study revealed antimicrobial activity of *mentha spicata plant leaves extract* but to a limited value against various microorganisms (Arumugam *et al*., 2010). The titanium-oxide Np's prepared from *mentha spicata plant* were less studied in health care systems. The current study involves the synthesis of titanium-oxide Np's via *Mentha spicata* medicinal plant. These nanoparticles were characterized for their shape, size, surface, texture, phase form, elemental composition and functional compounds in order to investigate the effects of their physico-chemical properties on antimicrobial sensitivity against resistant bacterial pathogens.

MATERIALS AND METHODS

Synthesis of titanium-oxide Np's by *Mentha spicata*: Fresh *Mentha spicata* leaves were collected from local garden, dried for seven days at room temperature and then, crushed in powder form. 1mg of plant powder was dissolved in 100ml of distilled water to form plant extract solution, which was boiled at 80°C for 5 minutes and allowed to cool down. 1 ml of Ti $(OH)_2$ (CAS number 20338-08-03 P.O. Box 3012, Columbus, Ohi-43210, USA) was added into 80ml of distilled water to get Ti $(OH)_2$ salt stock solution and 20ml of pure plant extract solution was added to it. This solution was kept in a shaking incubator (Memart, Germany) for 24 hours (28ºC temperature and 150rpm). Color changes from green to milky white confirmed formation of nanoparticles. These nanoparticles were dried in furnace (Thermoo electron LED-Gmbbh, Heraeuss Vacuthermm, D-83505) and calcinated at 500°C to get powder (Swathi *et al*., 2019).

Characterization of titanium-oxide Np's formed from *Mentha spicata*: The characterization of these Np's was done through atomic force microscope (Quesaants Universaal SSPMM, Ambioms Technologies, Santa Crus, CA-, USA), fourier transmission Infrared spectroscope (JAASCO-2 FTIRr-6600/s, Ultrecs Amsterdamn, AMSS-, Netherlands), scanning electron microscope and energy dispersive x-ray spectroscope (Novaananosems 430.00, FEAI company- 40220-261, 493912 S/A columns FGG stronmprepa, Hilsboro, OR-, USA),x-ray diffraction pattern spectroscope (DPP' MARX,24.00 diffractometer, Riggaku-Corporations, Akishimias, Tokyo-, Japan), dynamic Light scattering instrument (ZETA sizersNanoosZnSSApparaatus; ZENNS 36000.0, Malveernanalyticals, Malverns-, UK), and UV/vis diffuse reflectance spectroscope (PerkinnElmerr, UV/VIS/NIRR Spectrometer-A Lambdaa 950.0 Walthamm, MA-, USA) (Swathi *et al*., 2019). Powder form of titanium-oxide Np's was used for the analysis by x-ray diffraction pattern spectroscope, fourier transmission Infrared spectroscope, dynamic Light scattering instrument and UV/vis diffuse reflectance spectroscope. Suspensions of titanium-oxide Np's were prepared by dissolving 1mg of Titania (TiO_{2-} Nps) in 1ml of ethanol. One drop from each suspension of titanium-oxide Np's was placed on glass slide and dried by evaporation. These were then sputter coated for the investigations by scanning electron microscope and energy dispersive x-ray spectroscope. For atomic force microscope readings drop of titanium-oxide Np's suspension was placed on glass slab, covered with another glass slab and placed under cantilever tip.

Antimicrobial sensitivity of titanium-oxide Np's: The titanium-oxide Np's were tested against various pathogens for the investigation of antimicrobial sensitivity by disc diffusion. The bacterial strains taken from NIH were: *S. aureus* (ATCC®25923^{TM,}), E. coli (ATCC®35218^{TM,}), *P. aeruginosa* (ATCC®9027^{TM,}), and *E. faecalis* (ATCC®29212^{TM,}). Agar powder of 03.80g (Muller Hinton) was induced into 100.00 mL of distil water, then placed at 121°C for about 15.0 minutes in an autoclave (15 lbs) in order to get Muller-Hinton's agar sensitivity medium. The bacterial strains were incubated at 37°C for getting the bacterial suspensions, which were kept in broth of peptones for about three to four hours. The bacterial suspension was streaked on sterile petri-dish with the help of sterile cotton swab. The titanium-oxide Np's suspension (250µg/L) was used for the testing of the antimicrobial sensitivity. The (3mm) Whatman paper was saturated with suspension of Np's and was kept on petri-dishes streaked with standard bacterial strains. These petri-dishes were kept in an incubator at 37°C for twenty-four hours for the evaluation of the zone of inhibition (Swathi *et al*., 2019). All the readings were taken in triplicates and data was statistically analyzed**.**

RESULTS

Synthesis of titanium-oxide Np's from *Mentha spicata*: The Mentha spicata substrate solution was green in color which changed to white color after formation of titanium-oxide Np's.

Characterization of titanium-oxide Np's formed by *Mentha spicata:*

AFM analysis: AFM image was taken to estimate the surface morphology of these Np's. The surface of titanium-oxide Np's synthesized by *Mentha spicata plant* revealed their rounded shape with few irregularities (Fig. 1).

FTIR analysis: FTIR readings were observed to find the type of functional compounds present in these Np's. The characteristic peaks fabricated by FTIR of titanium-oxide Np's synthesized by *Mentha spicata plant* were 3667.21 cm-^{1}, 3654.09 cm-^{1}, 3361.36 cm-^{1}, 2729.43 cm-^{1}, 2663.75 cm-^{1}, 1996.56 cm-^{1}, 1703.69 cm-^{1}, 1656.11 cm-^{1}, 1546.93 cm-^{1}, 503.23 cm-^{1} and 464.17 cm^{-1}. Peaks generated at 3667.2, 3654.09 cm-^{1} and 3361.36 cm-^{1} were due to stretching vibrations of O-H groups of alcohol. Therefore, peaks produced at 2663.75 cm-^{1} and 1996.56 cm-^{1} revealed stretching vibrations of C-H groups of alkynes and aldehydes. The peaks observed at 1703.56 cm-^{1}, 1656.11 cm-^{1} and 1546.93 cm-1 corresponded to bending vibrations of O-H groups of hydroxyl compounds. Peaks obtained at 503.23 cm-^{1} and 464.17 cm-^{1} attributed to stretching and bending vibrations of Ti-O-Ti groups (Fig. 2).

SEM, DLS and XRD analysis: The SEM, DLS and XRD were performed for investigating the size, shape and phase of these Np's. The particle size revealed by SEM image of titanium-oxide Np's was found to be 39 nm having rounded shape with very few irregular particles. The round shape of these Np's was more eminent comparatively (Fig. 3a, b). The hydrodynamic particle size of titanium-oxide Np's calculated by DLS was found

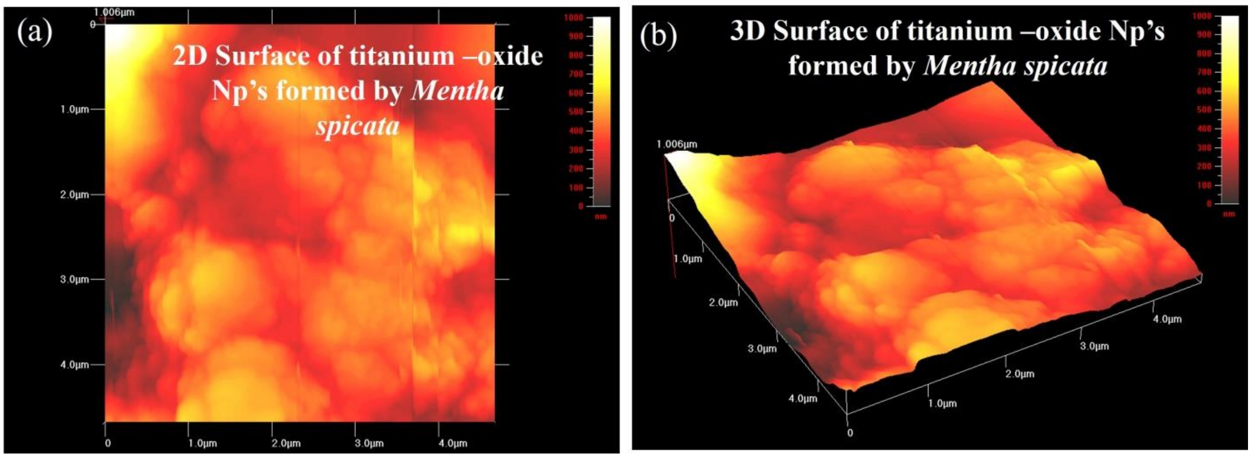

Fig. 1: 2D Vertical and 3D horizontal image is displaying the rounded shape and smooth surface of titanium-oxide Np's prepared by *Mentha spicata* in the AFM scan at (a) low and (b) high resolution powers.

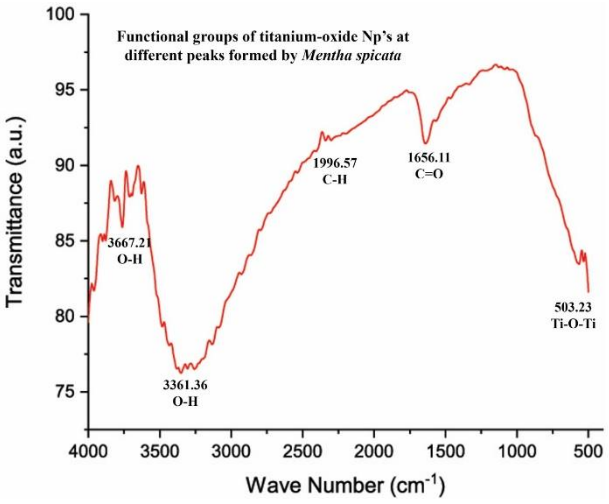

Fig. 2: Functional groups of O-H, C-H, C=O and Ti-O-Ti are displayed at different peaks between 4000-500cm^{-1} wavelengths in the FTIR spectrum of titanium-oxide Np's prepared by *Mentha spciata*.

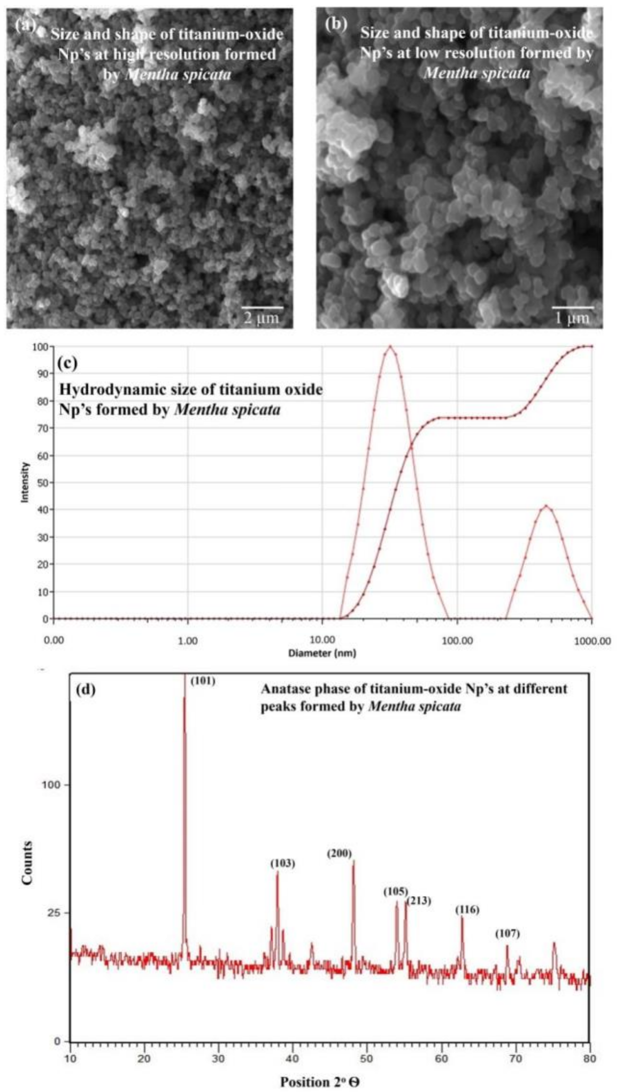

Fig. 3: Size, Shape and Phase of titanium-oxide Np's prepared by *Mentha spicata* confirmed: Particle size of about 39nm and spherical shape of these Np's in SEM at the magnifications of (a) 2000x (b) 1000x (c) Hydrodynamic size of about 49nm through peaks in the DLS scan and (d) Anatase phase formation at peaks of 2θ (101), (103), (200), (105), (213), (116), (107) was displayed in the XRD spectrum.

to be 49nm (Fig. 3c). XRD pattern of titanium-oxide Np's matched with JCPDS Card no: 00-001-0562 that showed existence of main peak (101), of anatase phase at 2Θ = 25.39°. Other peaks observed were (103) 37.89°, (200) 48.14°, (105) 53.97°, (213) 62.77°°, (116) 68.89°, and (107) 75.37°. The Np's were having pure anatase phase with crystalline size of 37.60 nm as calculated by Debyee - Scherrer's formula (Fig. 3d).

EDX analysis: The composition of these Np's was checked in the EDX spectra for purity. Intense peaks of titanium and oxygen were observed in EDX spectrum where Titanium was available in 78.30 weight % and 54.65 atomic % while oxygen was in 21.70 weight % and 45.35 atomic % (Fig. 4).

UV-VIS DRS analysis: The band gap energy and crystalline-size of these Np's was investigated by UV-VIS DRS pattern. The UV-Vis DRS spectrum confirmed the formation of titanium-oxide Np's (Fig. 5a). The band gap energy of these Np's was 3.2eV attributing to the crystalline size (Fig. 5b).

Antimicrobial activity of titanium-oxide Np's against drug resistant bacterial pathogens: The Whatman paper dipped with water (control group) and titanium-oxide Np's suspension (experimental group) was tested against bacterial strains of *S. aureus* (ATCC®25923TM,), *E. coli* (ATCC®35218TM,), *P. aeruginosa* (ATCC®9027TM,), and *E. faecalis* (ATCC®29212TM,). The zone of inhibition against these bacterial strains was calculated. The water dipped Whatman paper (control group) displayed 0 mm zone of inhibition (no zone) against all the bacterial strains used in the current study (*S. aureus*, *E. coli*, *P. aeruginosa*, and *E. faecalis*) in comparison to the experimental group. The titanium-oxide Np's suspension dipped Whatman paper (experimental group) revealed calculated zone of inhibition of about 17 mm against *S. aureus*, 24 mm against E. *coli*, 23 mm against *P. aeruginosa*, and 24 mm against *E. faecalis* (Fig. 6, Table 1).

DISCUSSION

The menace of antimicrobial resistance is attributed to large number of deaths globally and regarded as a silent pandemic. The emergence of AMR in low-to-middle income countries is associated with over-the-counter sale of antibiotics, use of antibiotics in animal production and animal food-chain. *Mentha spicata* is an ancient herbal plant cultivated in all the regions of the world due to its additional advantages. It is well known for its anti-microbial, anti-fungal, anti-parasitic, anti-diabetic, anti-oxidant, diuretic, anti-inflammatory, analgesic, anti-pyretic, anti-hemolytic, anti-inflammatory effects in medical fields whereas insecticidal advantages in agriculture (Menyiy *et al*., 2022). Previously, it has been enormously used as an aromatic and medicinal plant to treat various diseases in both the eastern and western world (Kee *et al.*, 2017). Multiple investigations confirmed the efficacy of *Mentha spicata plant* in throat ailments (Orch *et al*., 2020), diabetes, headache, tiredness,

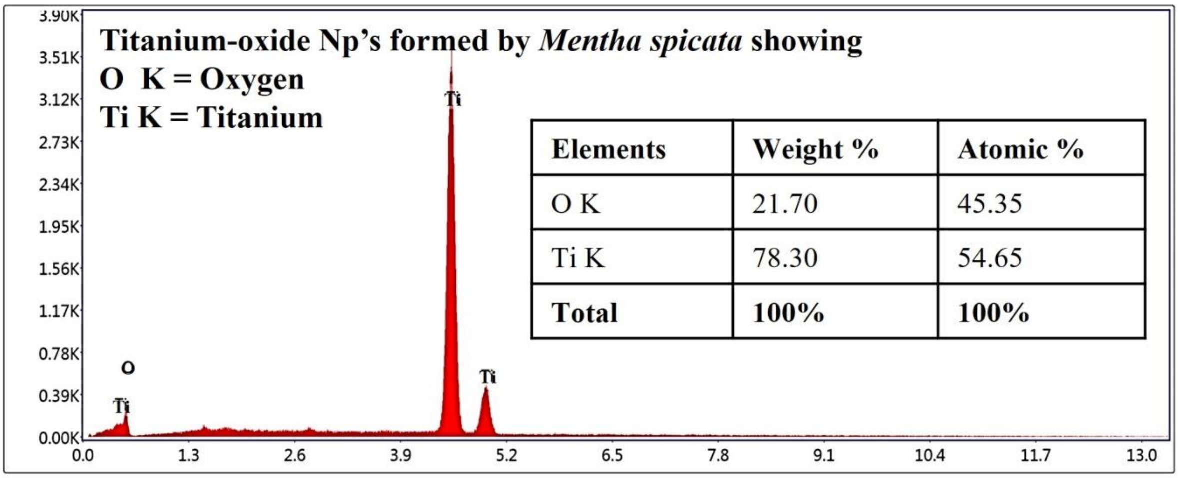

Fig. 4: Peaks of elemental composition of titanium-oxide Np's prepared by *Mentha spicata* containing pure weight% and atomic% of titanium and oxygen are displayed in the EDX scan.

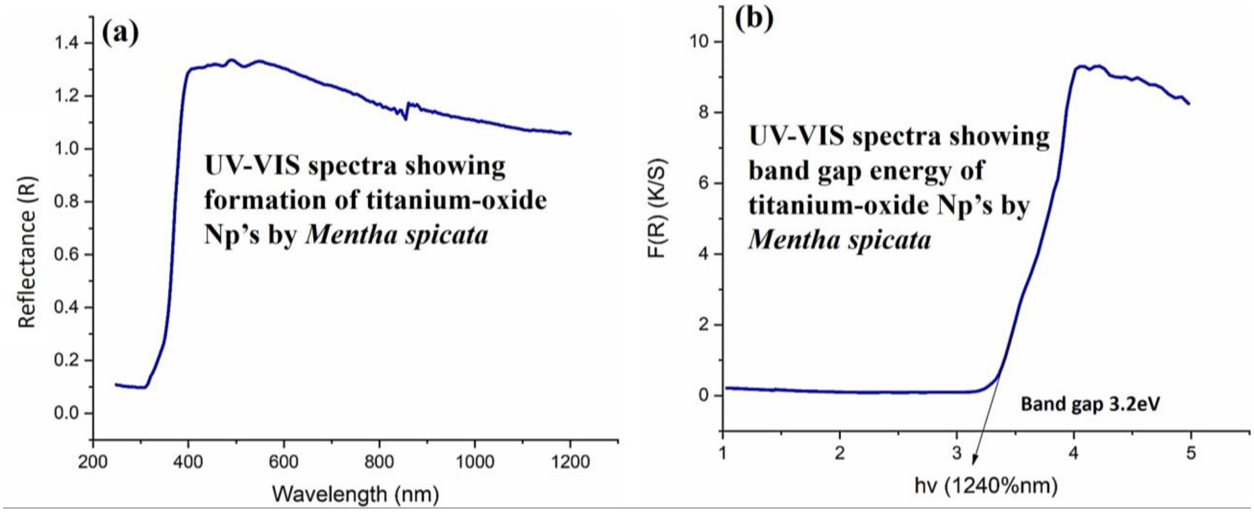

Fig. 5: Origin and formation of titanium oxide nanoparticles prepared by *Mentha spicata* between the wavelength of 200-400cm^{-1} (a) and 3.2 eV energy band gap of titanium-oxide Np's prepared by *Mentha spicata* confirming the medium crystalline size is displayed by the DRS pattern.

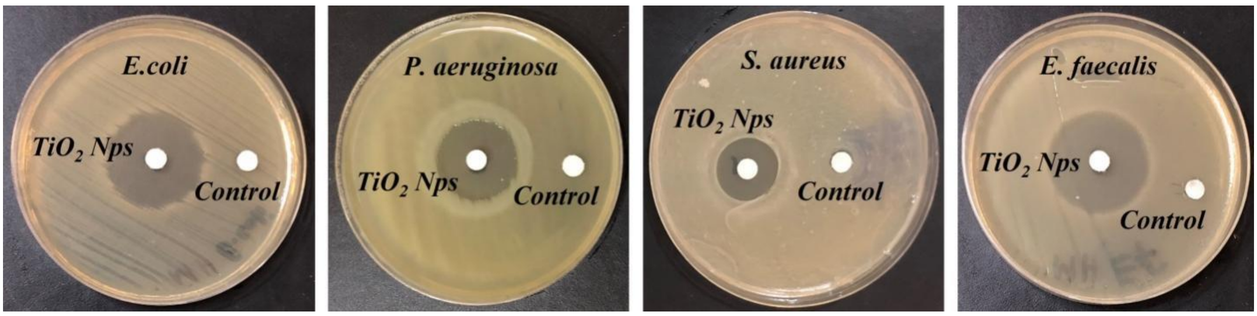

Fig. 6: Antimicrobial sensitivity displayed by control group (water) and titanium-oxide Np's prepared by *Mentha spicata* against disease producing pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus faecalis*.

Table 1: Titanium-oxide Np's prepared by Mentha spicata confirming the Zone of Inhibition against bacterial disease producing pathogens.

Ser	Bacterial Strain	Zone of Inhibition (mm)
1.	*Escherichia coli*	24 mm
2.	*Pseudomonas aeruginosa*	23 mm
3.	*Staphylococcus aureus*	17 mm
4.	*Enterococcus faecalis*	24 mm

asthma, chest pain, lung problems, bronchitis, kidney disorders, gastric orders, and skin diseases (Salhi *et al*., 2019). Additionally, this plant has been also useful in cold, aphrodisiac, headache, and flatulence. More importantly, it relieves the toothache and is used as tonic for the human beings (Labiad *et al*., 2020). The titanium-oxide Np's in this study were fabricated by a green route utilizing the *Mentha spicata* leaves which were then characterized via standard protocol. The change in color of *Mentha spicata plant* extract from green to white after 24 hrs of incubation occurred due to the bio-reduction of Ti^{+} and OH^{-2} ions to TiO_2 Np's (Patra and Baek, 2014). The phytochemicals are natural reducing agents present in plants responsible for reducing the titanyl hydroxide salts to titanium-oxide Np's. The phytochemicals present in *Mentha spicata plant* are alkaloids, flavonoids, aldehydes, and terpenoids that might have played a sole role in reducing, capping, and stabilizing the newly formed titanium oxide Np's without any other chemical being used.The AFM image demonstrated the round shape and smooth surface of these Np's. The prominent peak at 503.23 cm^{-1} in the FTIR spectrum is a characteristic peak of the Ti-O-Ti bending which is the confirmation of the titanium-oxide Np's between 600-400cm^{-1}. The absence of any peak at 2900 cm^{-1} (C-H stretching) justified the pure spectrum of these novel Np's as this particular peak is accountable for the presence of organic compounds, responsible for the entrapment of the impurities (Vetrivel *et al*., 2015). The phytochemicals in the *Mentha spicata plant* might have encouraged the appropriate binding, bridging and nucleation of these Np's in an orderly manner that might have resulted in their purity and other physico-chemical characteristics.

The SEM image showed the predominantly round shape of the titanium-oxide Np's with very few irregularly shaped particles in between having particle size of about 39nm while DLS scan calculated the hydrodynamic size of these newly formed Np's which was found to be 49nm. The XRD scan revealed pure anatase phase of novel titanium-oxide Np's having crystalline size of 37.6 nm. The EDX scan showed peaks of titanium and oxygen purely, with no other impurity in its composition. A study conducted by researchers reported the pure anatase phase of titanium-oxide Np's having nano-size of the particle, by utilizing other biological source (Verma *et al*., 2017). The DRS spectrum revealed the formation of titanium-oxide Np's at 329nm absorbance which was confirmed in literature between 200-600nm (Gautam *et al*., 2016). The band gap energy of about 3.2ev, was calculated by these novel Np's which is just equal to the standard value (3.2ev). This means that particle size attained by these Np's is neither too big nor too small because particle size and band gap energy are inversely proportional to each other as reported by Reddy *et al.* (2003). Thus, the particle size of titanium-oxide Np's produced in the current study might be helpful in enhancing the antimicrobial sensitivity of these Np's against the AMR pathogenic bacteria.

The titanium-oxide Np's synthesized by *Mentha spicata plant* in the current study displayed medium size, round shape, anatase phase, smooth surface having pure elemental composition and functional compounds which made them suitable enough to be utilized for preventive, diagnostic and therapeutic purposes in the fields of medicine and clinical dentistry. The medicinal and therapeutic effectiveness of these parameters (size, shape, phase form and surface) in relation to Np's was also reported (Chellappa *et al*., 2015). The green synthesis of Np's utilizing the extract of *Mentha spicata* which is a medicinal plant confirming their safe and compatible nature. The formation of these Np's at low temperature and pressure has resulted in their specifically beneficial structural and morphological entities (Wang *et al*., 2003). The usage of lower pressure and temperature during the preparation of titanium-oxide Np's might have activated the medicinal ingredients present in the phytochemicals of *Mentha spicata plant* that might have played a dominant role in reduction, and stabilization of these Np's thus, preventing them from getting toxic.

The Np's are utilized as antimicrobial agents against various AMR resistant microorganisms but the role of their physicochemical characteristics in establishing the potent antimicrobial sensitivity is still under discussion (Mansoor *et al*., 2022). The current study displayed strong inhibitory zones of these novel *Mentha spicata* plant-based titanium-oxide Np's against *P. aeruginosa* (24mm), *E. coli* (24mm), *S. aureus* (17mm), and *E. fecalis* (15mm). These pathogens are contagious and considered mainly responsible for inducing various diseases in both animals, and human beings as reported in a previous work performed (Yadav *et al*., 2017). Known AMR bacterial pathogens such as *P. aeruginosa, E. coli, S. aureus, S. pneumonae, A. baumanniii, K.pneumonae, S. Typhi, E. faecium, M. tuberculoses, S. agalactiae* and *E. fecalis* have been accountable for millions of deaths globally (Murray *et al.,* 2022). This increase in the deaths of the worldwide population has emerged as a threat in both the human beings and animals of this modern era.

The findings in the current study matched the work done previously by Velhal *et al.* (2014) that utilized another facile protocol for the synthesis of titanium-oxide Np's. The size, shape and anatase phase of these Np's might have easily enveloped the pathogenic bacteria and their organelles thus, enhancing their elimination. This might have become possible due to their size and spherical shape that could have attacked the pathogenic bacteria quite easily and more quickly because of their reactive anatase phase alone (Velhal *et al*., 2014). The antimicrobial sensitivity of Np's is greatly attributed to its physicochemical characteristics such as shape, size, surface, synthesis, composition, and functional compounds as reported by Pradhan *et. al.* (2015). The commendable size, predominant spherical shape and pure anatase phase of Np's in current study might have caused the disruption of cell walls of the bacteria resulting in their deaths via reactive oxygen species (ROS). These ROS's releases large amount of hydroxyl ions through the phospho-lipid perioxidation reaction responsible for the breakdown of the pathogenic bacterial cell walls and their organalles. This in turn activates the process of cellular disruptions that might lead to their ultimate deaths (Shah *et al*., 2008). The current study suggested that the facile green synthesis protocol and physico-chemical properties of novel titanium-oxide Np's displayed a well-established role in the potent antimicrobial sensitivity against pathogenic bacteria. Future studies are required to develop more Np's using the hidden biological routes and their association with physico-chemical properties in order to enhance their antimicrobial sensitivity for their employment in medical and dental applications. Further studies are required to investigate the insecticidal effects of these titanium-based Np's in food and agriculture industry for better crop production as well.

Conclusions: The current study concluded that novel titanium-oxide nanoparticles synthesized by medicinal *Mentha spicata plant* were safe, sustainable and compatible in nature because they were formed via green facile route. The 39nm particle size, anatase phase, round shape, smooth surface, pure elemental composition and functional compounds displayed by these novel Np's revealed potent antimicrobial sensitivity against pathogenic bacteria producing infections such as *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus and Pseudomonas aeruginosa.* This showed that novel titanium-oxide Np's formed by *Mentha spicata* are beneficial enough to be utilized in medical and dental applications.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Authors contribution: Conceptualization: Afsheen Mansoor, Asif Jamal; Methodology: Afsheen Mansoor, Asif Jamal, Mazhar Mehmood; Data Collection: Afsheen Mansoor, Syed Mujtaba Ul Hassan, Malik Badshah; Data Processing: Afsheen Mansoor, Syed Mujtaba Ul Hassan, Muhammad Ishtiaq, Malik Badshah; Data Analysis & Interpretation: Afsheen Mansoor, Mazhar Mehmood; Writing-Original Draft, Afsheen Mansoor, Asif Jamal; Writing-Review & Editing: Afsheen Mansoor, Asif Jamal, Mazhar Mehmood, Muhammad Ishtiaq; Supervision, Asif Jamal; Critical Review: Asif Jamal, Mazhar Mehmood, Syed Mujtaba Ul Hassan., Muhammad Ishtiaq.

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