

**Phytosynthesis of Titanium oxides from *Andrographis paniculata* and its antibacterial efficacy against isolate *S.mutans***

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**Abstract:** *Andrographis paniculata* Wall (*Acanthaceae* family) is a widely used medicinal herb with antimicrobial, cytotoxic, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, and anti-infective effects. Researchers are now mainly focused on synthesizing nanoparticles using eco-friendly methods to enhance their biological efficacy. This species has pharmacological properties such as antibacterial, antioxidant, anti-inflammatory, antiparasitic, antihyperglycemic, hypoglycemic, and antiallergic properties. *Andrographis paniculata* colonies were inoculated into Luria Bertani's (LB) Broth and incubated for 48 hours at 37 degree Celsius. The solution was centrifuged at 4500 RPM for 15 minutes at 25. The pellets and supernatant were collected afterwards. The synthesis of nanoparticles of TiO<sub>2</sub> showed antimicrobial activity against the oral pathogen *Streptococcus mutans* based on the findings the further study needed to explore the *Andrographis paniculata* Mediators synthesis TiO<sub>2</sub> nanoparticles as a future antibacterial oral mouth wash. In the another study The antibacterial activity of the synthesized TiO<sub>2</sub> nanoparticles was performed by the agar-well diffusion method. Fresh colonies of *Bacillus* sp., *E. coli*, *Salmonella* sp.

**Keywords:** Psychological harm, Psychological wellbeing, Public Health, Medicine.

**1.Introduction:** Nanotechnology has several applications in biology, chemistry, physics, and medicine. There are two types of nanomaterials: organic and inorganic. (1) The reduction/oxidation process, which is a bottom-up method, is the main reaction in the production of nanoparticles. (2) Metal and metal oxide nanoparticles are typically generated by microbial enzymes and plant phytochemicals with antioxidant or reducing capabilities. (3) The biological approach is used to create nanoparticles by combining bacteria, fungi, and plant extracts like neem, Coriandrum, Camellia sinensis, Nelumbo lucifera, Ocimum sanctum, and several more. (4) Titanium dioxide nanoparticles, among other nanoparticles, (5) have a wide range of applications since they boost agricultural plant development, yield, and are also utilised in food and cosmetics. (6) According to reports, TiO<sub>2</sub> NPs have a number of positive effects on the biochemical and morphophysiological characteristics of different crop plants. Recent decades have seen the establishment of specific protocols that aid in the control of bacterial flora, including suggestions for bettering oral hygiene (7), lowering daily carbohydrate intake, oral physiotherapy programmes that aid in improving mechanical removal of bacterial plaque (either individually or with assistance), (8) and also the use of substances based on natural products that aid in the control of bacterial plaque in order to reduce the amount of bacteria in the mouth. Since *S.mutans* was identified as one of the primary causative agents for dental caries, it has drawn attention as a potential target for the development of vaccines and antimicrobial treatments to prevent dental caries. (8,9) Patients would suffer if antibiotics were used to prevent and cure dental caries, and the emergence of bacterial strains that are resistant to several drugs was a possibility. (10) The research indicates that crop plants are affected by titanium dioxide nanoparticles in both beneficial and detrimental ways. (11) Through the creation of ROS, which lowers the amount of chlorophyll, high dosages of TiO<sub>2</sub> nanoparticles have toxicological effects on plants. (12) Low doses improve crop growth characteristics and output.

These applications may include efficient use of micronutrients, delivery of pesticides, nanosensors to monitor pesticide degradation, and more. (13) To our knowledge, several inorganic and organometallic oxide nanomaterials and some hybrid nanomaterials, such as TiO<sub>2</sub>, ZnO, CuO graphene oxide and Fe<sub>3</sub>O<sub>4</sub>-Ag core-shell magnetic nanoparticles, have demonstrated their excellent physics (14). Selected for chemical properties, high surface area. Their volume ratio and characteristic nano-sized structural features are increasingly used as alternative antimicrobial agents in biomedical applications.

Researchers are now mainly focused on synthesizing nanoparticles using eco-friendly methods to enhance their biological efficacy. (15) The plant species *Andrographis paniculata*, commonly known in India as the king of bitters and nilavembu, belongs to the family Apocynaceae. (15,16) This species has pharmacological properties such as antibacterial, antioxidant, anti-inflammatory, antiparasitic, antihyperglycemic, hypoglycemic, and antiallergic properties.

The crystal structure and average crystal size of the synthesized TiO<sub>2</sub> NPs were characterized by X-ray diffractometer using a Scifert diffractometer. (17) Synthesis of TiO<sub>2</sub> nanoparticles reduces titanium metal ions in solution of *Andrographis paniculata* leaf extract for characterization by Perkin-Elmer Lambda, a UV-Vis spectrophotometer. (18) The surface morphology of titanium nanoparticles was characterized by SEM and AFM. EDX analysis was performed to confirm the presence of titanium dioxide in the particles and to confirm other elemental compositions of the particles. (19) TGA analysis was performed to know how the biosynthesized titanium dioxide nanoparticles changed their physical and chemical properties at room temperature of 800 °C per minute at 20 °C.

**Objective:**

- 1 synthesis of TiO<sub>2</sub> nano particles from *Andrographis paniculata*
- 2 Characterisation TiO<sub>2</sub>-NPs with UV-Vis, SEM, EDAX, and FTIR
- 3 To determine Antibacterials Efficacy against *S. mutans*

**2.Material and methods:**

**2.1.Isolation of *Andrographis paniculata* species:**

*Andrographis paniculata* colonies were inoculated into Luria Bertani's (LB) Broth and incubated for 48 hours at 37 degree Celsius. The solution was centrifuged at 4500 RPM for 15 minutes at 25. The pellets and supernatant were collected afterwards.

**2.2.UV TEST:**

**2.2.1.Sample collection :**

*Andrographis paniculata* leaves were collected around Poonamallee, Chennai, Tamil Nadu, parts were separated and washed with distilled water.

**2.2.2.Preparation of Aqueous left extract:**

They are dried for 7 days and grinded to fine powder. Then, 1 g was added to 100 ml of distilled water and boiled at 60–70°C for about 10 min.

**2.2.3.Synthesis of nanoparticles:**

The crude extracts were filtered through Whatman No. 1 filter paper and the aqueous extract used for synthesis TiO<sub>2</sub> nanoparticles.

**2.2.4.Synthesis of TiO<sub>2</sub> nanoparticles :**

The synthesized nanoparticles further characterized using UV vis photometer, SEM, EDAX and FTIR. After characterisation synthesized nano particles carried out antibacterial efficacy

**2.3.Anti-bacterial test (Well diffusion method):**

**2.3.1.Preparation of sample:**

**2.3.1.1.Agar Preparation:** 20 ml of distilled water with 0.76g of Muller Hinton Agar.

**2.3.1.2.Sample preparation:** 100mg in 1 ml of sterile distilled agar.

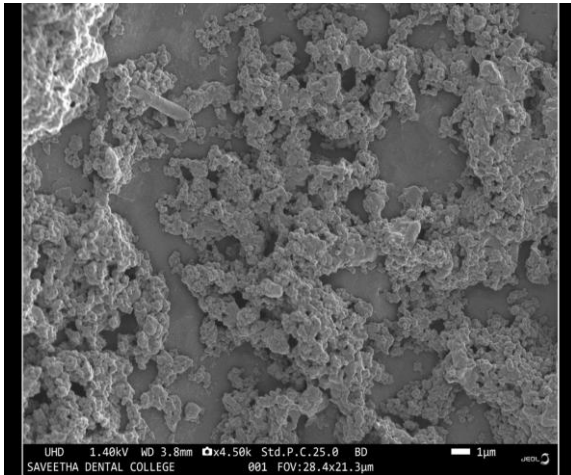
**2.3.1.3.Culture preparation:** 4 ml of saline is added to 1 ml of *S. mutans* culture.

**2.4.CHARACTERIZATION:**

The Mueller Hinton Agar powder is poured into sterile petri plates. After half an hour, a micro tip (gel puncture) was created. Then swab the culture in the plate and after five minutes NPS are added into the well from lower to higher concentration. Plates were then kept in an incubator at 37 degree Celsius overnight. The next day plates were observed and a Zone of inhibition was formed.

**3.RESULTS :**

**FIGURE 1:**

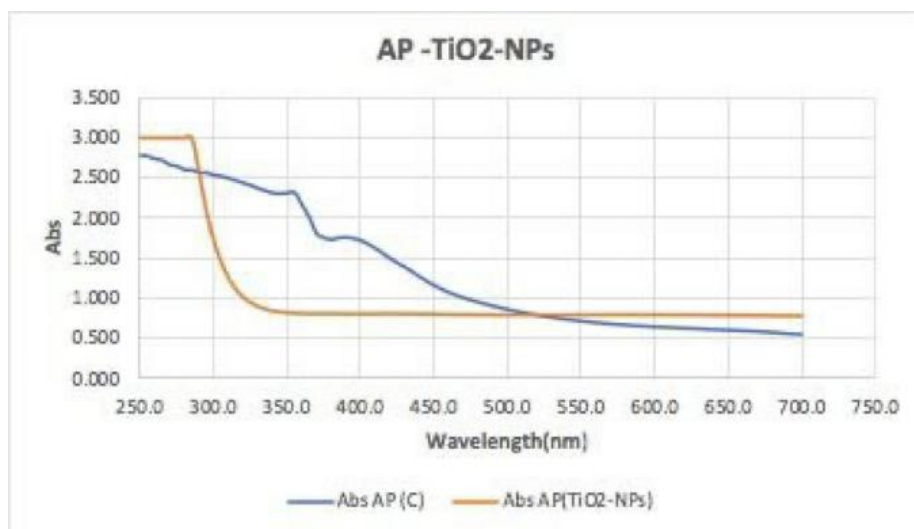


**FIGURE 1:** Morphological and topographical characterisation of TiO<sub>2</sub> NPs using SEM at 100 nm scale

**FIGURE 2:**



**FIGURE 2:** Antimicrobial efficacy study of synthesised TiO<sub>2</sub>-NPs against oral pathogen *S. mutans*



**FIGURE 3 :** T24 UV-vis spectrophotometry of TiO<sub>2</sub> NPS showing wavelength range of 350

FIGURE 4 :

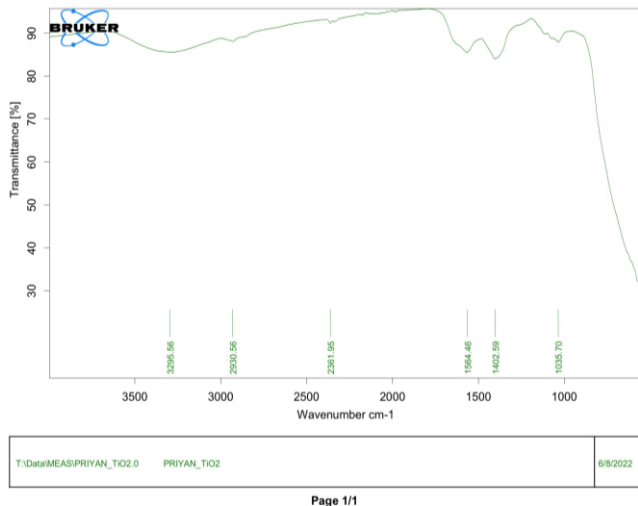


FIGURE 4: FTIR spectroscopy analyses TiO<sub>2</sub> NPS showing different functional groups present in the synthesised NPS

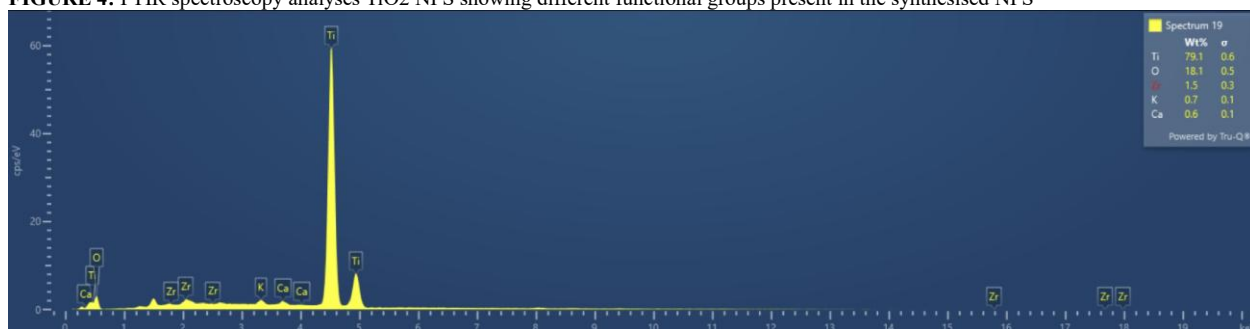


FIGURE 5: EDX used element of TiO<sub>2</sub> ps from *Andrographis paniculata*

**4.DISCUSSION** :According to earlier research conducted by Maryam, metal oxide nanoparticles, such as titanium dioxide nanoparticles, also exhibit strong antimicrobial activity by binding to microbial proteins and deoxyribonucleic acid (DNA). This prevents bacterial replication by avoiding metabolic enzymes of the bacterial electron transport chain,(20) which results in their inactivation. Zhang claims that TiO<sub>2</sub> nanoparticles can be chosen as an antibacterial material due to their superior qualities, which include high specific surface area and high activity to block a variety of pathogenic agents.(21)Despite the fact that titanium dioxide nanoparticles enhance the mechanical and antimicrobial qualities of traditional glass ionomer cements, there is ongoing debate regarding their biocompatibility. Titanium dioxide is now a commonplace substance.(4) Cosmetics, paints, dyes, and varnishes, textiles, paper, plastics, food, medications, and even paving stones are among the many consumer goods and everyday products that contain it. (4,12)Titanium dioxide's many shapes and sizes contribute to its remarkable adaptability. NPs of titanium dioxide have been investigated for a number of biomedical uses.

The primary cause of dental caries, particularly during the initiation and development stages, is *Streptococcus mutans*, a microbe that was first identified in 1924(14). Polyphenolic compounds, primarily monomeric catechin and epicatechin, gallic acid and polymeric, and oligomeric procyanidins, are thought to be abundant in grape (*Vitis vinifera*) seeds. Simple molecules like hydroquinone, pyrocatechol, caffeic acid, ferulic acid, p-coumaric acid, gallic acid, ellagic acid, and resveratrol are examples of grape phenolics. (22)Additionally, grape seed extract, also referred to as grape seed proanthocyanidins extract, is a rich source of various bioflavonoids.(20,23) It is well known that polyphenols have microbicidal properties against a wide variety of harmful bacteria.

In another study the antibacterial activity of the synthesized TiO<sub>2</sub> nanoparticles was performed by the agar-well diffusion method.(24) Fresh colonies of *Bacillus* sp., *E. coli*, *Salmonella* sp. Streaked on Mueller-Hinton agar plates.(25) 50 µl of titanium dioxide nanoparticles were placed in wells made of agar.

The nanoparticles' antibacterial activity and structure were connected.(26) Additionally, some researchers have noted that the suspension's actual particle size differed greatly from the starting powders' promised size.(25,27) The MgONPs employed in this study's analysis were broken down into their representative chemical and physical characteristics in this section. The morphology of the nanomaterials was first described using SEM micrograph.(28) According to Supplementary in the supporting documentation, the MgO microspheres displayed

## 5.CONCLUSION:

The synthesis of nanoparticles of TiO<sub>2</sub> showed antibacterial activity against the oral pathogen *Streptococcus mutans* based on the findings the further study needed to explore the *Andrographis paniculata* Mediators synthesis TiO<sub>2</sub> nanoparticles as a future antibacterial oral mouth wash. SEM test: Spherical and acclumulated TiO<sub>2</sub> nanoparticles were observed in the SEM images which proves that TiO<sub>2</sub> is synthesised from TiNO<sub>3</sub> by the soil bacteria *Andrographis paniculata* Different elements like magnesium, carbon, nitrogen and oxygen were identified in the EDAX spectral of TiO nanoparticles produced by *Andrographis paniculata*. Antibacterial test: Zone of inhibition formed which indicates that the nanoparticles have antibacterial activity.

## 6. REFERENCE :

1. Dinda B. Pharmacology and Applications of Naturally Occurring Iridoids. Springer; 2019. 296 p.
2. Fedlheim DL, Foss CA. Metal Nanoparticles: Synthesis, Characterization, and Applications. CRC Press; 2001. 348 p.
3. Qiu H, Zhao X, Jiang Y, Liang W, Wang W, Jiang X, et al. Design and synthesis of faspalyisin derivatives as inhibitors of FtsZ with potent antibacterial activity and mechanistic study. *Eur J Med Chem.* 2023 Apr 11;254:115348.
4. Akbarzadeh I, Rezaei N, Bazzazan S, Mezajin MN, Mansouri A, Karbalaieheidar H, et al. In silico and in vitro studies of GENT-EDTA encapsulated niosomes: A novel approach to enhance the antibacterial activity and biofilm inhibition in drug-resistant *Klebsiella pneumoniae*. *Biomater Adv.* 2023 Mar 15;149:213384.
5. Ann Preethy N, Somasundaram S. Safety and physiologic effects of intranasal midazolam and nitrous oxide inhalation based sedation in children visiting Saveetha Dental College and Hospitals, India. *Bioinformation.* 2022 Jan 31;18(1):26–35.
6. Sitovs A, Skadins I, Purvina S, Bandere D. In vitro and ex vivo antibacterial activity of levofloxacin against *Pasteurella multocida* and *Escherichia coli* isolated from rabbits (*Oryctolagus cuniculus*) - A preliminary study. *J Vet Pharmacol Ther [Internet].* 2023 Apr 15; Available from: <http://dx.doi.org/10.1111/jvp.13383>
7. Sathiyamoorthy S, Gheena S, Jain RK. Prevalence of oral mucocoele among outpatients at saveetha dental hospital, india. *Bioinformation.* 2020 Dec 31;16(12):1013–8.
8. Jie X, Shiu BC, Zhang Y, Wu H, Ye Y, Fang R. Chitosan-Urushiol nanofiber membrane with enhanced acid resistance and broad-spectrum antibacterial activity. *Carbohydr Polym.* 2023 Jul 15;312:120792.
9. Wu J, Liu F, Chen C, Zhao Z, Du Y, Shi X, et al. Long-term antibacterial activity by synergistic release of biosafe lysozyme and chitosan from LBL-structured nanofibers. *Carbohydr Polym.* 2023 Jul 15;312:120791.
10. Pradeep M, Balakrishnan N, Arvind TRP. Prevalence of usage of various removable appliances among undergraduate dental students in a private dental college. *J Adv Pharm Technol Res.* 2022 Dec;13(Suppl 2):S559–62.
11. Díez-Pascual AM. Antibacterial Activity of Nanomaterials. MDPI; 2018. 339 p.
12. Gobezie S. Antibacterial activity of four plant species used in traditional medicine practice of South Omo Zone, Southern Ethiopia. GRIN Verlag; 2021. 78 p.
13. Akaolisa C. Investigation of the Antibacterial Activity of three types of medicated soaps on *Staphylococcus aureus*. GRIN Verlag; 2018. 38 p.
14. Thakur M, Guleria P, Sobti RC, Gautam A, Kaur T. Comparative analysis of the antibacterial efficacy and bioactive components of *Thuja occidentalis* obtained from four different geographical sites. *Mol Cell Biochem [Internet].* 2023 Apr 15; Available from: <http://dx.doi.org/10.1007/s11010-023-04729-9>
15. Otong ES, Makena W, Solomon AY, Bazabang SA, Aminu A, Henry R. *Andrographis paniculata* protects against brain hippocampus and cerebellum from mercury chloride induced damage by attenuating oxidative stress. *Environ Anal Health Toxicol.* 2022 Dec;37(4):e2022027–0.
16. Selvaraj K, Gayatri Devi R, Selvaraj J, Jothi Priya A. In vitro anti-inflammatory and wound healing properties of and *Andrographis paniculata*. *Bioinformation.* 2022 Apr 30;18(4):331–6.
17. Chen X, Graetzel M, Li C, Davide Cozzoli P. Titanium Dioxide Nanomaterials: Volume 1352. Materials Research Society; 2011.
18. Karthik L, Vishnu Kirithi A, Ranjan S, Mohana Srinivasan V. Biological Synthesis of Nanoparticles and Their Applications. CRC Press; 2019. 463 p.
19. Chiu PWY, Yue GGL, Cheung MK, Yip HC, Chu SK, Yung MY, et al. The effect of *Andrographis paniculata* water extract on palliative management of metastatic esophageal squamous cell carcinoma-A phase II clinical trial. *Phytother Res [Internet].* 2023 Apr 12; Available from: <http://dx.doi.org/10.1002/ptr.7815>
20. Pourzal R, Agarwal P, Leurgans SE, McCarthy SM, Hall DJ, McDevitt CA, et al. Cobalt and titanium levels in the brain are associated with Alzheimer's disease pathology but not cognition: A study of older adults with and without total joint replacement. *Acta Biomater [Internet].* 2026 May 7; Available from: <http://dx.doi.org/10.1016/j.actbio.2026.05.006>
21. Shiraz M, Imtiaz H, Azam A, Hayat S. Phytogetic nanoparticles: synthesis, characterization, and their roles in physiology and biochemistry of plants. *Biometals.* 2024 Feb;37(1):23–70.
22. Mahboob S, Nivetha R, Gopinath K, Balalakshmi C, Al-Ghanim KA, Al-Misned F, et al. Facile synthesis of gold and platinum doped titanium oxide nanoparticles for antibacterial and photocatalytic activity: A photodynamic approach. *Photodiagnosis Photodyn Ther.* 2021 Mar;33:102148.
23. Peng S, Wang K, Ning X, Li B, Zhang M, Hu D, et al. Piezo-metastructured arrays enhance osteoblast mitochondrial quality control to promote osseointegration under osteoporotic conditions. *Biomaterials.* 2026 Apr 27;334:124248.
24. Komaikul J, Ruangdachsuan S, Wanlayaporn D, Palabodeewat S, Punyahathaikul S, Churod T, et al. Effect of andrographolide and deep eutectic solvent extracts of *Andrographis paniculata* on human coronavirus organ culture 43 (HCoV-OC43). *Phytomedicine.* 2023 Apr;112:154708.
25. Songvut P, Pholphana N, Suriyo T, Rangkadilok N, Panomvana D, Puranajoti P, et al. A validated LC-MS/MS method for clinical pharmacokinetics and presumptive phase II metabolic pathways following oral administration of *Andrographis paniculata* extract. *Sci Rep.* 2023 Feb 13;13(1):2534.
26. Zamparini F, Spinelli A, Tosco V, Gandolfi MG, Prati C. Single cone versus carrier-based obturation using a premixed bioceramic sealer in postgraduate master cohort: a 36 months retrospective evaluation. *Clin Oral Investig [Internet].* 2026 May 11;30(6). Available from: <http://dx.doi.org/10.1007/s00784-026-06900-0>
27. Phurailatpam A, Geetha KA, Satyabrata M. *Andrographis Paniculata* - An Important Medicinal Plant. LAP Lambert Academic Publishing; 2012. 184 p.
28. Tewari DN. "Andrographis paniculata" Wall Ex. Nees, Kalmegh, family : "Acanthaceae." 2001. 41 p.

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The authors declare no potential conflict of interest in the present study.

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