

**GREEN SYNTHESIS OF TITANIUM DIOXIDE (TiO₂) NANOPARTICLES WITH
STAR ANISE EXTRACT AND THEIR INTEGRATION INTO MOUTHWASH
FORMULATION**

PROJECT WORK

**Submitted in partial fulfilment of the requirements for the
award of Bachelor of Technology degree in Biotechnology**

By

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DEPARTMENT OF BIOTECHNOLOGY

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**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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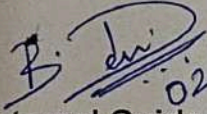
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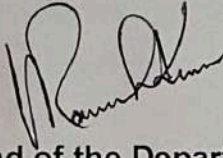
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **DHARSHINI S (40230008)** who carried out Project work entitled "**Green Synthesis of Titanium Dioxide (TiO₂) Nanoparticles with Star Anise Extract and Their Integration into Mouthwash formulation**" under my supervision from October 2023 to April 2024.


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DECLARATION

I, Dharshini S (**40230008**), hereby declare that the Project Report entitled “**Green Synthesis of Titanium Dioxide (TiO₂) Nanoparticles with Star Anise Extract and Their Integration into Mouthwash formulation**” done by me under the guidance of **Dr. B. Devi**, Assistant Professor, Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE: 05/04/2024

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ABSTRACT

The utilization of environmentally green methods for nanoparticle synthesis has gained considerable attention in recent years due to concerns regarding the ecological impact of conventional chemical processes. In this study, we present a green synthesis approach for the production of Titanium Dioxide (TiO₂) nanoparticles using star anise (*Illicium verum*) extract as a reducing and stabilizing agent. Star anise, a commonly utilized spice renowned for its rich in bioactive compounds like thymol and anethole, will be employed for the green synthesis of TiO₂ nanoparticles. The synthesis process was characterized by its simplicity, cost-effectiveness, and eco-friendliness, aligning with the principles of green chemistry. The synthesized TiO₂ nanoparticles were characterized using Scanning Electron Microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) techniques. The green-synthesized TiO₂ nanoparticles were integrated into a mouthwash formulation, aiming to harness their antimicrobial properties for oral hygiene applications. The mouthwash formulation was subjected to confirmatory tests including pH determination, antimicrobial activity assessment against *Staphylococcus Aureus* and cytotoxicity evaluation using the MTT assay with human fibroblast cell lines. This thesis aims to develop a sustainable and potentially more effective mouthwash formulation. Green synthesized TiO₂ nanoparticles may offer enhanced antimicrobial activity while mitigating environmental concerns. The findings will contribute to the advancement of eco – friendly nanomaterial for oral hygiene applications.

Keywords: Green synthesis, TiO₂ nanoparticles, Star anise extract, Mouthwash, Antimicrobial activity, Cytotoxicity.

LIST OF ABBREVIATIONS

TiO ₂	Titanium Dioxide
NPs	Nanoparticles
Nm	Nanometres
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X Ray Diffraction
UV	Ultra Violet
pH	Potential of Hydrogen
H ⁺	Hydrogen Ions
DNA	Deoxy Ribonucleic Acid
RNA	Ribonucleic Acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MRI	Magnetic resonance imaging
CT	Computed Tomography
ROS	Reactive Oxygen Species
DSSCs	Dye-sensitized solar cells
PEC	Photo Electrochemical Cells
PVD	Physical Vapour Deposition
M	Molarity
Mg	Milligram
g	Gram
ml	Millilitre
μl	Microlitre
rpm	Revolutions per minute
Zn O	Zinc Oxide
Ag	Argentum (Silver)
CO ₂	Carbon Dioxide
OH group	Hydroxyl Group

FWHM

Full Width at Half Maximum

HCT 116

Human Colorectal Carcinoma Cell line

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CHAPTER 1

INTRODUCTION

Nanotechnology, a rapidly evolving field, has revolutionized various industries with its ability to manipulate matter at the nanoscale, leading to the development of nanoparticles. Nanoparticles, defined as materials with dimensions ranging from 1 to 100 nanometres, exhibit unique physical, chemical, and biological properties compared to bulk materials. These characteristics make nanoparticles highly versatile and desirable for a wide range of applications across diverse sectors. *(Khanna V, 2019)*

Nanoparticles possess several distinctive characteristics owing to their nanoscale dimensions. Firstly, their large surface area-to-volume ratio enables enhanced reactivity and interaction with surrounding environments. Additionally, nanoparticles exhibit quantum effects and size-dependent properties, leading to novel optical, electronic, and magnetic behaviours. Moreover, their small size facilitates penetration into biological systems, making them promising candidates for biomedical applications. *(Khanna V, 2019)*

The advantages of nanoparticles are manifold, contributing to their widespread adoption in various fields. Firstly, nanoparticles offer tuneable properties, allowing to tailor their size, shape, composition, and surface chemistry to meet specific application requirements. Furthermore, nanoparticles exhibit superior mechanical, thermal, and electrical properties, enabling advancements in materials science and engineering. Additionally, nanoparticles offer increased efficiency and functionality in diverse applications, including catalysis, drug delivery, sensing, imaging, and environmental remediation. *(Service R.F, 2019)*

Among the numerous nanoparticles, Titanium Dioxide (TiO₂) nanoparticles stand out due to their remarkable properties and versatile applications. TiO₂ nanoparticles possess high stability, biocompatibility, and non-toxicity, making them indispensable in various fields. In addition to their inherent photocatalytic properties, TiO₂ nanoparticles exhibit excellent UV-blocking capabilities, making them ideal for sunscreen formulations and UV-resistant coatings. *(Chen X et al.2017)*

The applications of TiO₂ nanoparticles span across multiple industries, ranging from cosmetics and healthcare to environmental and energy sectors. In cosmetics, TiO₂ nanoparticles are used in sunscreens, cosmetics, and skincare products for their UV-blocking properties and skin-friendly nature. In healthcare, TiO₂ nanoparticles are explored for drug delivery, antimicrobial coatings, and biomedical implants, showcasing their potential in advancing medical technologies. (*Chen X et al. 2017*)

The unique properties of TiO₂ nanoparticles lies in their photocatalytic activity, which enables the degradation of organic pollutants, sterilization of surfaces, and purification of air and water. Additionally, TiO₂ nanoparticles exhibit photoelectrochemical properties, making them suitable for applications in solar cells, sensors, and photocatalytic water splitting for hydrogen production. (*Liu G et al 2016*)

Various synthesis methods are employed to produce TiO₂ nanoparticles, including physical, chemical, and biological approaches. Among these, chemical methods are commonly used due to their scalability and controllability. However, chemical methods often involve the use of hazardous chemicals, high temperatures, and energy-intensive processes, leading to environmental concerns and safety risks. (*Liu G et al 2016*)

To address these challenges, green synthesis methods have emerged as eco-friendly alternatives for nanoparticle production. Green synthesis utilizes natural sources such as plant extracts, microorganisms, and biomolecules as reducing and stabilizing agents, minimizing environmental impact and promoting sustainable practices. Star anise, a widely available natural product, has gained attention as a reducing agent for synthesizing TiO₂ nanoparticles due to its abundance, low cost, and eco-friendly nature. (*Khan S et al. 2016*)

The applications of star anise-based nanoparticles extend to oral hygiene products, particularly mouthwash formulations. The antimicrobial and antioxidant properties of star anise make it an attractive candidate for incorporating into mouthwash formulations. By applying the antimicrobial activity of star anise-based nanoparticles, mouthwash formulations can effectively inhibit oral pathogens,

promote oral health, and prevent dental diseases. Moreover, the eco-friendly nature of green synthesis aligns with the growing demand for sustainable and natural oral care products, highlighting the potential of star anise-based nanoparticles in mouthwash formulations. (*Huang Y et al. 2019*)

In summary, nanoparticles, particularly TiO₂ nanoparticles synthesized using green methods and star anise as a reducing agent, hold immense promise for various applications, including oral hygiene products. Through their unique properties and eco-friendly synthesis, these nanoparticles offer innovative solutions to address challenges in healthcare, cosmetics, environmental remediation, and energy sectors, contributing to the advancement of nanotechnology and sustainable development.

CHAPTER 2

LITERATURE SURVEY

2.1 NANOPARTICLES IN BIOTECHNOLOGY

Nanoparticles are incredibly small particles ranging in size from 1-100 nanometres (nm). For perspective, a human hair is roughly 80,000-100,000 nm wide. Due to their miniscule size, nanoparticles exhibit unique physical, chemical, and biological properties compared to their bulk counterparts. These properties make them highly attractive for various applications in biotechnology. (*Albert et al.2015*)

Nanoparticles offer several advantages over traditional materials in biotechnology due to their unique properties such as high surface area to volume ratio which allows for increased interaction with biological molecules, enhancing their effectiveness in drug delivery, biosensing, and bioimaging, tuneable properties which is controlling the size, shape, and surface chemistry of nanoparticles, researchers can tailor their properties for specific applications, enhanced permeability and retention effect which can passively accumulate the nanoparticles in tumour tissues due to their size and leaky vasculature, making them ideal drug delivery vehicles for cancer therapy. (*Duncan TV et al.2012*)

Nanoparticles have revolutionized various fields within biotechnology, with some prominent applications including drug delivery in which nanoparticles can encapsulate drugs and deliver them directly to diseased cells, minimizing side effects and improving therapeutic efficacy, gene therapy includes nanoparticles that is used to deliver genetic materials such as DNA or RNA into cells for gene editing or replacement therapies, biosensing in which nanoparticles can be functionalized with biomolecules to detect specific pathogens, biomarkers, or environmental contaminants with high sensitivity, bioimaging includes nanoparticles that can be used as contrast agents for imaging techniques like magnetic resonance imaging (MRI) or computed tomography (CT), allowing for better visualization of cells and tissues and tissue engineering in nanoparticles can be used to create scaffolds for tissue regeneration or to deliver growth factors that promote cell proliferation and differentiation. (*Peer D et al. 2007*)

2.2 TITANIUM DIOXIDE (TiO₂) NANOPARTICLES

Titanium dioxide nanoparticles (TiO₂ NPs) have become a ubiquitous material in nanotechnology due to their remarkable properties and extensive applicability across various industries. These incredibly small particles, typically ranging from 1-100 nanometres in size, exhibit unique physical and chemical characteristics compared to their bulk counterparts. This review delves into the fascinating world of TiO₂ nanoparticles, exploring their fundamental properties and their diverse applications across various sectors. (*K. Krishna Reddy et al.2017*)

The exceptional properties of TiO₂ nanoparticles originate from their unique size and crystal structure. They primarily exist in three crystalline phases: rutile, anatase, and brookite, with rutile and anatase being the most prevalent for technological applications. It has high surface area due to their miniscule size, TiO₂ nanoparticles possess an exceptionally high surface area relative to their volume. This translates to a significantly greater number of active sites available for interaction with other materials and molecules, making them ideal for applications involving catalysis, adsorption, and sensing. TiO₂ nanoparticles exhibit photocatalytic activity, meaning they can absorb light and initiate chemical reactions upon activation. Anatase, in particular, possesses a strong absorption band in the ultraviolet (UV) region, making it suitable for photodegradation of pollutants and various other light-activated processes. TiO₂ nanoparticles can demonstrate semiconducting behaviour, allowing them to control the flow of electricity under specific conditions. This property finds application in solar cells, photocatalysis, and other electronic devices. TiO₂ nanoparticles boast exceptional chemical stability, making them resistant to corrosion and degradation. This characteristic makes them suitable for long-term applications under harsh environmental conditions. (*D. Reyes-Coronado et al.2008*)

The unique properties of TiO₂ nanoparticles have fueled their exploration across a multitude of applications, transforming various industries. One of the most widely explored applications of TiO₂ nanoparticles lies in photocatalysis. Upon exposure to ultraviolet light, they can generate highly reactive oxygen species (ROS) such as superoxide radicals and hydroxyl radicals. These ROS can degrade organic pollutants, bacteria, viruses, and even odorous compounds present in air

and water (*M. R. Hoffmann et al. 2015*). This property holds immense potential for environmental remediation efforts, wastewater treatment, and air purification systems. (*Efstathiou et al. 2014*) demonstrated the efficacy of TiO₂ nanoparticles for degrading organic pollutants in wastewater, highlighting their potential contribution to environmental cleanup. (*Dunne et al. 2012*) investigated the antimicrobial properties of TiO₂ nanoparticles against various bacteria, suggesting their potential for air and surface disinfection.

TiO₂ nanoparticles incorporated into coatings can impart self-cleaning properties to surfaces. When exposed to sunlight, the photocatalytic activity of TiO₂ nanoparticles breaks down dirt, organic matter, and even some airborne pollutants, promoting a cleaner surface. (*Wang et al. 2012*) explored the self-cleaning properties of TiO₂ nanoparticle-coated glass surfaces, demonstrating their effectiveness in degrading organic contaminants. This application finds use in building materials, paints, and textiles, facilitating easier maintenance and potentially reducing cleaning frequency.

TiO₂ nanoparticles play a crucial role in the development of clean energy technologies. They are essential components in dye-sensitized solar cells (DSSCs) due to their light-harvesting and electron-transporting properties. (*Regan and Grätzel et al. 2011*) pioneered the use of nanocrystalline TiO₂ in DSSCs, paving the way for a new generation of solar cells with potential for lower production costs and higher efficiency compared to traditional silicon-based solar cells. Additionally, research is ongoing to explore the use of TiO₂ nanoparticles in photoelectrochemical (PEC) water splitting for hydrogen generation, a potential clean energy source.

The potential of TiO₂ nanoparticles in the biomedical field is vast and actively being explored. Their antimicrobial properties make them suitable for coatings on medical implants and devices to prevent infections. Furthermore, research suggests their potential application in drug delivery systems, biosensing, and even bone regeneration due to their biocompatibility and unique properties. (*Theisen et al. 2012*)

2.3 SYNTHESIS OF NANOPARTICLES

Titanium dioxide nanoparticles (TiO₂ NPs) have emerged as a cornerstone of nanotechnology due to their remarkable properties and diverse applications. Their miniscule size and unique crystal structures offer exceptional benefits in photocatalysis, photovoltaics, and various other fields. However, the synthesis method significantly impacts the properties and performance of TiO₂ nanoparticles. This review explores various methods for synthesizing TiO₂ nanoparticles, focusing on physical methods, chemical methods, and the increasingly prominent green synthesis approach.

2.3.1 PHYSICAL METHODS

Physical methods offer precise control over the size, shape, and crystal structure of TiO₂ nanoparticles. However, these methods often require specialized equipment and can be energy-intensive. This explores two prominent physical methods such as Physical Vapor Deposition (PVD) and Laser Ablation. PVD techniques involve the transformation of a solid titanium source into vapor and subsequent deposition onto a substrate. Common PVD techniques for TiO₂ nanoparticle synthesis includes sputtering which is high-energy beam that bombards a titanium target, ejecting atoms that condense on a substrate to form TiO₂ nanoparticles. This method allows for precise control over film thickness and composition. (*Liu et al., 2005*), evaporation is another technique, in this method, a titanium source is heated to a high temperature, causing it to evaporate. The vapor condenses on a cooler substrate, forming a thin film of TiO₂ nanoparticles. (*Chen & Ying, 2008*). Advantages of this includes high purity and crystallinity of TiO₂ nanoparticles, precise control over film thickness and morphology and scalable for large-scale production. Its disadvantages include high capital and operational costs, requires specialized equipment and vacuum environment and can be energy-intensive.

Laser Ablation technique utilizes a high-intensity laser beam to ablate a titanium target, generating a plume of titanium atoms, ions, and clusters. These particles condense on a substrate, forming TiO₂ nanoparticles with unique properties such as high surface area and crystallinity. (*Gattus & Mazumder, 2002*).

Advantages of laser ablation includes ability to generate a variety of morphologies like nanoparticles, nanowires, nanotubes, high purity and crystallinity of TiO₂ nanoparticles, relatively simple setup compared to other PVD techniques. Disadvantages of this includes limited production rate, unsuitable for large-scale synthesis, requires specialized laser equipment and can generate a broader size distribution of nanoparticles.

2.3.2 CHEMICAL METHODS

Chemical methods offer a broad range of options for TiO₂ nanoparticles synthesis, often with lower costs compared to physical methods. However, precise control over size and morphology can be challenging. This explores two common chemical methods such as sol-gel method and hydrothermal synthesis. Sol - Gel method is a versatile method which involves the hydrolysis and condensation of titanium precursors to form a sol (colloidal suspension). This sol is then gelled and subsequently calcined to obtain TiO₂ nanoparticles with desired properties. (*Brinker & Scherer, 2019*). Advantages of this includes relatively simple and cost-effective, allows for control over size, morphology, and doping of TiO₂ nanoparticles and scalable for large-scale production. Disadvantages includes precise control over size and morphology can be challenging, requires careful control of reaction parameters such as pH, temperature and may involve toxic solvents and generate hazardous waste.

Hydrothermal synthesis methods involve the reaction of titanium precursors in a sealed vessel at high pressure and temperature. The hydrothermal method utilizes water, while the solvothermal method employs organic solvents. These methods offer control over crystallinity and morphology of TiO₂ nanoparticles. (*Peng & Li, 2013*). Advantages of this method includes formation of high-quality crystalline TiO₂ nanoparticles, allows to control over morphology like nanorods, nanotubes and it relatively simple setup compared to some PVD techniques. Disadvantages of high pressure and temperature requirements, limited control over particle size distribution and potential for hazardous solvent use in solvothermal methods.

2.3.3 GREEN SYNTHESIS

The growing concerns regarding environmental impact and human health have led to the exploration of green synthesis methods for TiO₂ nanoparticles. These methods utilize natural resources like plant extracts, microorganisms, or biopolymers as reducing and capping agents, promoting sustainable and often cost-effective nanoparticle production. Advantages of this is environmentally friendly and non-toxic, utilizes renewable and readily available resources and often requires less harsh chemicals and generates less. Titanium dioxide nanoparticles (TiO₂ NPs) have emerged as a revolutionary material with vast applications in photocatalysis, photovoltaics, environmental remediation, and various other fields. However, traditional chemical methods for TiO₂ nanoparticles synthesis often rely on hazardous chemicals, generate toxic byproducts, and raise environmental concerns. Green synthesis, a rapidly evolving approach, offers a sustainable and environmentally friendly alternative.

Chemical synthesis of TiO₂ nanoparticles often involves the use of toxic solvents, strong acids, and heavy metals. These chemicals pose significant risks to human health and the environment during production, handling, and disposal. Green synthesis, on the other hand, utilizes natural resources like plant extracts, microorganisms, or biopolymers as reducing and capping agents. These readily available and renewable resources eliminate the need for harsh chemicals, significantly reducing the environmental footprint of TiO₂ nanoparticle production. Studies by (*Iravani and Zhu et al. 2011*) demonstrate the effectiveness of plant extracts like Aloe vera and green tea in synthesizing TiO₂ nanoparticles, highlighting the potential of this eco-friendly approach.

Chemical synthesis methods can introduce impurities and residual chemicals onto the surface of TiO₂ nanoparticles. These impurities and residual chemicals can be cytotoxic, raising concerns about their potential health risks. Green synthesis offers a solution by utilizing biocompatible natural resources, leading to TiO₂ nanoparticles with potentially lower cytotoxicity. Studies by (*Sintesa et al. 2014*) investigating the use of fungi for TiO₂ nanoparticles synthesis demonstrated improved biocompatibility compared to chemically synthesized nanoparticles. Chemical synthesis methods often require specialized equipment, high temperatures, and expensive starting materials. Green synthesis, on the other hand,

can be less demanding. The use of readily available natural resources and simpler reaction conditions can potentially reduce production costs. Additionally, green synthesis methods can be adapted for large-scale production, making them commercially viable. For instance, a study by (Philip 2014) explored the scalable production of TiO₂ nanoparticles using a green synthesis approach based on waste banana peels, demonstrating the potential for cost-effective and sustainable large-scale synthesis.

Green synthesis offers a unique advantage in tailoring the properties of TiO₂ nanoparticles for specific applications. Natural extracts and biomolecules can act as capping agents, influencing the size, shape, and surface functionalities of the nanoparticles. This control over properties allows for the development of TiO₂ nanoparticles with enhanced performance for specific applications. Studies by (Zhu *et al.* 2010) demonstrated how using green tea extract for synthesis led to TiO₂ nanoparticles with improved photocatalytic activity for degrading organic pollutants.

Chemical synthesis methods often require high temperatures and harsh reaction conditions, leading to significant energy consumption. Green synthesis, on the other hand, often utilizes milder reaction conditions and readily available resources, potentially leading to a lower environmental impact through reduced energy consumption. Studies by (Nadagouda *et al.* 2011) compared the energy consumption of green synthesis and conventional methods for TiO₂ nanoparticle production, highlighting the potential energy savings with the green approach.

2.4 STAR ANISE AS REDUCING AGENT

Green synthesis utilizes natural resources like plant extracts, microorganisms, and biopolymers as reducing and capping agents. These readily available resources eliminate the need for harsh chemicals, leading to a more sustainable and environmentally friendly production process. For instance, research by (Al-Qasmi *et al.* 2019) demonstrated the successful synthesis of magnetic nanoparticles using star anise extract, highlighting the potential of this natural resource in nanoparticle production.

(Duke, J. A. 2008) Star anise, a spice native to East and Southeast Asia, is known for its distinct flavour and medicinal properties. It is rich in various bioactive

compounds, including terpenes, phenols, and flavonoids. These biomolecules possess reducing properties, making star anise a potential candidate for green synthesis of metal nanoparticles.

The novelty of this project lies in exploring star anise extract as a reducing agent specifically for TiO₂ nanoparticle synthesis. While research suggests its effectiveness in synthesizing other nanoparticles like magnetic nanoparticles, no published studies have investigated its application in TiO₂ nanoparticle production.

(He X et al, 2016) This presents a unique opportunity to contribute to the field of green synthesis and explore the potential benefits of star anise for TiO₂ nanoparticle production. Utilizing star anise for TiO₂ nanoparticle synthesis offers several potential advantages such as it is a readily available and renewable resource. Its use eliminates the need for harsh chemicals, reducing environmental impact and promoting sustainability. Green synthesis approaches often lead to less toxic nanoparticles compared to those produced chemically. The biocompatible nature of star anise extract can potentially contribute to the development of less cytotoxic TiO₂ nanoparticles. It is a relatively inexpensive resource compared to some conventional starting materials used in chemical synthesis. This can potentially lower the production cost of TiO₂ nanoparticles. Biomolecules in star anise extract may act as capping agents, influencing the size, morphology, and surface functionalities of the synthesized TiO₂ nanoparticles. This allows for potential control over the properties of nanoparticles for specific applications.

(Al-Qasbi N, 2019) The properties of star anise-synthesized TiO₂ nanoparticles will depend on the specific synthesis conditions and resulting characteristics. However, based on the known properties of green-synthesized TiO₂ nanoparticles and the potential benefits of star anise, these nanoparticles could be explored for various applications which exhibit good photocatalytic activity for degrading pollutants and organic matter. Star anise-synthesized nanoparticles could be explored for environmental remediation applications. The antimicrobial properties of star anise extract could be translated to the synthesized TiO₂ nanoparticles making them suitable for antimicrobial coatings on surfaces. The biocompatible nature of star anise and green-synthesized TiO₂ nanoparticles suggests potential applications in drug delivery or biosensing.

CHAPTER 3

AIM AND OBJECTIVE

3.1 AIM

The main aim of this study is to synthesis Titanium dioxide (TiO_2) nanoparticles with star anise extract and integration into mouthwash formulation.

3.2 OBJECTIVE

- To optimize the green synthesis of TiO_2 nanoparticles utilizing star anise extract as a reducing and stabilizing agent.
- To characterize the synthesized TiO_2 nanoparticles using SEM, XRD, FTIR, etc.
- To evaluate the antioxidant and bioactive properties of the plant-mediated TiO_2 nanoparticles.
- To integrate synthesized TiO_2 nanoparticles into mouthwash formulation.

CHAPTER 4

MATERIALS AND METHODS

4.1 PREPARATION OF PLANT EXTRACT

- ❖ Star Anise is collected from local market.
- ❖ 20g of Star Anise is weighed.
- ❖ Grinded and powdered the star anise for preparation of extract.
- ❖ Mixed the powdered star anise in 100ml of distilled water and stirred in magnetic stirrer for 4 hours.
- ❖ Kept it in rest for 12 hours.
- ❖ Filtered the extract using Whatmann Filter paper and used for synthesis process.



Fig:4.1 Star Anise

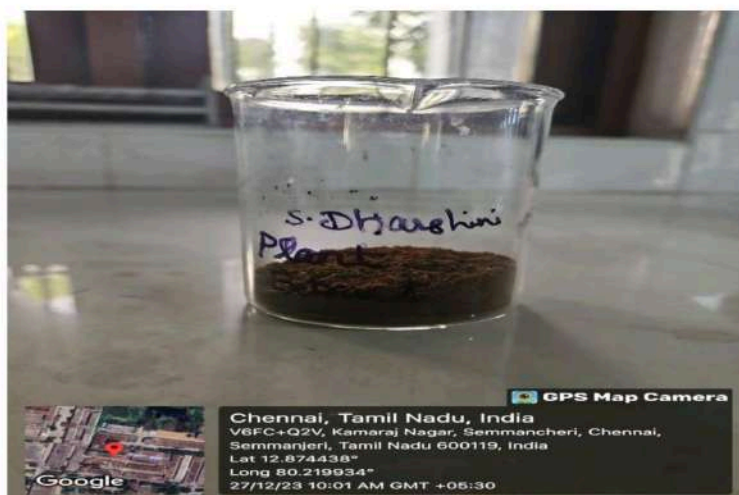


Fig:4.2 Star Anise (Powdered)



Fig: 4.3 Star Anise extract preparation

4.2 SYNTHESIS OF TiO₂ NANOPARTICLES

The titanium precursor (Titanium Tetrachloride) solution is taken in three different concentrations such as 0.1M (950 μ l), 0.5M (4740 μ l), 1M (9480 μ l) and mixed with 10ml of star anise extract and 50ml of ethanol respectively stirred for 24 hours individually. Allow the reaction to proceed until the formation of TiO₂ nanoparticles is complete. Purify the synthesized TiO₂ nanoparticles by centrifugation at 10,000rpm for 15 minutes and collected pellets. Dried the pellets in hot air oven at 70 °c and grinded the nanoparticles into powdered form.



Fig:4.4 Process Chart

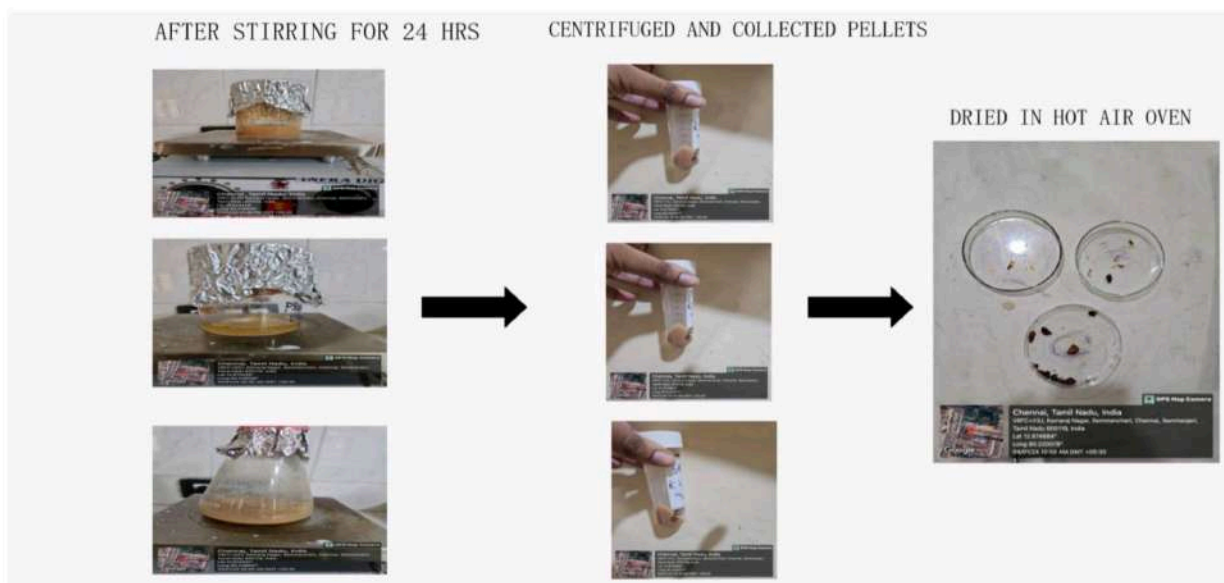


Fig:4.5 Process Chart

4.3 INTEGRATION INTO MOUTHWASH FORMULATION

4.3.1 Dispersion of TiO₂ Nanoparticles

TiO₂ nanoparticles is weighed and taken in three different concentrations such as 0.025g, 0.05g, 0.1g to incorporate into the mouthwash formulation and mixed with saline water. For dispersion of TiO₂ nanoparticles sonicate it for 20 minutes to mix them.

4.3.2 Preparation of Mouthwash Formulation

The mouthwash is formulated with antimicrobial agent (Hydrogen Peroxide) 0.06 ml, Flavouring agent (Peppermint oil) 0.03 ml, Humectant (Glycerol) 3 ml, pH adjuster (Citric Acid) 0.015g, Preservative (Ascorbic Acid) 0.03g, Surfactant (Sodium Lauryl Sulphate) 0.015g. Incorporate the synthesized TiO₂ nanoparticles into the mouthwash formulation under stirring to ensure uniform dispersion. (Huang, Y., et al. 2019)

4.4 CHARACTERIZATION STUDIES

4.4.1 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) stands as a pivotal technique for investigating the surface morphology and structural characteristics of materials at the nanoscale. In the context of Titanium Dioxide (TiO₂) nanoparticles, SEM proves indispensable for elucidating their morphology and size distribution, critical factors that influence their properties and performance across various applications. Given the nanoscale dimensions of TiO₂ nanoparticles, SEM's high-resolution imaging capabilities enable to observe individual nanoparticles, assess their structural characteristics, and optimize synthesis protocols for applications.

4.4.2 Fourier Transform Infrared Spectroscopy

Fourier-Transform Infrared Spectroscopy (FTIR) is used to characterize the chemical composition and molecular structure of materials based on their vibrational modes. By passing infrared radiation through a sample and measuring the resulting absorption spectra, FTIR provides valuable insights

into the presence of functional groups, chemical bonds, and molecular interactions within the material. In Titanium Dioxide (TiO₂) nanoparticles, FTIR spectroscopy plays a crucial role in identifying surface functional groups, adsorbed species, and chemical modifications that may occur during synthesis or subsequent processing. By analysing the characteristic absorption bands associated with TiO₂ nanoparticles, we can observe their surface chemistry, assess their stability, and optimize their performance for various applications such as photocatalysis, solar cells, and biomedical devices. Through FTIR analysis, we can understand the molecular composition and surface properties of TiO₂ nanoparticles, guiding the development of synthesis strategies and enhancing their functionality in diverse applications.

4.4.3 X-Ray Diffraction

X-ray Diffraction (XRD) is a powerful analytical technique used to probe the crystalline structure and phase composition of materials. By directing a monochromatic X-ray beam onto a crystalline sample and measuring the angles and intensities of the scattered X-rays, XRD generates a diffraction pattern that provides insights into the arrangement of atoms within the crystal lattice. In the context of Titanium Dioxide (TiO₂) nanoparticles, XRD analysis is crucial for identifying the crystal phases present (e.g., anatase, rutile) and quantifying their relative abundance. Additionally, XRD provides information about the crystallite size and lattice parameters of TiO₂ nanoparticles, offering valuable insights into their physical and chemical properties.

4.4.4 pH measurement

pH measurement serves as a fundamental tool for assessing the acidity or alkalinity of solutions, crucial for numerous applications across various scientific disciplines. In general, pH measurement provides insights into the concentration of hydrogen ions (H⁺) in a solution, influencing chemical reactions, biological processes, and the behaviour of substances in solution. In the case of Titanium Dioxide (TiO₂) incorporated mouthwash formulations, pH measurement is essential for evaluating the acidity or alkalinity of the solution, which directly impacts its effectiveness, stability, and compatibility with oral tissues. The pH of mouthwash formulations plays a critical role in

ensuring their safety and efficacy, as deviations from the optimal pH range may lead to adverse effects or reduced therapeutic benefits. By monitoring the pH of TiO₂ incorporated mouthwash formulations, researchers can assess their suitability for oral hygiene applications, optimize formulation parameters, and ensure consumer safety and satisfaction.

4.4.5 Antimicrobial Activity

Antimicrobial activity refers to the ability of a substance to inhibit the growth or kill microorganisms such as bacteria, fungi, viruses, or parasites. This property is essential for controlling and preventing infections in various settings, including healthcare, food preservation, and personal hygiene. Antimicrobial agents can act through different mechanisms, including disrupting cell membranes, inhibiting essential enzymes, or interfering with nucleic acid synthesis, leading to the suppression of microbial growth. Assessing antimicrobial activity involves testing the effectiveness of a substance against specific microorganisms through standardized methods such as agar diffusion assays or broth dilution methods. In the case of mouthwash formulations, evaluating antimicrobial activity is crucial for ensuring their efficacy in reducing oral microbial load and preventing oral infections. Specifically, testing the antimicrobial activity of mouthwash formulations against common oral pathogens such as *Staphylococcus aureus* provides valuable information about their potential to maintain oral hygiene and prevent dental diseases. *Staphylococcus aureus* is a Gram-positive bacterium commonly found in the oral cavity and is associated with various oral infections, including periodontitis and dental caries. Therefore, assessing the antimicrobial activity of mouthwash formulations against *Staphylococcus aureus* is essential for evaluating their suitability for oral hygiene applications and ensuring consumer safety and satisfaction. (Mohammed et al. 2010)

4.4.6 MTT Assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a widely used colorimetric assay that measures cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. This assay relies on the reduction of MTT tetrazolium salt by mitochondrial enzymes in

viable cells to form insoluble formazan crystals, which are then solubilized and quantified spectrophotometrically. Cells of interest (Human Colorectal Carcinoma cells) are cultured in appropriate growth medium under 37 °C, 5% CO₂ until they reach the desired confluence. Cells are seeded into multi-well plates and treated with mouthwash formulation at various concentrations for a specified duration. After treatment, the MTT reagent is added to each well and incubated with the cells for a defined period, typically 2-4 hours. Viable cells convert the MTT reagent into insoluble formazan crystals via mitochondrial enzymes. The formazan crystals are solubilized using a dimethyl sulfoxide to dissolve the coloured product. The absorbance of the solubilized formazan solution is measured using a microplate reader at a specific wavelength 570 nm, with higher absorbance indicating increased cell viability. For testing mouthwash formulations, the MTT assay can be performed to assess their cytotoxicity on human colorectal carcinoma cells. The mouthwash formulations are typically diluted to various concentrations and applied to cell cultures for a specified duration. After treatment, the MTT reagent is added to the wells, and the assay is carried out as described above. The absorbance readings are then used to calculate cell viability relative to untreated control cells, allowing researchers to determine the cytotoxic effects of the mouthwash formulations on human colorectal carcinoma cells.

4.5 STATISTICAL ANALYSIS

GraphPad Prism is a strong statistical analysis and graphing programme that is commonly used in scientific research. Its sample interface facilitates data visualisation, curve fitting and statistical analysis. Prism, with features like nonlinear regression and automated error bar creation, simplifies data analysis, making it a popular option among researchers. The tests that were performed are Antimicrobial Activity (Chi square Test) and MTT Assay (One way ANOVA).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 CHARACTERIZATION STUDIES

5.1.1 Scanning Electron Microscopy

The three different concentrations of TiO_2 nanoparticles such as 0.1M, 0.5M and 1M is collected and done SEM analysis.

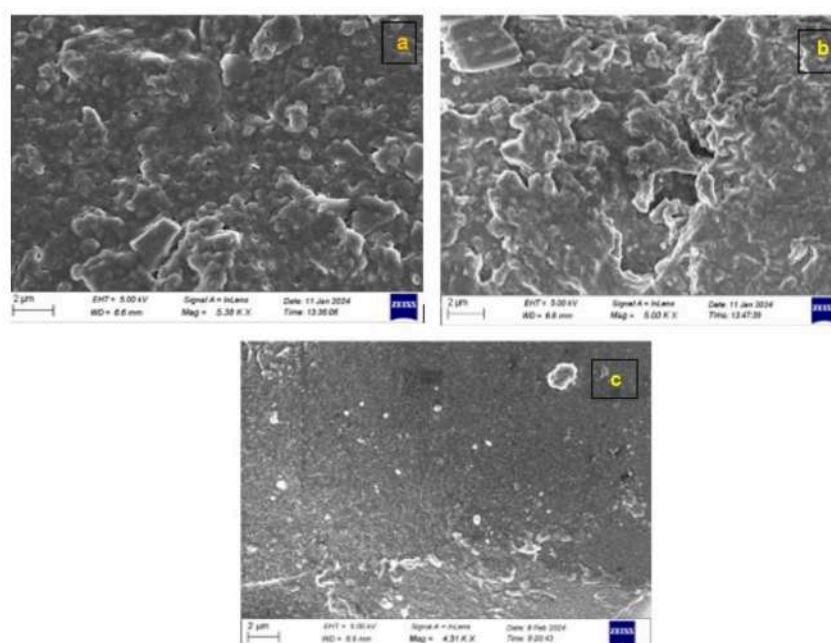


Fig:5.1 SEM Micrographs of TiO_2 Nanoparticles of three concentrations a) 0.1 M b) 0.5 M c) 1 M

The SEM Images in Fig 5.1 shows that there is agglomeration of nanoparticles where nanoparticles are clumped together. Whereas in 1 M concentration it showed some better results. Therefore did further analysis of SEM of this 1M concentration with high resolution, got best results with uniform distribution of nanoparticles and no agglomeration.

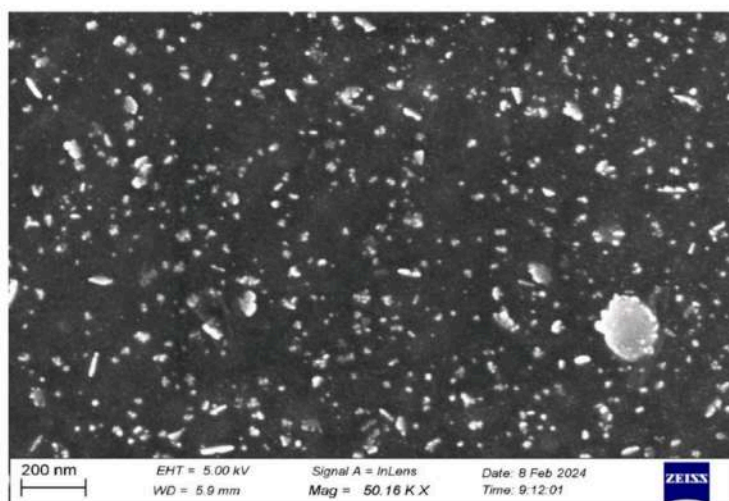


Fig:5.2 1M SEM Images at 50.16 K X

The SEM image suggests the presence of TiO_2 nanoparticles with good uniform distribution of nanoparticles and no aggregation.

5.1.2 Fourier Transform Infrared Spectroscopy

FTIR analysis of collected TiO_2 nanoparticles and reducing agent (star anise) peaks are given in the below figure.

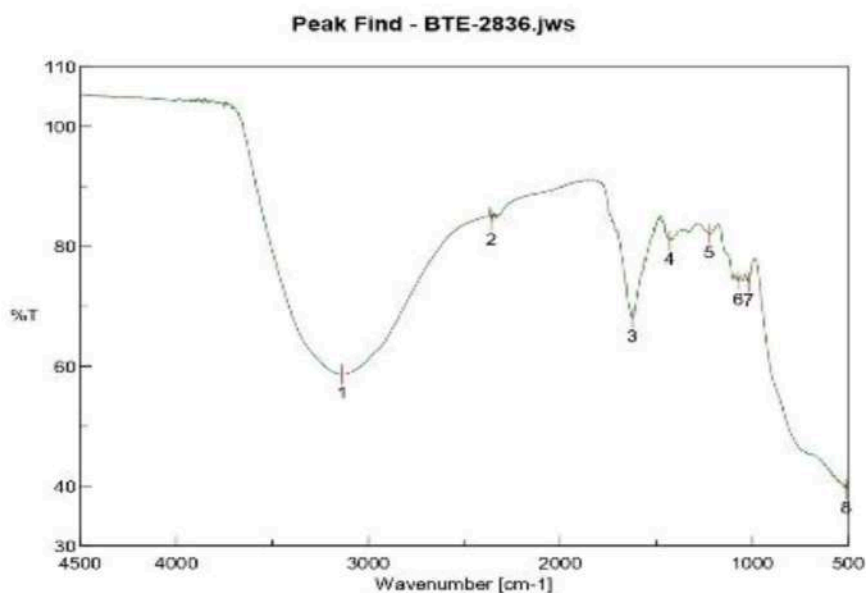


Fig:5.3 FTIR Peak of TiO_2

TABLE:5.1.2 FTIR Readings

No.	Position	Intensity
1	3138.58	58.5965
2	2359.48	84.1471
3	1624.73	68.0523
4	1428.99	80.9753
5	1223.61	82.0239
6	1074.16	74.2182
7	1018.23	74.0377
8	511 .044	39.4929

Peak Find - 2980.jws

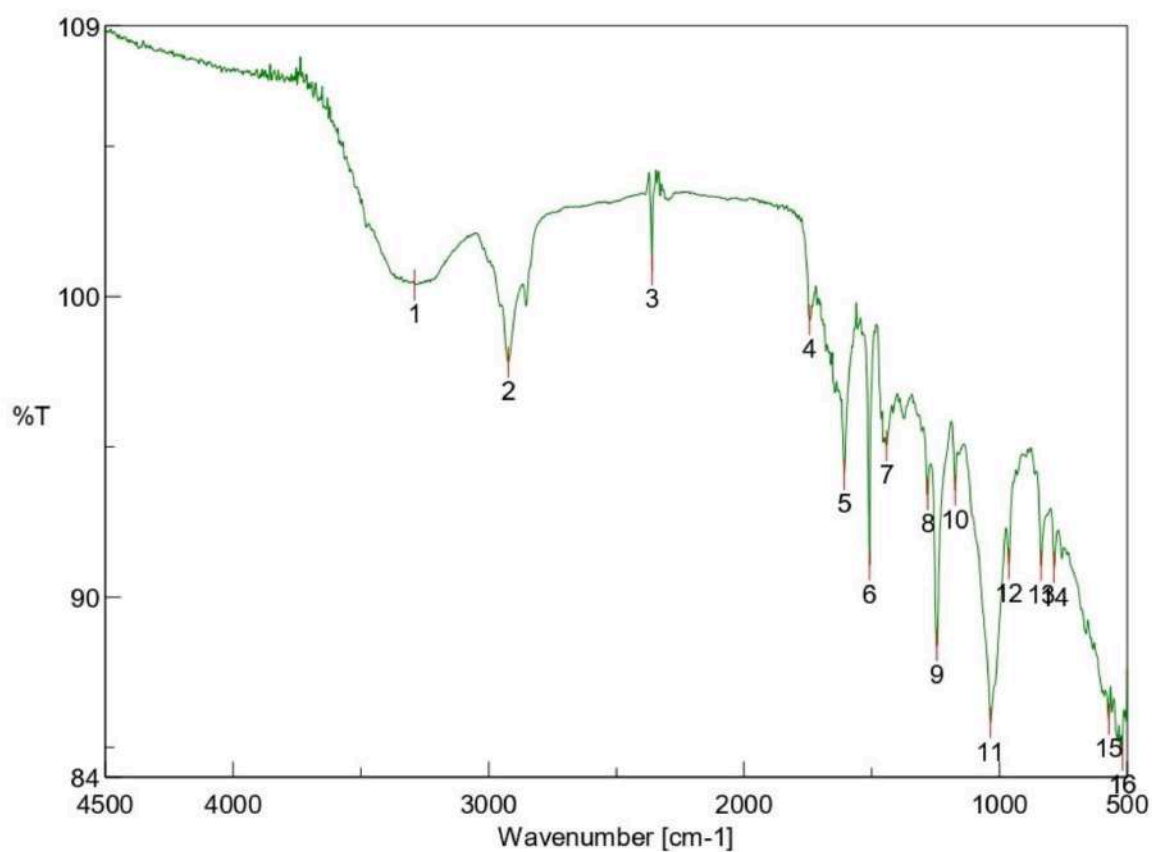


Fig:5.4 FTIR Peaks of Star Anise

TABLE:5.1.3 FTIR Readings

No.	Position	Intensity
1	3289	100.372
2	2922.59	97.8069
3	2360.44	100.871
4	1743.33	99.2189
5	1607.38	94.0594
6	1509.03	91.059
7	1442.49	95.0264
8	1281.47	93.4121
9	1244.83	88.3753
10	1173.47	93.5441
11	1035.59	85.816
12	963.269	91.0997
13	836.955	91.0491
14	785.85	90.9792
15	572.755	85.9321
16	518.758	84.7298

The FT-IR spectrum of Titanium Dioxide nanoparticles synthesized from *Illicium verum* is depicted in the above figure. FT-IR spectrum of Titanium Dioxide nanoparticles showed specific stretching vibrations. The stretching frequency observed in 511 cm^{-1} indicated an effective formation of Titanium Dioxide nanoparticles in peak 8. The broad range in 3138 cm^{-1} reveals stretching frequency of O-H group in peak 1. The peaks found in FTIR Star Anise is around 1750 cm^{-1} (C=O stretching) indicates ester group. Around 1600 and 1500 cm^{-1} (C=C stretching) indicates aromatic group. Around 3300 cm^{-1} (O-H stretching) indicates alcohol group and around $2900\text{-}2800\text{ cm}^{-1}$ (C-H stretching) indicates alkanes group. The vibrational frequencies in the range of $1018\text{-}2359\text{ cm}^{-1}$ will be attributed to flavonoids and polyphenols exist in *Illicium verum* solution in peak 2 to 7.

5.1.3 X-Ray Diffraction

XRD analysis of collected TiO₂ nanoparticles is given as pattern in below figure.

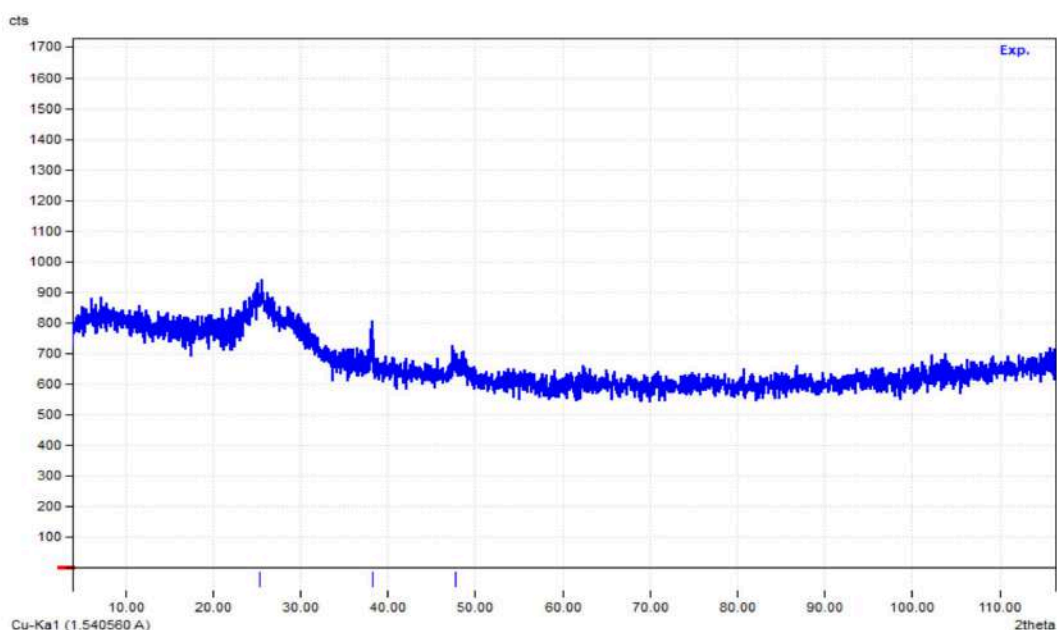


Fig:5.5 XRD pattern

The X-ray diffraction pattern of the synthesized Titanium Dioxide nanoparticles is shown and the peak details are in Table. The experimental XRD pattern agrees with anatase TiO₂. The 2θ at peak 25.28°, 38.28°, 47.70° confirms the TiO₂ tetragonal anatase structure. Strong diffraction peaks at 25 ° and 48 °. Debye- Scherrer equation used to calculate particle size is given below:

$$D = K \lambda / \beta \cos \theta$$

D is nanoparticles crystalline size

K represents the Scherrer constant (0.98)

λ denotes the wavelength (1.54)

β denotes the full width at half maximum (FWHM).

TABLE:5.1.4: XRD Readings

No.	2 THETA [°]	d[A]	I/10 (Peak height)	Counts (Peak Area)	FWHM	D
1	25.28	3.5196	Nan	1726.6	9.763	0.0117
2	38.28	2.3495	Nan	60.31	0.2905	0.2604
3	47.7	1.9051	Nan	543.34	9.221	0.0098

5.1.4 pH measurement

Determination of pH of the mouthwash formulation using pH indicator strips. pH is tested as 7 which should be around 6 to 8 for mouthwash.



Fig:5.6 pH

5.1.5 Antimicrobial Activity

The antimicrobial activity of the mouthwash formulation is analysed using standard microbiological methods (disc diffusion assay) against *Streptococcus Aureus*. Here Listerine is used as control (250mg/ml). It shows 1.2mm diameter of zone of inhibition for the mouthwash samples and 2mm diameter for control.

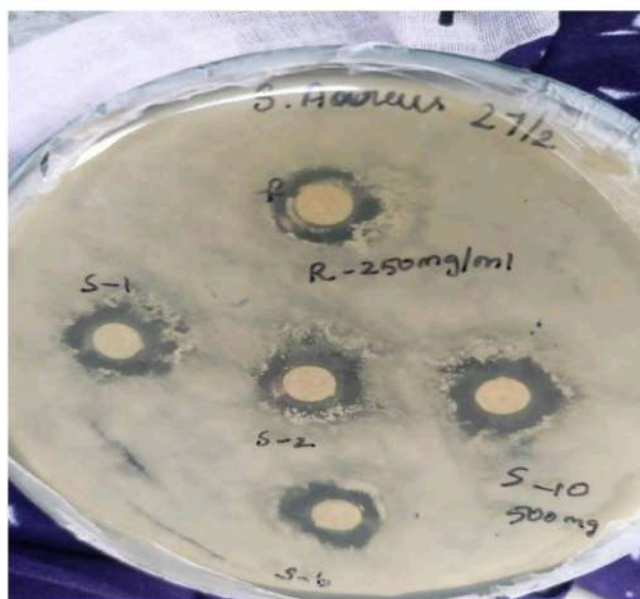


Fig:5.7 Antimicrobial Activity plate

TABLE:5.1.5: Antimicrobial Activity Readings

	Zone of inhibition
Control 1	2mm
Control 2	2mm
Treated 1	1.2 mm
Treated 2	1.2 mm
Treated 3	1.2 mm

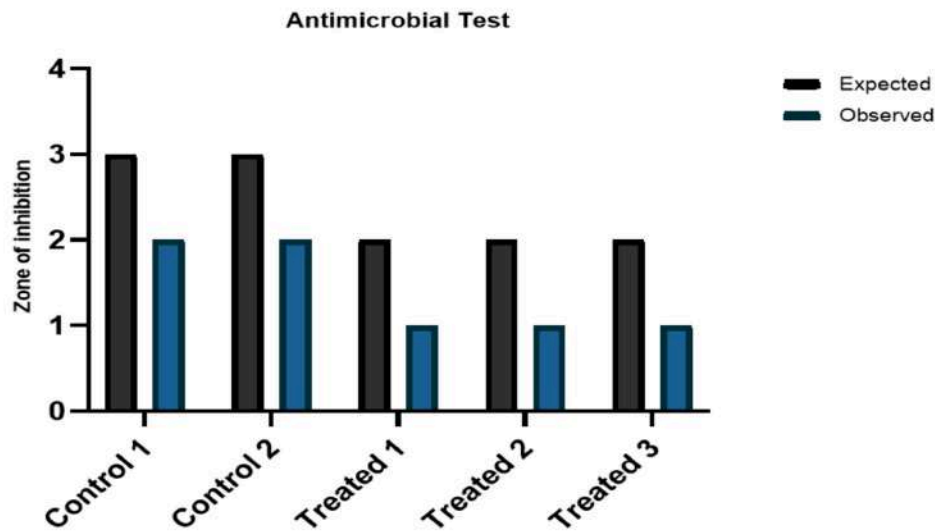


Fig:5.8 Antimicrobial (Zone of inhibition) Graph

Table Analyzed	Data 1
P value and statistical significance	
Test	Chi-square
Chi-square, df	0.09048, 4
P value	0.9990
P value summary	ns
One- or two-sided	NA
Statistically significant (P < 0.05)?	No
Data analyzed	
Number of rows	5
Number of columns	2

Fig:5.9 Chi square test for Antimicrobial Activity

The chi-square test (X^2) statistic (0.09048) with 4 degrees of freedom (df) results in a p-value of 0.9990, which is considered non-significant (ns).

5.1.6 MTT Assay

The cytotoxicity of the mouthwash formulation using cell viability assays such as MTT assay with human cell lines.

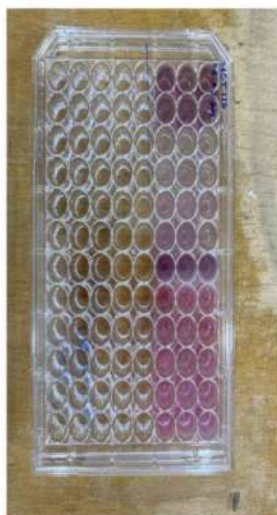


Fig: 5.10 MTT Assay Plate

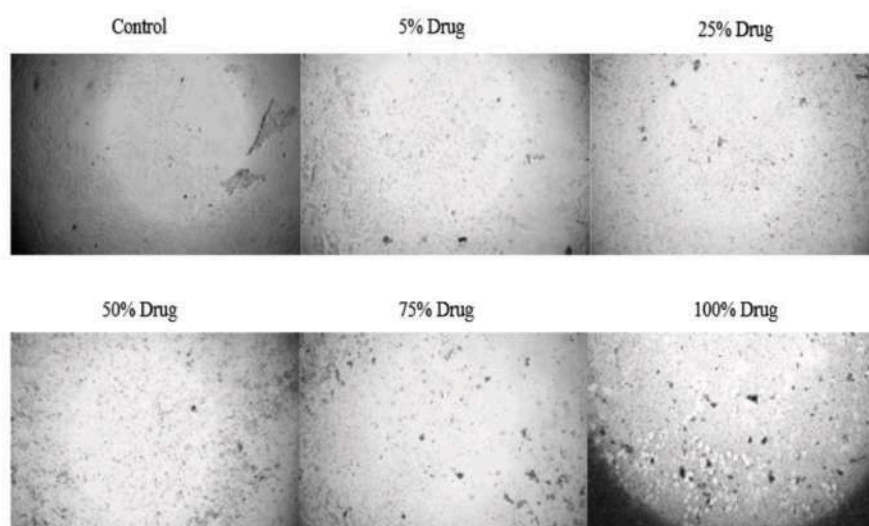


Fig:5.11 MTT Assay Drug Treated

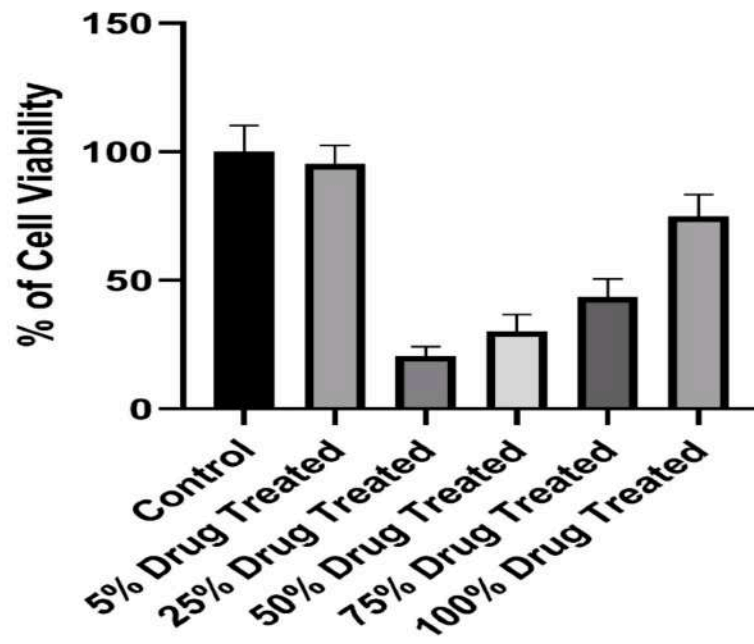


Fig:5.12 Mouthwash Vs HCT 116 Graph

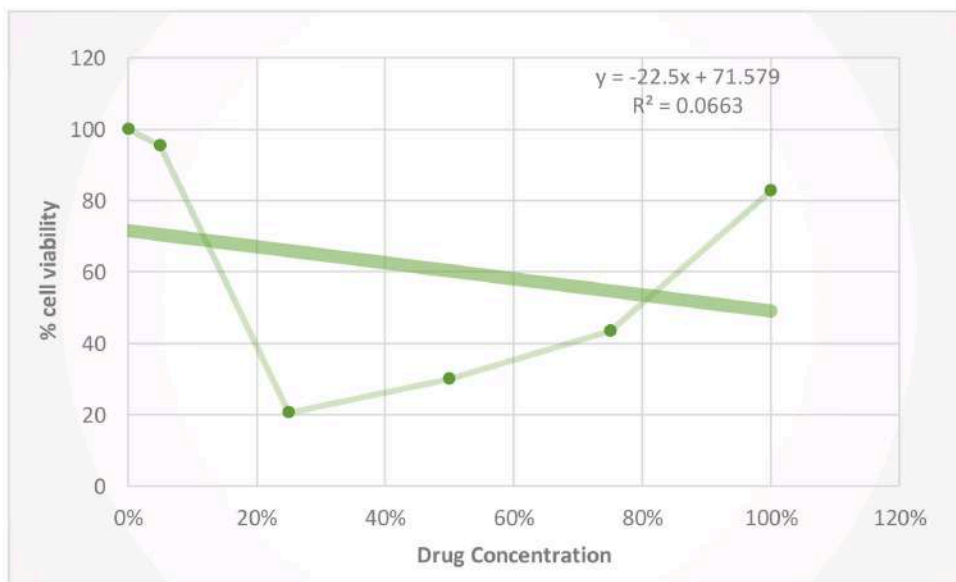


Fig:5.13 IC50 Graph

The IC50 value of mouthwash was found to be 0.959067 which proves that the mouthwash is potent.

TABLE:5.1.6: MTT Assay Readings

Mouth Wash vs HCT116 Results								
		Values			(individual value*100)/average value			Average
				Average.				
Control	0.5	0.405	0.47	0.456333	108.6924763	88.7509	102.557	100
5% Drug Treated	0.44	0.399	0.46		96.63988313	87.4361	101.68	95.252
25% Drug Treated	0.08	0.084	0.11		18.40759679	18.4076	24.7626	20.5259
50% Drug Treated	0.11	0.168	0.14		23.66691015	36.8152	29.8028	30.095
75% Drug Treated	0.2	0.168	0.23		42.95105917	36.8152	50.84	43.5354
100% Drug Treated	0.32	0.386	0.43		69.90504018	84.5873	93.572	82.6881

We can conclude that the control cells (0% drug treated) have 100% viability, but on the other hand all drug-treated groups (5%, 25%, 50%, 75%, and 100% drug treated) show a lower percentage of cell viability compared to the control. This suggests the drug has a cytotoxic effect on HCT116 cells, meaning it reduces their viability. As the mouthwash contains antimicrobial agents, they might be responsible for the cytotoxic effect on the cancer cells. These agents target and kill microorganisms but may also have some level of toxicity towards human cells at higher concentrations. Certain ingredients in mouthwash, like flavouring agents or surfactants, could potentially contribute to the observed cytotoxicity, especially at higher concentrations. Overall, the MTT assay results suggest that the mouthwash

exhibits promising anti-cancer activity against HCT116 colorectal cancer cells.
(Mosmann T, 2013)

Ordinary one-way ANOVA		
1	Table Analyzed	Data 1
2	Data sets analyzed	A-F
3		
4	ANOVA summary	
5	F	62.23
6	P value	<0.0001
7	P value summary	****
8	Significant diff. among means ($P < 0.05$)?	Yes
9	R square	0.9629
10		
11	Brown-Forsythe test	
12	F (DFn, DFd)	0.2034 (5, 12)
13	P value	0.9548
14	P value summary	ns
15	Are SDs significantly different ($P < 0.05$)?	No
16		
17	Bartlett's test	
18	Bartlett's statistic (corrected)	
19	P value	
20	P value summary	
21	Are SDs significantly different ($P < 0.05$)?	

Fig:5.14 ANOVA of MTT Assay

The above ANOVA of MTT Assay represents that P value is <0.0001 which is statistically significant.

CHAPTER 6

SUMMARY AND CONCLUSION

This project investigated the utilization of star anise extract for the green synthesis of titanium dioxide (TiO₂) nanoparticles, with a focus on their potential integration into a mouthwash formulation. Green synthesis offers a more sustainable approach, employing natural plant extracts as reducing and stabilizing agents. While there's no published research directly exploring star anise for TiO₂ synthesis, existing studies demonstrating successful green synthesis of nanoparticles using other plant extracts provide a promising foundation for further exploration. Techniques like X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR) is employed to characterize the size, morphology, and crystal structure of the synthesized nanoparticles. These characterizations are essential for understanding their potential applications in mouthwash, where the nanoparticles' size and structure directly influence their performance. The inherent antimicrobial properties of TiO₂ nanoparticles make them potentially beneficial for oral hygiene. The successful integration of TiO₂ nanoparticles necessitates addressing factors like stability, dispersion within the solution, and potential cytotoxicity. Safety assessments are crucial to evaluate potential adverse effects of nanoparticles in the oral cavity. By successfully navigating these challenges, this research could pave the way for sustainable and potentially more effective approaches to oral hygiene.

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ANNEXURE

Your GATE 2024 Result [BT]

Name

DHARSHINI S

Registration Number

BT24S57402202

Gender

Female

Parent's/Guardian's name

ANURADHA

Date of Birth (YYYY-MM-DD)

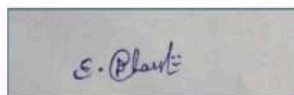
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Examination Paper

Biotechnology (BT)



Photograph



Signature

Marks out of 100[#]

9.33

Qualifying Marks^{##}

38.9

General

35

OBC-NCL/EWS

25.9

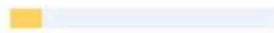
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1 message

Dr Girish Ratnaparkhi via <no-reply@ias.ac.in>

Reply to: Dr Girish Ratnaparkhi <girish.iiserpune@gmail.com>

To: Dharshini S <dharshanu07@gmail.com>, Arun Karthick S <arunskarthick@gmail.com>, Devi B <famidevi@gmail.com>

Dharshini S, Arun Karthick S, Devi B:

We have reached a decision regarding your submission to Current Science Bangalore, "Green Synthesis of TiO₂ Nanoparticles and Their Mechanism of Reduction – A Review".

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Name of the student : S. Dharshini

Register Number : 40230008

Title of the Thesis : Green Synthesis of Titanium Dioxide Nanoparticles with Star Anise Extract and Their Integration into Mouthwash Formulation

Internal Guide Name : Dr. B. Devi

External Guide Name :

PO / PSO / PEO / SDG	Justification
PO3	This study justifies that the mouthwash formulation addresses a complex oral hygiene need while prioritizing safety, user preferences, and environmental responsibility.
PSO1	This research project conceptualizes and implements a novel mouthwash formulation to address a technical challenge in oral healthcare, prioritizing improved efficacy and enhanced safety.