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Titanium oxide nanoparticles; Green synthesis; Medicinal plants; Antibacterial activity; Cytotoxicity; Nanomedicine.

Antioxidant, Antimicrobial activity, Anti-inflammatory and cytotoxic effect, Embryonic toxicology effect of Green synthesised Syzygium aromaticum and Thymus vulgaris Titanium oxide nanoparticles

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ABSTRACT: **Background**: Green synthesis of metal oxide nanoparticles using plant-based phytochemicals. The biological properties of Titanium oxide nanoparticles (TiO_2 NPs) synthesised with Syzygium aromaticum and Thymus vulgaris Formulation are correlated to the presence of natural bioactive compounds utilized as reducing and capping agents. Objective: This study aimed to synthesize and characterize TiO_2 NPs using clove and thyme extracts and determine their antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and embryonic toxicological effects for biomedical purposes.

Methods: TiO_2 NPs were obtained by a green synthesis from the aqueous extracts of clove and thyme particles, which was characterized by Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD) and Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM). DPPH and hydrogen peroxide scavenging assays were performed to assess antioxidant properties. Agar well diffusion method was used to evaluate antimicrobial efficacy against important oral pathogens. Brine shrimp lethality and human cell line assays were used to assess cytotoxicity. Embryonic toxicity was studied zebrafish embryos.

Results: The TiO_2 NPs obtained had a spherical morphology and anatase crystalline structure with a size between 20 and 50 nm. They showed considerable antioxidant and antimicrobial activities, particularly against Staphylococcus aureus and Candida albicans. They also exhibited dose-dependent anti-inflammatory effects and selective cytotoxicity, with biocompatibility observed at 50 µg/mL, but inducing embryotoxic effects at higher concentrations.

Conclusion: Green Synthesis of Syzygium aromaticum and Thymus vulgaris mediated TiO_2 NPs application in biomedical and dental therapeutics having remarkable multifunctional bioactivities.

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1. INTRODUCTION

Nanotechnology is advanced biomedical research and therapeutic tools, proposing new opportunities for some microbial infections, inflammation, oxidative stress, and cytotoxicity. Oral infections caused by microbial pathogens are a major global health risk (1). oral pathogens and fungi contributing to oral disease together comprise dental caries, periodontitis, and oral candidiasis, including *Streptococcus mutans, Lactobacillus sp., Staphylococcus aureus, Enterococcus faecalis, and Candida albicans* (2). The application of nanotechnology, which uses nanoparticles with antimicrobial activity, can provide a more specific, effective, to control oral pathogens. Nanoparticles have small size and high surface energy which promotes their disruption of biofilms and bacterial membrane which leads to cell disruption (3). Antimicrobial Mechanism of Nanoparticles The main antimicrobial mechanisms of nanoparticles involve the generation of reactive oxygen species, inhibition of essential enzymes and interference with quorum sensing (4).

Oral infections are one of the most common health problem, affecting millions of people. A microbial ecosystem exists in our oral cavity comprising many bacterial and fungal species influencing oral health and disease (5). The dental disease has been linked to chronic relapsing infections caused by pathogenic microbes such as S. *mutans, Lactobacillus sp., S. aureus, E. faecalis, and C. albicans*. Biofilms also increase pathogen protection from antimicrobials. Generation of biofilms in many case accounts for chronic persistence of infection and is a major challenge in treating infections (6). In addition, the global burden of antimicrobial resistance is rising, creating a demand for new therapeutic strategies like nanoparticle-based therapeutics (7). Titanium oxide nanoparticles have a biomedical applications owing to their biocompatibility, stability, and antimicrobial activity (8). TiO_2 NPs exhibit photocatalytic activity, with UV- visible-spectrometer irradiation, which boosts their antimicrobial effectiveness (9). TiO_2 NPs exhibit multiple functionalities which has made them popular in the biomedical field, with applications in dentistry and medicine S. Titanium oxide nanoparticles (TiO_2 NPs) have many applications in the biomedical field due to biocompatibility, stability, and multifunctional properties (10). Dental caries is important for maintaining optimal oral wellbeing, however in dentistry TiO_2 NPs can also be found in dental implants, restorative materials, and antimicrobial coatings to prevent biofilm formation and bacterial infections visibly improving oral health (11). The mechanical properties translate to enhanced durability and longevity of the dental materials (12). Therefore, TiO_2 NPs can also have potential applications beyond dentistry, due to their strong antioxidant properties that neutralize free radicals and reduce oxidative stress, consequently promoting tissue regeneration and preserving cellular health(13) . They also anti-inflammatory pathways, the regulation of cytokine production, which make them of great value in the treatment of inflammatory diseases such as periodontitis (14). TiO_2 NPs have another important biomedical function, according to their photocatalytic activity, this phenomenon can facilitate the generation of reactive oxygen species (ROS) under the light exposure, and ROS can lead to anti-microbial sterilization and environmental disinfection (15).

The green synthesis of nanoparticles from medicinal plant extracts are environmentally friendly and enriches them with biologically active compounds (16). Clove (*Syzygium aromaticum*) and Thyme (*Thymus vulgaris*) are two plants that have attracted great attention due to their antimicrobial, antioxidant, and anti-inflammatory activities (17). Therefore, clove is abundant with eugenol which acts as natural reducing and stabilizing agent during TiO_2 NP synthesis thereby increasing their stability and biological activity (18). Its anti-inflammatory and antimicrobial properties. Thyme has also interestingly high levels of thymol, flavonoids, and polyphenolic compounds that provide it with its antimicrobic and antioxidant properties. Phytochemicals are able to improve the biological compatibility of TiO_2NPs and thus contribute to their therapeutic effect against oral pathogenic microorganisms via breaking down biofilms and altering immune response (19). The use of Clove and Thyme for the synthesis of green nanoparticles thus presents a novel and effective method for generating improved biomarkers for biomedical applications (20). The Aim of the study is green synthesis of titanium oxide nanoparticles using clove and thyme formulation of antioxidant, anti-inflammatory activity, Anti-microbial, cytotoxicity and embryonic toxicology activity against oral pathogens (21).

2. MATERIALS AND METHOD

2.1 Preparation of plant extract

Fresh leaves of *Thymus vulgaris* and buds of *Syzygium aromaticum* were obtained from a local botanical garden. The plant materials were rinsed by double-distilled water to remove the soil particles as well as surface impurities. 1g each of S. aromaticum and T.

vulgaris were weighed out separately and 100mL of double-distillated water was added. Then, the mixtures were heated on a heating mantle, at temperatures between 45 and 75 °C for 15-20 minutes. The solutions were filtered by using Whatman No.1 filter paper, the filtrate were concentrated under gentle evaporation by a hot plate to a volume of approximately 5mL. the filter was stored at 4 °C for further synthesis of nanoparticles.

2.2 Green synthesis of titanium oxide nanoparticles (Ti02)

To synthesis the titanium oxide nanoparticles (TiO_2 NPs), 0.1g of titanium dioxide powder was mixed in 50mL of distilled water. This TiO_2 solution with 50mL of the synthesized plant extract that contains *Syzygium aromaticum* and Thymus *vulgaris* for the nanoparticle's synthesis. This solution was maintained at room temperature. The color and turbidity change indicated the formation of TiO_2 nanoparticles. The synthesis of TiO_2 NPs was confirmed by UV-Visible spectroscopy. The solution was centrifuged at 6000rpm for 10minutes.

2.3 Antimicrobial activity

Antimicrobial activity of green synthesized titanium oxide (TiO_2) nanoparticles was estimated by agar well diffusion method. Mueller Hinton agar plates were made and sterilized in an autoclave at 121°C for 15-20 minutes. The medium was then poured into sterile Petri dishes and allowed to solidify at room temperature after sterilization. Cultures of oral pathogens *Streptococcus mutans, Lactobacillus sp., Staphylococcus aureus, Enterococcus faecalis, and Candida albicans* were prepared in fresh overnight growth, and the buffer was used to make suspensions of each bacterium, which were diluted and evenly spread on the surface of agar plates with sterile cotton swabs. Wells were made in the agar, and their filled with different concentrations of TiO_2 nanoparticles (25, 50, and 100 μg/mL). Plates were incubated for 24 hours at 37°C.

2.4 Antioxidant activity

2.4.1 DPPH assay

Antioxidant Activity of Green Synthesized TiO_2 Nanoparticles by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay. A DPPH solution (0.1 mM) was prepared in methanol and kept at dark room. Then, the DPPH solution was mixed with TiO_2 nanoparticles in concentrations of 25, 50, and 100 μg/mL, and the mixture was incubated at room temperature in the dark for 30 minutes. The absorbance of the mixture was then measured at 517 nm using a UV-Visible spectrophotometer after the incubation. DPPH methanol was considered as control and ascorbic acid was taken as standard antioxidant. The following formula was used to calculate the percentage of DPPH radical scavenging activity:

% DPPH Scavenging Activity = [(Acontrol – Asample) / Acontrol] × 100

where A-control is the absorbance of the control and A-sample is the absorbance of the TiO_2

2.4.2 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the green-synthesized titanium oxide nanoparticles (TiO_2 NPs) was measured by the deoxyribose degradation method. The reaction mixture included phosphate-buffered saline (PBS) pH 7.4. TiO_2 NPs were prepared at various concentrations (10, 20, 30, 40, and 50 μg/mL) in PBS. The mixture was mixed with TiO_2 NPs of the desired concentration and incubated at 37°C for 1 hour. Absorbance of the obtained pink-colored complex was recorded at 532 nm using a UV-Visible spectrophotometer. ascorbic acid was a used as the control.

Hydroxyl radical scavenging activity (%) = [(Ablank − Asample)/Ablank] × 100

Where A blank is the absorbance of the control reaction, and A sample is the absorbance of the reaction with the sample.

2.5 Anti-inflammatory activity

2.5.1 Bovin serum albumin denaturation assay

The anti-inflammatory activity of green synthesized strontium nanoparticles (TiO_2NPs) was determined by Bovine Serum Albumin (BSA) denaturation assay. The control was dimethyl sulfoxide (DMSO) and the standard reference drug was diclofenac sodium. 0.45 mL of 1% BSA solution was mixed with various concentrations (10, 20, 30, 40, and 50 μg/mL) of

TiO_2. The reaction mixture was adjusted to pH 6.3. The Samples were incubated at room temperature for 10min and then heated in a water bath at 55°C for 30min to facilitate protein denaturation. The reaction mixtures were then cooled before measuring the absorbance at 660 nm using a UV-Visible spectrophotometer.

2.5.2 Egg Albumin denaturation assay

Egg albumin denaturation assay, 2.8 mL of 1X phosphate-buffered saline (PBS) was mixed with 0.2 mL of fresh egg albumin. the reaction mixture, various concentrations (10, 20, 30, 40, and 50 µg/mL) of *Syzygium aromaticum* and *Thymus vulgaris* formulated Titanium oxide were added. And the solution was neutralized to pH 6.3 and maintained at ambient temperature for 10 minutes. the mixture was incubated for 30 minutes at 55°C in a water bath to denature the proteins. Dimethyl sulfoxide (DMSO) was adopted for control, and then the standard drug was diclofenac sodium. The samples were analyzed by UV-Visible spectrophotometer at 660 nm.

2.6 Cytotoxicity effect

The brine shrimp lethality assay, 2 g of iodine-free salt was dissolved in 200 mL distilled water to make saline water. About 10-12 mL of the prepared saline solution was added to each of six-well ELISA plates. Ten freshly hatched Artemia nauplii were gently transferred to each well using a micropipette. The wells were treated with different concentrations (20, 40, 60, 80, and 100 µg/mL) of TiO2 nanoparticles synthesized from *Syzygium aromaticum* and *Thymus vulgaris*. The plates were kept at room temperature in constant light for 24 hours.

The plates were observed and noted for the number of live nauplii's present and calculated by using following formula,

number of dead nauplii + number of live nauplii×100

2.7 Embryonic toxicology effect

2.7.1 Fish maintenance

Wild-type zebrafish (*Danio rerio*) were purchased from certified aquaculture vendors and maintained in aerated tanks with controlled environmental conditions at 28±2 °C, 14:10-hour light/dark cycle, the pH range: 6.8-8.0. Fish were fed two times per day with a mixture of commercially available dry bloodworms. To collect embryos, one female and three males were confined in breeding tanks for one night. fertilized embryos were collected and washed three times with freshly prepared E3 medium. then transferred healthy embryos, chosen based on the uniformity of their blastocyst stage, into sterile 24-well culture plates. Synthesis of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated titanium oxide nanoparticles (TiO_2 NPs) was prepared at various concentrations (5, 10, 20,40, and 80 µg/mL) and sonicated for 15 minutes for homogenization. These concentrations were made fresh in E3 medium and used for treatment groups. The exposure time was 24 to 96 hours post-fertilization (hpf), and daily monitoring and recording of the process of development, malformation development, heartbeat and survival rate were performed. Control groups were kept under the same conditions but not exposed to the nanoparticles. All wells were incubated at 28 °C, wrapped with foil paper to prevent direct sunlight. Dead embryos were removed every 12 hours, and all experimental treatments were done in triplicate to ensure reproducibility and statistical robustness.

2.7.2 Zebrafish embryo evaluation

The zebrafish (*Danio rerio*) embryos were monitored under a stereo zoom microscope during the exposure time postfertilization. From 24 to 96 hours post fertilization (hpf), embryos were exposed to different concentrations of green-synthesized titanium oxide nanoparticles (5, 10, 20,40, and 80 µg/mL). Developmental progress was assessed every 24 hours to watch for potential toxic effects. This study includes, embryonic and larval mortalities, hatching rates and morphological deviations. The structural deformations such as pericardial edema, tail curvature, yolk sac edema, and spinal malformations were recorded. The hatching rate was calculated as the number of hatch embryos that were free from chorion at 72 and 96 hpf divided by the number of total embryos. Images were taken of affected embryos and larvae using a HL-10A light microscope. The percentage of abnormal embryos was quantified at each time point and the egg counts were compared between treated and control groups to examine any concentration dependent effects.

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3. RESULT

3.1 Visual observation

Fig: 1shows a visual observation confirmed the initial evidence of titanium oxide nanoparticle synthesis. The plant extract of *Thymus vulgaris* and *Syzygium aromaticum* were mixed with the titanium dioxide precursor and the reaction mixture initially displayed a pale yellow to light brown color. The solution gradually yielded a deep brown suspension, showing the formation of nanoparticles after a 24-h incubation period at room temperature. A change in color that is visible indicates bio-reduction of titanium ions and bio-stabilization of the nanoparticles, likely through phytochemicals such as eugenol, thymol, and flavonoids existing in the plant extract.

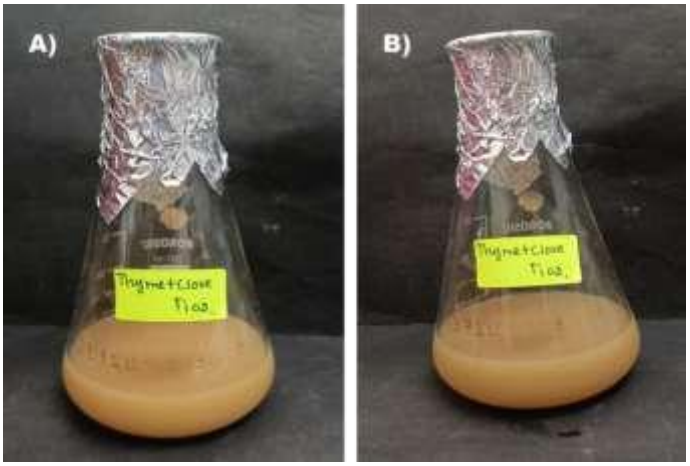


Fig: 1 Visual observation image of *Syzygium aromaticum* and *Thymus vulgaris* mediated Titanium oxide nanoparticles (Image A) Initial stage of *Syzygium aromaticum* and *Thymus vulgaris* mediated TiO2NPs. (Image B) Final stage of *Syzygium aromaticum* and *Thymus vulgaris* mediated TiO2NPs.

3.2 UV-visual spectrometer

Fig: 2 shows UV-Visual spectrometer of the synthesized Titanium oxide nanoparticles at 2 hours, 24 hours and 56 hours confirmed the formation of nanoparticles with characteristic absorption spectra. A distinctive absorbance maximum in the 350-380 nm region was detected, and intensity reached maximum at 2 h before a modest decline at 24 h. Specifically, the 56-hour sample displayed a slight rise in the 400-450 nm. Such a transition implies possible secondary growth, stabilization or slight aggregation of the nanoparticles.

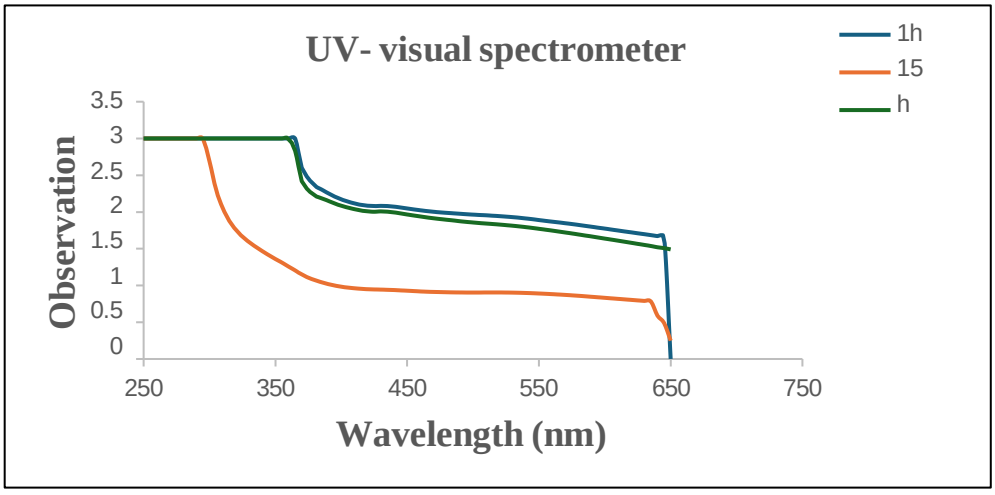


Fig: 2 UV-visual spectrometers of *Syzygium aromaticum* and *Thymus vulgaris* mediated Titanium oxide nanoparticles.

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3.3. X-ray diffraction

The green-synthesized TiO_2 NPs was obtained through XRD analysis, which displays the XRD pattern of TiO_2 NPs synthesized by *Thymus vulgaris* and *Syzygium aromaticum* formulation, respectively, Fig: 3 shows characteristic diffraction peaks were observed at 25.3°, 37.8°, and 48.0°, which were assigned to the (101), (004), and (200) indicating the successful synthesis of crystalline TiO_2 NPs. High purity of synthesized compounds and the peaks were broad, suggesting the particle size of the compound is of nanoscale. The average crystallite size was determined to be 20 nm using the Debye-Scherrer equation and falls within the expected range of sizes for biologically synthesized TiO_2 NPs. The existence of anatase phase with photocatalytic and biomedical activity also adds further support for potential uses of these NPs in antimicrobial, antioxidant and drug delivery systems. These results show that the green synthesis method led to the fabrication of nanoscale phase-pure TiO_2 materials suitable for subsequent biological testing.

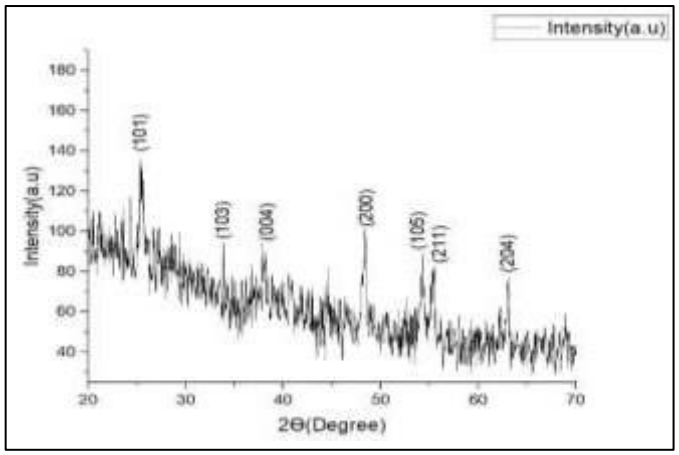


Fig: 3 X-ray diffraction graph of *Syzygium aromaticum* and *Thymus vulgaris* mediated Titanium oxide nanoparticles.

3.4 FTIR

In FTIR, the absorption of transmittance at particular wavenumbers due to resonant absorption of infrared light by quantised vibrational modes of the molecular bonds, is a technique for the determination of functional group composition. Fig: 4 shows the strong absorption at around 1700 cm^{-1} would be ascribed C=O stretching from carbonyl function and a broad intense absorption between 3200-3550 cm^{-1} would appear from O-H stretching modes, which have been attributed to alcohols, phenols and carboxylic acids. The absorption peaks, vibrational modes, and relative intensity values must be accurately recorded and systematically analyzed in order to perform a rigorous spectral interpretation and to probe the functional groups involved.

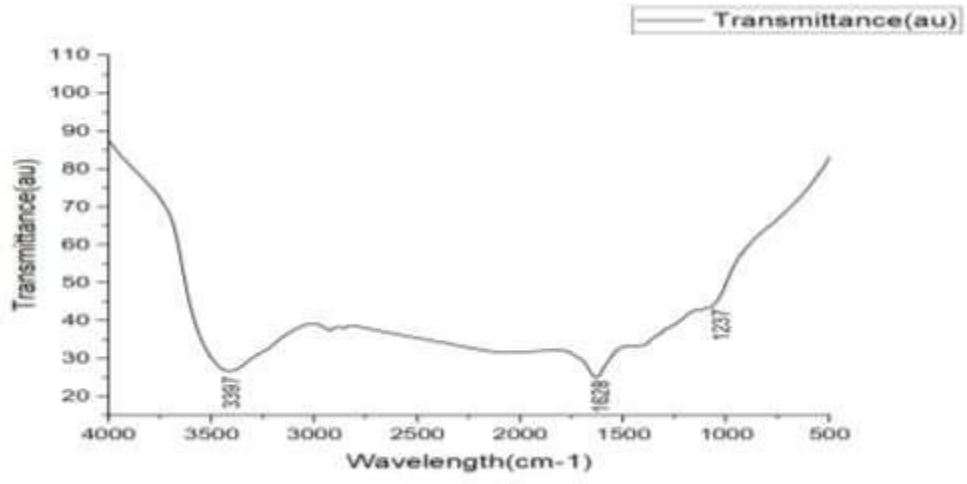


Fig: 4 FTIR Graph of *Syzygium aromaticum* and *Thymus vulgaris* mediated Titanium oxide nanoparticles.

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3.5 TEM Analysis of Titanium Oxide

Transmission Electron Microscopy (TEM) was used to characterize the morphology and structural properties of the synthesized TiO_2 nanoparticles. Fig: 5shows a TEM images of Panels A and B exhibit clean nanocrystalline lattice fringes, indicating the crystallinity of the particles, the anatase phase of TiO_2, which is well known to impart a powerful biological and photocatalytic property. In the image of C and D, the nanoparticles are predominantly spherical to slightly irregular and between 20 and 50 nm in size. The particles are relatively agglomerated but individual nanoparticles are present, Then the presence of natural stabilizers such as thymol and eugenol from thyme and clove extracts used in the synthesis. The TEM analysis, green synthesis of the TiO_2 nanoparticles with uniform size and crystalline structure.

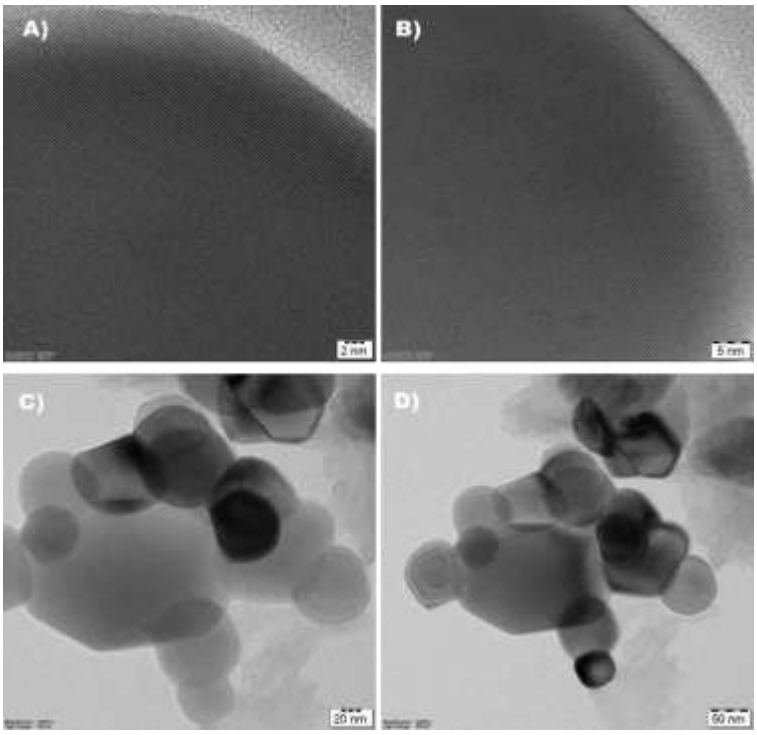


Fig: 5 TEM image of Green synthesis Titanium oxide nanoparticles A) Imageshows a 2nm, B) Image of 50nm scale, C) Scale of 100nm, D) Scale of 200nm of Titanium oxide nanoparticles.

3.6 Scanning Electron Microscope (SEM) of Titanium oxide

The surface morphology, of green-synthesized TiO_2 nanoparticles, SEM was performed by changing magnifications. Fig: 6 shows the image A shows the nanoparticles as irregular and crystalline aggregates with rough texture surface. image B, the same particle clusters are seen to intersperse, such clustering, although common in green-synthesized nanoparticles and influenced by the phytochemicals, which play the role of capping and reducing agents. Image C reveals a wider area of nanoparticle surface distribution, signified by the predominance of larger agglomerates in the field. The morphology of nanocomposite has rough surface morphology indicative of organic residues from plant extracts that helps in particle stabilization and bioactivity. The TiO_2 nanoparticles synthesized via green synthesis using T. vulgaris and S. aromaticum display a heterogeneous but compact morphology with particle agglomeration and surface roughness that may be attributed to phytochemicals capping.

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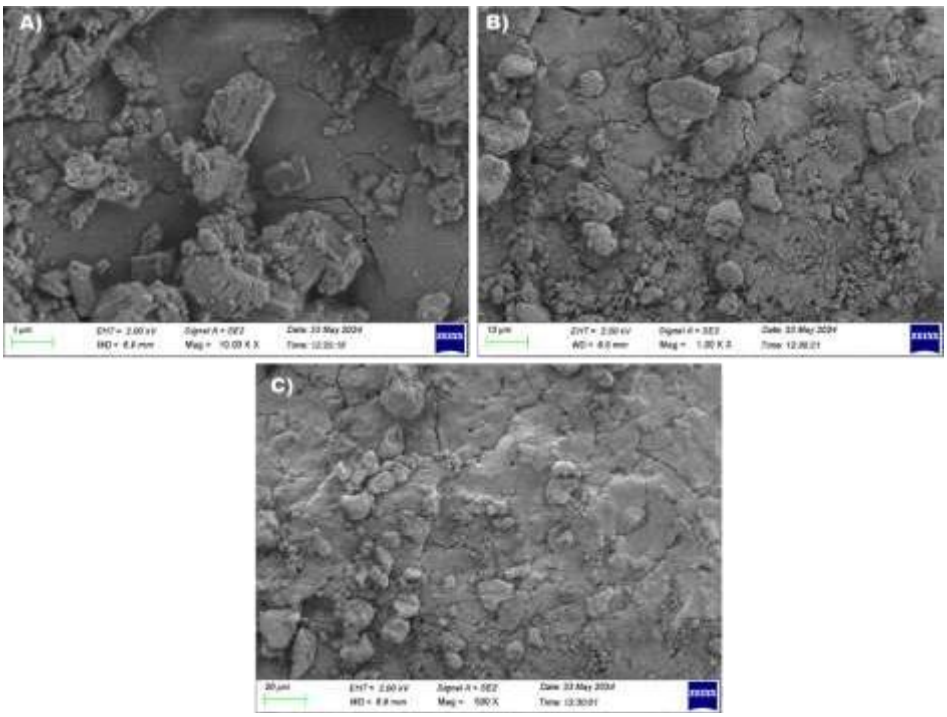


Fig: 6 SEM image of green synthesis of Titanium oxide nanoparticles

3.7 Antimicrobial activity

Fig: 7 shows, antimicrobial activity of green synthesized titanium oxide (TiO_2) nanoparticles of Thymus vulgaris and Syzygium aromaticum was determined by agar well diffusion method. Mueller-Hinton agar plates were autoclaved at 121 °C for 15-20 minutes. After cooling, agar was dispensed into sterile Petri dishes and allowed to solidify at room temperature. *Streptococcus mutans, Staphylococcus aureus, Candida albicans, Enterococcus faecalis, Lactobacillus sp.* was used for the assay. Fig: 8 shows Agar surface was uniformly streaked with sterile cotton swabs with established bacterial and fungal suspensions. Using a sterile micropipette tip, wells of 9 mm diameter were created in the agar. 100 µL of TiO_2 nanoparticle suspension (25, 50, and 100 µg/mL) was added to each well. Plates were incubated at 37°C for 24 hours for bacterial strains and up to 48 hours for fungal cultures. After a period of incubation, each well's zone of inhibition was measured with a clear ruler and recorded in millimetres. The results were considered according to the size of the clear zones that indicate the antimicrobial activity of nanoparticles.

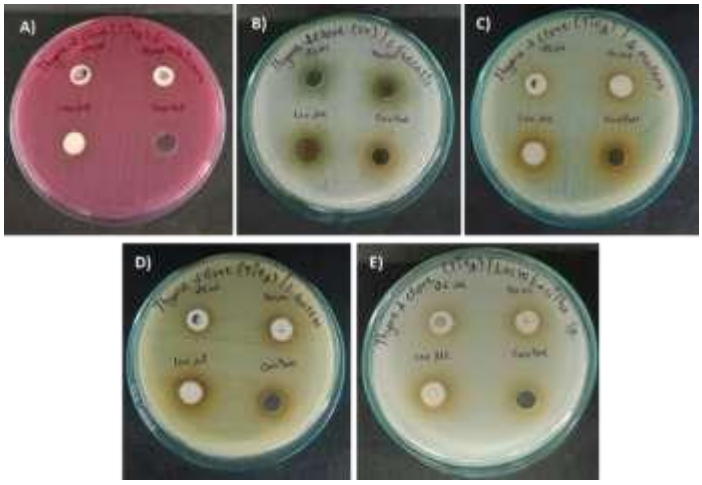


Fig: 7 Antimicrobial activity of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles against oral pathogens, A) C.albicans, B) E.faecalis, C) S.mutans, D) S.aureus, E) Lactobacillus.

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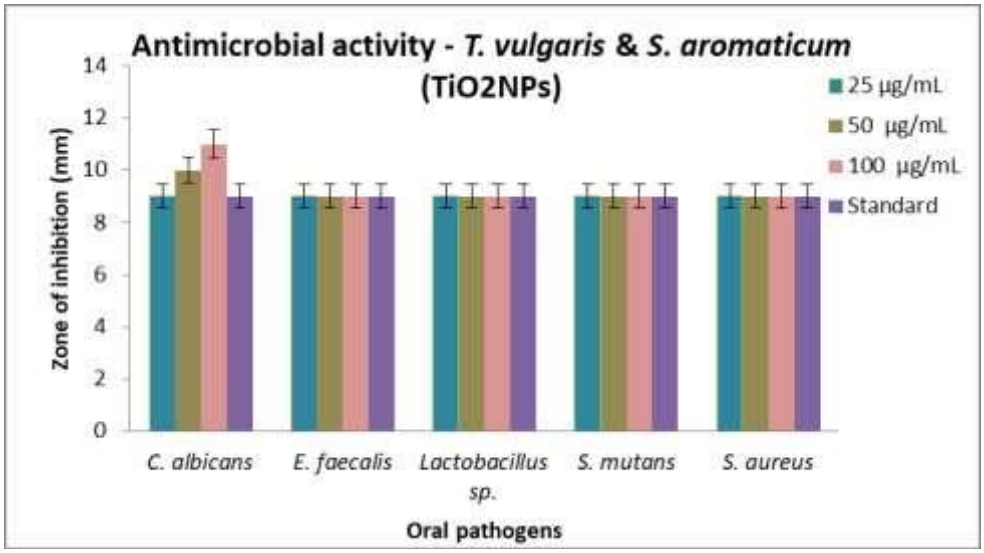


Fig: 8 Graph demonstrating antimicrobial activity of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles against oral pathogens.

3.8 Antioxidant activity

3.8.1 DPPH

The DPPH radical scavenging assay showed that the green-synthesized titanium dioxide (TiO_2) nanoparticles based on extracts of *Thymus vulgaris* and *Syzygium aromaticum* have high antioxidant activity. The inhibitory effect increased with increasing concentration of nanoparticles (10,20,30,40 and 50 µg/mL). Fig: 9 shows at the maximum concentration of (50 µg/mL), the TiO_2 NPs exhibited the highest degree of DPPH scavenging relevance, comparable to that of ascorbic acid, standard antioxidants. The strong potential of these nanoparticles in the neutralization of DPPH free radicals through the transfer of hydrogen atoms or electrons demonstrates a strong action against oxidative stress. The phytochemicals in *Thymus vulgaris* and *Syzygium aromaticum* formulation were involved in reduction and stabilization of TiO_2 NPs, which imparts considerable antioxidant capacity to the biobased Nanoparticles.

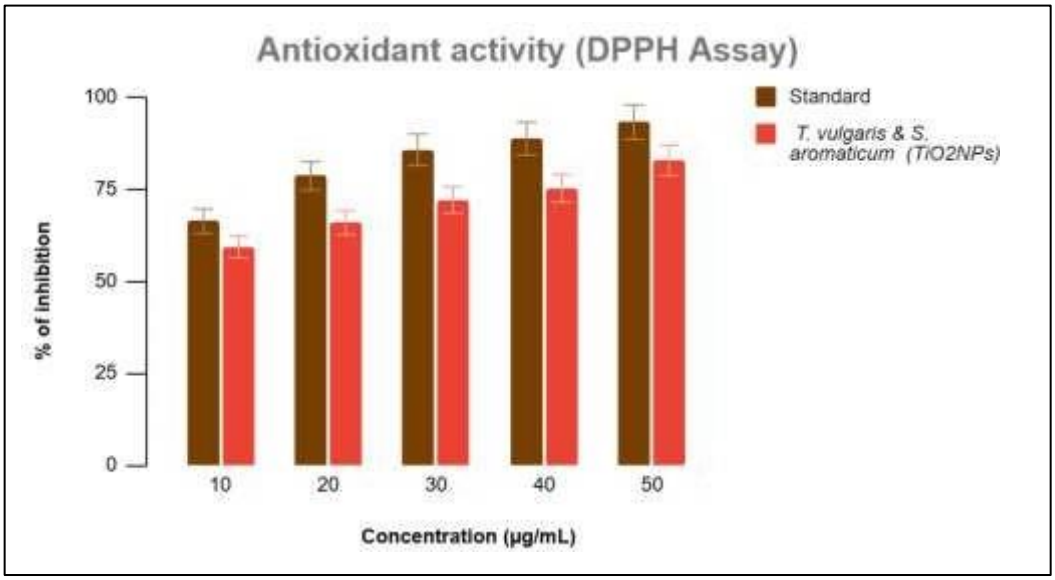


Fig: 9 DPPH assay of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles performed at different concentrations.

3.8.2 H_2O_2 assay of Titanium oxide

The green-synthesized titanium dioxide (TiO_2) nanoparticles of phytogenic mediation of *Thymus vulgaris* and *Syzygium aromaticum* formulation displayed concentration of antioxidant activity as revealed through a hydrogen peroxide (H_2O_2) scavenging assay. Fig: 10 shows Increasing the concentration of the nanoparticles from 25 to 100 µg/mL progressively improved the scavenging efficiency. The TiO_2 NPs exhibit approximately lower activity than the standard antioxidant but effectively scavenged free radicals too as indicated by the ability to scavenge H_2O_2 and the significant radical scavenging effect.

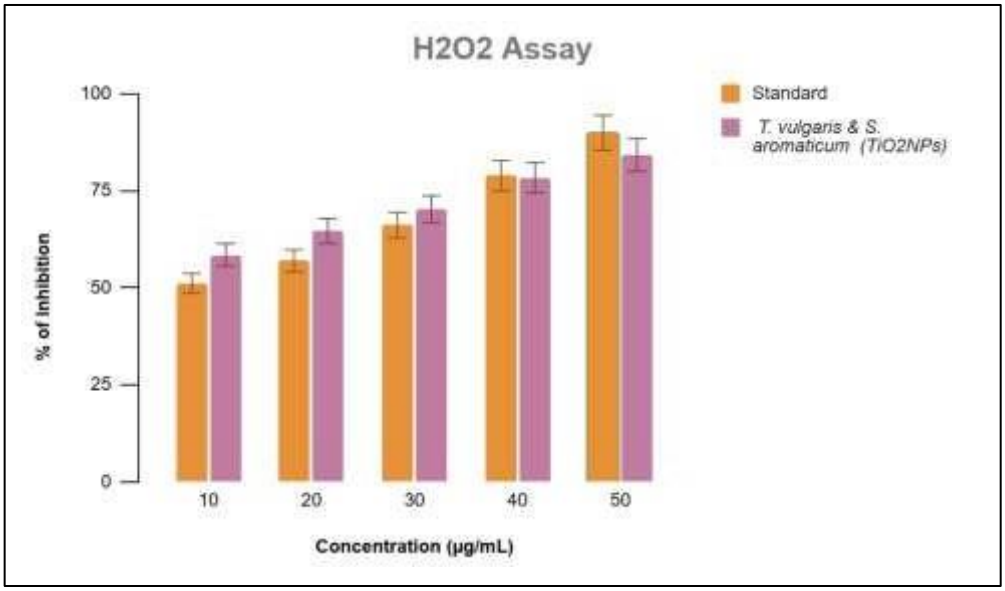


Fig: 10 H_2O_2 assay of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles performed at different concentrations.

3. 9 Anti-inflammatory activities

3.9.1 Bovin serum albumin (BSA) assay

The anti-inflammatory activity of the green-synthesized TiO_2 nanoparticles using the Bovine Serum Albumin (BSA) denaturation method, an in vitro technique to assess the ability of various compounds to inhibit protein denaturation, Fig: 11 shows the TiO_2 NPs, at lower concentrations (10–20 µg/mL), the nanoparticles showed mild inhibition, However, the inhibition percentage was significantly improved by increasing the concentrations from 30 to 50 µg/mL. the potential for the TiO_2 NPs to actively stabilize proteins and inhibit denaturation, which is a fundamental process of inflammation. The anti-inflammatory activity of TiO_2NPs surface and antioxidant-rich phytochemicals. Thus, the results, thyme and clove mediated TiO_2 NPs as a natural anti-inflammatory mediator.

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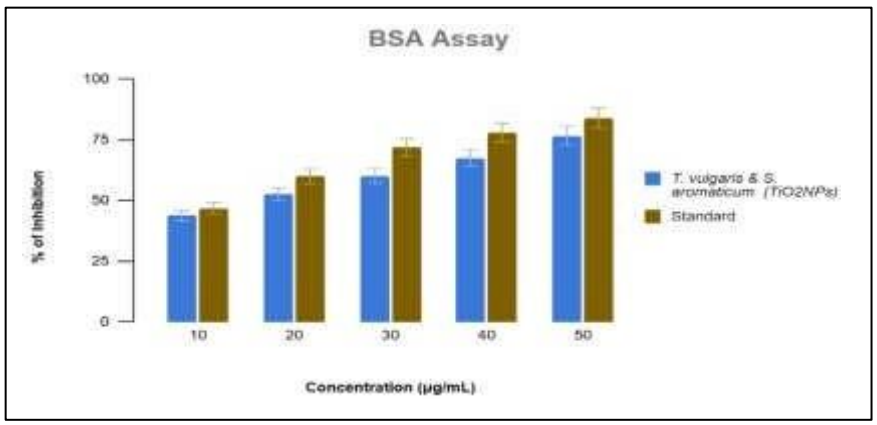


Fig: 11 Bovin serum albumin assay of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles.

3.9.2 Egg albumin assay

The anti-inflammatory activity of *Thymus vulgaris* and *Syzygium aromaticum* mediated TiO_2 NPs was evaluated in the egg albumin denaturation assay. Fig: 12 shows At 10-20 µg/mL concentrations, inhibition of TiO_2 NPs was in the moderate range of nearly 45% to 55% and thus lower in potency compared to the standard. However, increasing the concentration from 30 to 50 µg/mL significantly increased the anti-inflammatory activity. At 50 µg/mL, the inhibition percentage was nearly 80%, very similar to the standard, showing the significant anti-inflammatory potential. The TiO_2 NPs as natural and phytogenic anti-inflammatory agents, oral pathogens.

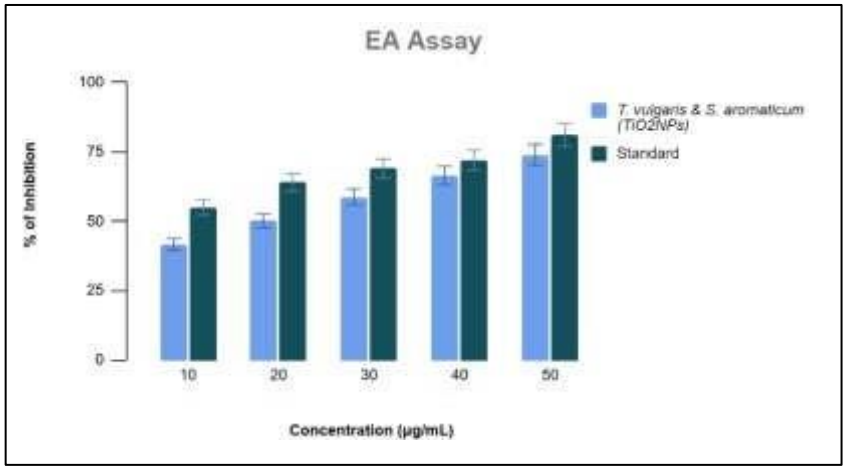


Fig: 12 Egg albumin activity of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles.

3.10 Cytotoxicity

The cytotoxic activity of green-synthesized titanium dioxide (TiO_2) nanoparticles using *Thymus vulgaris* and *Syzygium aromaticum* formulation. Fig: 13 shows the concentration and time-dependent response on brine shrimp nauplii. TiO_2 nanoparticles exhibited low toxicity at lower concentrations (5,10, 20, 40 and 80 µg/mL), with approximately 80% of nauplii still alive after 24 and 48 h. At the highest concentration (80 µg/mL), survival decreased to less than 50% by the second day, indicating increased toxicity at higher doses.

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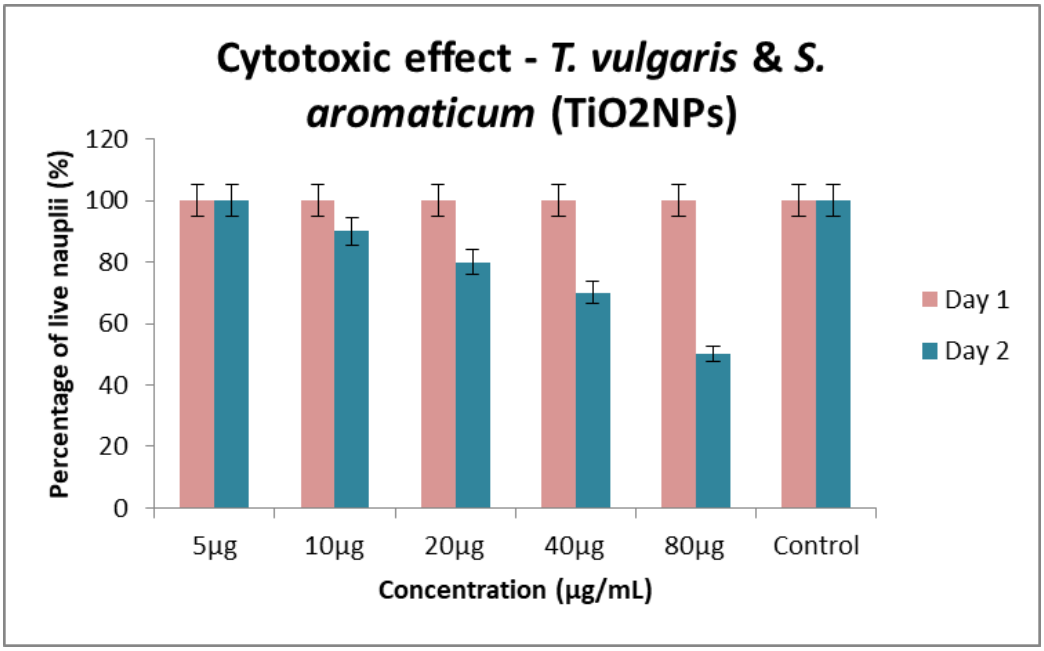


Fig: 13 Cytotoxic effects of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles.

3.11 Embryonic toxicology

The embryotoxicity effects of the green-synthesized nanoparticle formulation were evaluated the invitro and in vivo tests such as zebrafish embryonic hatching, cell viability, and morphological studies. Fig: 14 shows the cytotoxicity assessed by a cell viability assay showed that cell viability tended to decrease dose dependently, with higher concentration of 20 µg/mL significantly decreasing cellular viability compared with the untreated control group with a maximal viability 65 %. Corresponding lower concentrations (5–10 µg/mL) exhibited moderate cytocompatibility effects, indicating a potential therapeutic limiting concentration for the future biomedical application. Fig:14 shows the cell hatching assay in zebrafish embryos also revealed a concentration dependent reduction in hatching frequency. Hatching in control embryos was more than 60%, whereas exposure to 20 µg/mL and greater led to a significant inhibition of hatching to 30%, suggesting interference with developmental progression, which could be attributed to oxidative stress induced by the nanoparticles. Fig: shows Embryos from the control and low dose (A, B and E) groups developed normally and showed good development, such as somites formation, retinal pigmentation, and blood circulation. In contrast, zebrafish embryos treated with high doses showed severe teratogenic effects such as yolk sac edema, axial malformations and retarded development. at lower concentrations, the Nanoparticle formulation becomes cytotoxic and embryotoxic at increased doses and calls for careful dose optimization in future biomedical and dental applications

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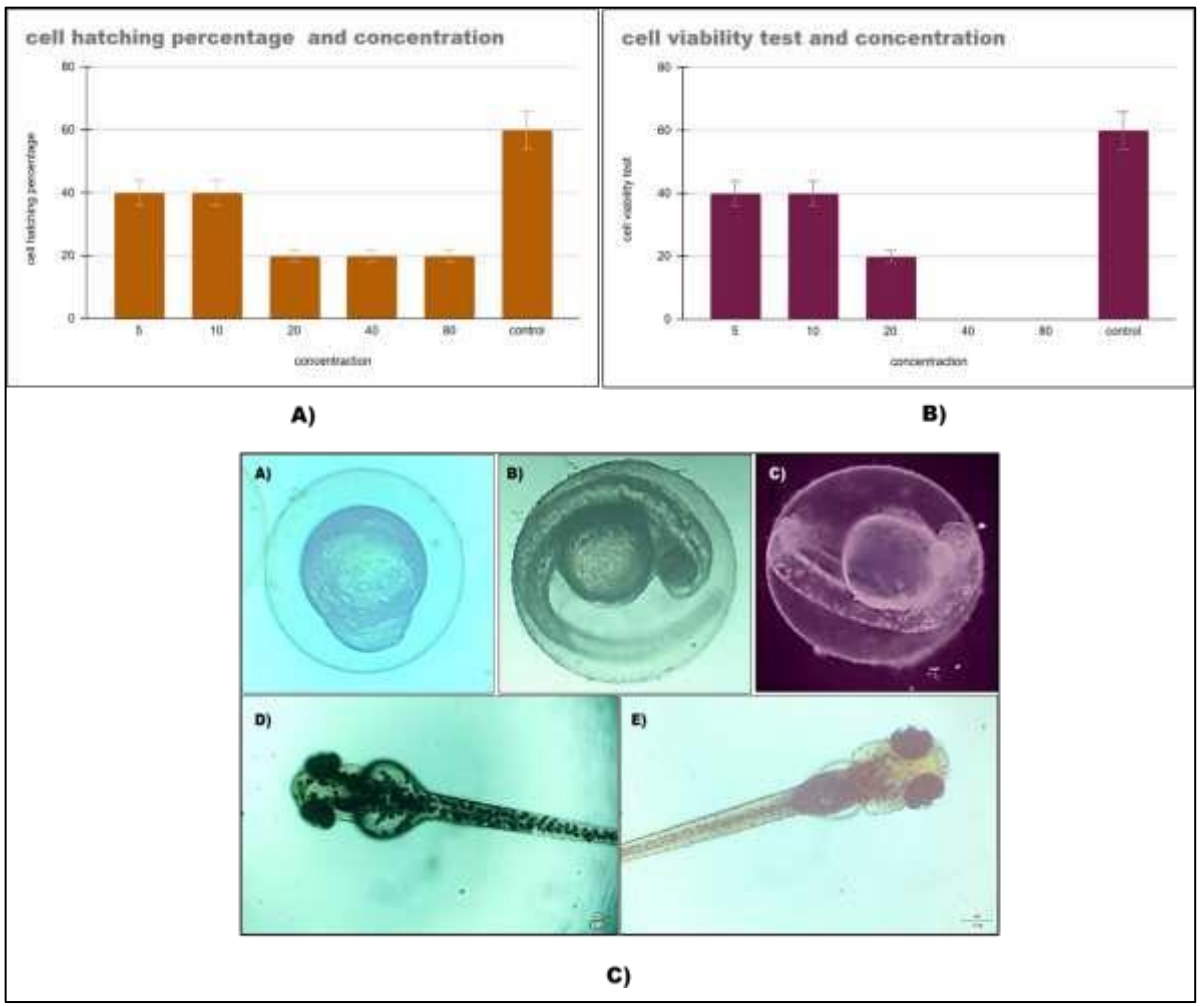


Figure: 14 Zebrafish embryonic toxicology evaluation of Titanium oxide Nanoparticles A) cell viability test of *Syzygium aromaticum* and *Thymus vulgaris* formulation mediated Titanium oxide nanoparticles. B) Cell hatching percentage of TiO2 C) Morphology observation of zebrafish embryo.

4. DISCUSSION

Green synthesis of titanium dioxide (TiO_2) nanoparticles by using *Thymus vulgaris* and *Syzygium aromaticum* extracts and it was characterized by several techniques. UV-Visible analysis of strontium nanoparticles demonstrated a symmetric peak in the range 350-380 nm at 2 hours indicating an early formation of nanoparticles, while trace of peak was noticed at 24 and 56 hours. This development indicates that the nanoparticles mature, aggregate, or undergo secondary growth during the aging period. The XRD patterns demonstrated that TiO_2 nanoparticles formed were crystalline in nature with anatase phase peaks at 25.3°, 37.8°, and 48.0° and average crystallite size of about 20 nm. FTIR analyses identified the functional groups hydroxyl, carbonyl, phenolic groups responsible for reduction and stability of nanoparticles. TEM and SEM images revealed that the particles were mostly spherical in shape with a size of 20–50 nm approximately and presented some agglomeration, probably owing to the plant capping agents as thymol and eugenol. The biological tests reported that the green synthesized TiO_2 NPs have effective antimicrobial activity against the most important oral pathogens and the inhibition zone is then dose dependent. Antioxidant activity became apparent using the DPPH and hydrogen peroxide trapping assays for free radical quenching at higher nanoparticle concentrations. Anti-inflammatory studies of BSA and egg albumin assays demonstrated considerable inhibition of protein denaturation especially at higher concentrations, suggesting, the prospects of the nanoparticles in inflammation control. Cytotoxic effect and embryotoxic effects showed that lower doses (up to 10 μg mL −1) of TiO2 NPs ensure excellent biocompatibility, whereas higher doses cause decrease of cell viability and developmental anomalies in zebrafish embryos.

These results indicate the importance of fine tuning the doses in biological applications. the TiO_2 nanoparticles by using *T. vulgaris and S. aromaticum* may provide a human-safe, eco-friendly opportunity to develop multifunctional nanoparticles with strong antimicrobial, antioxidant, and anti-inflammatory activities, these would be applicable for oral or therapeutic purposes.

5. CONCLUSION

In the conclusion, Titanium dioxide (TiO_2) nanoparticles using green synthesis using *Thymus vulgaris* and *Syzygium aromaticum* extracts have been reported and structural, nanoscale and biological activity, phytochemical based capping has been confirmed by an advanced study of UV-Vis, XRD, FTIR, TEM and SEM. The biosynthesized TiO_2 NPs demonstrated good crystalline nature with anatase phase and the phyto-capping functional groups, which confirmed their stability and bioactivities. Biological assessments showed that TiO_2 NPs exert strong antimicrobial effects on all tested oral pathogens and show high antioxidant and anti-inflammatory activities. Concentration-dependent toxicity was observed in brine shrimp and zebrafish embryos in the cytotoxicity assay low concentrations were still highly biocompatible with adverse effects seen only at high concentration, Cell viability experiments also demonstrated a therapeutic dose window where cells underwent stress and morphological destruction. In this study, the potential of green synthesized TiO_2 NPs as in oral and biomedical uses as they have multi-functional nature. the synthesis of green nanoparticles of therapeutic significance with low-toxic effect on environment.

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