Green Synthesis of TiO_2 Nanoparticles using Averrhoa Bilimbi Fruit Extract and their Applications in Photocatalytic Dye Degradation and Anti-bacterial Activities

Dhanya S[1*], Jayaprabha K N[2], Anupama S[3]
[1*,2,3]PG & Research Department of Chemistry, NSS Hindu College, Changanacherry, Kerala, India– 686102.
***Corresponding Author: Dhanya S[1*]**
Email: [1*]dhanya.ark@gmail.com

Abstract:
Due to their numerous distinctive characteristics, nanostructured materials are currently a crucial topic of research. One of the most promising materials today are TiO_2 nanostructures, which are one among the transition metal oxides. Due to the absence of any potentially dangerous ingredients and commercially viable procedure, green approaches of nanoparticle synthesis have significant benefit over traditional approaches. The present work reported, the photocatalytic as well as anti-bacterial activity of green synthesized titanium dioxide (TiO_2-NPs). An aqueous fruit extract of Averrhoa bilimbi as a capping as well as reducing agent, is utilized for the synthesis of TiO_2-NPs. The prepared TiO_2-NPs have been characterized by spectral methods using powder XRD (X-ray diffraction) and FTIR (Fourier transforms infrared spectroscopy). Morphological characterization by SEM analysis showed that the synthesized was spherical structure with an average particle size of 60 nm. The photocatalytic dye degradation and anti-bacterial activity have been performed and the result revealed the impressive capability of TiO_2-NPs. Hence, the current research work offers a futuristic outlook on a green photocatalysts for profound applications.

Keywords: Green Synthesis, Averrhoa Bilimbi, Titanium Dioxide, Photocatalytic Activity, Methylene Blue, Anti-Bacterial.

1. Introduction

In nanoscale material sciences, metal oxide nanoparticles are a captivating class with an exponential increase in their diverse applications. Several metallic elements tend to react with oxygen under several conditions to form metal oxides with various structural forms [1]. Oxide nanoparticles can exhibit unique properties including electronic, optical and chemical properties due to their limited size and a high density of corner or edge surface sites. Metal oxides with nanoscale size range showed essential applications, such as fluorescence and optical sensing [2,3], catalysis [4,5], photovoltaics [6] etc. Because of their unfilled d-shells, transition metal ions allowed for reactive electronic transitions, high dielectric constants, wide band gaps and superior electrical characteristics [7]. Innumerable applications such as batteries, catalysis, chemical sensing, drug delivery, solar cells and magnetic storage media are recognized with metal oxide nanoparticles, make them a potential candidate in future era.

Among the metal oxide NPs, TiO_2-NPs. show great potential for its use as a photocatalyst and biological activities due to its unique and desirable properties like simplicity, low cost, non-toxic, high degradation efficiency and excellent stability [8]. There are three common polymorphs of TiO_2 crystal found in nature: brookite, anatase, and rutile. TiO_2, is a chemically stable, biocompatible, non-toxic, oxidizing agent with high photocatalytic activity. TiO_2 is a semiconductor with a band gap of 3.2 eV (for anatase). TiO_2 with band gap energy of 3-3.5 eV promotes the photo catalytic behavior in a considerable way [9]. The electron transfer mechanism from valance band to conduction band in TiO_2 during exposure to UV or visible light generates a hole (h+), which further contacts with water to form an OH radical. This OH radical will act as a strong oxidizing agent and stands responsible for removing organic pollutant dyes, pesticides and heavy metals through photo oxidation mechanism [10]. Thus, due to its exceptional catalytic, antibacterial and unique semiconducting proficiencies, TiO_2 is a substance of tremendous relevance. TiO_2 nanoparticles were synthesized with regulated crystal phases, which makes them particularly suitable for a variety of high-performance applications.

There are several physical and chemical synthesis methods available for metal oxide nanoparticles, but synthesis through green route is less toxic, simple, economical and eco-friendly way to synthesize nanoparticles from various extracts of plants, microbes, enzymes, etc. [11]. The major benefit of using plant extracts for synthesizing NPs is that it allows the selection of an environmentally benign stabilizing agent, reducing agent, and solvent medium for NPs [12]. A wide variety of compounds such as flavanones, terpenoids, alkaloids, pigments, proteins, amines, amides and phenolics are present in the plant extract, which help in the stabilization as well as reduction and of metal ions during NP synthesis by green method [13,14].

Several research works on green synthesized metal oxide nanoparticles using various fruit extract *Citrus sinensis* and *Musa acuminata* [15], Orange Fruit [16], North Arcot (*Syzygium alternifolium*) [17], Guava (*Psidium guajava L*) [18] are reported. Therefore, this study was intended to synthesize, titanium dioxide nanoparticles from the fruit extract of Averrhoa bilimbi using a simple, green approach. Previous reports are available for the synthesis of noble metal nanoparticles, but the synthesis of a metal oxide such as TiO_2 nanoparticles from $TiCl_3$ using Bimbili fruit extract was not reported yet. Further, the synthesized NPs can be explored for dye degradation by photo catalytic method and antibacterial activity. In the present work, we are going to explore the potential use of TiO_2-NPs in dye degradation by synthesising them utilising a green method that it just requires a few inexpensive chemicals and straight forward steps.

2. Experimental

2.1 Materials

$TiCl_3$, NaOH (Nice chemicals, India), All of the chemicals were analytical grade and used as purchased without further purification. The solutions were prepared in distilled water. Bilimbi fruits were collected from the local market of Kottayam district, Kerala, India. All the above chemicals were used without further purification.

2.2 Synthesis

2.2.1 Preparation of Bimbili Fruit Extract

The fruits were cleaned thoroughly 2–3 times using distilled water and cut into small pieces. Then 50 g of Bimbili fruits was grinded along with100 mL distilled water and the extract has been gathered in separate conical flask using the conventional filtration process using Whatman filter paper (40). The filtered fruit extract was collected and stored in refrigeration for further usages.

2.2.2 Synthesis of Titanium Dioxide Nanoparticles by Green Method

A solution was made by adding 10 ml Bimbili fruit extract and 10 ml of 15% $TiCl_3$ and diluted to 50 ml with distilled water, stirred well using a magnetic stirrer with high rpm for 10 – 15 minutes. Then 100 ml water is added and heated on a hot plate with a stirrer. During heating, the colour of the solution changed from violet to white and eventually, after 1 h, a white solid phase precipitate is obtained. Finally, the precipitate was centrifuged for 3–4 times using distilled water, washed, dried and the nanoparticles were collected.

2.3 Characterization

Synthesized TiO_2-NPs were studied for their physical and structural properties using various techniques. Surface chemistry and organic functional groups were studied by by using Perkin Elmer FT-IR Spectrometer (ATR, Lab India Instruments Pvt. Ltd) in the range of 4000-400 cm^{-1}. The crystalline TiO_2 NPs structure was characterized by X-ray diffraction profiles at an acquisition rate of 2°/ min at 40 kV and 30 mA, using XRD (Rigaku Miniflex 600). Surface morphological characterizations were observed by SEM (Carl Zeiss EVO Edn: 18 Cryo Environmental SEM) instrument. Absorbance measurements were done using a Colorimeter (Systronics, India).

2.4 Photocatalytic Studies

After successful confirmation of TiO_2-NPs synthesis, they were used for the methylene blue dye decolorization under direct sunlight. Methylene blue dye concentration of 10^{-6} M (50 mL) was added to 3.00 g of synthesized TiO_2-NPs. A sample was also maintained containing only dye. The mixture was stirred magnetically for 30 min in dark condition prior to exposure to direct sunlight irradiation. At specific intervals (every 30 min), an aliquot of 5ml of the suspension was taken and centrifuged for 10 min at 10,000 rpm to obtain a clear supernatant and the absorbance was measured using a colorimeter in the wavelength range of 650 nm for 2hours. Then a graph was plotted between concentration vs time of incubation to check the dye degradation capacity of the synthesised TiO_2-NPs.

2.5 Anti-bacterial studies

The antibacterial activity of titanium dioxide nanoparticles was tested by the agar disc diffusion method. First, the nutrient agar was uniformly spread in the Petri dish plate. Then fix the 5 mm diameter well, which is used to study the inhibition zone. Place 20 µg/ml and 10 µg/ml of TiO_2-NPs in 6 mm diameter well. The culture medium was incubated at 37 °C for 24 h under aerobic conditions. The zone of inhibition layer was measured using the millimeter region using a zone reader. The Zone of inhibition results in the antibacterial activity of TiO_2 -NPs.

3. Results and Discussion

The one-step green synthesis of titanium dioxide nanoparticles (TiO_2-NPs) utilizing an aqueous fruit extract of *Averrhoa bilimbi* was accomplished at room temperature. The reaction mixture of containing $TiCl_3$ and aqueous Bimbli fruit extract changes its colour from violet to white and eventually a white the solid phase precipitate is obtained. The change in colour of reaction mixture was considered as the preliminary conformation for the formation of TiO_2-NPs. The mechanism of TiO_2-NPs formation via biogenic synthesis is not well elucidated yet. The plausible mechanism is that the phytochemicals present in the fruit extract converts the chloride salt of titanium to corresponding hydroxides and then to the oxides NPs.

3.1 XRD Studies

X-ray diffraction spectroscopy was employed for the phase, purity, and structural average crystallite size values of the as-synthesized TiO_2-NPs using bimbli fruit extract. All the results indicate that pure TiO_2-NPs were successfully synthesized. Figure1 represents the X-Ray diffraction pattern of TiO_2-NPs in powder form. The sample was analysed at a range of angles from 0° to 100° with Cu Kα radiation at a scanning speed of 2˚/min. The crystallite size was determined by Scherrer's formula, $t = (0.9\ \lambda/\beta \cos \theta)$ where λ is the wavelength characteristic of the Cu Kα radiation, β is the full width at half maximum (in radians) and θ is the angle at which 100 intensity peak appears.

Significant peaks in a 2θ range of $10° < 2\theta < 80°$ were observed at 25.07^0, 35.92^0, 40.99^0, 53^0, 54.02^0 and 63^0 and suitably matches to the Miller indices (hkl) values:(101), (004), (112), (105), (211) and (204) respectively, to confirm the anatase phase. The peaks corresponds to Brookite phase also appear and a mixture of both the anatase and brookite phases of TiO_2 existed. Obtained results were justified with JCPDS (Joint Committee on Powder Diffraction Standards) XRD patterns of TiO_2 anatase (JCPDS card no. 21-1272) and TiO_2 brookite (JCPDS card no. 29-1360). High crystalline nature of the NPs which was indicated by a sharp peak, favours the photo catalytic activity. The size of the nanoparticles was calculated by adopting Debye-Scherrer's equation using the (101) plane and results confirms that the nanoparticles were in the average crystallite size of 18±2 nm. As a result, it is explained that TiO_2 nanoparticles possess a large surface area, which lead to excellent photocatalytic performance.

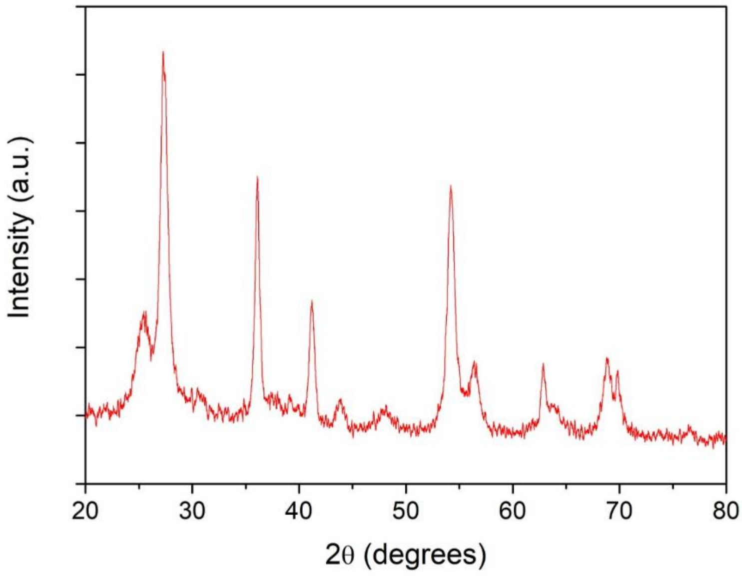

Figure 1: XRD pattern of synthesised TiO_2-NPs

3.2. FT-IR Studies

FT-IR gives the composition and formation of functional groups of the synthesized TiO_2-NPs. It also suggests that the formation of TiO_2-NPs is due to the interaction of the phenolic compounds, alkynes, terpenoids, and flavonoids present in the fruit extract. Figure. 2 represents FT-IR spectra of the synthesized TiO_2-NPs in the range 400–4000 cm^{-1}. The functional groups were responsible for reducing Ti ions to TiO_2, which was observed as bands. The bands in the IR spectrum indicate the stretching modes that correspond to the functional groups responsible for the observed reduction of Ti ions into TiO_2.The broad peak identified at 3000-3500cm^{-1} correspond to O-H stretching of phenolic compounds present in the fruit extract. The alkene group's presence was attributed to 1700cm^{-1}, and the band at 1300 cm^{-1} corresponds to C–N stretching bonds of the amines. The peak at 500 cm^{-1} corresponds to the Ti–O functional group was also identified in the FT-IR spectrum.

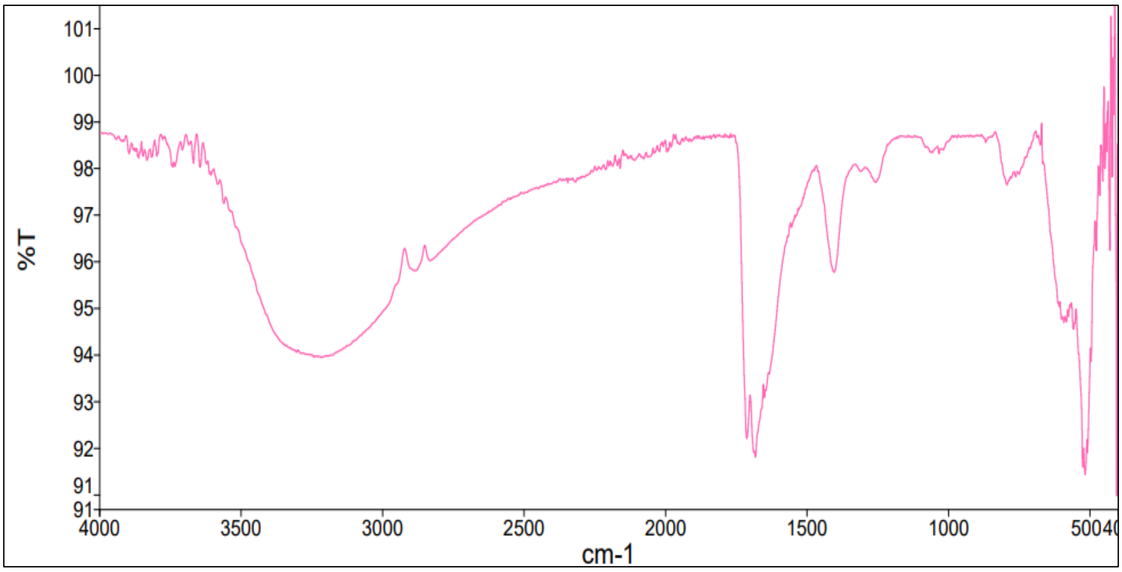

Figure 2: FT-IR pattern of synthesized TiO_2-NPs

3.3. SEM Studies

The SEM image of the synthesised TiO_2-NPs synthesized using aqueous Bimbli fruit extract is presented in Figure 3. From the SEM analysis, it is evidenced that these nanoparticles show spherical morphology and the average particle size was found in the range of 60 nm. The particle size obtained from SEM images exhibit good correlation with the average crystallite size from XRD. The smaller particle size of the TiO_2-NPs well explained its antibacterial activity [19, 20].

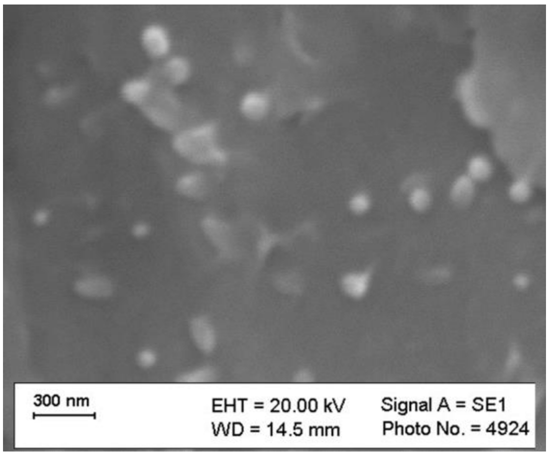

Figure 3: SEM images of synthesized TiO_2-NPs

3.4 Photocatalytic activity

The photocatalytic activity of TiO_2-NPs was evaluated by the photodegradation of methylene blue dye under visible light at a wavelength of 650nm. Figure 4 shows the degradation of methylene blue dye intensity with time. The reaction was carried out for a period of 180 min. From the figure, it was evident that the degradation of dyes was proportional to the time of exposure to TiO_2-NPs. The degradation of dye was achieved almost completely. Thus, the biogenic preparation of TiO_2-NPs using bimbli fruit extract was effective in degrading methylene blue and was in agreement with the previous reports.

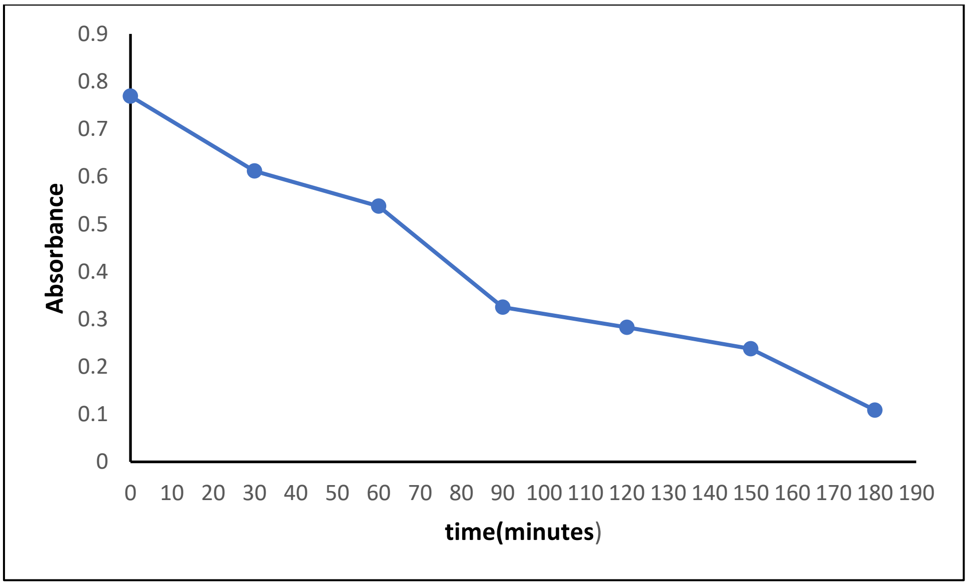

Figure 4: Reduction of methylene blue dye absorbance intensity with time usingTiO_2-NPs.

3.5 Anti-bacterial activity

The antibacterial activity of titanium dioxide nanoparticles was examined by gram-negative bacteria Ecorechia Coli. Figure 5 shows anti-bacterial activity of titanium dioxide nanoparticles. The zone of inhibition layer of the

TiO_2-NPs against Ecorechia Coli. is measured in mm scale. The zone of inhibition (ZOI) of prepared TiO_2-NPs at two different concentrations S1(20µg/ml) and S2(10µg/ml) are 9mm and 8 mm respectively and hence exhibit a moderate antibacterial activity. The antibacterial activity of the NPs is due to their high surface area, which cause more effective destruction of bacterial cell wall [21, 22].

Figure 5: Effect of TiO_2-NPs and antibiotic against E.coli.

4. Conclusion

In the present work, an eco-friendly method to synthesise TiO_2-NPs, using a green approach was established. An aqueous fruit extract of Averrhoa bilimbi mediated synthesis of TiO_2-NPs effectively utilised the properties of phytochemicals in nanoparticles synthesis. The spectral and morphological results confirmed the formation of spherical shaped TiO_2-NPs with an average particle size of 60 nm. The synthesized nanoparticles were anatase form. The synthesized TiO_2-NPs were evaluated for photocatalytic as well as anti-bacterial activity. Methylene blue dye was completely degraded by the use of green synthesised TiO_2-NPs as photocatalytic agents within 4 hours. The synthesized TiO_2-NPs effectively inhibited the growth of Gram-negative bacterial pathogen. Thus, the vital advantage of the current study is that the TiO_2-NPs made from Bimbli fruit can be used as a promising contender degradation of pollutant dyes and antibacterial applications.

Acknowledgements

Authors are grateful to SAIF MGU, Kottayam for characterization studies.

References

[1] Garcia, M. and Rodriguez, J. (2007) *Metal oxide nanoparticles encyclopedia of inorganic and bioinorganic chemistry.* John Wiley & Sons; Hoboken, NJ, USA.

[2] Maruthupandy, M., Zuo, Y., Chen, J. S., Song, J. M., Niu, H. l., Mao, C. J., Zhang, S.Y. and Shen, Y. H. (2007) Synthesis of metal oxide nanoparticles (CuO and ZnO NPs) via biological template and their optical sensor applications. *Applied Surface Science*, **397**, 167-174.

[3] Syal, A. and Sud, D. (2018) Development of highly selective novel fluorescence quenching probe based on Bi_2S_3-TiO_2 nanoparticles for sensing the Fe(III). *Sensors & Actuators, B: Chemical,* **266**,1-8.

[4] Bahal, M., Kaur, N., Sharotri, N. and Sud, D. (2019) Investigations on amphoteric chitosan/TiO_2 bionanocomposites for application in visible light induced photocatalytic degradation. *Advances in Polymer Technology,* **2019***,* 2345631-2345639.

[5] Yu, L., Xu, L., Lu, L., Alhalili, Z. and Zhou, X. (2022) Thermal properties of Mxenes and relevant applications. *ChemPhysChem,* **23**, e202200203.

[6] Bykkam, S., Prasad, D., Maurya, M. R., Sadasivuni, K.K. and Cabibihan, J. J. (2021)Comparison study of metal oxides (CeO_2, CuO, SnO_2, CdO, ZnO and TiO_2) decked few layered grapheme nanocomposites for dye-sensitized solar cells. *Sustainabililty,* **13**, 7685-7702.

[7] Mavrou, G., Galata, S., Tsipas, P., Sotiropoulos, A., Panayiotatos, Y., Dirnoulas, A., Evangelou, E. K., Seo, J. W. and Dieker, Ch. (2008) Electrical properties of La_2O_3 andHfO_2/La_2O_3 gate dielectrics for germanium metal-oxide-semiconductor devices. Journal of Applied Physics, **103**(1), 014506.

[8] Sethy, N. K., Arif, Z., Mishra, P. K. and Kumar, P. (2020) Green synthesis of TiO_2 nanoparticles from *Syzygium cumini* extract for photo-catalytic removal of lead (Pb) in explosive industrial wastewater. *Green Processing and Synthesis*, **9**(1), 171-181.

[9] Marien, C.B.D., Marchal, C., Koch, A. *et al.* (2017) Sol-gel synthesis of TiO_2 nanoparticles: effect of Pluronic P123 on particle's morphology and photocatalytic degradation of paraquat. *Environmental Science and Pollution Research,* **24**, 12582–12588.

[10] Abd-Elsalam, K., Periakaruppan, R. and IRajeshkumar, S. (2022). *Agri-waste and microbes for production of sustainable nanomaterials*.

[11] Ajitha, B., Reddy,Y. A. and Reddy, P. S. (2015) Biosynthesis of silver nanoparticles using Momordica charantia leaf broth: Evaluation of their innate antimicrobial and catalytic activities. J Photochem Photobiol B. **146**, 1-9.

[12] Kumar, H., Bhardwaj, K., Kuca, K. et al. (2020) Flower-based green synthesis of metallic nanoparticles: Applications beyond fragrance. Nanomaterials, **10**(4), 766-781.

[13] Kumar, H., Bhardwaj, K., Dhanjal, D.S., Nepovimova, E. et al. (2020) Fruit extract mediated green synthesis of metallic nanoparticles: a new avenue in pomology applications. *International Journal of Molecular Sciences,* **21**(22), 8458.

[14] Khanna, P., Kaur, A. and Goyal, D. (2019) Algae-based metallic nanoparticles: Synthesis, characterization and applications. Journal of Microbiological Methods, **163**, 105656

[15] Olana, M. Hirko., Sabir, F. K., Bekele, E. T. and Gonfa B. A. (2022) *Citrus sinensis* and *Musa acuminata* peel waste extract mediated synthesis of TiO_2/rGO nanocomposites for photocatalytic degradation of methylene blue under visible light irradiation. *Bioinorganic Chemistry and Applications*, Article ID 5978707.

[16] Ganapathi Rao, K., Ashok, Ch., Venkateswara Rao, K., Shilpa Chakra, Ch. and Rajendar, V. (2015) Synthesis of TiO_2 nanoparticles from orange fruit waste, *International Journal of Multidisciplinary Advanced Research Trends,* II(I), 2349-7408.

[17] Yugandhar, P., Vasavi, T., Rao, Y. J., Devi, P.U.M., Narasimha, G. and Savithramma, N. (2018) Cost effective, green synthesis of copper oxide nanoparticles using fruit extract of Syzygium alternifolium (Wt.) Walp., characterization and evaluation of antiviral activity. *Journal of Cluster Science*, **29**, 743–755.

[18] Carloling, G., Priyadharshini, M. N., Vinodhini, E., Ranjitham, A.M. and Shanthi, P. (2015) Biosynthesis of copper nanoparticles using aqueous guava extract-characterisation and study of antibacterial effects. *International Journal of Pharmacy and Biological Sciences*. **5**, 25–43.

[19] Tulip, D. R. E., Aishwarya, Surya, K. K., Krishna Devi, K. and Kousalya, R. (2012) Biosynthesis of silver nanoparticles using Morinda Citrifolia l. as capping and reducing agents. *International Journal of Engineering Trends and Technology (*IJETT), **3**(4), 24-34.

[20] Pal, M., García Serrano, J., Santiago, P. and Pal, U. (2007) Size-controlled synthesis of spherical TiO_2 nanoparticles: morphology, crystallization, and phase transition. *The Journal of Physical Chemistry C*, **111** (1), 96-102.

[21] Bindhu, M. R., Rekha, P.V., Umamaheswari,T. and Umadevi, M. (2014) Antibacterial activities of Hibiscus cannabinus stem-assisted silver and gold nanoparticles. *Materials Letters*, 131, 194-197.

[22] Fattahi, F. S. and Zamani, T. (2020) Synthesis of polylactic acid nanoparticles for the novel biomedical applications: a scientific perspective. *Nanochemistry Research*, **5**(1), 1-13.