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The Application of Biotechnology in the Synthesis of Metal/Metal Oxides Nanoparticles: Review

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Abstract: This paper is aimed at reviewing the application of biological extracts from plants, fungi and bacteria in the synthesis of metal/metal oxide nanoparticles with reference to Ag and TiO_2 nanoparticles as a case study. The procedures for the extraction of the biological extracts as well as the chemical instrumentation analysis of the extracts and metal/metal oxide nanoparticles were explained in this review. It was reported that standard chemical test confirms the presence of tannins, alkanoids, flavonoids and terpenoids in various plant extracts, while FTIR characterization of various biological extracts confirms the presence of organic compounds as a capping and reducing agents in the biosynthetic process for the preparation of the nanoparticles. The use of other instrumental techniques such as FESEM, XRD, SEM, EDX, TEM and UV-Visible spectroscopy in the characterization of biosynthesized metal/metal oxide nanoparticles were also explained in this review.

Keywords: Plants, Metal, Metal, Metal oxide

1. Introduction

Biotechnology is affiliated with the utilization of living organisms such as plant and microbes to carry out certain scientific or industrial process. The physical and chemical synthetic methods which have earlier been used for the preparation of metal/metal oxide nanoparticles (NPs) are associated with severe challenges such as high cost, chemical toxicity, high pressure, long refluxing time and high energy requirement [1-2]. The demerits of physical and chemical synthetic methods for the preparation of metal/metal oxide nanoparticles has lead to the quest in the utilization of biological synthetic method (biosynthesis or green synthesis), which is environmentally friendly, safe to handle, biocompatible and cost-effective, where the use high pressure, high energy, high temperature or toxic chemicals for the synthesis of metal/metal oxide NPs is not necessary because most biosynthesis occurs under ambient temperature and pressure, thereby resulting in vast energy savings, high yield and low cost of operation [3-8].

Biosynthesis is considered as a bottom-up technique which involves an oxidation/reduction reaction in the synthesis of metal/metal oxide NPs by the utilization of plant extracts and microbes [1, 5, 7, 9-11]. In contrast to biosynthesis, chemical synthesis leads to the formation of toxic chemical species which are adsorbed on the surface of nanoparticles, thereby making the use of such

nanoparticles highly incompatible for medical application. This has resulted to the high preference of biosynthetic method over chemical synthetic method for the preparation of metal/metal oxide NPs for pharmaceutical and biomedical applications [12-14].

The source of material that can be used for biosynthesis of nanoparticles include plant extract, bacteria, yeast, fungi and biomolecule, however, it has been postulated that the success of biosynthesis in the preparation of metal/metal oxide NPs is attributed to the antioxidant and reduction properties of microbial enzymes and key phytochemicals such as terpenoids, alkaloids, glycosides, citric acid, ascorbic acid and phenolic compounds (flavonoids, tannins ubiquinones, etc). Phenolic acids are classified as a powerful antioxidant which possesses carboxyl and hydroxyl groups that are able to bind metals. The active hydrogen may responsible for reduction of metal ions into formation of metal NPs [1, 2, 5, 8, 11-13, 15, 16]. This review is aimed at explain the application of biotechnology in the synthesis of metal/metal oxide NPs as well as the various fields of application of these nano materials.

2. Biosynthesis of Metal/Metal Oxide Nanoparticles

The biosynthesis of metal/metal oxide nanoparticles such as Ag, TiO_2, Cu, Au and others has been reported by many researchers, however, the application of few biological extracts in the synthesis of Ag and TiO2 nanoparticles are reviewed below.

2.1 Biosynthesis of Titanium Dioxide Nanoparticles

The aqueous leaf extract of Eclipta prostrate obtained as a filtrate from boiling 10 g of the leaves in 100 ml of distilled water at 60^0 C for 10 min has been successfully used in the synthesis of TiO_2 NPs by adding 15 ml of the E. prostrate extract to 85 ml of 5 mM TiO2 at room temperature under stirring condition for 24 hours, it was reported that XRD characterization revealed a crystallographic plane of rutile phase while atomic force microscopy (AFM) revealed an uneven surface morphology which indicates the presence of both individual and agglomerated TiO_2 NPs. Also, it was observed that FTIR peak indicated the role of carboxyl group O-H stretching and amine N-H stretching in the formation of TiO_2 nanoparticles, while FESEM analysis revealed that TiO_2 NPs exhibited spherical clusters which was quite poly disperse in the size range from 36 to 68 nm with calculated average size of 49.5 nm [4].

Sundrarajan and Gowri, reported the synthesis of TiO_2 NPs by Nyctanthes Arbor-Tristis leaves extract obtained as filtrate by mixing 1g of the dried leaves with 50 ml of ethanol and extracted under reflux condition at 50^0 C for 5 hours. The biosynthesis of TiO^2 NPs was carried out by starring 0.4M of titanium tetraisoproxide in ethanolic leaf extract at a temperature of 50°C for 4 hours. The formed TiO2 NPs were subjected to centrifugation and calcinate at 500^0 C before analytical characterization was carried out using XRD, scanning electron microscopy (SEM) and particle size analyzer (PSA). The authors posited that the XRD analysis revealed that the TiO_2 NPs are cubic with high degree of purity and crystallinity while the SEM and PSA reveal that the TiO_2 NPs exhibit a spherical morphology with average size in the range of 100-150 nm with inter particle distance [1].

A research by Chatterjee et al., reported the biosynthesis of TiO_2 NPs with the use of Vigna Radiata extracts obtained as filtrate by boiling 10 g of crushed dried sprouted green grams in 100 ml of distilled water for 30 min. The biosynthetic process was conducted by stirring a mixture of 20 ml of Vigna Radiata extracts and 80 ml of 1 mM TiO_2 solution at room temperature for 24 hours and the filtrate was calcined to obtained the TiO_2 NPs which

was subjected to analytical characterization by Fourier transform infrared spectroscopy (FTIR) and SEM. The SEM analysis revealed a oval shaped smooth surfaced TiO_2 NPs, while the FTIR spectrum measured in a range of 500-40000 cm^{-1} revealed peaks around the regions of 1631.78 - 1641.42 cm^{-1} , 3000 cm^{-1} , 1400-1460 cm^{-1} , indicating O-Ti-O bond, -OH stretching and the Ti-O stretching vibration respectively. The FTIR spectrum also revealed peaks in the region of 1020-1250 cm^{-1} and 1552.70 cm^{-1} which depicts the existence of aliphatic amines and nitro compounds in the biosynthesized TiO_2 NPs [14].

A research by Santhoshkumar et al., on the synthesis of TiO_2 NPs by using Psidium guajava extract obtained as a filtrate from boiling 20 g of freshly amassed leaves of Psidium guajava in 250 ml of distilled water at 60^0 C for 15 min has been successfully conducted. The biosynthesis of TiO_2 NPs was done by stirring a mixture of 20 ml of aqueous leaf extract of Psidium guajava and 80 ml of $TiO(OH)_2$ in an Erlenmeyer flask for 24 hours showed a colour change to light green, indicating the formation of TiO_2 NPs which was characterized by FTIR, FESEM and XRD. However, the content of phenolic compounds (mg//g) in leaf aqueous extract and synthesized TiO_2 NPs were found to be 85.4 and 18.3 mgTA/g, respectively. FTIR spectra of the synthesized TiO_2 NPs was reported to exhibited prominent peaks at 3410 cm^{-1} (alkynes), 1578 cm^{-1}, 1451 cm^{-1} (alkanes), and 1123 cm^{-1} indicating alkynes, alkanes and C-O absorption respectively. The morphological characterization of synthesized TiO_2 NPs was analysed by FESEM which showed spherical shape and clusters with an average size of 32.58 nm [2].

Isolated fungi Fusarium oxporum has been effectively used in the biosynthesis of TiO_2 NPs by Siva and co-worker, the fungus Fusarium oxysporum was grown in 500 ml Erlenmeyer flasks each containing 100 ml of MGYP media at 25-28°C on a shaker at 200 rpm for 96 hours. The mycelial mass was separated from the culture broth by 5000 rpm centrifugation at 10^0 C for 20 min and the settled mycelia were washed thrice with sterile distilled water. 20 g of the harvested mycelia mass was added into 500 ml Erlenmeyer flasks containing 20 ml of aqueous 0.025 M titanium dioxide solution and kept on a shaker (200 rpm) at 27 0 C for 96 hours. After incubation, nanoparticles containing fungal mycelia were filtered under laminar flow through Watman filter paper and subjected to calcinations at 180^0 C for 5 hours for crystallization of TiO_2 NPs, which were analysed by FTIR and UVvis spectrophotometer [17]. The FTIR spectrum of the biosynthesized TiO_2 NPs showed the presence of a resonance at ca. 600-1100 cm^{-1} and a weak band at 834.67cm^{-1} peaks corresponding to Ti-O-Ti vibrational mode and Ti-O antisymmetric stretching of Ti-O-Ti bonds. The presence of protein in the biosynthesized TiO_2 NPs was also reported by the appearance of absorption bands at ca. 1540.39 cm^{-1} indicated by the amide band in the particle. However, the uvvisble spectra revealed TiO_2 surface plasmon band occurs at ca. 257 nm and recorded a steady increase in the intensity as a function of the time of reaction for the complete oxidation of the TiO_2 solution by Fusarium oxysporum for 96 hours of reaction [17]. The biological synthesis of anatase TiO_2 NPs using Arnicae anthodium aqueous extract has been successfully investigated. This was done by stirring a mixture of 30 ml of aqueous extract of A. anthodium and 30 ml of 5ml M TiO_2 at room temperature for 24 hours. After this period, the solution was heated to heated to 100 °C for the time 30 minutes and filtered by 0.45 μm Milipore membrane filter, followed by 0.2 μm Millipore membrane filter in order to obtained the synthesized TiO2 NPs which was characterized using uv-visible spectrometer, SEM and FTIR [5]. XRD analysis revealed the particle size of 30 nm while the monitoring of the resulting solutions as a function of reaction

time in order to observed the increase in absorbance with different time interval of 3, 24, 48 and 72 hours after preparation of the solution was done by using uv-visible spectra analysis, which showed a peak at 321 nm corresponding to a characteristic band for TiO_2 [5]. The FTIR analysis of the synthesized TiO_2 NPs revealed strong IR absorption peaks at 1369, 1258 and 1020 cm^{-1}, which may be attributed to – C–O and –C–O–C stretching for alcohols, carboxylic acids, esters and ethers modes. Strong IR absorption peaks were also reported at 3269 and 2924 cm^{-1} correspond to -OH stretching and aliphatic methylene group –C-H stretching respectively. The FTIR spectrum also revealed peaks at 416 cm^{-1} and 591 cm-11 which indicated the presence of TiO2 NPs and C=C hydrocarbon respectively, it may therefore be said that the FTIR analysis confirmed the presence of TiO_2 NPs as well as flavonoids and phenolic acids groups, which are responsible for the biosynthesis of the nanoparticles [5]. The use of Curcuma longa extracts for the synthesis of TiO_2 NPs has been adequately studied by using two methods. The plant extracts was obtained as a filtrate from heating 15g of the powder C.longa in a soxlet extractor containing 300 ml of distilled at 40^0 C for 3-4 hours. In the first method, 50 ml of the filtrate was mixed with 2.5 ml of 50 mg/ml TiO_2 solution on a magnetic steer hot plate at 50^0 C and 1000 rpm for 5 hours. The second method involves the mixing of 50 ml of the filtrate with 5 ml of 50 mg/ml TiO_2 solution under the same condition as the first method but for a period of 8 hours [6]. It was observed that the first method resulted in the production of TiO_2 in both supernatant (colloidal solution) and precipitate (nanopowder), while the second method resulted in the production of precipitate only. The colloidal solution was kept for characterization while the precipitate was washed with distilled water and subjected to centrifugation at 15, 000 rpm for 10 min and dried at room temperature for 24 hours [6]. XRD characterization revealed an average crystallite size which was calculated by Scherer's equation, it was reported to be 43.088 and 22.881 nm for nanopowder and colloidal solution respectively for the first method while 45.808 nm for nanopowder in the second method [6]. The use of isolated strain of Bacillus mycoides in the synthesis of TiO_2 NPs has been successfully conducted by adding 40 mL of a 25 mM titanyl hydroxide solution to a culture of 200 µL of B. mycoides grown overnight (12h). The mixture was incubated at 37 °C for 24 hours with constant shaking. After this time, the solution was incubated at room temperature for 8 h and the appearance of a white precipitate was formed, which indicated the production of TiO_2 NPs. The precipitate was removed from the culture by centrifuging 15 min at 3820 × g. Finally, the biosynthesis product was washed and resuspended by successive centrifugations in Mili-Q ultrapure water [18]. The characterization of the biosynthesized TiO2 NPs was done by using TEM, FTIR and UVvisible spectroscopy, the TEM analysis revealed the morphology of the TiO_2 NPs to be spherical with a particle size between 40-60 nm and a size distribution which indicate high polydispersity of the sample, which is common of NPs prepared by biosynthetic methods, while the uv-vis spectra of the biosynthesized TiO_2 NPs was reported to produce a broad absorption band in the UV range of wavelength near 380 nm with a band gap of 3.27 eV which confirms that B. mycoides produced TiO_2 NPs in the anatase crystalline structure [18]. The FTIR spectrum for the biosynthesized NPs was observed to show a peak in the range of 450–700 cm^{-1} as a result of the vibration of Ti-O-Ti bond, which is a characteristic signal for TiO_2 NPs. The FTIR spectrum also revealed various peaks which could be attributed to biomolecules such as peptides or carbohydrates produced by B. mycoides during biosynthesis of TiO_2 NPs, some of these peaks were found at 3431 cm^{-1} which corresponds to the O-H stretching due to the alcoholic group while peaks in the regions of

1646, 1554, 1462 and 1400 cm^{-1} correspond to the characteristic signals of C=O and N-H vibrations due to the presence of amide and amine groups [18]. More peaks were observed in the region of 1246 cm^{-1} (C-O stretch vibrations, possibly due to the presence of an alcohol or carboxylic acid group), 1047 cm^{-1} (C-N stretching vibrations of aliphatic amines) and 2985 cm^{-1} are assigned to the symmetric stretch (C–H) of CH^2 and CH^3 groups of aliphatic chains. Finally of the FTIR spectrum, the peaks recorded in the region of 1554 and 1400 cm^{-1} was reported to indicate the C=C ring stretching and bending vibration of CH^2. The biomolecules produced by B. mycoides were reported as part of the cell envelope of the bacterium which act as stabilizing and capping agents by providing support for the nucleation of the NPs [18].

2.2 Biosynthesis of Silver Nanoparticles

The use of extracts from Azadirachta indica (Neem) leaves and Acorus calamus (Sweet flag) rhizome for the green synthesis of Ag NPs has been reported. Extracts from the individual plants were prepared by incubating a mixture of 1 % of the plant extracts with deionized water for 30 min, after incubation it subjected to centrifugation at 5000 rpm and the supernatant was filtered using a filter paper with the aid of a vacuum filter [19]. The green synthesis was carried out by adding 0.17 % of 1 mM $AgNO_3$ metal ion into the prepared plant extract such that the concentration ratio of plant extract to metal ions were 30:1, 60:1,120:1 and 240:1, thereby increasing the concentration of plant extract in the biosynthetic process. The bioreduction process of the aqueous component was measured with UV-Vis spectra of the solution while the biosynthesized TiO_2 NPs was characterized using UV-Vis, FTIR, XRD, SEM as well as Dynamic light scattering (DLS) and Zeta-Potential Analyses [19].

The UV-Vis spectra analysis revealed that a sharp bands of Ag NPs were observed around 421 nm in case of Azadirachta indica while the bands for Acorus calamus were observed around 384 nm, while the bioreduction of Ag ions to Ag NPs by the plant extracts was observed as a result of colour change. On the basis of bioreduction comparison between the two plant extracts, the UV-vis data revealed that A. indica reduces metal ions better than A. calamus [19].

The SEM analysis revealed the morphology and particle size of the green synthesized Ag NPs, it was reported that the morphology and particle sizes of the Ag NPs vary with the concentration ratio of plant extract to metal ion. It was observed that only the concentration ratio of 60:1 produces a particle size in nanometer, while other concentration ratio produced sizes in several micrometers. The result of the SEM analysis also revealed spherical shape for Ag NPs synthesized with the concentration ratio of 30:1, 120:1 and 240:1, while sheet shape was reported for the Ag NPs synthesize with the concentration ratio of 60:1 [19].

Report on the DSL was used to predict the particle size distribution (PSD) of the green synthesized Ag NPs in the different concentration ratio of plant extract to metal ion, the DSL revealed that the concentration ratio of 60:1, 120:1, 240:1 gave a uniform distribution of particles while the concentration of 30:1 ratio does not give uniform distribution. However, the concentration ratio of 60:1 was reported to be very appropriate because it gave the lowest average NPs size [19]. The zeta potential (surface potential) was used to determine the stability o the synthesized Ag NPs, the zeta potential reported measurement of 15.5, 1.92, 6.12, and 2.45 mV for the concentration ratio of 30:1, 60:1, 120:1 and 240:1 respectively. From the analysis, it was observed that the order of stability of the Ag NPs were in the

concentration ratio is 30:1> 120:1 > 240:1 > 60:1 [19].

FTIR analysis was used for identifying the biomolecules responsible for capping and stabilizing the synthesized NPs, for the concentration ratio of 60:1 and 120:1, the spectra band observed between 3490-3500 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols, while the peaks found around 500-1550, 1450-1500 and 500- 550 cm^{-1} were attributed to stretch for C-H, N-H and Ag-NPs bond respectively. The FTIR study was therefore reported to reveal that the synthesized Ag-NPs were surrounded by proteins, reducing sugar and metabolites such as terpenoids, however the author posited that carbonyl groups from the protein and amino acid residues have a strong ability to bind Ag-NPs [19]. XRD spectrum of the synthesized Ag-NPs showed distinct diffraction peaks around 380 which might have resulted due to the stabilization of the NPs by capping agent, it was however suggested that the crystallization of the bio-organic phase occurs on the surface of the AgNPs or vice-versa. All the characterization techniques proved that the concentration ratio of plant extract to metal ion plays a vital role in morphology and particle size determination of biosynthesized Ag-NPs [19].

Rodríguez-León et al., reported the synthesis of Ag NPs by the use of Rumex hymenosepalus plant extracts, which was prepared as a filtrate by adding 100 ml of an ethanol/water solution (70:30 v/v) to 15 g of a dried R. hymenosepalus sample in a flask, which was stored at room temperature and the extraction was allowed to proceed for several days by monitoring the visual appearance of the liquid on daily basis. The reaction was posited to be completed after 15 days because there was no noticeable change in the colour of the solution [8].

The biosynthesis of the Ag NPs was conducted for 96 h by mixing a fixed volume of the plant extract (VRh = 200 µl) with different volumes of 0.1 M stock solution of $AgNO_3$ in order to prepare different $AgNO_3$ concentrations of 2.5, 5, 7.5, 10, and 15 mM. The extract concentration was 5% v/v in all the samples and the total volume of each sample was adjusted to 4 ml by adding the necessary amount of ethanol in order to prepare samples with different $AgNO_3$ concentrations [8]. UV-Vis spectroscopy was used for the visual inspection of the sample for every 24 h, it was observed that the appearance of absorbance peak in the range of 277-280 nm indicated a characteristic polyphenol molecules. On the other hand, the spectra obtained from the use of 1H NMR analysis for the characterization was reported to display signal which corresponds to polyphenol molecules, which has the ability to act as a reducing agent in the synthesis of NPs [8].

The UV-Vis spectroscopy also revealed that for all the $AgNO_3$ concentrations, the visual appearance of the sample changes shortly after the addition of the plant extract with different time interval after the reaction started. It was observed that the colour of the reacting mixture changes from a slightly yellowish liquid; as the reaction proceeded, the solutions became orange, red, and brown indicating the reduction of Ag ion to Ag NPs. The UV-Vis spectra was reported to revealed a peak around 425 nm which corresponds to the absorbance due to the surface plasmons in the Ag NPs, while the increase in peak intensity was proportional to the concentration of $AgNO_3$ [8]. TEM analysis of the biosynthesized Ag NPs revealed a particle size with diameter in the range of 2 to 40 nm, while the use of high-resolution transmission electron microscopy (HR-TEM) and fourier transform analysis revealed that the biosynthesized Ag NPs structures were mainly of face-centered cubic and hexagonal crystal structures [8].

The eco-friendly synthesis of Ag NPs by commercially available plants such as such as Solanum tricobatum, Syzygium cumini,

Centella asiatica and Citrus sinensis has been investigated by Logeswari and coworkers. The characterization of the biosynthesized Ag NPs was done by UV-vis spectrophotometer, XRD, Atomic Force Microscopy and (FTIR) spectroscopy. The individual plant extracts were prepared as filtrate by boiling 1.5 g of the powder of the individual plants in 100 ml of deionized water [16]. Green synthesis of the Ag NPs was conducted by adding 2.5 ml of ammonium solution were added to 5 ml of 1 mM $AgNO_3$ solution, which was followed by the addition of 10 ml plant extract, and the final volume was adjusted to 50 ml with de-ionized water. The Erlenmeyer flasks were incubated at 37°C under agitation (200 rpm) for 24 - 48 hours, the solution was observed to turn from yellowish to bright yellow and to dark brown [16].

Phytochemical screening of the individual plant extracts by conventional methods revealed the presence of alkaloids, steroids, flavonoids and tannins, which plays a significant role in the reduction of metal ions to metal NPs, absorbance peak at 420 nm corresponding to a characteristic Ag NPs was reported when UV-Vis spectrophotometer was used to monitor the synthesized Ag NPs, the increased in the absorbance was noted to be proportion to various time interval of 1, 24 and 48 hours. XRD analysis revealed the crystalline nature of the Ag NPs as well as their particle size ranges of 48, 34, 43 and 33 nm corresponding to S. cumini, C. sinensis, S. tricobatum and C. asiatica respectively. However, AFM revealed and irregular shape of Ag NPs in the particle sizes of 53, 41, 52 and 42 nm, corresponding to S. cumini, C. sinensis, S. tricobatum and C. asiatica, respectively while the FTIR confirms the presence of peaks at 1620, 1633, 1641 and 1637 cm^{-1}, corresponding to S. cumini, C. sinensis, S. tricobatum and C. asiatica, respectively. Thereby indicating carbonyl stretching in the amide linkages of the protein and confirms the presence of protein as the stabilizing agent surrounding the Ag NPs [16].

Green synthesis of Ag NPs by the use of Coffea arabica seed extract has been successfully reported by Dhand et al., the seed extract of C. Arabica was obtained as a filtrate by boiling and stirring 10 g of finely crushed dried roasted seeds in 100 ml of ethanol/water (1:1) at 60^0 C for 1h. While the biosynthesis of Ag NPs was carried out by using a magnetic stirrer to continuously stir a mixture of hydro-ethanol C. Arabica and $AgNO_3$ solution for 10 min, this was later incubated at room temperature for 2 hours. A color change from light brown to dark brown indicated the completion of the reaction, while the supernatant was subjected to centrifugation at 4000 rpm for 5 min in other to ensure purity of the Ag NPs [13].

The UV–vis spectroscopy of the synthesized Ag NPs showed maximum absorption at 459 nm, which represents the characteristic surface plasmon resonance of Ag NPs, while X-ray crystal analysis showed that the Ag NPs are highly crystalline and exhibit a cubic, face centered lattice with characteristic (111), (200), (220) and (311) orientations. TEM analysis revealed that the Ag NPs exhibit spherical and ellipsoidal shaped structures, while the composition analysis obtained from SEMEDX confirmed the presence of elemental signature of Ag. Finally, FTIR spectra of the biosynthesized Ag NPs recorded a downward shift of absorption bands between 800-1500 cm^{-1} indicting the formation of Ag NPs, while DLS revealed that the mean particle size of the Ag NPs was found to be in between 20- 30 nm [13].

3. Conclusion

The application of biotechnology in the synthesis of Ag and TiO_2 nanoparticles has been explained and it can be deduced that the active component that stimulate the bio reduction of metal ions to metal/metal oxide nanoparticles are biomolecules and phytochemical substances such as alkaloids,

flavonoids, tannins and terpenoids. This was supported by FTIR characterization, which confirms the presence of organic compounds as an active ingredient in the bio reduction process for the preparation of metal/metal oxide nanoparticles. Biosynthesis or green synthesis was also reported to be preferable to physical and chemical syntheses of metal/metal oxide because biosynthesis of does not involve the use of toxic chemicals and costly instrument for the preparation of metal/metal oxide nanoparticles.

4. References

[1] M. Sundrarajan, S. Gowri, Green Synthesis of Titanium Dioxide Nanoaparticles by Nyctanathes Arbor-Tristis Leaves Extract, *Chalcogenide Letters*, 8 (2011) 447-451.

[2] T. Santhoshkumar, A.A. Rahuman, C. Jayaseelan, G. Rajakumar, S. Marimuthu, A.V. Kirthi, K. Velayutham, J. John Thomas, J. Venkatesan, Se-Kwon Kim, Green synthesis of titanium dioxide nanoparticles using Psidium guajava extract and its antibacterial and antioxidant properties, *Asian Pacific Journal of Tropical Medicine*, 7(2014) 968-976.

[3] A.A Zahir, I.S Chauhan, A. Bagavan, C. Kamaraj, G. Elango, J. Shankar, N. Arjaria, S.M. Roopan, A.A. Rahuman, N. Singh, Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in Leishmania donovani, *Antimicrobial Agents and Chemotherapy*, 8 (2015) 4782-4799.

[4] G. Rajakumar, A. Abdul Rahuman, B. Priyamvada, V. Gopiesh Khanna, D. Kishore Kumar, P.J. Sujin, Eclipta prostrata leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles, *Materials Letters*, 68 (2012) 115-117.

[5] R. Dobrucka, The biological synthesis of anatase titanium dioxide nanoparticles using Arnicae anthodium extract, *Bulgarian Chemical Communications*, 49 (2017) 595 – 599.

[6] D.H. Raghad, A. Abdul Jalill, R.S. Nuaman, A.N. Abd, Biological synthesis of Titanium Dioxide nanoparticles by Curcuma longa plant extract and study its biological properties, *WSN*, 49 (2016) 204- 222.

[7] A. Umer, S. Naveed, N. Ramzan, M. S. Rafique, Selection of Suitable Method for the Synthesis of Copper Nanoparticles. *NANO: Brief Reports and Reviews*, 7 (2012) 1-18.

[8] E. Rodríguez-León, R. Iñiguez-Palomares, R.E. Navarro, R. Herrera-Urbina, and J. Tánori, C. IñiguezPalomares, A. Maldonado, Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts), *Nanoscale Research Letters*, 8 (2013) 1-9.

[9] H.R. Ghorbani, A.A. Safekordi, H. Attar, and S.M.R. Sorkhabadi, Biological and Non-biological Methods for Silver Nanoparticles Synthesis, *Chemical and Biochemical Engineering Quarterly*, 25 (2011) 317-326.

[10] V.G. Pozzato, A.G. Pereira, L.G.L. Gerolis, L.S. Gonçalves, T.A. Pedrosa, M.J. Neves, Gadolinium Nanoparticles: Chemical Synthesis and Biogenic Synthesis by Saccharomyces cerevisiae, 23rd Congress of the International Union for Biochemistry and Molecular Biology, 44th

Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology, (2015) Foz do Iguaçu, PR, Brazil.

[11] S. Kandasamy, R.S. Prema, Methods of synthesis of nano particles and its applications, *Journal of Chemical and Pharmaceutical Researc*h, 7 (2015) 278-285.

[12] H. Rafiee, F. Serri, S. Yari, M. Pourghadir, V. Abdousi, A review: Silver Nanoparticles Synthesis through plants in comparison with chemical and biological methods, *Fire Journal of Engineering and Technology*, 1(2015) 46-50.

[13] V. Dhand, L. Soumya, S. Bharadwaj, S. Chakra, D. Bhatt, B. Sreedhar, Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity, *Materials Science and Engineering C*, 58 (2016) 36-43.

[14] A. Chatterjee, D. Nishanthini, N. Sandhiya, A. Jayanthi, Biosynthesis of Titanium Dioxide Nanoparticles Using Vigna Radaita, *Asian Journal of Pharmaceutical and Clinical Research*, 9(2016) 85-88.

[15] A.G. Ingale, A.N. Chaudhari, Biogenic Synthesis of Nanoparticles and Potential Applications: An EcoFriendly Approach, *Journal of Nanomedicine & Nanotechnology*, 4 (2013) 165.

[16] P. Logeswari, S. Silambarasan, J. Abraham, Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties, *Scientia Iranica*, 20 (2013) 1049-1054.

[17] S.G. Subramanyam, k. Siva, Bio-Synthesis, Characterization and Application of Titanium Oxide Nanoparticles by Fusarium oxysporum, *International Journal of Life Sciences Research*, 4 (2016) 69-75.

[18] N.A. Órdenes-Aenishanslins, L.A. Saona, M.D. DuránToro, J.P. Monrás, D.M. Bravo, J.M. Pérez-Donoso, use of titanium dioxide nanoparticles biosynthesized by Bacillus mycoides in quantum dot sensitized solar cells, *Microbial Cell Factories*, 13 (2014) 90.

[19] T. Panigrahi, (2013) Synthesis and Charactierization of Silver Nanoparticles using Leaf Extract of Azadirachta indica, Master Thesis, *National Institute of Technology Rourkela*-769008, Orissa, India.

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