Applied Ecology and Environmental Sciences, 2020, Vol. 8, No. 5, 273-277
Available online at http://pubs.sciepub.com/aees/8/5/13
Published by Science and Education Publishing
DOI:10.12691/aees-8-5-13

Synthesis and Characterization of Mn_3O_4 Nanoparticles for Biological Studies

Murugan Perachiselvi, Muthiah Sakthi Bagavathy, J. Jenson Samraj, E. Pushpalaksmi, G. Annadurai*

Division of Nanoscience, Sri Paramakalyani Centre for Excellence in Environmental Sciences,
Manonmaniam Sundaranar University, Alwarkurichi - 627412, India
*Corresponding author: gannadurai@msuniv.ac.in

Received June 01, 2020; Revised July 02, 2020; Accepted July 10, 2020

Abstract Nowadays nanoparticles comprising the diversity of applications have been synthesized and used up in various fields. In our research, Manganese tetroxide NPs (Mn_3O_4) was synthesized by precipitation method. In the present investigation, antibacterial activity, as well as In-vitro cytotoxic effects of Mn_3O_4 NPs, have been evaluated. The cytotoxicity studies on the Vero cell line (African green monkey kidney cell line) were studied by using MTT assay at 72 hrs. The Cell viability which was observed during the process depends upon the time exposure and concentration. The antibacterial activity was evaluated against the bacteria such as *Bacillus species*, *Escherichia coli*, and *Enterobacter sp.* The synthesized nanoparticles were characterized using X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), Dynamic Light Scattering (DLS), and Fourier Transform Infra-Red Spectroscopy (FTIR). Moreover, In-vitro cytotoxic effects and the antibacterial activity of Mn_3O_4 NPs showed a better result at the concentration of 50 µg/100µl in Vero cell and the Zone of inhibition was measured for *Enterobacter*. This proves Mn_3O_4 NPs as promising biocompatible material.

Keywords: *Mn_3O_4 NPs, cytotoxicity effects, Vero cell, and antibacterial activity*

Cite This Article: Murugan Perachiselvi, Muthiah Sakthi Bagavathy, J. Jenson Samraj, E. Pushpalaksmi, and G. Annadurai, "Synthesis and Characterization of Mn_3O_4 Nanoparticles for Biological Studies." *Applied Ecology and Environmental Sciences*, vol. 8, no. 5 (2020): 273-277. doi: 10.12691/aees-8-5-13.

1. Introduction

Nanotechnology exhibits rapid development in current research [1]. The nanoparticles which are extreme Ultrafine particles were exploited over 17 centuries. Due to its tremendous applications, healthcare, food technology, cosmetics, pharmaceutics, modify membrane, biomedicine, electrochemistry, energy science, sensor, optics, catalysis [2-11] owing to its high surface area, structural stability and unique physiochemical properties. The properties of nanomaterials in the biological field were increased superior compared to their bulk counterparts. However, simultaneously as the nanoparticle number and its application increases, the studies to characterize their consequences after exposure and to deal with their prospective toxicity are few in contrast. In the scientific discipline especially, nanoparticles are being utilized in diagnostic and therapeutic tools to better understand, detect, and deal with human diseases [13].

Metal oxide nanoparticles are extremely exciting in semiconducting materials and promote an ideal opportunity for the development of biological studies. Tri manganese tetroxide is one of the important metal oxide nanoparticles and consists of different crystal structures built from MnO_6 octahedra were, MnO_2, Mn_2O_3, Mn_3O_4 [14]. Mn_3O_4 is a stable mixed oxide material and possesses a spinel structure, It has multitudinous advantages such as low cost, environmentally friendly and effective catalysts, which are producing various applications in human exposure, like biosensor, water treatment, imaging contrast agent, cancer treatment, drug delivery, biomarker, etc [15,16,17]. Manganese is considered an essential element in metabolism and their homeostasis has been well regulated by biological systems [18,19] and Mn_3O_4 NPs exhibit higher antibacterial properties and less toxicity. The mn^{2+} ions produce toxic-free radicals, which play a crucial role in clinical disorders like heart disease, stroke, diabetes mellitus, Alzheimer's, sclerosis, etc [20,21,22,23,24].

There are several reports to produce manganese tetroxide nanoparticles such as Sol-gel [25], Co-precipitation [26], Hydrothermal [27] Freeze-drying [28], and solvothermal [29]. In a present study, we have synthesized Manganese tetroxide nanoparticles (Mn_3O_4NPs) by precipitation method [30]. To demonstrate their application in the cytotoxic studies using Vero (African green monkey kidney) cell line. As well as antibacterial activity investigated from *Bacillus species*, *E. Coli* and *Enterobacter*. In this present investigation, synthesis of manganese tetroxide nanoparticles (Mn_3O_4 NPs) using chemical precipitation method and evaluation of *in-vitro* studies such as antibacterial efficacy and cytotoxicity is reported [31].

2. Materials and Methods

Manganese chloride, Citric acid, and Sodium hydroxide were purchased from Sigma-Aldrich. *Escherichia coli, Enterobacter, Bacillus species* were purchased from IMTECH at Chandigarh. Vero Cell lines were purchased from the National Centre for Cell Science (NCCS). All the reagents used for this study were of analytical grade.

2.1. Synthesis of Mn_3O_4 NPs

The manganese tetroxide nanoparticles (Mn_3O_4 NPs) were prepared using manganese chloride according to the previous study with few optimizations. 0.1 M manganese chloride was added into 50 ml of distilled water and stirred well using a magnetic stirrer, then 0.5 ml of citric acid was added to the $MnCl_2$ solution. Finally, NaOH was added to the suspension to increase the pH at 9, the mixture was stirred for 20 minutes. After drying at 60°C for 12 hours the final product was calcined at 600°C for 4 hours to obtain Mn_3O_4 NPs.

2.2. Antibacterial Activity

Pathogenic bacteria such as *Bacillus sp, E. coli, and Enterobacter* were used to evaluate the Antibacterial activity of synthesized manganese tetraoxide (Mn_3O_4 NPs). Muller-Hinton agar was used as a nutrition medium and a well diffusion method was performed. Fresh bacterial culture was swabbed over the agar medium using sterile cotton and the wells were made by puncturing the agar using a micropipette tip. Then, Mn_3O_4 NPs added to the wells at various concentrations such as 20, 40, and 80 µl. Petri dishes were incubated at 37°C for 24 hrs and the resulting zone of inhibition was measured.

2.3. Cell Line and Culture

The *Vero cell line was* obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

2.4. In Vitro Cytotoxic Activity of Mn_3O_4 Nanoparticles

The biocompatibility evaluation of synthesized Mn_3O_4 must be justified before *in-vivo* procedures. The cells were maintained in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C. The synthesized Mn_3O_4 nanoparticles were added in various concentrations such as (50, 100, 200, 400, 500 µg/mL) and incubated at 37°C for 24, 48, and 72 h.

The biocompatibility (MTT) test was performed to determine the toxic behavior of synthesized Mn_3O_4 nanoparticles. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added with the cells and incubated for 4 h. 1 mL of dimethyl sulphoxide (DMSO) was added to dissolve the formazan crystals and the OD (optical density) values were recorded at 570 nm using ELISA plate reader (Bio-Rad 680, USA).

3. Result and Discussion

3.1. Characterization Techniques

The X-ray Diffraction (XRD) of the sample was recorded using the *PhillipsPW1800* diffractometer with Cu Kα radiation maintained at 40 kV for the crystalline phase identification. The particle size of Mn_3O_4 NPs was determined by Dynamic Light Scattering (DLS) analysis using *nano plus micromeritics*. The morphology was observed by Scanning Electron Microscopy (SEM) with TESCAN (Model WEGA11), operated at an acceleration voltage of 25.0 kV. Fourier Transform Infrared Spectroscopy (FTIR) analysis of Mn_3O_4 NPs performed in *Perkin-Elmer spectrometer*. Optical properties of Nanoparticles of Mn_3O_4 NPs were studied from *Shimadzu Japan UV- spectrometer*.

The XRD pattern was obtained for the Mn_3O_4 nanoparticles as shown in Figure 1, in which all the diffraction peaks are matched with standard JCPDS Card No (24-0734) [32]. The peak positions (2θ) were obtained at 15.7°, 37.2°, 45.4°, 56.4°, 66.1° and 75.2° corresponding to crystal planes (101), (112), (200), (211), (220),) (303), (400) and (413). The results indicate that synthesized Mn_3O_4 NPs are in the tetragonal structure. The crystalline size was calculated by using Scherrer's formula

$$D^{1/4}k\lambda = \beta \cos \theta$$

Where D represents grain size, K is an empirical constant to 0.9. λ is the X-ray wavelength of the CU. Kα radiation. β is the full width half maximum value and θ is the Bragg's angle. The average crystalline size of Mn_3O_4 NPs is about 73nm.

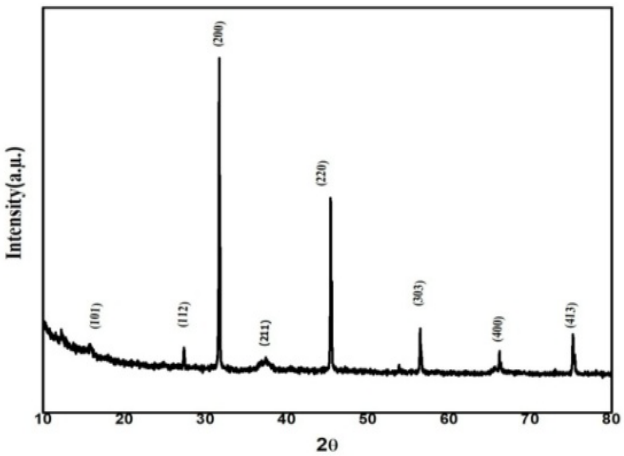

Figure 1. X-ray diffraction (XRD) analysis

3.3. Fourier-Transform Infrared Spectroscopy

Figure 2 shows the FTIR spectra of the Mn_3O_4 NPs after calcinated at 450°C for 12 hrs. The absorption band at 3436 cm^{-1} is attributed to O-H stretching mode and the absorption peak exists near around 1631, 1452, and 1384 cm^{-1} could be attributed to O-H bending vibration combined with Mn atoms. The absorption band located at 606, 636, and 571cm^{-1} are associated with the coupling mode between the Mn-O vibration mode of tetrahedral and the peaks at 470 and 493 cm^{-1} correspond to octahedral sites [33].

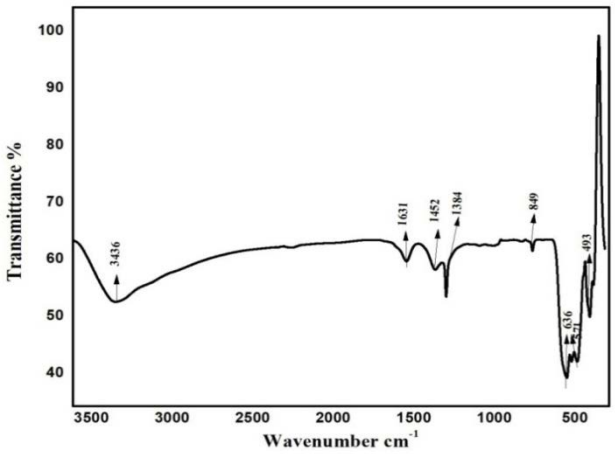

Figure 2. FTIR analysis of Mn3O4 NPs

3.4. FESEM, DLS, and EDAX

The morphology of Mn_3O_4 Nanoparticles was observed by FESEM is shown in Figure 3 (a). Shows spherical nanoparticles with aggregation and Figure 3 (b). DLS results revealed the average size of synthesized nanoparticles is found to be 165 nm. Figure 3 (c). Shows EDAX Spectrum. It can be seen that the manganese and oxygen atom of Mn_3O_4 NPs.

$$Mn^{2+} + 2\,OH^- \rightarrow Mn(OH)_2 \quad (1)$$

$$Mn(OH)_2 \rightarrow MnO + H_2O \quad (2)$$

$$3MnO + \frac{1}{2}O_2 \rightarrow Mn_3O_4 \quad (3)$$

The manganese ions were reduced in alkaline solution, which forms manganese hydroxide then it was converted into MnO. Finally, MnO was oxidized into Mn_3O_4 by atmospheric oxygen.

3.5. Optical Properties Investigations of Mn_3O_4 NPs

Mn_3O_4 NPs were prepared using a chemical precipitation method. Figure 4 (a) shows the fluorescence (FL) spectra with an intense emission band at 384.16nm and 684.36 nm. Figure 4 (b) UV band emission at 197 nm results from the recombination process due to electron excited from the valance band (h^{vb+}) to conduction band (e^{CB-}) as this result suggests, the nanoparticles enhance biological activity due to surface defects.

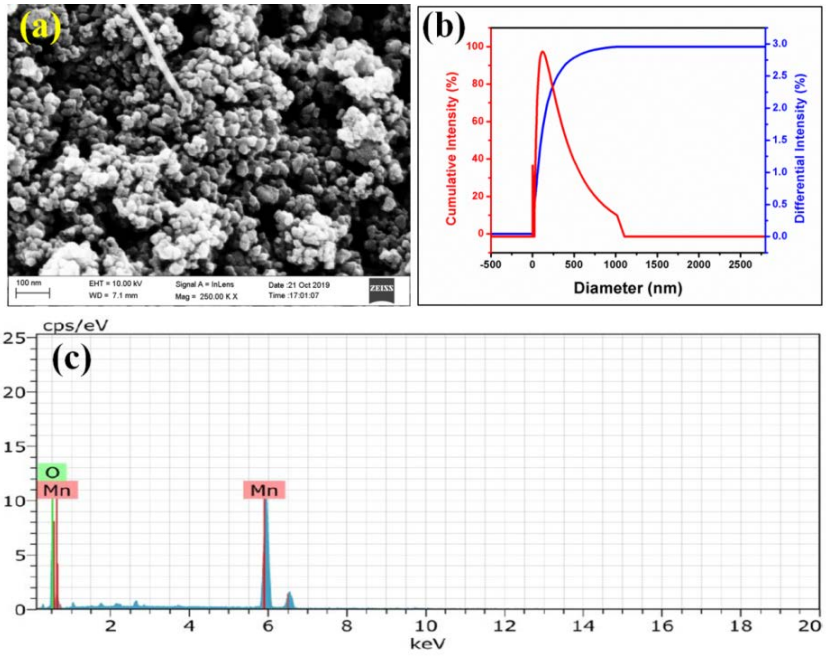

Figure 3. (a) SEM morphology of Mn_3O_4 NPs, (b) DLS analysis of Mn_3O_4 NPs, and (c) EDAX analysis

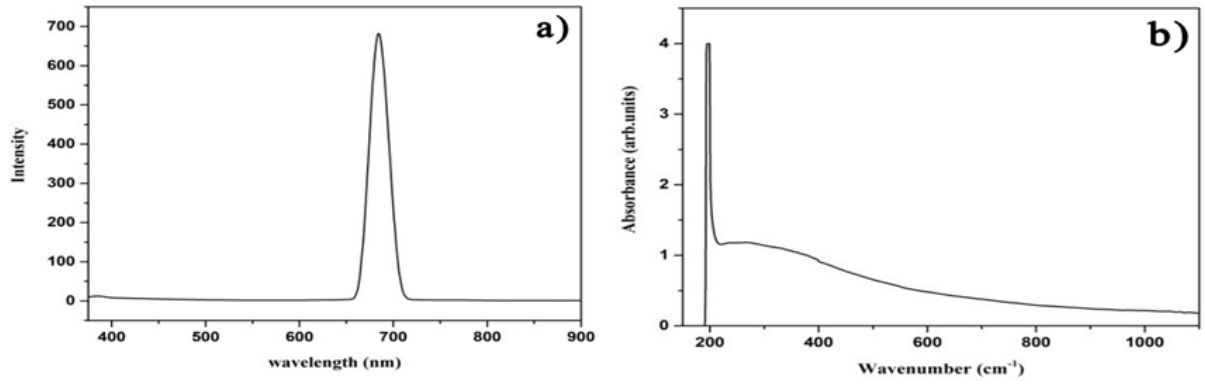

Figure 4. (a) FL spectra of Mn_3O_4 NPs, and (b) UV-Vis Spectra of Mn_3O_4 NPs

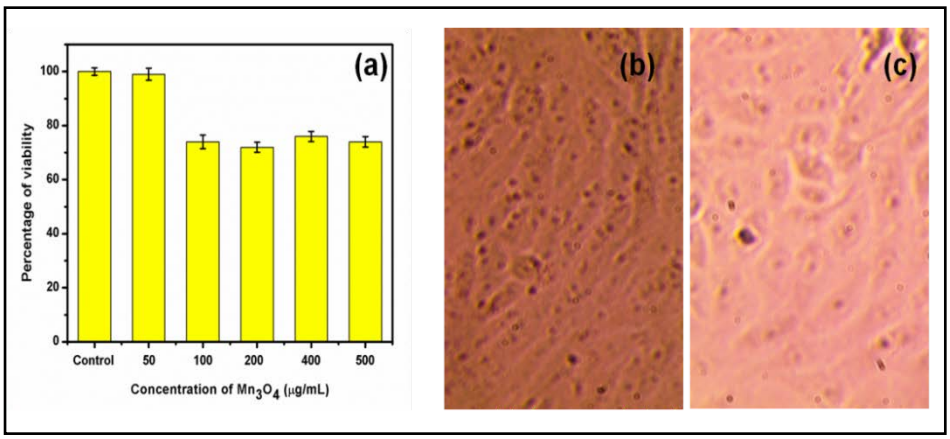

Figure 5. Cell viability of Mn_3O_4 NPs

3.6. In Vitro Cytotoxic Effect of Mn_3O_4 NPs

The toxicity studies of nanoparticles on Vero cells are investigated due to the increasing nanotoxicity. The present toxicity studies reveal the biocompatibility of the synthesized Mn_3O_4 nanoparticles and the obtained results are displayed in Figure 5. The obtained histogram shows the percentage of viability in various concentrations of 50, 100, 200, 400, 500 (µg/mL). Cell viability decreased when the concentration and exposure time of Mn_3O_4 NPs increased and untreated wells were kept as control. For 50 µg/mL few cell death was observed because this particular concentration could be highly biocompatible with Vero cells and while increasing the concentrations of Mn_3O_4 above 100 µg/mL shows slight toxicity behavior due to attachment of a large number of superoxide and Mn^+ ions on cell membranes, thus damaging the genetic material and organelles of the cells. Similarly, the effect of Mn_3O_4 on the morphological changes in the cell lines was studied using an optical microscope and the images are shown in Figure 5 (b and c). In that, Figure (b) refers to the control, and Figure (c) refers to the 72 hrs of incubation of Cell morphology. The morphology of the Cells is found to be irregular and elongated. Based on the above results, the synthesized Mn_3O_4 nanoparticles show the better biocompatibility after the exposure of 72 hrs. However, more studies to be done to understand other biological properties.

3.7. Antibacterial activity

Figure 6 (a) and Figure 6 (b) show the Antibacterial efficacy of the synthesized Mn_3O_4 NPs and the resulting exhibit the antibacterial activity against *Bacillus sp*, *E. coli*, and *Enterobacter* due to the size of the particles arranged at nanocomplex and it possesses unique properties. The increase in the Zone of inhibition corresponds to the increase of the nanoparticle dosage as shown in Figure 6 (b). The highest antibacterial activity was observed in *Enterobacter* than *Bacillus sp* and *E. coli*.

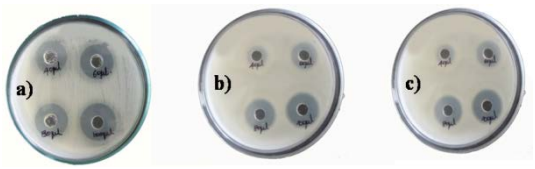

Figure 6. (a) Zone of inhibition of *Enterobacter* (a), *E. coli* (b), and *Bacillus subtilis* (c)

4. Conclusion

Mn_3O_4 nanoparticles were successfully synthesized from chemical precipitation method. The formation of the Mn_3O_4 phase was confirmed by XRD. The sharp peaks indicate the good crystalline nature of the nanoparticles. The FL emission at 384.16nm and 684.36 nm and UV absorption band emission at 197 nm confirmed the presence of defect states, which trigger the antibacterial property and cytotoxic activity, and it leads to the formation of electron-hole pairs, thereby generating ROS which kill the cells. The result shows, the zone of inhibition proves that the synthesized nanoparticles having effective antibacterial properties.

The cytotoxic activities were assessed in the Vero cell line using MTT assay. This study indicated a direct relationship between exposure time and cell viability maximum cell viability was observed at 50µg/100 µl concentration after 72 hrs post-exposure. The regular and smooth surface as controlled cells whereas irregular cell surface was observed for cells treated with Mn_3O_4 NPs due to the internalization of nanoparticles. The cell viability study revealed Mn_3O_4 NPs are biocompatible nanoparticles and can be used for clinical applications.

However, more studies have to be done to understand the complete biological properties of Mn_3O_4 nanoparticles.

Acknowledgments

Authors are thankful to Management and Head, Department of Environmental Science, Manonmaniam Sundaranar University for providing our required and facilities to complete this project successfully.

References

[1] Ahmed, K.A.M. and Huang, K., "Formation of Mn_3O_4 nanobelts through the solvothermal process and their photocatalytic property," *Arabian Journal of Chemistry*, 12 (3). 429-439. 2019.

[2] Bello, A., Fashedemi, O.O., Lekitima, J.N., Fabiane, M., Dodoo-Arhin, D., Ozoemena, K.I., Gogotsi, Y., Charlie Johnson, A.T. and Manyala, N., "High-performance symmetric electrochemical capacitor based on graphene foam and nanostructured manganese oxide," *AIP Advances*, 3 (8). 082118. 2013.

[3] Chen, G., Roy, I., Yang, C. and Prasad, P.N., "Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy," *Chemical Reviews*, 116 (5). 2826-2885. 2016.

[4] Chen, H. and He, J., "Facile synthesis of monodisperse manganese oxide nanostructures and their application in water treatment," *The Journal of Physical Chemistry C*, 112 (45).17540 -17545. 2008.

[5] Chen, J., Wu, X., Gong, Y., Wang, P., Li, W., Tan, Q. and Chen, Y., "Synthesis of Mn3O4/N-doped graphene hybrid and its improved electrochemical performance for lithium-ion batteries," *Ceramics International*, 43(5). 4655-4662. 2017.

[6] Dhaouadi, H., Ghodbane, O., Hosni, F. and Touati, F., "$Mn3O_4$ Nanoparticles: Synthesis, Characterization, and Dielectric Properties," *ISRN Spectroscopy*, 82-86. *2012*.

[7] Fang, M., Tan, X., Liu, M., Kang, S., Hu, X. and Zhang, L., "Low-temperature synthesis of Mn 3 O 4 hollow-tetrakaidecahedrons and their application in electrochemical capacitors," *CrystEngComm*, 13(15).4915-4920. 2011.

[8] Fernandes, C., Benfeito, S., Fonseca, A., Oliveira, C., Garrido, J., Garrido, E.M., and Borges, F., "Photodamage and photoprotection: toward safety and sustainability through nanotechnology solutions," In *Food Preservation* (pp. 527-565). *Academic Press*. 2017.

[9] Frewer, L.J., Gupta, N., George, S., Fischer, A.R.H., Giles, E.L., and Coles, D., "Consumer attitudes towards nanotechnologies applied to food production," *Trends in food science & technology*, 40(2).211-225. 2014.

[10] Fritsch, S., Sarrias, J., Rousset, A. and Kulkarni, G.U., "Low-temperature oxidation of Mn3O4 hausmannite," *Materials Research Bulletin*, *33*(8).1185-1194. 1998.

[11] Hafez, A.A., Naserzadeh, P., Ashtari, K., Mortazavian, A.M. and Salimi, A., "Protection of manganese oxide nanoparticles-induced liver and kidney damage by vitamin D," *Regulatory Toxicology and Pharmacology*, 98.240-244. 2018.

[12] Jiménez-Pérez, Z.E., Singh, P., Kim, Y.J., Mathiyalagan, R., Kim, D.H., Lee, M.H. and Yang, D.C., "Applications of Panax ginseng leaves-mediated gold nanoparticles in cosmetics relation to antioxidant, moisture retention, and whitening effect on B16BL6 cells," *Journal of ginseng research*, 42(3). 327-333. 2018.

[13] Khan, S., Ansari, A.A., Khan, A.A., Abdulla, M., Al-Obeed, O. and Ahmad, R., "In vitro evaluation of anticancer and biological activities of synthesized manganese oxide nanoparticles," *MedChemComm*, 7(8).1647-1653. 2016.

[14] Khedkar, M., Michael, P.E. and Khan, S.R., "Copper and Copper Oxide Nanoparticles: Applications in Catalysis," 2019.

[15] Kumar, S., Kaur, R., Rajput, R. and Singh, M., "Bio-Pharmaceutics Classification System (BCS) Class IV Drug Nanoparticles: Quantum Leap to Improve Their Therapeutic Index," *Advanced pharmaceutical bulletin*, 8(4).617.624. 2018.

[16] Li, L., Seng, K.H., Liu, H., Nevirkovets, I.P. and Guo, Z., "Synthesis of Mn3O4-anchored graphene sheet nanocomposites via a facile, fast microwave hydrothermal method and their supercapacitive behavior." *Electrochimica Acta*, 87, 801-808. 2013.

[17] Li, W.N., Yuan, J., Shen, X.F., Gomez‐Mower, S., Xu, L.P., Sithambaram, S., Aindow, M. and Suib, S.L., "hydrothermal synthesis of structure‐and shape‐controlled manganese oxide octahedral molecular sieve nanomaterials," *Advanced Functional Materials*, 16(9). 1247-1253. 2006.

[18] Liu, M., Wang, Y., Cheng, Z., Zhang, M., Hu, M. and Li, J., "Electrospun Mn2O3 nanowrinkles and Mn3O4 nanorods: Morphology and catalytic application," *Applied Surface Science*, 313.360-367. 2014.

[19] Luo, J.D., Wang, Y.Y., Fu, W.L., Wu, J. and Chen, A.F., "Gene therapy of endothelial nitric oxide synthase and manganese superoxide dismutase restores delayed wound healing in type 1 diabetic mice," *Circulation*, 110(16).2484-2493. 2004.

[20] Markides, H., Rotherham, M. and El Haj, A.J., "Biocompatibility and toxicity of magnetic nanoparticles in regenerative medicine," *Journal of Nanomaterials*, 8,213-216.2012.

[21] Masdor, N.A., Altintas, Z. and Tothill, I.E., "Sensitive detection of Campylobacter jejuni using nanoparticles enhanced QCM senso,". *Biosensors and Bioelectronics*, 78.328-336. 2016.

[22] Mo, C.M., Li, Y.H., Liu, Y.S., Zhang, Y. and Zhang, L.D., "Enhancement effect of photoluminescence in assemblies of nano-ZnO particles/silica aerogels," *Journal of applied physics*, 83(8).4389-4391. 1998.

[23] Nicoloff, G., Blazhev, A., Petrova, C. and Christova, P., "Circulating immune complexes among diabetic children," *Journal of Immunology Research*, 11(1).61-66. 2004.

[24] Pi, J.K., Yang, H.C., Wan, L.S., Wu, J. and Xu, Z.K., "Polypropylene microfiltration membranes modified with TiO2 nanoparticles for surface wettability and antifouling property," *Journal of Membrane Science*, 500.8-15.2016.

[25] Raj, B.G.S., Asiri, A.M., Wu, J.J. and Anandan, S., "Synthesis of Mn3O4 nanoparticles via chemical precipitation approach for supercapacitor application," *Journal of Alloys and Compounds*, 636.234-240.2015.

[26] Ranjan, M., Bhatnagar, M. and Mukherjee, S., "Localized surface plasmon resonance anisotropy in template aligned silver nanoparticles: A case of biaxial metal optics," *Journal of Applied Physics*, 117(10).103106.2015.

[27] Reddy, R.N. and Reddy, R.G., "Sol-gel MnO2 as an electrode material for electrochemical capacitors," *Journal of Power Sources*, 124(1).330-337.2003.

[28] Shaik, D.P., Pitcheri, R., Qiu, Y. and Hussain, O.M., "Hydrothermally synthesized porous Mn3O4 nanoparticles with enhanced electrochemical performance for supercapacitors," *Ceramics International*, 45(2).2226-2233.2019.

[29] Shukla, S., Jadaun, A., Arora, V., Sinha, R.K., Biyani, N. and Jain, V.K., "In vitro toxicity assessment of chitosan oligosaccharide coated iron oxide nanoparticles," *Toxicology reports*, 2. 27-39. 2015.

[30] Tholkappiyan, R., Naveen, A.N., Vishista, K., and Hamed, F., "Investigation on the electrochemical performance of hausmannite Mn3O4 nanoparticles by ultrasonic irradiation assisted co-precipitation method for supercapacitor electrodes," *Journal of Taibah University for Science*, 12 (5).669-677.2018.

[31] Ullah, A.A., Kibria, A.F., Akter, M., Khan, M.N.I., Maksud, M.A., Jahan, R.A. and Firoz, S.H., "Synthesis of Mn3O4 nanoparticles via a facile gel formation route and study of their phase and structural transformation with distinct surface morphology upon heat treatment," *Journal of Saudi Chemical Society*, 21(7).830-836. 2017.

[32] Vélez, M.A., Perotti, M.C., Santiago, L., Gennaro, A.M. and Hynes, E., "Bioactive compounds delivery using nanotechnology: design and applications in dairy food In *Nutrient delivery"*, *Academic Press*, 221-250.2017.

[33] Versiani, A.F., Andrade, L.M., Martins, E.M., Scalzo, S., Geraldo, J.M., Chaves, C.R., Ferreira, D.C., Ladeira, M., Guatimosim, S., Ladeira, L.O. and da Fonseca, F.G., "Gold nanoparticles and their applications in biomedicine," *Future Virology*, 11(4). 293-309. 2016.