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Green Synthesis And Characterization Of Iron Oxide and Copper Oxide Nanoparticles Using Blumea Lacera Leaf Extract And Evaluation Of Antibacterial Activity

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ABSTRACT

An iron and copper oxide nanoparticles manufacturing technique was prepared using a leaf extract of *Blumea lacera*. The green-synthesized iron and copper nanoparticles were analyzed using UV-visible absorption spectrophotometry, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and EDS. The results revealed that iron oxide nanoparticles exhibit an uneven spherical form, with sizes ranging from 15.73 nm to 32.37 nm. The antimicrobial efficacy of iron and copper oxide nanoparticles made using green methods was tested against four bacterial human diseases. The iron and copper oxide nanoparticles produced using green synthesis showed the highest antibacterial effectiveness at a concentration of 20 mm/disc and the lowest effectiveness at 13 mm/disc concentrations. The current study emphasizes that iron and copper oxide nanoparticles generated by green methods have the potential to be used as antibacterial agents.

Keywords: *Blumea lacera*, Iron and copper Oxide Nanoparticles, Antibacterial Activity, and Human Pathogens.

1. INTRODUCTION:

Nanoparticles are atomic clusters with sizes ranging from 1 to 100 nm, where "nano" represents one billionth of a meter[1]. Nanoparticles are produced using physical and chemical methods, however, they have limitations including the use of toxic compounds, the preparation of hazardous byproducts, high energy consumption, and high cost. Green synthesis of nanoparticles using plants is a subject of intense research due to its environmentally friendly nature and suitability for different uses such as drug delivery, medical diagnostics, cancer treatment, agriculture, and environmental purposes. Plant extract reduces metal ions more quickly than other environmentally friendly ways. Nanoparticles can be generated in a short time, ranging from minutes to hours, based on the plant species and the level of phytochemicals present[2]. An annual plant with a potent scent, Lettuce-Leaf Blumea grows up to 2,000 meters above sea level in the plains of northwest India[3-7]. Experts in Ayurveda define blumea as hot, pungent, and bitter; it is also an excellent antipyretic and is beneficial for blood illnesses, fevers, burning sensations, bronchitis, and other ailments. It is thought that keeping the root in the mouth might treat oral illnesses. The herb is used to repel insects such as fleas in the Konkan region of India[8-10]. It is prescribed in the homeopathic system for enuresis, neuralgia, headaches, and cold-induced cough[11-14]. The current work aimed to investigate the green synthesis and characterization of iron and copper oxide nanoparticles using *Blumea Lacera* leaf extract and assess its antibacterial effectiveness against human bacterial infections.

2. MATERIALS AND METHODS

2.1. Collection and identification of the plant

Healthy leaves of *Blumea lacera* were gathered at Karjat Local Park. The India Biodiversity Portal records Blumea Lacera with the taxonomic number 1768.

2.2. Preparation of *Blumea lacera* aqueous leaf extract

Approximately 100 grams of fresh healthy leaf from *Blumea Lacera* were air-dried in the shade and then ground into a powder using a kitchen blender. The powdered leaves were soaked in 200 ml of double distilled water overnight at 4°C in a fridge, then the washed mixes were boiled for 10 minutes. The extracts were cooled to room temperature and subsequently filtered using Whatman filter paper (No.41).

2.3. Synthesis of iron oxide nanoparticles using *Blumea lacera* extract

Synthesis of iron oxide nanoparticles involved dissolving $FeCl_3$ and $FeCl_2.5H_2O$ in a 1:2 molar ratio in 100 ml of double distilled water in a 250 ml beaker. The solution was then heated to 80°C with gentle stirring using a magnetic stirrer under air pressure. After 10 minutes, 20 ml of the aqueous solution of *Blumea lacera* extract was added to the mixture, causing a rapid shift in color from light green to dark black. After 10 minutes, 20 ml of an aqueous solution of sodium hydroxide was added to the solutions at a rate of 3 ml per minute to allow for uniform precipitation of iron oxide. The mixture was cooled to room temperature, and the iron oxide nanoparticles were collected using decantation to prepare magnetite. The magnetites that were generated underwent three washes with double distilled water and three washes with ethanol before being air dried at room temperature.

2.4. Synthesis of copper oxide nanoparticles using *Blumea lacera* extract

A 250 ml beaker was used to dissolve $CuCl_2.5H_2O$ in 100 ml of double distilled water for the synthesis of copper oxide nanoparticles. After that, the mixture was brought up to 80°C while being gently stirred with a magnetic stirrer and air pressure. The mixture's color changed from pale green to deep black in a matter of minutes after the addition of 20 ml of *Blumea lacera* leaves extract aqueous solution (10 minutes later). To ensure consistent precipitation of copper oxide, 20 ml of an aqueous sodium hydroxide solution was added to the solutions at a rate of 3 ml per minute after 10 minutes. Magnetite was prepared by allowing the liquid to settle to ambient temperature and then collecting the copper oxide nanoparticles using decantation. The produced magnetites were washed three times with ethanol and twice with double distilled water before being allowed to air dry at room temperature.

2.5. Characterization of iron and copper oxide nanoparticles

The sample's absorbance spectra were examined using a UV-Vis double-beam bio-spectrophotometer JASCO V650 within the wavelength range of 250-700 nm. The size of magnetic iron and copper oxide nanoparticles was determined using laser diffractometry with a Nano Size Particle Analyzer, ranging from 0.6 nm to 6.0 µm. The structure and crystalline size of nanoparticles were analyzed using X-ray diffraction (XRD) with a SHIMADZU XRD-6000 model. The nanoparticles' functional group was analyzed using FT-IR spectroscopy (Brucker, IR Affinity 1, India) within a scan range of 4000 to 500 cm^{-1} and a resolution of 4 cm^{-1}. Magnetic iron and copper oxide nanoparticles were analyzed morphologically using a FEI Nova Nano SEM 450 scanning electron microscope (SEM).

2.6. Screening of antibacterial activity

2.6.1. Bacterial strains

The clinical isolates of various bacteria were acquired from the Konkan Gyanpeeth Rahul Dharkar College and Pharmacy and Research Institute. The bacteria include *B. subtilis*, *E. coli*, *S. aureus*, and *P. aeruginosa*.

2.6.2. Antibacterial analysis by disc diffusion method

The antimicrobial efficacy of iron and copper oxide nanoparticles generated by green methods was assessed using the disc diffusion technique[15]. Sterile 6 mm discs (Hi-media) were loaded with varying amounts of iron and copper nanoparticles: 10 µg/disc (10 µg/µl), 15 µg/disc (15 µg/µl), 20 µg/disc (20 µg/µl), 25 µg/disc (25 µg/µl), and 30 µg/disc (30 µg/µl). The culture plates were prepared by pouring 20 mL of Mueller-Hinton agar (MHA) medium onto them. A bacterial suspension was then swabbed onto the plates using a sterile cotton swab, and the plates were set aside for a few minutes. The discs were delicately compressed and placed upside down to incubate for 24 hours at 37°C. *Ciprofloxacin* discs (20 µg/disc) were inserted on the MHA plates as the positive control. Following the incubation period, the test organisms' susceptibility was assessed by measuring the diameter of the zone of inhibition with a Himedia zone scale. The data were then collated for evaluation.

3. RESULTS AND DISCUSSION

3.1. Green synthesis of iron and copper oxide nanoparticles using *Blumea lacera* extract

The iron and copper oxide nanoparticles were generated in the current study employing a more environmentally friendly process including an aqueous extract of *Blumea lacera*. Iron and copper oxide nanoparticles of different sizes and shapes have been produced through environmentally friendly methods using plant extracts like *Musa ornate, Averrhoa bilimbi, Juglans regia, Platanus orientalis,* and *Asparagus racemosus*. The preparation of iron and copper oxide nanoparticles was verified by closely monitoring the color alteration during the reaction. The aqueous extract of *Blumea lacera* leaf extract was initially pale green but turned black upon the addition of Fe^{+3} and Fe^{+2} along with NaOH solution acting as a reducing agent.

3.2. UV-visible spectroscopy analysis

UV-visible spectroscopy is a useful method for analyzing the structure of iron and copper oxide nanoparticles. Metal nanoparticles' optical absorption spectra are primarily influenced by surface plasmon resonances[16]. The analysis of **Figure 1** revealed two absorption peaks at 268-270 nm and 328-339 nm in plant extracts. The peak is found at about 261 nm and 378 nm due to the surface plasma resonance of the produced iron and copper oxide nanoparticles. The results are similar to the UV-visible spectra of iron and copper oxide nanoparticles produced by *Averrhoa bilimbi,* which exhibited absorption maxima at 440 and 465 nm[17]. Iron and copper oxide nanoparticles produced from plant extracts have absorption maxima in the UV-visible spectrum from 400 nm to 500 nm, as indicated in previous studies[18].

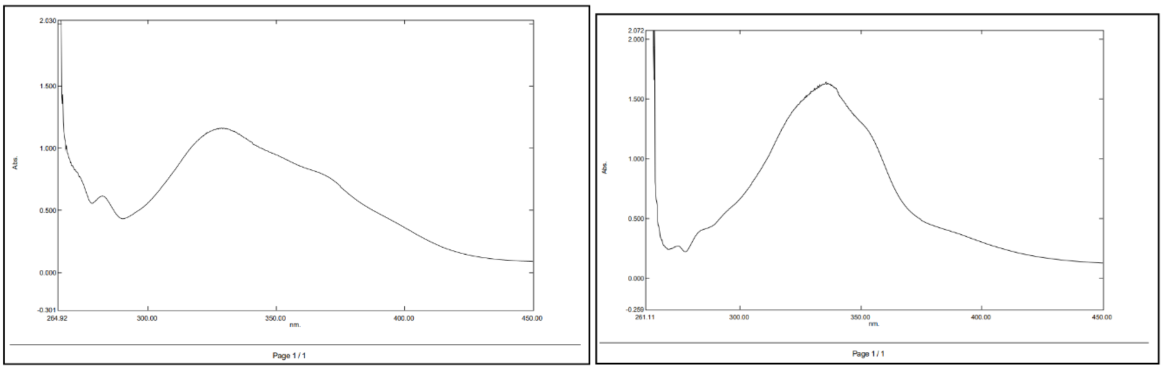

Figure 1. UV-vis spectra of Fe_2O_3-NPs and CuO-NPs

X-ray diffraction (XRD) was used to analyze the phase, purity, and crystallite size of the iron and copper oxide nanoparticles. The X-ray diffraction pattern of iron and copper oxide nanoparticles synthesized by *Blumea lacera* is displayed in **Figure 2**. The powder XRD pattern of the iron and copper oxide indicates a rhombohedral crystal structure. The prominent peaks of iron and copper oxide nanoparticles corresponded to crystal faces (016), (105), (110), (111), (331), (026), (111), (116), (211), and (300) of iron and copper oxide. Malarkodi *et al*. found that the XRD pattern of the peaks indicates that the iron and copper oxide nanoparticles produced using *Blumea lacera* officinalis extract are crystalline and have a rhombohedral structure[19].

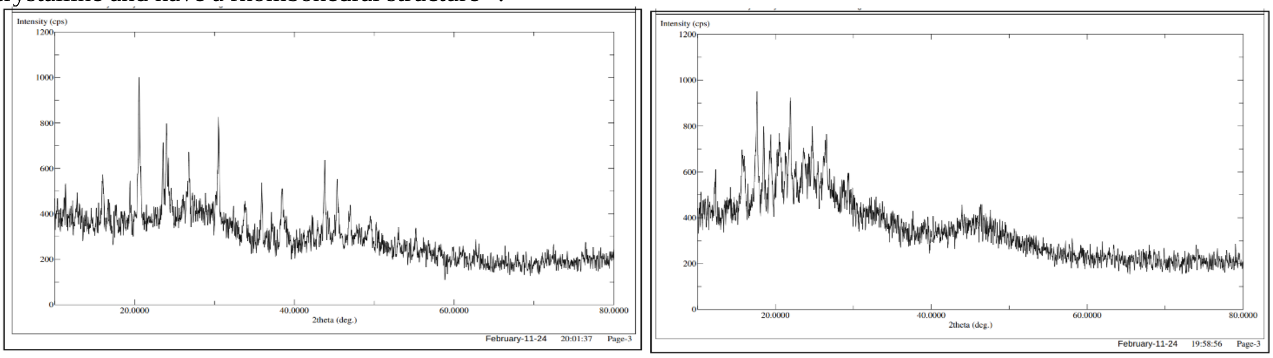

Figure 2. XRD spectra of Fe_2O_3-NPs and CuO-NPs

3.3. FT-IR analysis

Figure 3 analyzed the FT-IR spectra of iron and copper oxide nanoparticles produced from *Blumea lacera* extract. The absorption peaks in the FT-IR spectra of nanoparticles were primarily localized at 3213-3268 cm^{-1}, 2188-2190 cm^{-1}, 2081-2107 cm^{-1}, and 1990-2009 cm^{-1} and 1631-1634 cm^{-1}. The peak at 3213-3268 cm^{-1} corresponds to strong stretching vibrations of the –OH bond, 2188-2190 cm^{-1} to C≡N stretching vibrations, 2081-2107 cm^{-1} to aliphatic C-H stretching, and 1990-2009 cm^{-1}. The frequency is 1631-1634 cm^{-1} for conjugated carbonyl (–C=O) group stretching vibration and O-H bending. The functional groups were previously found using FT-IR analysis of different green-synthesized iron oxide nanoparticles[20-21].

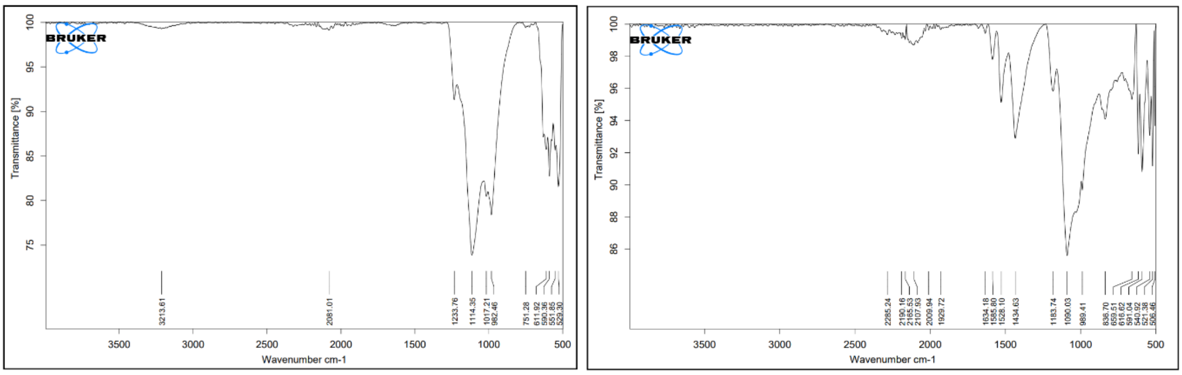

Figure 3: FT-IR spectra of Fe_2O_3 and CuO NPs

3.4. SEM analysis

The morphology of the iron and copper oxide nanoparticles generated using green methods was examined using SEM. Scanning electron microscope pictures showed that the iron and copper oxide nanoparticles were clustered in quasispherical forms, ranging in size from 15.73 nm to 574.3 nm. The results align with Vijay Kumar et al.'s findings, which indicated that α-Fe_2O_3 has a spherical morphology with a consistent size ranging from 30 to 40 nm in diameter[21]. Veeramanikandan et al. examined[22] the morphology of iron and copper oxide nanoparticles manufactured using green methods. The nanoparticles displayed irregular rhombic forms ranging in size from 117 µm to 1.29 mm.

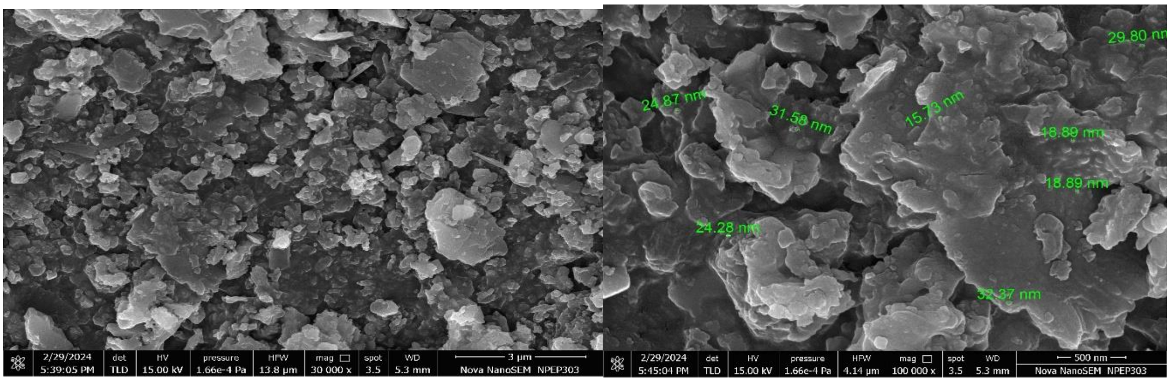

Figure 4: FE-SEM images of Fe_2O_3-NPs with a magnification (3µm and 500nm)

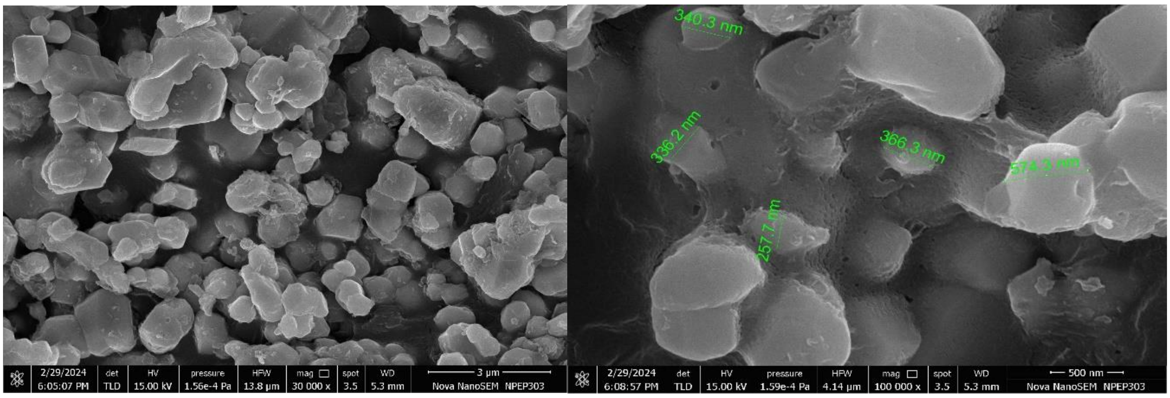

Figure 5: FE-SEM images of CuO-NPs with a magnification (3µm and 500nm)

3.5. Screening of antibacterial activity of iron and copper oxide nanoparticles synthesized by *Blumea lacera* aqueous extract

The antimicrobial capabilities of iron and copper oxide nanoparticles produced by green methods were tested against both Gram-positive and Gram-negative bacterial strains using the agar well diffusion technique. The antibacterial activity results from iron and copper oxide nanoparticles made using green methods. Iron oxide nanoparticles effectively suppressed the development of *B. subtilis* (20 mm), and *E. coli* (18 mm) among the investigated bacterial strains at a dose of 25µg. However, copper oxide nanoparticles showed moderate growth inhibition of *E. coli* (15 mm) and *S. aureus* (13 mm) at a dose of 25 µg. The zone of inhibition created by the commercial antibiotic *ciprofloxacin* (20 µg/disc) ranged from 11 to 20 mm and exceeded the sizes of those produced by iron and copper oxide nanoparticles. The results align with the findings of Veeramanikandan *et al*. demonstrated[22] significant antibacterial activity through the green manufacture of iron and copper oxide nanoparticles using *Blumea lacera* leaf extract.

The concentration of produced iron and copper oxide nanoparticles significantly influenced their antibacterial activity. Kiruba Daniel and colleagues used a leaf extract from the *perennial shrub Dodonaea viscosa* to create iron nanoparticles[23]. The resulting nanoparticles demonstrated notable antibacterial effects against human pathogens such as *E. coli, K. pneumonia, S. aureus, and B. subtilis*.

4. CONCLUSION

Iron and copper oxide nanoparticles were generated in this study utilizing an aqueous extract of *Blumea lacera*. The nanoparticles exhibit distinct shapes and sizes as confirmed by XRD, SEM, XRD, UV, and FT(IR) spevtra. The study's findings indicate that iron and copper oxide nanoparticles made using green methods have shown significant antibacterial efficacy against several bacterial infections. They could serve as substitutes for typical antibacterial drugs in treating bacterial infections.

5. REFERENCES:

1. Sudarenkov, V. (2013). Nanotechnology: Balancing benefits and risks to public health and the environment. Strasbourg: Council of Europe, Committee on Social Affairs, Health and Sustainable Development.
2. Herlekar, M., Barve, S. and Kumar, R. (2014). Plant-mediated green synthesis of iron nanoparticles. J. Nanopart., ID 140614, 9.
3. Khair, A., Ibrahim, M., Ahsan, Q., Homa, Z., Kuddus, M. R., Rashid, R. B., & Rashid, M. A. (2014). Pharmacological activities of Blumea lacera (Burm. f) DC: a medicinal plant of Bangladesh. British Journal of Pharmaceutical Research, 4(13), 1677.
4. Pattewar, A. M., Dawalbajea, A. B., Gundalea, D. M., Pawarb, P. B., Kavtikwara, P. G., Yerawara, P. P., ... & Patawar, V. A. (2012). Phytochemistryical and anthelmintic studies on Blumea lacera. Indo Global J Pharm Sci, 2, 390-96.
5. Khandekar, U., Tippat, S., & Hongade, R. (2013). Investigation on antioxidant, anti-microbial and phytochemical profile of Blumea lacera leaf. Int J Biol Pharm Res, 4(11), 756-761.
6. Akter, R., Uddin, S. J., Tiralongo, J., Grice, I. D., & Tiralongo, E. (2015). A new cytotoxic steroidal glycoalkaloid from the methanol extract of Blumea lacera leaves. Journal of Pharmacy & Pharmaceutical Sciences, 18(4), 616-633.
7. Mishra, P., Irchhiaya, R., & Mishra, S. K. (2015). Phytochemical investigation and spectral studies of isolated flavonoid from ethanolic extract of whole plant Blumea lacera DC. Journal of Pharmacognosy and Phytochemistry, 4(2), 01-04.
8. Yadav, V. K. (2018). Phytochemical and pharmacognostical studies of Blumea lacera (Roxb.) DC. International Journal of Green Pharmacy (IJGP), 12(01).
9. Mishra, P., Irchhiaya, R., & Mishra, S. K. (2015). Phytochemical investigation and spectral studies of isolated flavonoid from ethanolic extract of whole plant Blumea lacera DC. Journal of Pharmacognosy and Phytochemistry, 4(2), 01-04.
10. Kundu, P., Debnath, S. L., & Sadhu, S. K. (2022). Exploration of pharmacological and toxicological properties of aerial parts of Blumea lacera, a common weed in Bangladesh. Clinical Complementary Medicine and Pharmacology, 2(3), 100038.
11. Khair, A., Ibrahim, M., Ahsan, Q., Homa, Z., Kuddus, M. R., Rashid, R. B., & Rashid, M. A. (2014). Pharmacological activities of Blumea lacera (Burm. f) DC: a medicinal plant of Bangladesh. British Journal of Pharmaceutical Research, 4(13), 1677.
12. Kundu, P., Debnath, S. L., & Sadhu, S. K. (2022). Exploration of pharmacological and toxicological properties of aerial parts of Blumea lacera, a common weed in Bangladesh. Clinical Complementary Medicine and Pharmacology, 2(3), 100038.
13. Ahmed, F. A., Rahman, A., & Mubassara, S. (2014). Phytochemical composition, antioxidant activity and cytotoxicity of Blumea lacera Linn. from two different habitats. Jahangirnagar University Journal of Biological Sciences, 3(1), 37-45.
14. Ashrafi, S., Alam, S., Islam, A., Emon, N. U., Islam, Q. S., & Ahsan, M. (2022). Chemico-biological profiling of Blumea lacera (Burm. f.) DC.(Family: Asteraceae) Provides new insights as a potential source of antioxidant, cytotoxic, antimicrobial, and antidiarrheal agents. Evidence-Based Complementary and Alternative Medicine, 2022.
15. Bauer, A.W., Kirby, W. M., Sherris, J. C. and Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45(4) : 493–496.
16. Mahdavi, M., Namvar, F., Ahmad, M. and Mohamad, R. (2013). Green Biosynthesis and Characterization of Magnetic Iron Oxide (Fe3O4) Nanoparticles using Seaweed Sargassum multicum Aqueous Extract. J.Mole., 18 : 5954-5964.
17. Rosli, I. R., Zulhaimi, H. I., Ibrahim, S. K. M., Gopinath, S. C. B., Kasim, K. F., Akmal, H. M., Nuradibah, M. A. and Sam, T. S. (2018). Phytosynthesis of Iron Nanoparticle from Averrhoa bilimbi Linn. In IOP Conference Series: Materials Science and Engineering (Vol. 318, No. 1, p. 012012).
18. Mittal, A., Cristi, K. and Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. J. Biotechnol. Ad., 31: 346-356.
19. Malarkodi, C., Malik, V. and Uma, S. (2018). Synthesis of Fe2O3 using Emblica officinalis extract and its photocatalytic efficiency. Mat. SciInd. J. 16(1) : 125.
20. Adeleye, T. M., Kareem, S. O., & Kekere-Ekun, A. A. (2020, March). Optimization studies on biosynthesis of iron nanoparticles using Rhizopus stolonifer. In IOP Conference Series: Materials Science and Engineering (Vol. 805, No. 1, p. 012037). IOP Publishing.
21. Vijay Kumar, P. P. N., Pammi, S. V. N. and Shameem, U. (2018). A Green approach for the synthesis of iron oxide nanoparticles by using roots of Asparagus racemosus and its degradation of dye methyl orange. Inter. J. Pharma. Drug Anal., 6 (1): 22 – 28.

22. Irshad, R., Tahir, K., Li, B., Ahmad, A., Siddiqui, A. R. and Nazir, S. (2017). Antibacterial activity of biochemically capped iron oxide nanoparticles: A view towards green chemistry. J. Photochem. Photobiol. B: Biol., 170: 241-246.
23. Kiruba Daniel, S. C. G. and Vinothini, G., Subramanian, N., Nehru, K. and Sivakumar, M. Biosynthesis of Cu, ZVI, and Ag nanoparticles using Dodonaea viscosa extract for antibacterial activity against human pathogens. J. Nanopart. Res., 15 (1): article 1319.