NATURE INSPIRED SYNTHESIS, PHYSICO-CHEMICAL CHARACTERIZATION OF Zn DOPED Fe₃O₄ NANO PARTICLES USING ANDROGRAPHIS PANICULATA (BURM. F.) NEES LEAF EXTRACT AND ASSESSMENT OF IN VITRO PANCREATIC ALPHA AMYLASE INHIBITORY ACTIVITY

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ABSTRACT

Objective: Magnetite (Fe₃O₄) nanoparticles (NPs) have gained considerable attention in the Biomedical filed. Evolution of new magnetic material based on the transition metal-doped magnetite has become the subject of increasing research interest. The main aim of the current investigation was to improve the diabetic potential, optical, magnetic, structural properties of magnetite nanoparticles and hence Fe₃O₄ NPs were doped with a divalent transition element such as Zinc.

Methods: Zinc doped magnetite nanoparticles (Zn-Fe₃O₄ NPs) were obtained through Co-precipitation methods using aqueous plant extract of Andrographis paniculata as an efficient stabilizer and a reducing agent. The structure, morphology, crystalline, optical and magnetic property of synthesized Zn-Fe₃O₄ NPs were evaluated by X-ray diffraction (XRD), Scanning electron microscopy with Energy dispersive x-ray spectroscopy(SEM-EDX), Fourier transform infrared spectroscopy (FTIR), Ultraviolet-Visible (UV-Vis) Spectrophotometer and Vibrating scanning magnetometer (VSM).

Results: In XRD analysis, the average crystallite size of the synthesized Zn-Fe₃O₄ NPs was found to be 5 nm exhibiting super paramagnetic behavior, which composes it an appealing possibility for biomedicines. The Zn-Fe₃O₄ NPs have strongly inhibited the alpha (α)-amylase enzyme and had proved their therapeutic role.

Conclusion: In conclusion, Zn-Fe₃O₄ NPs is an excellent anti-diabetic agent to control type 2 diabetes mellitus.

Keywords: Alpha-amylase, Andrographis paniculata, SEM-EDX, VSM, Zn-Fe₃O₄ NPs

INTRODUCTION

Iron oxide nanoparticles (IONPs) are widely used in bioremediation systems because of their cost efficiency, magnetic strength, biocompatibility, easy water separation and surface modifiability. Maghemite (γ-Fe₂O₃), Magnetite (Fe₃O₄) and hematite (α-Fe₂O₃) are the most common IONPs found in nature [1]. Among these, Magnetite (Fe₃O₄) is the strongest magnetic mineral on earth [2]. Fe₃O₄ NPs (magnetite nanoparticles) have a property that is ferromagnetic at RT (Room temperature) [3]. However, the magnetic behavior of magnetite nanoparticles depends largely on the preparation methods. In addition, the crystallite size and then surface morphology of Fe₃O₄ crystal play a crucial role, which influences the magnetic properties of Fe₃O₄ NPs [4, 5].

Fe₃O₄ consists of Fe⁺²and Fe⁺³ in particular spinel format. Fe⁺³ species resides at octahedral sites and Fe⁺² species are distributed between octahedral as well as tetrahedral sites. Structural Fe⁺³ ion in Fe₃O₄ plays a major role part as an electron donor to start the reduction process [6]. Structural Fe⁺³ inserts Fe₂O₃ with reducibility besides environmental contaminants. Even though Fe₂O₃ stoichiometry ratio (Fe⁺³/Fe⁺²) becomes lesser at the time of the reduction, the proximity of an active reducer, i.e., aqueous Fe⁺², can efficaciously recharge Fe₂O₃ [7] and recuperate its reducibility. Due to its non-toxicity, Magnetite nanoparticles have attracted considerable interest in various applications [8].

After many research works, it has been proved that the IONPs concert can be improved further by doping. The synthesis of magnetite in the presence of divalent and trivalent cation of transition metals (Zn²⁺, Mn²⁺, Co²⁺, Ni²⁺, Li⁺) can modify structural, optical, electrical, magnetic, saturation magnetization properties of iron oxide [9]. In an anoxic nature, Zn²⁺ can be accessed into the Fe₂O₃ structure by the reduction of Fe⁺³ compounds with reducible iron bacteria, e.g., Clostridium sp and Geobacter sulfurreducens. The magnetic moment of Fe₂O₃ increases with the addition of a small amount of Zinc, but decreases dramatically with an increase in Zinc content, whichever is related to conversion in cation site order. At low Zinc level, tetrahedral site Fe⁺² species is replaced by Zn²⁺ species, which is coupled with the oxidation of octahedral site Fe⁺³ to Fe²⁺ state. Thus, it controls the spinel structure as well as it also enhances the magnetic property by bettering the Fe⁺²-Fe⁺³ species interaction [10].

Andrographis paniculata (AP) (Burm. f.) Nees has been used to cure a wide range of biomedical applications, including hepatitis, sexual dysfunctions, anti-diarrheal, anti-hyperglycemic, anti-malarial, antioxidant, cardiovascular, inflammatory activity, microbial activity, cancer activity, hepatoprotective, anti-HIV and immunostimulatory [11]. After a detailed literature study, it has been found that the preparation of nanoparticles by using Andrographis paniculata (plant) has not been attempted yet by any researcher.

The principle of this study was to improve its diabetic potentiality of Fe₃O₄ NPs; we report here synthesis and characterization of Zn-Fe₃O₄ NPs by using Andrographis paniculata leaf extract without any stabilizers, distributors and oxidants. The obtained zinc doped magnetic nanoparticles (Zn-Fe₃O₄ NPs) were characterized using physicochemical techniques such as UV-Vis, VSM, FTIR, SEM, XRD and EDX, respectively. The synthesized Zn-Fe₃O₄ NPs were evaluated for their anti-diabetic potential by alpha-amylase inhibitory activity.

MATERIALS AND METHODS

Materials

The chemicals required were purchased from the following sources: Iron sulphate, Zinc chloride, Iron chloride and ammonium hydroxide (NH₄OH) from Sigma-Aldrich with 99% purity.

Preparation of AP extract

Indian medicinal herb, Andrographis paniculata (Burm. f.) Nees leaves were collected from local area of Nagercoil in the month of March and botanical identity was confirmed by Dr. Mahesh,
Assistant professor, Department of botany, ST. Hindu College, Nagercoil. The Voucher specimen number of Andrographis paniculata (STHCH10) was deposited in the institute for future reference. The fresh leaves were first cleaned with tap water followed by de-ionized (DI) water to remove adhering soil and unwanted dust particles, cut into fine pieces and dried out at RT.

Approximately 12g of the leaves were weighed separately and shifted into 400 ml beaker containing 150 ml of DI water and heated for 25 min, followed by filtering through Whatman filter paper to remove biomaterials. Finally, Andrographis paniculata extract was stored at 4 °C in Erlenmeyer flasks for the preparation of Zn-Fe₃O₄ Nps [12].

**Phytochemical screening**

Fresh and healthy leaves were selected for phytochemical analysis. The phytochemical screening of *Andrographis paniculata* was carried out by the standard method that was previously discussed [13].

**Synthesis of Zn-Fe₃O₄ nanoparticles**

Zinc doped magnetite nanoparticles were carried out by an improved co-precipitation method. The standard stock solutions of FeCl₃.7H₂O, FeSO₄.7H₂O and ZnCl₂.6H₂O were added in the ratio of 2: 1: 0.2. This solution was heated and maintained at 35-45 °C under a mild stirring using a magnetic stirrer for 20 min. After 10 min, *Andrographis paniculata* extract was added slowly into the solution. After 1 h, 1N ammonium hydroxide was added into the solution drop by drop for uniform precipitation of zinc doped magnetite nanoparticles. The final pH of the mixture solution was reached to 11. The mixture solution was left undisturbed and allowed to settle down at room temperature. The black coloured Zn-Fe₃O₄ NPs got deposited at the base of the conical flask. The deposited nanoparticles were repeatedly washed with DI water. Then the solution is centrifuged at 6000 rpm and the colloidal solution is retained. The colloidal solution acquired is then transferred to the Petri plate and dried in a dryer oven machine at 150 °C for 10 h. The dried sample is again calcinated using a muffle furnace [14]. The resulting nanoparticles are subjected to characterization and application (fig. 1).

**In vitro pancreatic alpha-amylase inhibitory assay**

The inhibitory activity of zinc doped magnetite nanoparticles against the alpha-amylase enzyme was studied, according to previous resources [15]. Acarbose is a standard pharmacological drug to cure type-II diabetes mellitus. The positive control was to be used as a pharmacological drug (pharmacological inhibitor).

**Characterization methods**

The phase purity and crystalline structure of the prepared nanoparticles were investigated with an XRD machine (PANalytical, Phillips PW 1830) using CuKα radiation in the 2θ ranges from 10°-80°. SEM attached with EDX were done using Quanta FEG 250 instrument; while electronic and FT-IR spectra were investigated using Shimadzu model (UV-1800) and Perkin Elmer model (version 10,300) FTIR spectrometer, respectively. Magnetic behavior of the Zn-Fe₃O₄-NPs was examined using a vibrational sample magnetometer (YSM, Gryoergic, UK).

![Fig. 1: Schematic diagram of synthesized zinc doped magnetite NPs](image)

**RESULTS AND DISCUSSION**

**Phytochemical screening for AP extract**

The leaf extract of *Andrographis paniculata* was analyzed by phytochemical screening for the evaluation of bioactive compounds. Ten phytochemical tests were tested, out of which seven were confirmed (table 1) in the *Andrographis paniculata* (AP) extract.

The bioactive compounds detected in the leaf extract act as a reducer and stabilizer in the nanoparticle synthesis, which includes alkaloids, flavonoids, terpenoids, saponins, phlobatannins, tannins and phenols, respectively [16]. The aqueous *Andrographis paniculata* leaf extract was found to have these constituents, suggesting that they are the excellent starting material for synthesizing zinc doped magnetite nanoparticles.

**UV-Vis absorption spectra**

The stability as well as the formation of Zn-Fe₃O₄ NPs in colloidal suspension, is examined by using UV-Visible spectroscopy. Extinction spectra of *Andrographis paniculata* and synthesized Zn-Fe₃O₄ Nps are
depicted in fig. 2. The absorption peak was not observed in *Andrographis paniculata* extract and however, the green synthesized zinc doped magnetite nanoparticles reveals that a significant absorption peak was observed at 380 nm resulting from the surface Plasmon resonance. The optical absorption maximum is found to be increased in doping Zn$^{2+}$ metal ion with bulk magnetite nanoparticles [17].

### Table 1: Phytochemical analysis of *Andrographis paniculata* extract

| Phytochemicals | Aqueous extract |
|----------------|-----------------|
| Alkaloids      | +               |
| Carbohydrates  | -               |
| Terpenoids     | +               |
| Cardiac glycosides | -           |
| Phlobatannins  | +               |
| Tannins        | +               |
| Glycoside      | -               |
| Saponins       | +               |
| Flavonoids     | +               |
| Phenols        | +               |

Presence (+): Absence (-)

![Fig. 2: Ultraviolet-visible absorption spectra of plant extract (AP) extract and Zn-Fe$_3$O$_4$NPs](image2.png)

**Fig. 2:** Ultraviolet-visible absorption spectra of plant extract (AP) extract and Zn-Fe$_3$O$_4$NPs

![Fig. 3: Optical direct energy band gap detection of Zn-Fe$_3$O$_4$NPs](image3.png)

**Fig. 3:** Optical direct energy band gap detection of Zn-Fe$_3$O$_4$NPs

![Fig. 4: Optical indirect energy band gap detection of Zn-Fe$_3$O$_4$NPs](image4.png)

**Fig. 4:** Optical indirect energy band gap detection of Zn-Fe$_3$O$_4$NPs
Band gap calculation

The energy band gap for the prepared nanoparticles is evaluated by Tauc relation

\[\alpha h \gamma = I(h \gamma - E_g)^2\]

Where \(\alpha\), \(I\), \(h\), \(\gamma\) and \(E_g\) are linear absorption coefficient, proportionality constant, Planck's constant, photon frequency and optical energy band gap. The optical energy of the band gap can be evaluated by plotting \((\alpha h \gamma)^2\), \((\alpha h \gamma)^{1/2}\) and photon frequency will be made (fig. 3 and 4). The energy of band gap for Zn-Fe3O4Nps was found to be 1.9eV (direct band gap) and 1.3eV (indirect band gap), which is smaller than bulk Fe3O4NPs [18]. The outcome confirmed that zinc metal ion plays a significant role to reduce the energy band gap of the magnetite nanoparticles.

FTIR spectral analysis

FTIR spectra were studied to analyze the bioactive functional groups of Andrographis paniculata, which acted as a reducer, capping and stabilizing agent in the preparation of Zn-Fe3O4Nps. The spectrum of the Andrographis paniculata indicated a high intense absorption peak at 3436, 2071, 1634, 1119, 666 cm\(^{-1}\), whereas the intense absorption peaks of synthesized Zn-Fe3O4Nps were indicated at 3446, 2340, 1615, 1111, 616, and 435 cm\(^{-1}\) (fig. 5). The absorption band at 3446 cm\(^{-1}\) in the Andrographis paniculata extract was attributed to the O-H stretches [19], while absorption peak at 1634 cm\(^{-1}\) and 1119 cm\(^{-1}\) were contributed to C=O stretches [20] and C-O-C stretches, respectively. All the bands were shifted, indicating the bioactive group involvement and interactions of NPs with the Andrographis paniculata extract, presence of bioactive compounds on the surface of Zn-Fe3O4NPs has an influence on the FTIR peaks. Two strong absorption peaks were found at 552 cm\(^{-1}\) and 435 cm\(^{-1}\) in the spectrum of synthesized nanoparticles, which is related to the Fe-O stretching vibration mode of magnetite (Fe3O4). The absorption peak at 552 cm\(^{-1}\) can be contributed to the intrinsic stretching vibration of metal at the tetrahedral site, whereas the peak found at 435 cm\(^{-1}\) is corresponds to octahedral stretches of Fe-O [21]. The weak absorption band at 616 cm\(^{-1}\) is associated with Zn-O stretching vibration [22]. The weak absorption band at 552 cm\(^{-1}\) is associated with Zn-O stretching vibration [22]. The above information confirms the formation of Zn-Fe3O4NPs. Based on FTIR, the bioactive compounds found in the Andrographis paniculata extract strongly suggested the conformation of alkaloids, terpenoids, flavonoids, saponins, phlorotannins, phenols and tannins. These bioactive compounds acted as a reducer and efficient stabilizing agent for Zn-Fe3O4NPs in the solution.

Fig. 5: FTIR spectra for the plant extract (AP) and Zn-Fe3O4NPs

Fig. 6: XRD pattern of Zn-Fe3O4NPs synthesized using Andrographis paniculata extract
XRD spectral studies

The crystalline size and structural identification of the material were detected by using powder XRD spectral studies. Fig. 6 demonstrates the XRD spectral data of Zn-Fe3O4 NPs. The crystallite size of zinc doped magnetite nanoparticles can be evaluated using the Debye Scherrer equation; the required equation is

\[ D = \frac{0.9\lambda}{\beta \cos \theta} \]

The estimated size of the Zn-Fe3O4 nanoparticles was calculated to be 5 nanometres, respectively. The synthesized material showed the major diffraction peaks for prepared crystalline nanoparticles at 2θ values of 30.27, 35.60, 43.23, 53.87, 57.23, 62.78 degrees corresponding to the crystal planes at (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), (4 4 0) respectively. All these diffraction peaks clearly coincide with the diffractions of iron oxide nanoparticles (magnetite phase). No other secondary phase is detected to prove the formation of other zinc-based structures such as simonkolleite, hydroxyzine, etc. So, we might conclude that Fe3O4 NPs has been formed with high phase crystalline purity and Fe ions have successfully replaced by zinc ions. Furthermore, this XRD spectral pattern is well-matched with early reported zinc metal-doped iron oxide nanoparticles [23]. The XRD spectral pattern is matched with pure cubic magnetite nanoparticles with the Joint Committee of Powder Diffraction Standards (JCPDS File no 01-074-1910).

SEM-EDX analysis

The powder sample was analyzed for the morphological character of the Zinc doped magnetite nanoparticles by using SEM at different magnifications, including 2 µm, 5 µm and 10 µ. The SEM images of zinc doped magnetite nanoparticles using Andrographis paniculata leaf extract are shown in fig. 7. SEM micrographs reveal that the surface morphology of the nanoparticles is cubic in shape [24]. The intense peak obtained from EDX spectra is Zn, Fe and O (fig. 8). The composition of zinc, iron and oxygen element is about 5.26, 35.71 and 52.18 % for Zn-Fe3O4 NPs. EDX spectra also detect the presence of C and S, which may originate from the biomolecules present in the Andrographis paniculata extract. Therefore elemental studies confirm that the synthesized nanoparticles are pure without any impurity peaks.

![SEM micrographs of the synthesized nanoparticles obtained through co-precipitation method using Andrographis paniculata extract, at different magnifications](image)

![EDX spectra of synthesized Zn-Fe3O4NPs](image)

VSM study

The magnetic behavior of zinc doped magnetite nanoparticles was evaluated by vibrating scanning magnetometer. The applied magnetic field was between -15,000 Oe to 15,000 Oe. The magnetization curve of the synthesized nanoparticles is depicted in fig. 9. The measured magnetic saturation value of Zn-Fe3O4 NPs is 70 emu/g at 300 k temperature, which is higher than that of bulk magnetite (Fe3O4) [25]. The obtained result reveals that the synthesized Zn-Fe3O4 nanoparticles are super paramagnetic (particle size 5 nm) at room temperature.
Antidiabetic activity

The inhibition activity of zinc doped magnetite nanoparticles against alpha-amylase

Diabetes mellitus is a major metabolic disease in which there are high blood sugar levels over an extended period. A medical approach to control hyperglycemia is to suppress the alpha-amylase enzyme. The carbohydrate digestive enzyme (α-amylase) is used for the conversion of carbohydrates into monosaccharides, which is the major reason for increasing of high blood sugar levels in the human body. Therefore, synthesizing compounds having strong inhibitory activities towards digestive enzymes is an easy route to cure diabetes [26]. The inhibitory activity of green synthesized Zn-Fe₃O₄ NPs in combination with commercially used pharmacological inhibitor, Acarbose, was examined. The results indicate that the digestive enzyme was significantly inhibited with various concentrations of Zn-Fe₃O₄ nanoparticles.

Comparison of pancreatic alpha-amylase inhibition of plant extract (AP), Acarbose, and Zn-Fe₃O₄ nanoparticles were shown in fig. 10. The percentage inhibition of zinc doped magnetite nanoparticles at various concentrations (10, 50, 100, 250 and 500) was found to be 19.93, 33.3, 45.2, 65.32, and 90.91 %, respectively. Hence the biomolecules present in the *Andrographis paniculata* extract likely improved the diabetic character of the synthesized nanoparticles [14]. Based on the results, blank < Acarbose < Zn-Fe₃O₄ NPs inhibited the alpha-amylase enzyme and thus prevented the further hydrolysis of carbohydrates and controls the high blood sugar level in type-2 diabetes patients.

CONCLUSION

The zinc doped magnetite nanoparticles were successfully obtained by using a simple, eco-friendly technique from the selected natural plant (*Andrographis paniculata*) extract. This study aims at investigating Zn doping effects on optical, magnetic, and structural properties of magnetite nanoparticles. Based on the obtained results, Optical studies confirm that band gap energy decreases while doping with a transition metal ion (Zn²⁺). FTIR spectral data indicated that the phytochemical constituents found in the *Andrographis paniculata* extract acts as an efficient stabilizer and reducing agent for the synthesized Zn-Fe₃O₄ NPs. XRD spectral analysis results indicate that the Zn-Fe₃O₄ NPs are in the magnetite phase, cubic in structure. The secondary phase is not detected to prove the formation of other zinc-based structures such as simonkolleite and hydroxyzincite. VSM reveals the formation of a superparamagnetic structure with a high saturation magnetization value of 70emu/g. The major elements present in the EDX spectra are Fe, O and Zn in the Zn-Fe₃O₄ NPs. The presence of S and C as minor elements was associated with the elemental content of the *Andrographis paniculata* extract. The morphological character of the...
obtained sample was investigated by SEM measurements. The antidiabetic activity indicated that the synthesized Zn-Fe3O4 NPs exhibited an improved inhibitory potential against the alpha-amylase enzyme, it also offers a novel approach in nanomedicine for diabetes (type-2) management. The environmentally friendly synthesized Zn-Fe3O4 NPs are suitable in various application fields, especially in biosensing and pharmacological applications.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICT OF INTERESTS
The author reports no conflict of interest. The responsible for the writing and content of the article is just the author

REFERENCES

1. Sebastian A, Nangia A, Prasad MNV. A green synthetic route to pharmocics fabricated magnetite nanoparticles from Coconut husk extract: implications to treat metal-contaminated water and heavy metal stress in Oryza sativa L. J Cleaner Prod 2018;174:355–66.

2. Harrison RJ, Dunin Borkowski RE, Putnis A. Direct imaging of nanoscale magnetic interactions in minerals. Proc Natl Acad Sci 2002;99:16556–61.

3. Teja AS, Koh PY. Synthesis, properties and applications of magnetic iron oxide nanoparticles. Prog Chem Growth Charact Mater 2009;55:22–45.

4. Lin CR, Chu YM, Wang SC. Magnetic properties of magnetite nanoparticles prepared by mechanochemical reaction. Mater Lett 2006;60:447–50.

5. Song M, Zhang Y, Hu S, Song L, Dong J, Chen Z, et al. Influence of morphology and surface exchange reaction on magnetic properties of monodisperse magnetite nanoparticle. Colloids Surf A 2012;408:114–21.

6. Lu X, Xu J, Jiang G, Tang J, Xu X. Highly active nanoscale zero-valent iron (nZVI)-F (d), nanocomposites for the removal of chromium (VI) from aqueous solutions. J Colloid Interface Sci 2012;369:460–9.

7. Gorski CA, Scherer MM. Influence of magnetite stoichiometry on Fe(II) uptake and nitrobenzene reduction. Environ Sci Technol 2009;43:3657–80.

8. Yew YP, Shanmugam S, Mijake M, Kuwano N, Ahmad Kairinud NB, Bt Mohamad SE, et al. Green synthesis of magnetite particle (Fe3O4) nanoparticles using seaweed (Kappaphycus alvarezii) extract. Nanoscale Res Lett 2016;11:61–7.

9. Irina Vedernikova, Alla Koval, Alina Fatyliya. Thermogravimetric and structural studies of zinc-doped magnetite nanoparticles for pharmaceutical applications. J Chem Pharm Res 2018;10:109-12.

10. Zhang J, Zhang C, Wei G, Li Y, Jiang X, Chu W, et al. Reduction removal of hexavalent chromium by zinc-substituted magnetite coupled with aqueous Fe(II) at neutral pH value. J Colloid Interface Sci 2017;500:20–9.

11. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J Radiat Res Appl Sci 2016;6:9–17.

12. Arinton H, Kolegan H, Wuntu AD. Synthesis of silver nanoparticles using aqueous extract of medicinal plants’ (Impatiens balsamina and Lantana camara) fresh leaves and analysis of antimicrobial activity. Int J Microbiol 2019;1–8. Doi:10.1155/2019/8642303.

13. Jyoti Pereira, Pankaj S. Wasim Raja. Evaluation of phytochemical analysis of Andrographis paniculate leaf and stem extract. World J Pharm Life Sci 2019;5:180–90.

14. Krishnaveni K, Sakti Athithan AS, Jeyasundari J, Jacob YB, Renuga D. Biosynthesis and characterization of zinc doped iron oxide nanoparticles from Pedulum murex and its new avenues in pharmacological applications. IOSR J Pharm Biol Sci 2018;13:67–74.

15. Yousefi A, Yousefi P, Pahali F, Sarikhan S, Zolgahdr AR, Bahaoedini A, et al. Novel curcumin-based pyran [Z-3-

pyrimidine anti-oxidant inhibitors for α-amylase and α-glucosidase: implications for their pleiotropic effects against diabetes complications. Int J Biol Macromol 2015;78:46–55.

16. Nagajothi S, Mekala P, Raja A, Raja MJ, Senthilkumar P. Andrographis paniculata: quantitative and qualitative phytochemical analysis. J Pharmcogn Pytochem 2018;7:1251-3.

17. Chaki SH, Malek TJ, Chaudhary MD, Tailor JP, Deshpande MP. Magnetite Fe3O4 nanoparticles synthesis by wet chemical reduction and their characterization. Adv Nat Sci Nanosci Nanotechnol 2015;6:035009.

18. Mohanraj K, Sivakumar G. Synthesis of γ-Fe2O3, Fe3O4 and copper doped Fe3O4 nanoparticles by sonochemical method. Keral Jur Phys Sci 2019;136:105246.

19. Sutriyo S, Iswandana R, Ivariaini FM. Synthesis and stability of resveratrol-gold nanoparticle-polyethylene glycol-folic acid conjugates. Int J Appl Pharm 2020;12:237–41.

20. Saiwatakekar A, Kakde UB. Green synthesis of copper nanoparticles using aspergillus flavus. J Crit Rev Sci 2020;7:1083-90.

21. Erruta A, Chirita M. Highly crystalline porous magnetite and vacancy-ordered maghemite microcrystals of rhombohedral habit. J Cryst Growth 2013;380:182–6.

22. Suresh J, Prathheesh G, Alexramani V, Sundrarajan M, Hong SI. Green synthesis and characterization of zinc oxide nanoparticle using insulin plant (Costus pictus D. Don) and its new avenues in pharmacological applications. Adv Nat Sci Nanosci Nanotechnol 2019;9:015008.

23. Jouyandeh M, Ali JA, Aghazadeh K, Saeb MR, Ranbar Z, et al. Curing epoxy with electrochemically synthesized α-alumina nanoparticles. Prog Org Coat 2019;136:105246.

24. Mahdavi M, Namvar F, Ahmad M, Mohamad R. Green biosynthesis and characterization of magnetic iron oxide (Fe3O4) nanoparticles using seaweed (Sargassum muticum) aqueous extract. Mol 2013;18:5954–64.

25. Maboudi SA, Shojaosadati SA, Arpanaei A. Synthesis and characterization of multilayered nano-bio hybrid magnetic nanoparticles for biomedical applications. Mater Des 2017;115:317–24.

26. Saratate RG, Shin HS, Kumar G, Benelli G, Kim DS, Saratale GD. Exploring antidiabetic activity of silver nanoparticles synthesized using Punica granatum leaves and anticancer potential against human liver cancer cells (HepG2). Artif Cells Nanomed Biotechnol 2017;45:211–22.