Original Article

Magnetite Fe_3O_4 Nano-oxide from Aqueous leaf Extract of *Coccinia grandis* (L.) Voigt: Synthesis, Characterization, Magnetic studies and Anti-cancer Evaluation

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ABSTRACT

Aim: To synthesize and investigate the anticancer activities of the metal nanoparticles derived from the ethanolic solution of the *Coccinia grandis* (L.) Voigt leaves. **Background:** *Coccinia grandis* (Ivy gourd) belongs to the Cucurbitaceae family is known for its own significance especially in conventional medicines, including Indian Ayurveda. **Materials and Methods:** The title iron oxide nanoparticles from *Coccinia grandis* leaf extract has been synthesized as reported in the literature and characterized using spectroscopic techniques like FT-IR, GC-MS, VSM, SEM and EDAX. Pharmacological evaluation like *in-vitro* anti-cancer activity has been done using MTT assay. **Discussion and Conclusion:** The powder XRD pattern of the synthesized Fe_3O_4-NPs showed its high purity crystalline nature and also proved it to be approximately cubic in structure. By comparing the powder XRD patterns and the standard JCPDS database (file no: 01-089-3850), the obtained Fe_3O_4 nanoparticles were found to be Fe_3O_4, and not γ-Fe_2O_3 (maghemite). The SEM images of the nanoparticle showed their cubic nature and their magnetic studies were carried out using VSM data. The anticancer activities of the magnetite nanoparticles have been evaluated by using *in vitro* assays and found to be 35.83μM and were compared to standard anticancer drugs such as cisplatin, Doxorubicin.

Key words: Magnetite, Anti-Cancer, Metal nanoparticle, VSM, *Coccinia grandis.*

INTRODUCTION

Nanoparticles have versatile characteristics and their applications are in the diverse fields like pharmaceutical, energy, food science, environmental, and so on. The size, surface characteristics and inner structure of these particles are the factors which determine their application in electronics, optics, drug delivery and other fields. The field of nanotechnology is usually inter-related with all branches of science like life sciences, physics and materials, chemistry, and engineering sciences, in developing novel bioactive nanomaterial's for therapeutic applications.[1] Nanomaterial's can be synthesized by chemical precipitation, pyrolysis, ion implantation and hydrothermal synthesis.[2-4] on combining with biochar, magnetite can be used as an adsorbent to remove toxic heavy metal ions from polluted water. Oyang reported the synthesis of nanomagnetite-biochar composite and their application as an active persulphate to degrade 1, 4 dioxane, an organic pollutant.[5-7] In recent years, there is a threat to human life, in the form of various dreadful diseases, which makes the researchers in the field of biology and chemistry to think of a novel drug, which will be ecofriendly, non-toxic and at the same time to have a targeted drug delivery. Two dimensional nanomaterial's can be used as cancer nanotheranostics, as they have applications in cancer diagnostics, imaging and treatments.[8] It includes the magnetic bio-separation from magnetite

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nanoparticles, that involves in cell separation, which is useful in diagnosing cancer cells.[9] Synthesis of nanoparticles by greener method is an emerging trend and the secondary metabolites available in the plant extracts help in bio-reduction of metal ions, to form nanoparticles, thereby stabilizing them. These significant applications of iron nanoparticles made us to synthesize the magnetite nanoparticle by using plant extract as shown in the literature, eg. caricaya papaya.[10] Phytomedicines and their nanoparticles have a great therapeutic potential and can be a rich source of drug that can be blended with existing drugs to form a good elevated pharmaceutical agent in efficacy and safety. *Coccinia grandis* is one among the phytomedicines that is used in various therapeutic agents like anti-diabetic, anti-oxidant, anti-leishmanial, antiiglycation and insulotrophic properties.[11-14] The leaf itself is used as anti-diabetic agent in patients with type 2 diabetes. The leaves extract reduces the cholesterol and has beneficial effects in preventing the rise of liver enzymes and has some anti-oxidant property. Tamilselvan *et al.* clearly stated the biological properties of *Coccinia grandis* as anti-bacterial, hepatoprotective, hypoglycemic, anti-helminthic, anti-ulcer, anti-tissue, anti-dyslipidemic, anti-oxidant, anti-inflammatory, analgesic, anti-pyretic, α-amylase inhibitor and so on.[15] The biologically active *Coccinia grandis* leaf can be used in the biosynthesis of nanoparticles. Wang *et al.* syntheized, characterized and studied the cytotoxicity of gold nanoparticles for cataract treatment.[16] Tura *et al.* studied the anti-microbial and anti-oxidant assay of ZnO nanoparticles of tuber extract of Coccinia abyssinica.[17] Silver nanoparticles obtained from the biogenic synthesis using *Coccinia grandis* leaf extract is used for the degradation of dyes like Brilliant blue G-250, a triphenyl methane dye under UV light.[18] Copper nanoparticles derived from *Coccinia grnadis* fruit extract were used in the catalytic reduction of toxic organic compounds like p-nitrophenol.[19] The biological application of leaf extract of *Coccinia grandis* and their nanoparticles are significant in the earlier reported works and so the present work involves the green synthesis and characterization of the Magnetite Iron nanoparticles from the leaf extract of *Coccinia grandis* (ivy gourd) which are commonly found in tropical climates and their pharmaceutical evaluation.

MATERIALS AND METHODS

$FeCl_2 \cdot 4H_2O$ (Iron (II) chloride tetrahydrate), $FeCl_3 \cdot 6H_2O$ (Iron (III) chloride hexahydrate) and Sodium hydroxide were purchased from Sigma Aldrich and used without purification. The *Coccinia grandis* leaves were collected from residential areas in Coimbatore. The aqueous solutions were prepared by using deionized water. The nanoparticles synthesized, were characterized using several analytical techniques like FT-IR(IR Affinity-1S Fourier Transform Infrared Spectrophotometer), GC-MS (Agilent GC-MS instruments), Powder XRD (Malvern Panalytical Diffractometer) SEM and EDAX (FE_SEM Sigma HV-Carl Zeiss with Bruker Quantax 200). Pharmacological evaluation like *in-vitro* anti-cancer activity has been done using MTT assay.

Preparation of *Coccinia grandis* Extract and Fe_3O_4-Nanoparticles

Coccinia grandis leaves were collected from the local residents in Coimbatore, they were cleaned in running water, to get rid of dust and other dirt particles. The cleaned leaves were dried without the presence of sunlight in room for 3 days and then powdered. The extract of *Coccinia grandis* leaves were prepared by heating the dried powder in hot water. Figure 1 shows the leaf extract. Fe_3O_4 nanoparticles from the leaf extract has been synthesized as reported in the literature.[20]

RESULTS AND DISCUSSION

Magnetite iron particles have significant importance in the literature, as potential drug carrier for various diseases whose therapy can be combined with the magnetic properties, environmental applications like adsorption of heavy metal ions, effluent treatment of aquaculture, degradation of dyes, electro catalyst, pharmacological and activities like anti-microbial, antioxidant and so on. In view of the application of magnetic particles in various fields, an attempt has been made to synthesize iron oxide nanoparticles using greener method. i.e from the leaf extract of *Coccinia grandis*, which have a well-known pharmacological activity. The mixture of Ferrous and Ferric iron salts, after the addition of NaOH and stirring for one hour, the colour of the reaction mixture

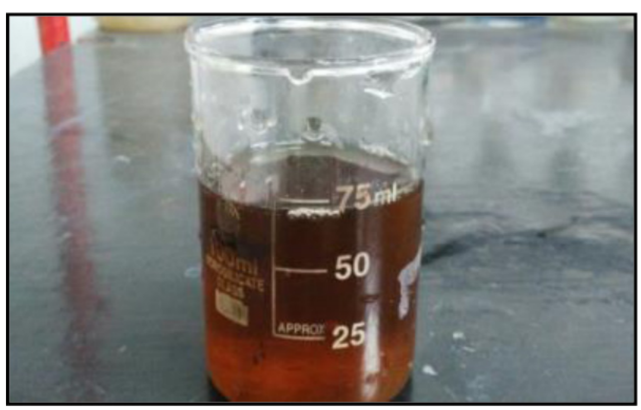

Figure 1: Leaf extract of *Coccinia grandis.*

changes from green to light brown which indicates the product formation (Fe_3O_4 nanoparticles). They are black (or a brownish black), while Fe_2O_3 nanoparticles are typically red to reddish-brown. To 2:1 molar ratio of Fe^{2+} and Fe^{3+}, a base was added and it resulted in iron oxide nanoparticles. The chemical reaction for the overall precipitation is given in the following equation:

$$\text{C.grands} + H_2O + Fe^{2+}(aq) + Fe^{3+}(aq) \xrightarrow{\text{Stirring}} \text{C.grandis} - Fe^{2+}/Fe^{3+}$$

$$\text{C.grandis} - Fe^{2+}/Fe^{3+} + 8\,OH^- \xrightarrow{\text{Stirring}} [\text{C.grandis}/Fe_3O_4]\downarrow + 4H_2O$$

The precipitation occurs because of the high tendency of the iron oxide particles to agglomerate. The possible mechanism of Fe_3O_4 formation as explained by Yen Ping Yu *et al.* as a precipitation reaction, through the formation of hydroxides is given above.

GC – MS Analysis of the Plant Extract

The GC-MS analysis of the plant extract done to determine the possible chemical constituents showed that the extract mainly contained the following compounds like triethoxy esters, tetradecanoic acid, L {+} ascorbic acid, 2,6, dihexadecanoate, oleic acid, octadecadonic acid, D-Galactopyranose, D-Mannofuranoside (competitive inhibitor of E. Coli) including phytol, a diterpene alcohol which is mainly used in the synthesis of Vitamin E, as a food additive and also in medicinal field. L {+} ascorbic acid, 2,6, dihexadecanoate that has been reported for important anti-oxidant, anti-inflammatory properties and in many medicinal applications.[21,22] Ascorbic acid also acts as a reducing agent in the synthesis of nanoparticles and the sugar units present in the leaf extract acts as stabilizing agent (eg: D-Galactopyranose or D-Mannofuranoside). Figure 2 the GC-MS analysis of the plant extract and Figure 3 is the schematic diagram showing the stabilizing agents from the plant extract that stabilizes the nanoparticle.

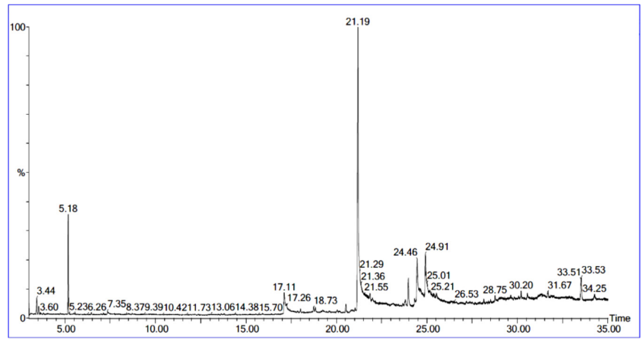

Figure 2: GC-MS of plant extract.

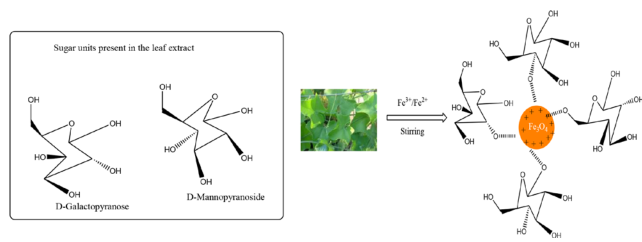

Figure 3: Schematic diagram which shows the stabilizing agents of Iron nanoparticles.

FT-IR Spectrum of Leaf Extract and the Iron oxide Nanoparticles

The Chemical constituents present in the plant extarct like sugars, carboxylic acids, esters, alcohols and so on act as stabilizers and capping agents in the synthesis of nanoparticles. FT-IR spectroscopy is an useful technique which helps for identifying the functional groups present in the extract. The FT-IR spectra of the *Coccinia grandis* leaf extract shows a strong absorption band at 3365 due to O-H stretching vibrations present in alcohols, sugars and carboxylic acid. The peak at 2900 cm^{-1} is attributed to aliphatic $-CH_2$ group while band at 1643 and 695 cm^{-1} are attributed to >C-H bending vibration of the $-CH_2$ functionality. The absorption frequency at 1287 cm^{-1} is due to the presence of C-O in the sugar unit. Comparing both the FT-IR spectra, leaf extract and Fe_3O_4-Nps, the bands of the nanoparticle are shifted due to the weak interactive force with the Fe_3O_4 moiety as stabilizing agents. Two new bands at the region 402 and 409 cm^{-1} are obtained which confirms the presence of Fe-O bond and also the formation of nanoparticles (Fe_3O_4). Figure SI 1a and 1b shows the FT-IR spectrum of the leaf extarct and the nanoparticle respectively. The formation of Fe_3O_4 nanoparticles was confirmed with the characteristic peaks laying in the region of 400-450 cm^{-1}.[23] A schematic diagram (Figure 3) shows the possible interactions that can exist between the stabilizing agents and the metal nanoparticles.

EDAX Spectrum

The energy dispersive X-ray analysis spectrum of the synthesized nanoparticles is Figure 4. The EDX spectrum indicates that the iron oxide structures are composed of Fe and O. However, a small percentage of chlorine is present which might be due to the ferric and ferrous precursor used.

Powder XRD

Powder XRD revealed that the synthesized iron oxide nanoparticles was in approximately cubic phase. The O to Fe ratio of the NP was observed from SEM-EDAX analysis and approximated to be Fe_3O_4. 2.69 Angstrom (311 plane) is the characteristic peak for Fe_3O_4 and the other one at 2.53 Angstrom is characteristic peak for Fe_2O_3. The 2θ diffraction peaks were detected at

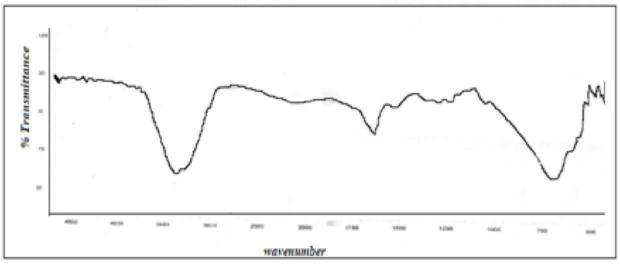

Figure SI 1a: FT-IR spectrum of leaf extract.

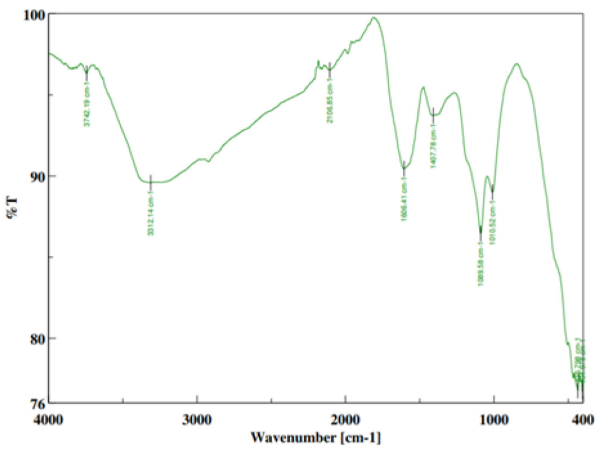

Figure SI 1b: FT- IR spectrum of iron oxide nanoparticles.

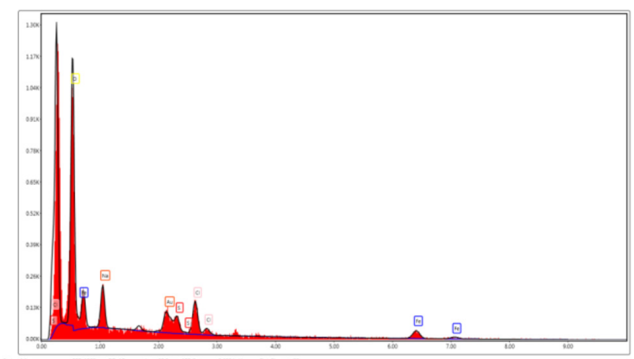

Figure 4: EDAX Spectrum of Iron Oxide nanoparticles.

31.74°C, 34.29°C, 45.50°C, 53.94°C, 56.54°C, and 75.35°C. and correlate well with the XRD patterns of standard magnetite with JCPDS file no: 00-003-0863, confirming the cubic phase crystallographic system, which was further confirmed from SEM images.[24,25]

The difference in the powder XRD patterns of γ-Fe_2O_3 and Fe_3O_4 NP is the number of peaks, the former has more peaks than the latter. The Debye-Scherrer equation is given as follows:

$$\text{Crystallite size } L = \left(\frac{K \times \lambda}{\beta \times \cos\theta}\right)$$

Using the above equation, the crystallite size of Fe_3O_4-NPs has been calculated and was found to be 5.21 nm. Figure 5 shows there is no impurity peak was observed, proving high purity crystalline nature of the synthesized Fe_3O_4-NPs

The SEM image of Figure SI. 2 clearly indicates that the products are composed of non-uniform cluster like features and in some places cubic structure. The magnified image in Figure 6, shows that the clusters are cubic in structure which clearly confirms the existence of Magnetite nanoparticle. The uniformity in the synthesis of magnetite still remains a challenge.[26]

Magnetic Studies

The VSM graph of the Fe_3O_4 nanoparticle was shown in Figure 7. The sample behaves like a classical paramagnet at relatively high temperature. The coercivity of Fe_3O_4 is 5.4498 G and the saturation magnetization is 23.084×10^{-3} emu. The magnetization of bulk magnetite will be ferromagnetic in nature generally, due to the tetrahedral sites aligned parallelly

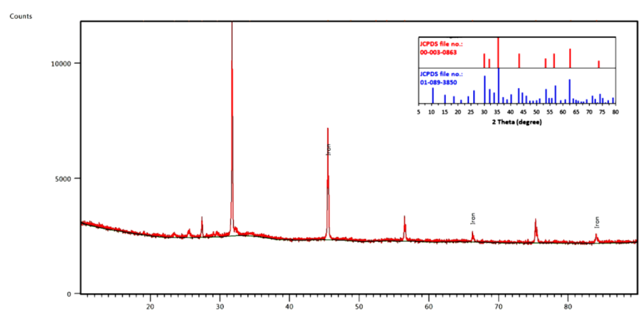

Figure 5: Powder XRD spectrum of Fe_3O_4 from JCPDS and the synthesized oxide SEM.

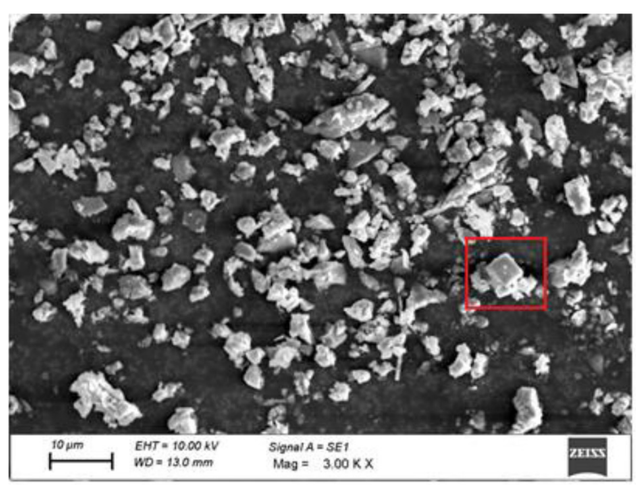

Figure SI 2: SEM image of iron oxide nanoparticles.

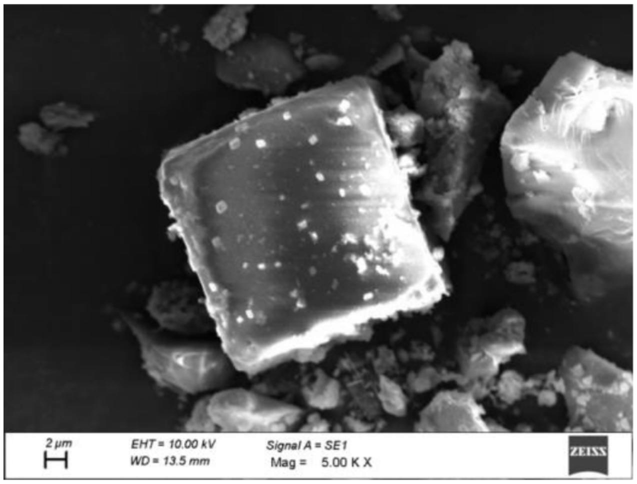

Figure 6: SEM image of Iron Oxide nanoparticles (magnified image).

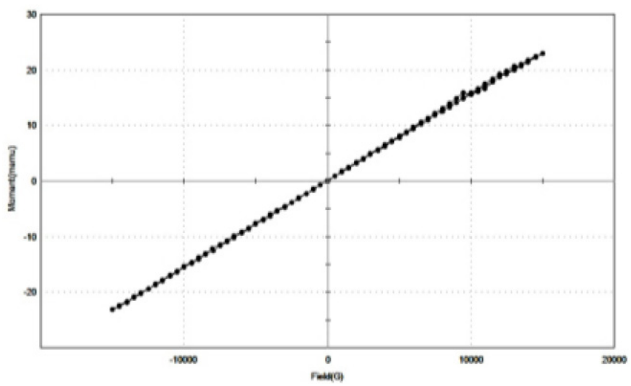

Figure 7: VSM graph of Magnetite material.

and anti-parallel alignment of ferrous and ferric spins on octahedral sites. When the crystallite size of the magnetite is reduced to nano size, the hysteresis loop expected for a ferromagnetic material is reduced with reduced coercivity. The reduction in the particle size demonstrates this behavior and if the magnetite is a bulk material, the presence of hysteresis loop will be there and the absence of which shows the existence of nanomaterials with reduction in size.[27]

Pharmacological Activity

In-vitro anti-cancer activity

The significant biological applications of iron nanoparticles and *Coccinia grandis* leaf in the literature as anti-cancer agents induced an interest to analyze the magnetite nanoparticle, which was done using MTT assay. The results were analyzed by means of cell viability curves given in Figure 8 and expressed with IC_{50} values in the studied concentration range from 0.1 to 100μM. The synthesized material has an IC_{50} value of 35.83 μM.

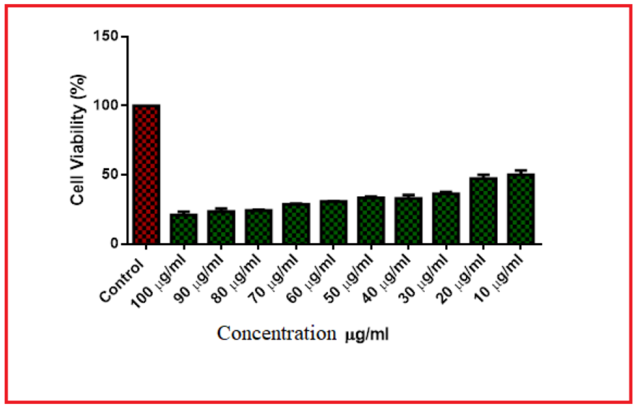

Figure 8: Cell Viability Curves of the magnetite.

Though the nanoparticles were active against tumor cell lines under *in-vitro* cytotoxicity experiments, the value is lesser than that of the more effective standard drug cisplatin. But the magnetite synthesized is water soluble, and if its toxicity is verified and functionalized to reduce the IC_{50} value it can be developed into a good anti-cancer drug.[28]

CONCLUSION

In this study, iron nano metal oxide has been synthesized and analyzed using various spectral techniques like FT- IR, SEM - EDX, VSM and powder XRD. The synthesized material has a cubic structure which is confirmed from the powder XRD and SEM images, but the particles are dispersed and non-uniform. The conditions should be optimized to make the structures uniform. The synthesized iron oxide has been screened for the *in-vitro* anti-cancer activity and it was found to be 35. 83 μM. The present study has revealed that the extract of *Coccinia grandis* showed good cytotoxic activity. Though in this case the results are moderate, these reports would definitely help in improving the biological property of magnetic nanoparticles.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IC_{50}: Half maximal inhibitory concentration; **FWHM:** Full width at Half Maximum; **GC-MS:** Gas

Chromatograph Mass Spectroscopy; **NP:** Nanoparticle; **SEM:** Scanning electron Microscope; **XRD:** X-Ray Diffraction; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **VSM:** Vibrating Sample Magnetometer.

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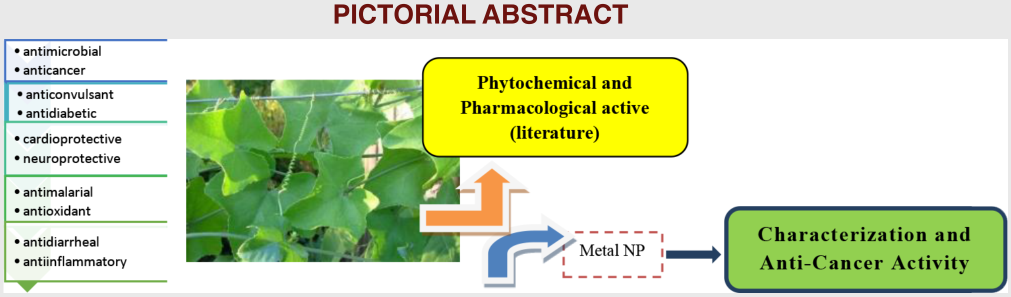
PICTORIAL ABSTRACT



SUMMARY

Phytosynthesis of Magnetite Fe_3O_4 nanoparticles were carried ot using *Coccinia grandis* leaf extract. The synthesized material was characterized using analytical techniques like FT-IR, GC-MS, Powder XRD, SEM, VSM. The pharmacological studies of the nanoparticle was carried out and found to be good.

About Authors

Dr. Suyambulingam Jone Kirubavathy, Assistant Professor in Chemistry, PSGR Krishnammal College for Women, is an expert in the field of metal organic materials. She has successfully completed two research projects with 30 publications in reputed scopus indexed journals with h-index 6. Her achievements include NET, SET and GATE qualifications with 13 years of teaching experience. She has guided more than 30 M. Sc projects, 6 M. Phil Scholars and presently 4 Ph.D Scholars are working in the area of Metal Organic Frameworks/Nanomaterials and their Clinical/ Environmental applications.

Dr. Natarajan Arunadevi, Assistant Professor, Department of Chemistry, PSGR Krishnammal College for Women is an expert in the field of solid state Chemistry and metal oxide nano particles. She has 19 years of experience in teaching and research and completed two research projects with one research project ongoing. She has published 30 research articles in reputed journals with h-index -5. She has guided 2 M.Phil and guiding 4 Ph.D scholars.

Ms. Ponnusamy Pavithra was an undergraduate student at PSGR Krishnammal College for Women during 2016-2019. She pursued her post graduate programme in PSGRKCW during 2019-2021. She is a hardworking, enthusiastic and knowledgable student and completed her project in the field of Nanomaterials.

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