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IN -VITRO CYTOTOXICITY OF GREEN SYNTHESIZED Ag-Fe NANOPARTICLES AGAINST HUMAN BREAST CANCER CELLS

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ABSTRACT

Developing an eco- friendly process for synthesis of nanoparticles is a noteworthy step in the field of green nanotechnology. It involves the tailoring of materials at the atomic level to achieve unique properties, which can be rightfully operated for the required therapeutic applications for various diseases. In the present work, bimetallic silver-iron bimetallic nanoparticles (BMNPs) are synthesized from silver nitrate ($AgNO_3$) and ferrous sulphate ($FeSO_4$) precursor solutions using aqueous leaf extract of medicinal plant *Aerva lanata*. The phyto molecules of the leaf extract are functioned as bio-reducing, stabilizing and capping agents. The synthesized BMNPs are characterized using UV-Vis spectroscopy, FTIR, SEM, EDX, XRD and TEM analytical techniques. The cytotoxic response is assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. These biogenic Ag-Fe BMNPs is found to be significantly toxic to MCF-7 cells (human breast cancer cells) via induction of apoptosis.

Keywords: **Bimetallic nanoparticles (BMNPs), *Aerva lanata* (AL), Phyto molecules, cytotoxicity**

1. INTRODUCTION

Breast cancer is the most common malignancy in mankind that causes major mortality worldwide. Over the past decenniums, treatments to this notable life-threatening cancer has become more challenging owing to the prevalence of multiple drug resistance, detrimental side effects and the lack of innovative approaches **[1]**. Nanomedicine is a hopeful and exciting field that could potentially lead to improvements in cancer treatment procedures, offering a modern perspective on tumour identification, prevention and treatment **[2]**. The successful application of nanoparticles as an anticancer drug is due to their exclusive qualities like large surface area for volume, porosity, solubility, increased bioavailability and different structural properties. Ultimately this can improve the stability and durability of the drugs, moreover it will offer many biomedical perceptions for clinical level applications **[3]**. Another interesting feature of nanoparticles, it can easily cross the cellular barriers and strongly interact with functional biomolecules **[4]**. Silver nanoparticles are among the most common and applicable nanostructures, according to their distinctive catalytic, therapeutic activities and stability as well as development of nanodevices and therapeutic preparation for diagnoses and treatment of cancer **[5]**. The treatment of a variety of cancers with silver NPs has been well documented **[6]**. Antitumor potentiality (cytotoxicity) of the silver NPs is expressed through oxidative stress as well as inflammation through production of reactive oxygen species that lead to DNA damage and mitochondrial membrane potential disorder, releasing cytochrome c and resulting in mitochondrial related apoptosis and necrosis to cell proliferation and carcinogenesis **[7]**.

Aerva lanata is a medicinal plant that belongs to the family *Amaranthaceae*. It is wealthy source of secondary metabolites that have antibacterial **[8]**, antifungal **[9]**, antioxidant **[10]**, cytotoxic **[11]**, anti-HIV **[12]**, anti tumour **[13]**, anti-diabetic **[14, 15]** and anticancer **[16]** activities. Moreover, the green synthesized nanoparticles are found to be more potent antitumour agents than the uncoated nanoparticles as the former are capped with bioactive phytomolecules **[17]**.

2. EXPERIMENTAL

2.1. Preparation of *Aerva lanata* leaf extract: 100g of fresh *Aerva lanata* leaves (**Figure 1(a)** are taken and cleaned with running tap water to remove dust on surface of leaves followed by deionised water to eliminate other contaminants from leaves and dried up under shade for ten days. The dried

leaves are made into powder by using home blender. Now 200 mL deionised water is taken in 500 mL beaker to this 10g leaf powder is added. The contents in the beaker heated for 30 minutes at 50^{o} C with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth is filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4^{o}C. This is taken as leaf extract for the experimental studies.

Figure 1(a): ***Aerva lanata*** **leaves**

2.2. Synthesis of Ag-Fe bimetallic nanoparticles:

Equimolar (25 mM) concentrations of silver nitrate ($AgNO_3$) and ferrous sulphate ($FeSO_4$) aqueous solutions are prepared separately in 100 mL volumetric flask by dissolving 0.4246 g, 0.6950 g weight of $AgNO_3$ and $FeSO_4$ in water respectively. Synthesis of Ag-Fe BMNPs is done by taking 100 mL of $AgNO_3$ solution in a 500 mL beaker. To this 90 mL of leaf extract and 100 mL of $FeSO_4$ solution are added drop wise by simultaneous addition process. During addition process beaker is placed on a magnetic stirrer for continuous agitation. Now this mixture is stirred at 40^{o} C for 40 minutes at pH 7 on magnetic stirrer. These synthesized BMNPs are separated out by doing centrifugation at 4500 rpm for 40 minutes. The obtained BMNPs are washed with using deionized water for two times to remove unwanted constituents and dried in oven at 90^{0} C for two hours. The resultant BMNPs particles are collected (**Figure: 1(b)**) and used for characterization.

Mechanism of green synthesis of Ag-Fe bimetallic nanoparticles:

Aerva Lanata leaf extract + Ag (I)-Fe (II) precursor salt solutions

↓ Reduction

Ag (0)-Fe (0) bimetallic nanoparticles

Growth and stabilization ↓

Capped Ag (0)-Fe (0) bimetallic nanoparticles

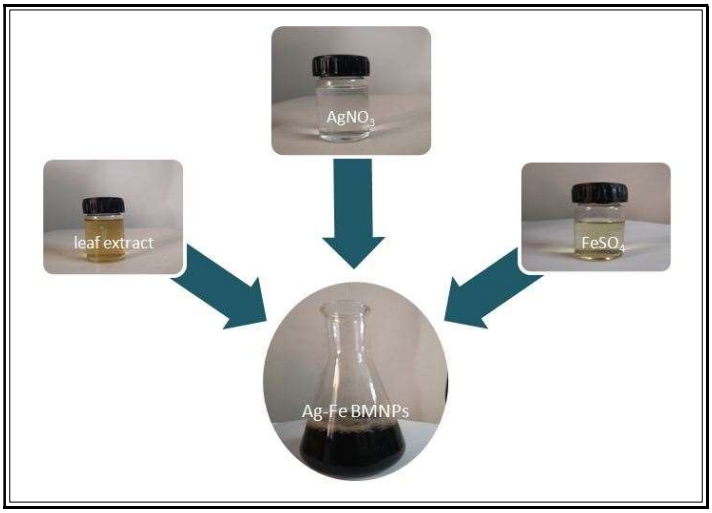

Figure: 1(b): Synthesis of Ag-Fe BMNPs from precursor solutions

2.3 Characterization:

Formation of Ag-Fe BMNPs is confirmed by UV-Visible absorption spectra using UV-2450 SHIMADZU double beam spectrophotometer, FTIR using Bruker, FESEM, EDX studies are done by using Hitachi S-3700N machine and the morphology of BMNPs is elucidated by HRTEM analysis with FEI Technai machine.

UV-Visible absorption spectrum of Ag-Fe BMNPs is revealed in **Figure 2(a)**. The characteristic surface plasmon resonance (SPR) band at around 441 nm is observed in Ag-Fe BMNPs which confirms the nano size of the synthesized particles **[18]**. FTIR

spectrum of Ag-Fe BMNPs is shown in **Figure 2(b)**. The band at 3553cm $^{-1}$ corresponds to –OH stretching hydrogen bonded alcohols and phenols. The peak at 3363cm^{-1} corresponds to amine functional groups. The peak at 1782 cm^{-1} corresponds to symmetric stretching of carbonyl group, the bands seen at 1466 cm^{-1}, 1381cm^{-1} corresponds to –CH stretching. The peak at 1306 cm^{-1} indicates geminal methyl groups. From the above analysis, it is confirmed that carbonyl and hydroxyl groups are present on the surface of BMNPs as capping agents **[19, 20]**.

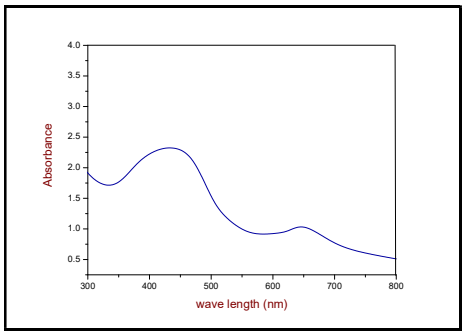

Figure 2(a): UV-Vis spectrum of Ag-Fe BMNPs

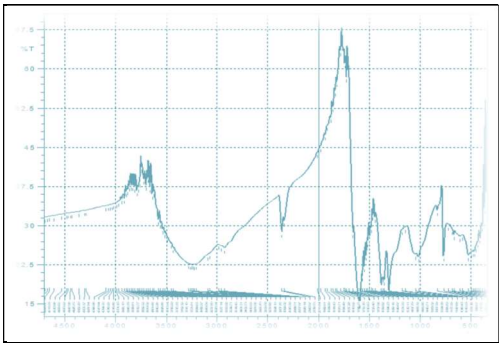

Figure 2(b): FTIR spectrum of Ag-Fe BMNPs

EDX spectrum (**Figure 2(c)** indicates the presences of Ag and Fe which confirms the formation of Ag-Fe bimetallic nanoparticles. This is also supported by the EDX study which gives quantitative data of Ag and Fe compositions in BMNPs**.** As per field emission scanning electron microscopic (FESEM) images of Ag-Fe BMNPs (**Figure 2(d)** it is noticed that nanoparticles are in the size range of 30-100 nm in Ag-Fe BMNPs. **Figure 2(e)** represents the HRTEM images for synthesized Ag-Fe BMNPs leaf extract. From these images, it is observed that Ag-Fe BMNPs are formed with spherical morphology and crystalline structure below 100 nm in size. Indeed more explicitly the two metals nanospheres appear to be placed adjoining to each other giving a overall bilobal structure. This is also in good agreement with FESEM images. The XRD spectrum of green synthesized Ag-Fe BMNPs from leaf extract is shown in **Figure 2(f)**. The peaks appeared at 2θ values of 32.123^0 ·37.972^0, 44.122^0, 64.253^0, 77.216^0 corresponding to the Bragg's reflections of (220), (111), (200), (220) and (311) planes respectively of face centered cubic crystal structure **[21]**.

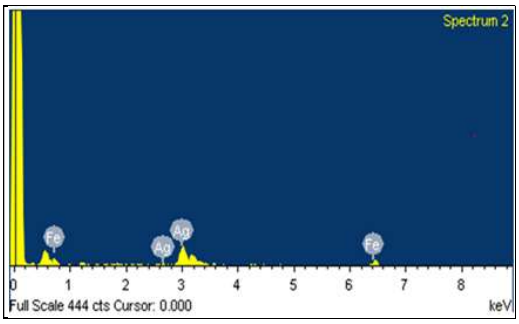

Figure 2(c): EDX spectrum of Ag-Fe BMNPs

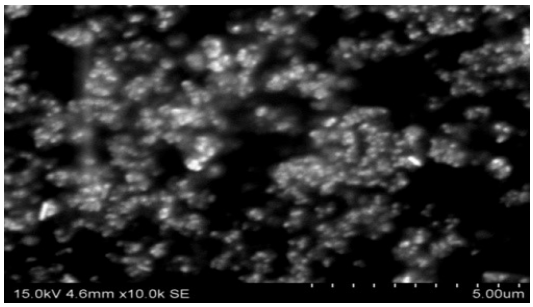

Figure 2(d): FESEM image of Ag-Fe BMNPs

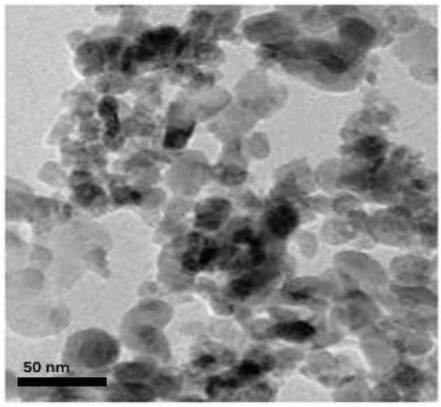

Figure 2(e): HRTEM image of Ag-Fe BMNPs

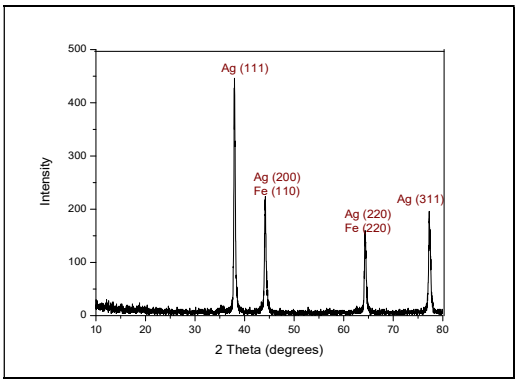

Figure 2(f): XRD spectrum of Ag-Fe BMNPs

2.4 In vitro Cytotoxic Activity by MTT assay

2.4.1 Materials and Methods

Apparatus and chemicals used

- Spectrophotometer
- Incubator
- Inverted microscope
- Centrifuge
- MCF cells (purchaged from NCCS, Pune, India)
- 96 well microplates
- Micropipette
- Doxorubicin
- MTT (1 mg/mL)
- DMSO

MTT assay is a quantitative colorimetric assay for evaluating cellular growth, cell proliferation and cell survival derived from the ability of living cells. The assay is conducted using (3-(4, 5- dimethyl thiazol-2yl) - 2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells resulting a measurable purple product formazan. The amount of formazan formed is directly proportional to the viable cell count and is inversely proportional to the extent of cytotoxic activity. The effect of in vitro

cytotoxicity of Ag-Fe nanoparticles on Breast cancer cell lines (MCF 7 cell line) is recorded at 24 hours and 48 hours.

2.4.2 Preparation of nanocompounds for the assay

Five hundred micro litre of stock (100 mg/ mL) nanoparticles are dissolved in 4.5 mL of DMSO for a concentration of 10 mg/ mL. Prior to the assay, the new working suspension is filtered through a 0.45 µm membrane filter. Five gradient concentrations (2 mg, 4 mg, 6 mg, 8 mg and 10 mg) of are used for this analysis. 500 µL of 48 h culture of MCF 7 cell lines at a concentration of 10^5 cells/ mL is applied to each well. Two control wells received only cell suspensions without nanoparticles, also the drug, doxorubicin used as positive control at same concentrations. The plate is placed in a humidified CO_2 incubator for 4 - 6 h at 37°C. Microscopically, the plate is tested for confluent cell monolayer, turbidity and toxicity.

2.4.3 Assay Process

After incubation, the medium from the well is carefully aspirated and then disposed. Each well is ished with Eagle's Minimum Essential Medium (EMEM) without Fetal Calf Serum (FCS). 200 µL of MTT solution (5mg MTT/ ml of PBS, pH 7.2) will be added to each and every well. In a CO_2 incubator with 5 percent CO_2, the plate is incubated for 6-7 h at 37°C. 1 mL of DMSO is applied to each well after incubation, combined with a pipette and left at room temperature for 45 seconds. In the wells, purple formazan is developed [22]. In order to compare full cell viability in cytotoxicity and antitumor activity assessments, cell control and solvent controls are used in each assay. The suspension is moved to a cuvette of the spectrophotometer and the optical density (OD) is calculated as blank at 540 nm using DMSO. With the following formula, cell the %viability is determined.

Cell viability % = Mean OD of wells receiving each plant extract dilution / Mean OD of control wells x 100.

The determination of IC50, the compound concentration needed to inhibit 50 percent cell growth, is calculated by plotting a log graph (extract concentration) vs. percent cell inhibition. A line drawn on the Y axis from the 50% value meets the curve and interpolates to the X axis. The value of the X axis gives the log value (concentration of the compound). The IC50 value is given by the anti-log of that value.

3. RESULTS AND DISCUSSION

The synthesized Ag-Fe BMNPs are investigated for their cell viability assay and cytotoxic activity against human breast cancer cell line (MCF-7) are assessed by applying

standard MTT assay and doxorubicin is used as a standard drug. The compounds are treated with MCF-7 cell line at five different concentrations (2mg, 4mg, 6mg, 8mg and 10mg). The cytotoxic activities of Ag-Fe nanocompound and doxorubicin drug against MCF-7 Cell Line at different concentrations are depicted in **Table 3.1 and Table 3.2**. The Linear graphs of percent inhibition of Ag-Fe nano compound and doxorubicin are shown in **Figure 3.1 and Figure 3.2**. The results clearly demonstrate that the Ag-Fe nano compound exhibits a maximum of 28.38 percentage inhibition at 10 mg concentration and a IC50 of **20.55** mg/mL. Figure 3.3 shows the morphological analysis of materials treated with MCF-7 cells.

The results clearly make obvious that the synthesized compound Ag-Fe (for 24 and 48 hrs) has shown moderate to significant cytotoxicity with values ranging from 2 mg to 10 mg respectively. So, it is confirmed that all the novel synthesized derivatives exhibit cytotoxicity and this activity has been increasing as the concentration of the BMNPs in the solution increases. Based on these results, compound Ag-Fe exhibited remarkable cytotoxicity comparable to standard drug, it showed 28.38% inhibition at 10 mg concentration and the IC50 for Ag-Fe is **20.55** mg.

Table 3.1: Cytotoxicity Activity of Ag-Fe nanoparticles against MCF-7 Cell Line at Different Concentrations by MTT Assay

Concentration (mg/mL)	OD at 540 nm	Percent inhibition	IC50 (mg/mL)
2	1.32	10.81	20.55
4	1.26	14.86	
6	1.2	18.92	
8	1.15	22.30	
10	1.06	28.38	

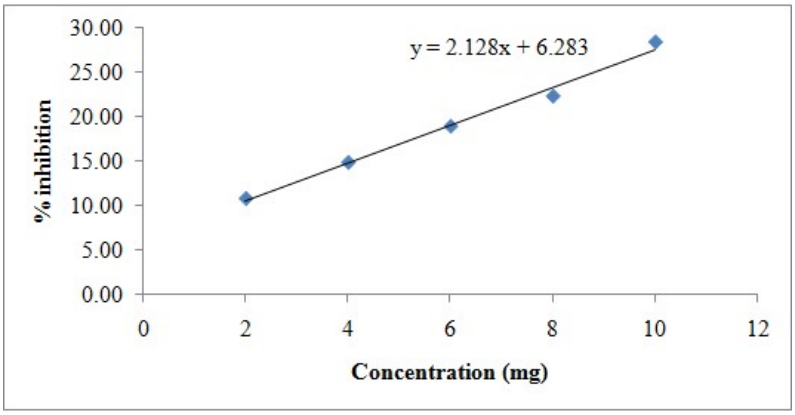

Figure 3.1: Linear graph for Ag-Fe nanoparticles percent inhibition

Table 3.2: Cytotoxicity Activity of positive control (Doxorubicin) against MCF-7 Cell Line at Different Concentrations by MTT Assay

Concentration (mg/ml)	OD at 540nm	Percent inhibition	IC 50 (mg/ml)
2	0.81	45.27	2.74
4	0.63	57.43	
6	0.49	66.89	
8	0.31	79.05	
10	0.19	87.16	

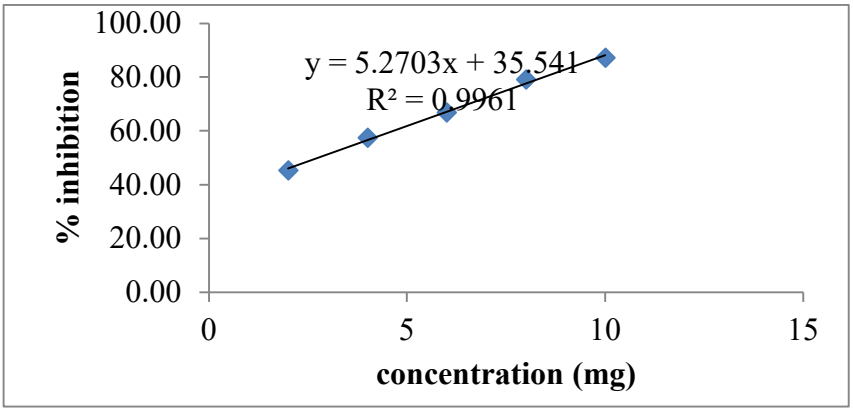

Figure 3.2: Linear graph for Doxorubicin percent inhibition

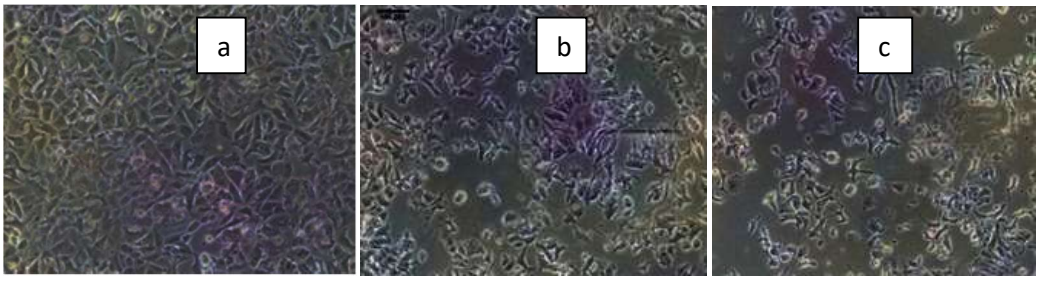

Figure 3.3: Morphological analysis of nano compounds treated MCF-7 cells.
a- control; b- Ag-Fe treated at 10 mg/mL concentration; c- Doxirubicin treated at 10 mg/mL concentration

4. CONCLUSIONS

As per the present study phytomolecules of *Aerva lanata* leaf extract are involved in the bioreduction, formation and stabilization of nanoparticles, the future studies might move towards the optimization of the reaction parameters for generation of high amount of biomolecules to stabilize and cap the formed nanoparticles.

In conclusion, this study implies that green synthesized Ag-Fe nanoparticles may be effective for treatment of MCF-7 cells of human breast cancer. A linear correlation is observed between the number of the tumor cells and the dose-dependent cytotoxic effects the synthesized nanoparticles. From the results, it is concluded that these nanoparticles can be used for the development of new measures for the therapy of tumors. The future

research may be intended for for the genetic manipulation of plants to amplify their cytotoxic activity and metal tolerance.

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REFERENCES

[1] Ranjita, Misra , Sarbari, Acharya & Sanjeeb K Sahoo (2010). Cancer Nanotechnology: Application of Nanotechnology in Cancer Therapy. *Drug Discov Today*.15(19-20):842-50.

[2] Kumar Teli, M., Mutalik, S. & Rajanikant, G. (2010). Nanotechnology and nanomedicine: going small means aiming big. *Curr Pharm Des* 16, 1882–1892.

[3] M. Goldberg, R. Langer, X. Jia, J. (2007). Nanostructured Materials for Applications in Drug Delivery and Tissue Engineering. *Biomater. Sci. Polym. Edn*. 18 (2007) 241–268.

[4] C.R. Patra, R. Bhattacharya, D. Mukhopadhyay, P. Mukherjee (2010). Fabrication of Gold Nanoparticles for Targeted Therapy in Pancreatic Cancer. *Adv. Drug Deliv. Rev*. 62: 346–361.

[5] Maksym V Yezhelyev , Xiaohu Gao, Yun Xing, Ahmad Al-Hajj, Shuming Nie & Ruth M O'Regan (2006). Emerging use of nanoparticles in diagnosis and treatment of breast cancer. *Lancet Oncol* 7, 657–667.

[6] R Sriranjani, B Srinithya, V Vellingiri, P Brindha, S Anthony Philip, Sivasubramanian, Aravind Muthuraman and Meenakshi Sundaram (2016). Silver nanoparticle synthesis using *Clerodendrum phlomidis* leaf extract and preliminary investigation of its antioxidant and anticancer activities. *Journal of Molecular Liquids.* 220:926-930.

[7] Datkhile, K. D., Durgawale, P. P. & Patil, M. N. (2017). Biogenic silver nanoparticles are equally Cytotoxic as Chemically Synthesized silver nanoparticles. *Biomed Pharmacol J* 10, 337–344.

[8] Indira, P. D. A. (2015). Evaluation Of Antimicrobial Activity Of *Aerva Lanata* Along With Preliminary Phytochemical Screening. *International Research Journal of Pharmacy*, *6*(6), 374–376

[9] Vidhya, R., & Udayakumar, R. (2017). Antifungal efficacy of leaf, flower and

root extracts of *Aerva lanata* (Linn.) against some selected fungal pathogens. *Journal of Immunology and Clinical Microbiology*, *2*(1), 7

[10] Dinnimath, B. M., & Jalalpure, S. S. (2018). Antioxidant and Antiurolithiatic Efficacy of *Aerva lanata* (L) Fractions by in vitro and in vivo Screening Techniques. *Indian Journal of Pharmaceutical Education and Research*, *52*(3), 426–436

[11] Chowdhury, D., Sayeed, A., Islam, A., Bhuiyan, M. S. A., & Khan, G. A. M. (2002). Antimicrobial activity and cytotoxicity of *Aerva lanata*. *Fitoterapia*, *73*(1), 92–94

[12] Gujjeti, R. P., & Mamidala, E. (2016). Anti-HIV Activity of Phytosterol Isolated from *Aerva lanata* Roots. *Pharmacognosy Journal*, *9*(1), 112–116

[13] Nevin, K., & Vijayammal, P. (2003). Effect of *Aerva lanata* on solid tumor induced by DLA cells in mice. *Fitoterapia*, *74*(6), 578–582

[14] Akanji, M. A., Olukolu, S. O., & Kazeem, M. I. (2018). Leaf Extracts of *Aerva lanata* Inhibit the Activities of Type 2 Diabetes-Related Enzymes and Possess Antioxidant Properties. *Oxidative Medicine and Cellular Longevity*, *2018*, 1–7

[15] Vetrichelvan, T., & Jegadeesan, M. (2002). Anti-diabetic activity of alcoholic extract of *Aerva lanata* (L.) Juss. ex Schultes in rats. *Journal of Ethnopharmacology*, *80*(2-3), 103–107

[16] Noolvi, M. N., Bhanot, A., Sharma, R., Singh, S., & Singh, S. (2013). In vitro Anti Cancer Activity of Ethanol Extract Fractions of *Aerva lanata* L. *Pakistan Journal of Biological Sciences*, *16*(22), 1612–1617

[17] Suresh, A. K., Pelletier, D. A., Wang, W., Morrell-Falvey, J. L., Gu, B., & Doktycz, M. J. (2012). Cytotoxicity induced by engineered silver nanocrystallites is dependent on surface coatings and cell types. *Langmuir*, *28*(5), 2727-2735

[18] Shirmardi, M., Mahvi, A. H., Hashemzadeh, B., Naeimabadi, A., Hassani, G., & Niri, M. V. (2013). The adsorption of malachite green (MG) as a cationic dye onto functionalized multi walled carbon nanotubes. *Korean Journal of Chemical Engineering*, *30*(8), 1603-1608.

[19] Joseph, S., & Mathew, B. (2015).Microwave-assisted green synthesis of silver nanoparticles and the study on catalytic activity in the degradation of dyes. *Journal of Molecular Liquids*, *204*, 184-191.

[20] Appapalam, S. T., & Panchamoorthy, R. (2017). *Aerva lanata* mediated phytofabrication of silver nanoparticles and evaluation of their antibacterial activity against wound associated bacteria. *Journal of the Taiwan Institute of Chemical Engineers*, *78*, 539-551.

[21] Tamuly, C., Hazarika, M., Borah, S. C., Das, M. R., & Boruah, M. P. (2013). In situ biosynthesis of Ag, Au and bimetallic nanoparticles using *Piper pedicellatum* C. DC: green chemistry approach. *Colloids and surfaces B: Biointerfaces*, *102*, 627-634.

[22] Thakkar, K., Prasad, A. K., Nayak, J., Iyer, S. V., & Kumar, S. (2014). Antioxidant and in vitro cytotoxic activity of extracts of aerial parts of *Cocculus hirsutus* (L) using cell line cultures (breast cell line). *The Journal of Phytopharmacology*, *3*(6), 395-399.