Synthesis, Characterization & Bacterial Evaluation of Cobalt Nanoparticles Using Drumstick Leaf Extract via Green Route

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SYNTHESIS, CHARACTERIZATION & BACTERIAL EVALUATION OF COBALT NANOPARTICLES USING DRUMSTICK LEAF EXTRACT VIA GREEN ROUTE.

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ABSTRACT: The cobalt nanoparticles are synthesized using drumstick leaf extract and cobalt chloride. The leaves extract of drumstick is act as reducing and capping agent. The cobalt nanoparticles synthesize using drumstick leaves extract are characterized using different recent characterization techniques such as SEM, FTIR and UV-Vis spectroscopy also characterized by using EDX which gives much information. Drumstick leave extract mediate nanopartices show significant antibacterial activity against both gram negative and gram positive bacteria such as *E. coli* and staphylococcus bacteria. The importance of research is as its cobalt that absorb and process vitamin B12. These synthesized nanoparticles have much importance in medicinal and drug chemistry as it may be a good precursor or drug carrier.

Key Words: NPs, FTIR, UV-Vis, SEM, EDX

1.1 Introduction

The term "nano-particle" [1] first appeared in the 1980s [2]. Nanotechnology is a completely new technology with lots of applications. The particles having size ranges between 1 and 100 nanometer are known as nanoparticles. Nanotechnology "It is study of manipulating of matter on an atomic and molecular scale". Nanotechnology has two classes on the basis of living and nonliving things, Wet nanotechnology & Dry nanotechnology. On the basis of their chemical formation, nanoparticles are basically of two types, Inorganic (Au, Ag, Ti & Zn) & Organic (Carbon & Fullerene) nanoparticles. The nanoparticles are classify into three classes on the basis of diameter i.e. Ultrafine, Fine & Coarse nanoparticls (diameter ranges from 1 to 10,000 nm).

On the basis of physical and chemical nature nanoparticles are classified as Carbon-based Nanoparticles, Fullerenes, Metal Nanoparticles, Ceramics Nanoparticles, Semiconductor Nanoparticles, Polymeric Nanoparticles & Lipid-based Nanoparticles. Taking dimensions into consideration, nanoparticles can be Zero (0D), One (1D), Two (2D) & complex structures.

Due to extensive research on nanoparticles in recent decade, there are handful amount of synthesis method that can be used for synthesis of nanoparticles. These include Top-down method, Dry grinding, Wet grinding, Bottom-up method, Chemical vapor deposition method (CVD) , Physical vapor deposition method (PVD), Liquid phase methods, Chemical reduction of metal ions, Sedimentation methods & Sol gel process. Some common features to all the methods are control of particle shape, size and crystal structure, improvement in the purity of NPs, regulation of the aggregation, steadiness of physical properties & increase in the reproducibility.

Techniques that are mainly in use for characterization of NPs include X-ray diffraction (XRD) [3], Scanning Electron Microscope(SEM), Transmission Electron Microscope(TEM), Infrared (IR), Polarized optical microscopy (POM), X-Ray Photoemission Spectroscopy (XPS), Energy dispersive X-ray (EDX) [4], Brunauer–Emmett–Teller (BET), Zieta size analyzer, Energy Dispersive X-Ray (Spectroscopy) (EDX), X-Ray Photoemission Spectroscopy (XPS), FT-IR and Raman spectroscopies, Dynamic light scattering (DLS) [5] & Nanoparticle tracking analysis (NTA) [6]. Physicochemical properties of NPs that make them attractive to use include their Electronic and optical properties, Magnetic, Mechanical & Thermal properties. Other alluring applications of NPs include Applications in medicine from being used in Fluorescent biological labeling, In Drugs[7] and gene delivery [8], In Biological detection of pathogens [9], Useful in Finding of proteins [10], In Probing of the DNA structure [11], For Tissue and cellengineering [12], Used for tumor destruction through heating (hyperthermia), Helpful for separation and purification of biological cells and molecules[13], In MRI improvement [14], In Phagokinetic studies [15] to Cellular imaging[16]. NPs also find their applications in other fields as Chemical Catalyst, in Micro-wiring, Paints, Food and Agriculture, electronics, energy harvesting, mechanical industries, Batteries & in environmental Protection

Certain Limitations of Nanoparticles include difficult handling, toxicological effects on plants, and adverse health effects in humans because of the delayed exposure at several concentration levels [17].

Cobalt having atomic number 27 belongs to 4^{th} period of Periodic table is a naturally occurring metal. Its only stable isotope is ^{59}Co. Main ores of cobalt include Cobaltite, Glaucodot, Erythrite, & Skutterudite. Cobalt is a brittle and silver-colored shiny metal. Cobalt in compounds form was used for blue dyes in glass, pottery and coatings [18]. Its melting point is high (i.e. 2723°F or 1495°C). Cobalt is the one from three magnetic metals that are naturally occurring and shows oxidation states as 0, +2 & +3 being a member of transition metals. Important salts of cobalt include Cobalt (II) Chloride ($CoCl_2$), Cobalt (II) Chloride hexahydrate ($CoCl_2$ $6H_2O$), Cobalt (II) sulphate ($CoSO_4$), Cobalt (II) sulphate heptahydrate ($CoSO_4$ $7H_2O$), Cobalt (II) nitrate hexahydrate ($Co\ (NO_3)_2$ $6H_2O$) & Cobalt (II) carbonate ($CoCO_3$).

Cobalt chemicals are present in the rechargeable batteries as metallic cathodes, in petrochemical catalysts, in ceramic, glass decolorizers, motors, magnetic recording media and generators, as a binding material in hard metals, in the manufacturing of turbine blades for jet engines, exhaust valves, hard facing parts of machine and the gun barrels. It is used for the production of permanent magnets, i.e. samarium cobalt magnets. The most important application of cobalt is in the wear resistant alloys like Vitallium. These types of alloys are essential for orthopedic and dental implants, along with prosthetic knees and hips.

Cobalt nanoparticles have unique thermochemical, physical and optical properties. They are great interest for the optical physics and optoelectronics. Cobalt NPs show a vast difference relative to gold or silver nanoparticles because the spectral dependencies of cobalt nanoparticles' scattering and absorption efficiency factors display wide bands or continuum spectrum. Their Boiling point is 2870°C (5198°F) and Melting point is1495°C (2723°F). Cobalt nanoparticles have sphericalmorphology. The appearance of Co NPs is a black and grey powder.

Cobalt nanoparticles are used in dyes, high-speed optical devices, microwave absorption materials, electromagnetic-wave absorption, magnetic inks, drug delivery, coating, cobalt batteries & in biomedical stents. They also find their applications as antistatic plastic bags, medical sensors, ferro fluids, super alloys & as light filters.

Green Chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products. Green chemistry can described as sustainable chemistry & can inhibit pollution at molecular level. Green chemistry finds its importance as it energy efficient, has low cost production, protects environment, economical & produces lesser waste, therefore it is also environmental friendly. Microorganisms are rapidly being used for the formation of nanoparticles because these can be easily grown in large amount [19]. Besides this, there is no use of toxic, harsh and expensive chemicals in biosynthesis of metal NPs. It is the environmental friendly method [20]. The synthesis of metal NPs by using the plant extracts is also an inexpensive method. The production of metal NPs by plant extract is an economic and valuable method. Use of plant extracts is a safe green method for synthesis of metal NPs. It is also an easy method to produce well dispersed metal nanoparticles at industrial level.

In following research drumstick tree leaves are used which goes by common names as Ben oil tree or benzoil tree, Horseradish tree. It belongs to Family *Moringaceae* and has scientific name as *Moringa oleifera*.

Drumstick tree is a fast growing and drought resistant tree, widely cultivated because its leaves used as vegetables and herb [21]. It helps in water purification [22] & has tendency to root in waterlogged soil. [23]. Drumstick leaves are used as an iron rich source [24]. Leaves extract have polyphenols [25] that has many potential properties [26] in research.

2.1 Materials Used:

Material used in all the research work includes Cobalt Chloride ($CoCl_2$) anhydrous, ≥98.0% (KT) from Sigma Aldrich & Drumstick (*Moringa Oleifera)* leaves were collected from outer boundary of University of Engineering and Technology, Lahore.

3.1 Synthesis of Co NPs:

3.1.1 Preparation of Leaf extract

10 g of washed fresh leaves were taken and dipped into 100ml double distilled water at almost 50 to 60^{o}C to prepare leaves extract and extract was obtained after 2 hours as the color of the solution changed from colorless to yellowish brown color. After the preparation of extract, the flask was removed from water bath. The whattman filter paper No 1.is used for the filteration of mixture. Then extract was stored into refrigerator at 4^{o}C for further use.

3.1.2 Preparation of Cobalt Chloride ($CoCl_2$) solution

To prepare 1000 ml solution of 0.001M cobalt chloride, dissolve 0.1298 gram of cobalt chloride in 1000 ml distilled water. As the solubility of cobalt chloride in water is high so it readily dissolved in water and a homogeneous solution was prepared in which cobalt chloride was completely soluble. The solution was stored in a 1000 ml measuring flask covered with the aluminum foil. This solution saved at room temperature for further research work.

3.1.3 Sample Preparation

20 ml leaf extract was taken in a beaker and 1mM solution of cobalt chloride was filled in a burette for drop wise addition. The beaker was placed on hot plate with continuous stirring for 48 hours. The cobalt chloride solution was added into the leaves extract drop wise with continuous stirring. The samples of different volume ratio were prepared. When the reduction of cobalt was done, colours of the solution changed from yellow color to pink indicating cobalt nanoparticles were obtained. The above mixture was continuously heated and stirred on hot plate till paste was formed. This paste dried into oven at 60C till black color Co nanopowder was formed.

3.2 Characterization of synthesized Nanoparticles

3.2.1 Scanning Electron Microscope (SEM)

Solution of NPs was transformed into a dry powder. Then dry powder is further fixed on a sample holder. This holder is coated with a conductive metal such as gold, using a sputter coater. With a focused fine electrons beam, whole sample is finally analyzed by scanning.

3.2.2 UV-Visible Spectrometer

The sample for UV-Vis was taken as dispersed particles appear in nanosolution. The UV-analysis was supported by using double beam spectrophotometer. 1cm path length of cuvet was used. Two solutions run at same time, for UV-analysis. The first reference solution which was distilled water and other was sample solution.

3.2.3 Infrared spectroscopy (IR)

Cobalt nano-powder directly runs for IR analysis. While $CoCl_2$ were in the form of solution, KBr pallet was prepared and then 1or 2 drops of cobalt nano-solution were added.

3.2.4 Biological Activity

Bacterial activity is mostly confirmed by using well or spreading method by using agar media. Equipment used in this process was sterilized. After sterilization, Agar was spread on Patri dish and then samples on it. After some time, sample prepare wells that represent the activity of given sample.

4.0 RESULTS & DISCUSSION

4.1 UV Spectra

In the case of green synthesized cobalt nanoparticles, we get two peaks at 275 and 350 nm in UV spectroscopy. These are the characteristic peaks for cobalt nanoparticles. While if the main prominent peak is obtained at 378 nm then it indicates the formation of Co nanoparticles (low dimensional beta form). UV-Visible spectrum for green synthesized coblt nanoparticles is given below.

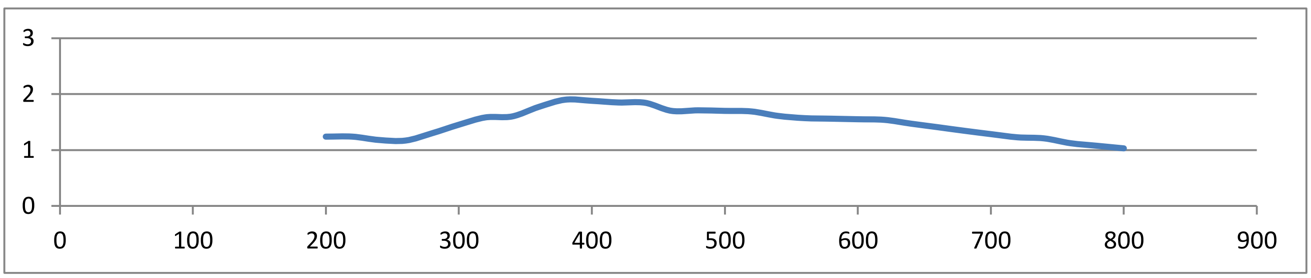


Fig-XII UV-Visible Graph of CoNP's with Drumstick.

In the absorption, spectroscopic studies cobalt nanoparticles exhibit a broader line near to 380nm that confirms the formation of CoNPs.The absorption peaks at 270-380nm indicates the formation of cobalt nanoparticles. Above mentioned spectra indicate a fine peak at 378nm that confirms the formation of cobalt nanoparticles. The absorption peaks at 275-378 nm regions because of excitation of the surface plasmon vibrations in cobalt NPs indicated the formation of cobalt nanoparticles.

4.2 FTIR Analysis

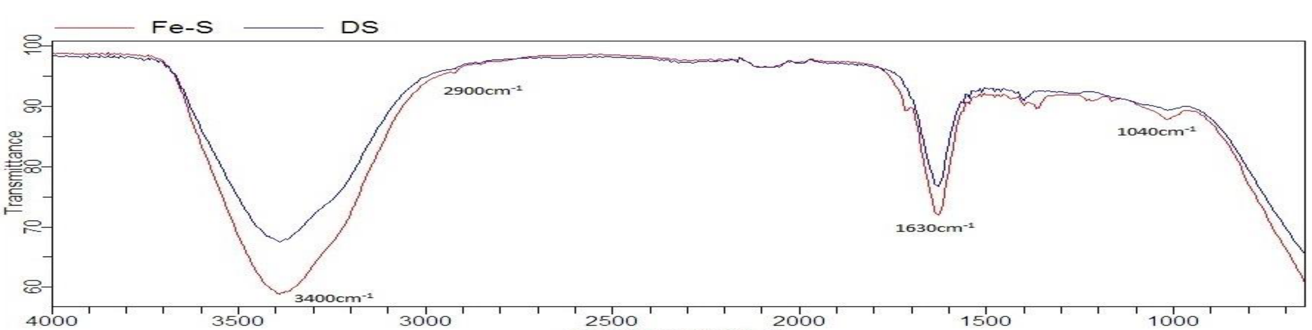


Fig-XIII FTIR Scan of CoNP's with Drumstick tree

Characteristic combined IR spectra for extract and Nano solution is given above.D ifferent peaks are obtained that shows functional groups present in sample.

1040cm-1 peak represents Primary alcohols

1630cm-1 peak represents C=C bending

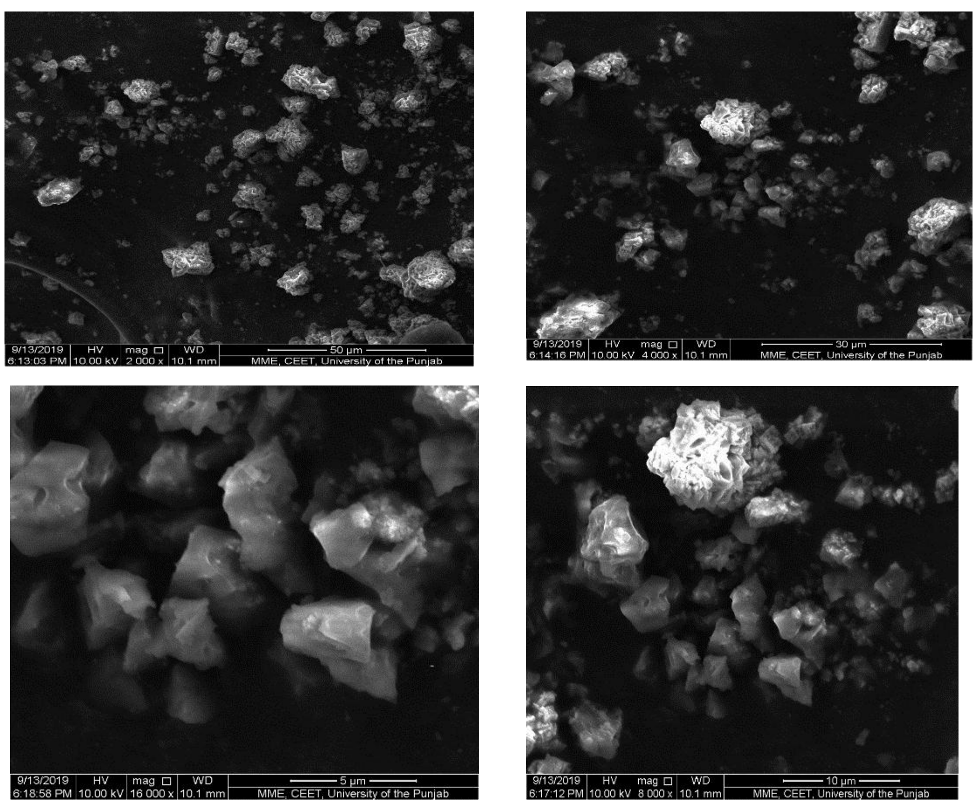
2900cm-1 peak represents Alkyl C-H

3400cm-1 peak represents Alcohol/Phenol O-H Strength

All these groups are present in cobalt nanoparticle solution. These groups were initially present in extract solution that plays a role of capping, reducing and stabilizing agent in nanoparticle formation. These are responsible to reduce cobalt into cobalt nanoparticles.

4.3 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopic images of green synthesized cobalt nanoparticles by using Drumstick tree leaf extract is given below.



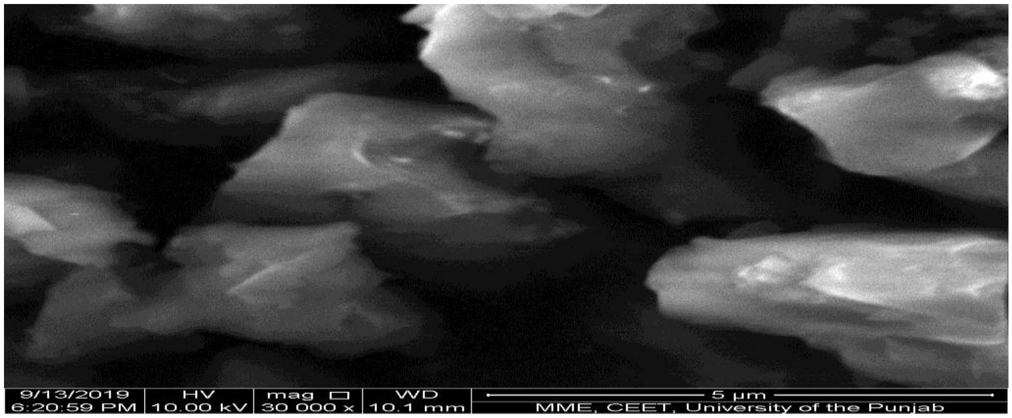


Fig-XIV SEM images of CoNP's with Drumstick leaves

SEM images are obtained at different resolutions; different images were obtained so that we can study them properly. The minimum size calculated from these SEM images is 27.3 nm. While different sized nanoparticles are obtained but the smallest particle have this size. For the calculation of size image software is used.

4.4 Energy Dispersive X-Ray (Spectroscopy) (EDX)

Energy Dispersive X-Ray for Co NPs is given as

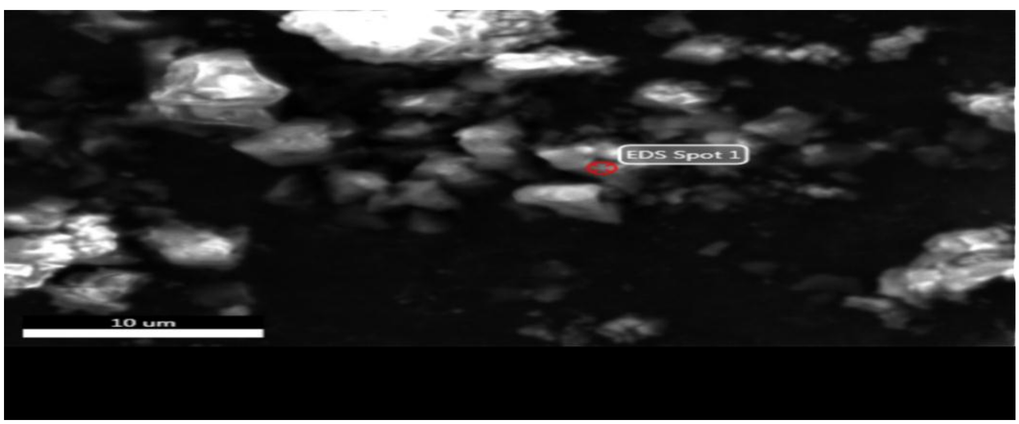


Fig-XV Energy Dispersive X-Ray (EDX) of CoNPs

5.0 Antibacterial Activity

Antibiotics inhibit the cell division of microorganism. Unfortunately, the dosage of antibiotics must be increased and the treatment can prolong due to the low MIC of antibiotics. Our synthesized cobalt nanoparticles are also subjected to check their bacterial activity and we get superfine results. The activity of Ecoli and staphylococcus is checked and got good results that indicate that these cobalt nanoparticles have greater bacterial activity then the extract used to prepare these particles.

Following table shows the activity of E coli and staphylococcus in CoNP's synthesized by using Drumstick leaf extract while the reference is extract itself.

Sample	Staphylococcus (+) Gram-Positive Bacteria	E coli (-) Gram-Negative Bacteria
Reference/ Extract	10 mm	7nm
Sample/ Drumstick CoNPs	15nm	8nm

These results clearly show that the bacterial activity of CoNp's is greater than simple extract. The bacterial activity of cobalt nanoparticles is greater than reference i.e. simple extract. These synthesized nanoparticles can also use in medical sciences because of nanoscale they can easily penetrate in human body organs like lungs and skin. So, they can be a good drug carrier in near future.

6.0 Discussion

Cobalt nanoparticles have unique thermochemical, physical and optical properties. Cobalt NPs have the great interest for the optical physics and optoelectronics. Cobalt NPs are synthesized by green synthesis because it is an environmental friendly method. Leaves extract of Drumstick tree used for the synthesis of Co NPs. In nanoparticle synthesis, extracts of plants may play role as reducing and capping agents. The production of metal nanoparticles by plant extract is an economic and valuable method. We can produce NPs in the large amount through this method, at very low cost. Different techniques are used for the characterization of synthesized Co NPs.

7.0 CONCLUSION

Cobalt nanoparticles were synthesized by using Drumstick tree leaves. The nanoparticles were also characterized with the help of many different techniques which are UV-vis spectroscopy, FTIR spectroscopy and SEM. The characteristic UV peak of Co nanoparticles was obtained. The UV absorption peaks for Cobalt at 378 that confirm the synthesis of Co nanoparticles. Similarly the UV absorption obtain at 378 nm which confirm the synthesis of Co NPs. Phytochemicals plays the important role as capping and reducing agent in nanosynthesis. SEM result revealed the size of Co synthesized nanoparticles. The size of Co nanoparticles ranged from 168 nm to 295 nm shown by SEM images. Cobalt synthesized nanoparticles were showed a significant antibacterial activity. Thus drumstick mediated Co nanoparticles can be used in antibiotic drugs.

REFERENCES

[1] Morse, M. D. (1986). Clusters of transition-metal atoms. Chemical Reviews, 86(6), 1049-1109.

[2] Henglein, A. (1989). Small-particle research: physicochemical properties of extremely small colloidal metal and semiconductor particles. Chemical Reviews, 89(8), 1861-1873.

[3] Khan, I., Ali, S., Mansha, M., &Qurashi, A. (2017). Sonochemical assisted hydrothermal synthesis of pseudo-flower shaped Bismuth vanadate (BiVO4) and their solar-driven water splitting application. Ultrasonicssonochemistry, 36, 386-392.

[4] Avasare, V., Zhang, Z., Avasare, D., Khan, I., &Qurashi, A. (2015). Room-temperature synthesis of TiO2 nanospheres and their solar driven photoelectrochemical hydrogen production. International Journal of Energy Research, 39(12), 1714-1719.

[5] Sikora, A., Shard, A. G., &Minelli, C. (2016). Size and ζ-potential measurement of silica nanoparticles in serum using tunable resistive pulse sensing. Langmuir, 32(9), 2216-2224.

[6] Filipe, V., Hawe, A., &Jiskoot, W. (2010). Critical evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. Pharmaceutical research, 27(5), 796-810.

[7] Mah, C., Zolotukhin, I., Fraites, T. J., Dobson, J., Batich, C., & Byrne, B. J. (2000). Microsphere-mediated delivery of recombinant AAV vectors in vitro and in vivo. MolTher, 1, S239.

[8] Pantarotto, D., Partidos, C. D., Hoebeke, J., Brown, F., Kramer, E. D., Briand, J. P., ...&Bianco, A. (2003). Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses. Chemistry & biology, 10(10), 961-966.

[9] Edelstein, R. L., Tamanaha, C. R., Sheehan, P. E., Miller, M. M., Baselt, D. R., Whitman, L., & Colton, R. J. (2000). The BARC biosensor applied to the detection of biological warfare agents. Biosensors and Bioelectronics, 14(10-11), 805-813.

[10] Nam, J. M., Thaxton, C. S., &Mirkin, C. A. (2003). Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. science, 301(5641), 1884-1886.

[11] Mahtab, R., Rogers, J. P., & Murphy, C. J. (1995). Protein-sized quantum dot luminescence can distinguish between" straight"," bent", and" kinked" oligonucleotides. Journal of the American Chemical Society, 117(35), 9099-9100.

[12] Ma, J., Wong, H., Kong, L. B., & Peng, K. W. (2003). Biomimetic processing of nanocrystallite bioactive apatite coating on titanium. Nanotechnology, 14(6), 619-621.

[13] Molday, R. S., & Mackenzie, D. (1982). Immunospecific ferromagnetic iron-dextran reagents for the labeling and magnetic separation of cells. Journal of immunological methods, 52(3), 353-367

[14] Weissleder, R., Elizondo, G., Wittenberg, J., Rabito, C. A., Bengele, H. H., & Josephson, L. (1990). Ultrasmallsuperparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging. Radiology, 175(2), 489-493.

[15] Parak, W. J., Boudreau, R., Le Gros, M., Gerion, D., Zanchet, D., Micheel, C. M., ...&Larabell, C. (2002). Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks. Advanced Materials, 14(12), 882-885.

[16] Roco, M. C., Williams, R. S., &Alivisatos, P. (Eds.). (2000). Nanotechnology research directions: IWGN workshop report: vision for nanotechnology in the next decade. Springer Science & Business Media.

[17] Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., ...&Behra, R. (2008). Toxicity of silver nanoparticles to Chlamydomonasreinhardtii. Environmental science & technology, 42(23), 8959-8964.

[18] Agarwal, S., Zaman, T., Murat Tuzcu, E., & Kapadia, S. R. (2011). Heavy metals and cardiovascular disease: results from the National Health and Nutrition Examination Survey (NHANES) 1999-2006. Angiology, 62(5), 422-429.

[19] Ankamwar, B., Damle, C., Ahmad, A., &Sastry, M. (2005). Biosynthesis of gold and silver nanoparticles using Emblicaofficinalis fruit extract, their phase transfer and transmetallation in an organic solution. Journal of nanoscience and nanotechnology, 5(10), 1665-1671.

[20] Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., ...& Hong, J. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomumcamphora leaf. Nanotechnology, 18(10), 105-114.

[21] Kalibbala, H. M., Wahlberg, O., &Hawumba, T. J. (2009). The impact of Moringaoleifera as a coagulant aid on the removal of trihalomethane (THM) precursors and iron from drinking water. Water Science and Technology: Water Supply, 9(6), 707-714.

[22] Kalibbala, H. M. (2012). Removal of Natural Organic Matter and Control of Trihalomethanes Formation in Water Treatment (Doctoral dissertation, KTH Royal Institute of Technology).

[23] Radovich, T. (2011). Farm and forestry production and marketing profile for Moringa (Moringaoleifera). Permanent Agriculture Resources (PAR), Holualoa, Hawai, 1-10.

[24] Owusu, D., Ellis, W. O., &Oduro, I. (2008). Nutritional potential of two leafy vegetables: Moringaoleifera and Ipomoea batatas leaves.

[25] Madukwe, E. U., Ezeugwu, J. O., &Eme, P. E. (2013). Nutrient composition and sensory evaluation of dry Moringaoleifera aqueous extract. Int. J. Sci. Basic Appl. Res, 13, 100-102.

[26] Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of Moringaoleifera leaves in two stages of maturity. Plant foods for human nutrition, 64(4), 303-310.