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GREEN SYNTHESIS OF IRON NANOPARTICLES FROM CASSIA FISTULA LINN LEAF EXTRACT AND ITS ANTI-BACTERIAL ACTIVITY

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ABSTRACT
Nanotechnology is an emerging field in Biotechnology and it has vast applications in all fields. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature, pressure and easily scaled up. Iron nanoparticles are reported to have magnetic, catalytic, optical, anti-inflammatory and antibacterial activity. In this study iron nanoparticles were successfully synthesized by reduction of ferric chloride solution using aqueous solution of *Cassia fistula* Linn leaf extract. The method was non-lethal, simple, eco-accommodating and relatively inexpensive. The resulting iron nanoparticles were characterized by physical color changes, UV-Vis spectroscopy, Scanning Electron Microscopy (SEM) and Fourier-transform infrared (FTIR) spectroscopy. The reaction mixture of aqueous ferric chloride and *Cassia fistula* Linn leaf extract displayed black color confirming the synthesis of iron nanoparticles. UV–Vis spectra showing an absorption band at 220nm characteristic of the iron nanoparticles in deed revealed the presence of nanoparticles. Images of the biosynthesized iron nanoparticles at different magnifications from SEM showed that the particles were spherical shaped and size of the nanoparticles ranged from 20-50 nm. Fourier transform infrared (FTIR) spectroscopy showed the contribution of O-H group, sulphate group, biopolymers, N-H group in primary amines and carboxylic groups in the synthesis procedure. The synthesized iron nanoparticles had antibacterial activity against pathogenic bacteria like *Staphylococcus* sp, *Pseudomonas* sp, *Bacillus* sp and *Klebsiella* sp by well diffusion method.

KEYWORDS: *Cassia fistula* Linn, Iron nanoparticles, Ferric chloride, UV, SEM, FTIR, Antibacterial activity.

INTRODUCTION
India is one of the most important countries in the world in term of floristic diversity. India has a rich culture of medicinal herbs and spices which includes about more than 2000 species and has a vast geographical area with high potential abilities for traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal values.[1] Medicinal plants are the "backbone" of traditional medicines.[2] The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare.[3-5] These medicinal plants are considered as rich resources of ingredients which can be used in synthesis and drug development.[6] Besides that these plants play critical role in the development of human cultures around the whole world.[7]

Cassia fistula Linn (Family Fabaceae) is native to southern Asia but now widely grown in tropical and subtropical areas as an ornamental plant due to its beautiful, bright yellow flowers.[8] Other vernacular names include golden shower, Indian laburnum and pudding pine tree. In Tamil, it is called as Konrai.[9] The golden shower tree is a medium-sized tree which grows up to 10–20 m tall with fast growth. The leaves are deciduous, 15–60 cm [5.9–23.6 inch] long, and pinnate with three to eight pairs of leaflets. Each leaflet is 7–21 cm [2.8–8.3 inch] long and 4–9 cm [1.6–3.5 inch] broad. The flowers are produced in pendulous racemes.[10,11] The fruit is a legume, with a pungent odor and contains several seeds.

Iron nanoparticles are nanoparticles of iron which are in the range of 1 - 100 nm in size. Many methods, a chemical reduction method,[12] a polyol method.[13] and radiolytic process.[14] have been applied to synthesize metallic nanoparticles of specific sizes and morphologies. The problem with these methods is that the synthesis is expensive and may also have toxic substances absorbed onto them that may have adverse application in medical fields. To overcome this, the biological method provides a feasible alternative. The major biological systems involved are bacteria, fungi and plant extracts. The use of environmentally benign material synthesis of nanoparticles using biological

entities has great interest due to their unusual optical, chemical, photo electro-chemical and electronic properties. The use of environmental friendly materials like plants, bacteria and fungi for the synthesis of iron nanoparticles which has numerous benefits such as eco friendliness, easily scaled up for large scale synthesis and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis.[15]

In recent couple of years, different engineered strategies have been created to deliver iron nanoparticles, adjust the nanoparticles surface properties and improve the productivity for field conveyance and responses. While several types of iron nanoparticles are available in the market, information on the nanoparticles synthesis and properties are still limited in literatures. So, the aim of the present study is to synthesize iron nanoparticles from *Cassia fistula* Linn leaf extract.

METHODOLOGY

Collection of plant sample

Cassia fistula leaf was collected from the campus of Dr. N.G.P. Arts and Science College, Coimbatore and it was identified and authenticated by Botanical Survey of India, Coimbatore (Plant authentication no: BSI/SRC/5/23/2018/Tech/3224).

Sample Preparation and Extraction

The collected *Cassia fistula* leaf was washed with distilled water to remove dust particles and shade dried for seven days. The dried leaves were ground into powder. About 5 g of powder was weighed and mixed with 50 ml of distilled water and kept in orbital shaker overnight at 120 rpm. The solution was filtered through Whatman no 1 filter paper. The filtrate was then stored at 4°C for further use.

Phytochemical Screening

Phytochemical screening was done by using standard procedure as described by Rohit Kumar Bargah (2015).[16]

Test for Alkaloids

The presence of alkaloids in extracts was tested by using Wagner reagent that was prepared by dissolving 2 g of iodine and 6 g of potassium iodine in 100 ml of water. Two ml of Wagner's reagent was added to 2 ml of extracts. The formation of reddish brown color indicated the presence of alkaloids.

Test for Phenols

The extract (500mg) was dissolved in 5ml of distilled water.To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Test for Proteins

2ml of extract was mixed with 2ml of water and 0.5 ml of conc. nitric acid. The formation of yellow color indicated the presence of protein.

Test for Steroids

2ml of extract dissolving in 2ml of chloroform and 2ml of conc. sulphuric acid was added. Appearance of red color in upper layer and yellow with green fluorescence indicate the presence of Steroids.

Test for Terpenoids

2ml of extract dissolved in 2ml of chloroform and 2ml of acetic anhydride added following addition of 2ml of conc. sulphuric acid was added. The formation of greyish color indicates the presence of terpenoids.

Test for Flavonoids

To 1 ml of extract, 1ml of 10% lead acetate solution wasadded. The formation of a yellow precipitate was taken as a positive test for presence of flavonoids.

Test for Saponins

5 ml of extract was shaken vigorously with 5 ml of distilledwater in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Cardiac glycoside

2ml of extract mixed with 0.4ml glacial acetic acid containing trace amount of Fecl3 and 0.5ml of conc. sulphuric acid. The formation of persistent blue color indicated the presence of Cardiac glycoside.

Synthesis of Iron Nanoparticles

0.1M ferric chloride solution was prepared in distilled water. For the synthesis of iron nanoparticles, 50 ml leaf extract was taken in a conical flask and placed at 55ºC for 3 min in a heating mantle then added 1-2 ml of ferric chloride solution. The formation of black color indicated the presence of iron nanoparticles. The iron nanoparticle solutions thus obtained were centrifuged at 10000 rpm for 20 minutes. The supernatant was discarded and the pellet collected was then air dried.[17]

Characterization studies

The characterization of iron nanoparticles synthesized from *Cassia fistula* leaf extract was done by using Ultraviolet Visible Spectroscopy (UV-VIS), Scanning Electron Microscopic (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).

Ultraviolet-Visible Spectroscopic analysis

The bioreduction of Fe ions in solution was monitored by measuring the sample in UV- visible spectrophotometer. The optical absorbance was measured in the range of 200 -800 nm wave length. The scanning speed was 1000nm per min. Distilled water was used as a reference. (Jasco UV, model V-670).[18]

Scanning Electron Microscopy (SEM)

The solution containing iron nanoparticle was centrifuged at 10000 rpm for 20 min and the supernatant was discarded and the pellet was dissolved in deionized water. The pellet was mixed properly with water and placed on glass cover slip and air dried. The cover slip containing dried sample was used for SEM analysis. The samples were then coated with carbon coated copper. The images of iron nanoparticles were obtained in scanning electron microscope (Hitachi -S 3400N).[19]

Fourier Transform Infrared Spectroscopy (FT-IR)

The powdered sample of iron nanoparticles were used and examined by Infra-red (IR) spectrum at the spectral range of 400-4000 cm^{-1} by Fourier Transform Infrared Spectroscopy (Thermo Nicolet 6700 FTIR spectroscopy) using KBr pellets to identify the functional groups bound to the iron surface.[20]

Antibacterial Assay

Four pathogens *Bacillus* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Klebsiella* sp were obtained from the Department of Microbiology, Dr.N.G.P Arts and Science College, Coimbatore. The bacterial cultures were maintained in nutrient agar slants at 37°C and it was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.Agar well diffusion assay[21] was the key process used to evaluate the antibacterial potential of iron nanoparticles synthesized from *Cassia fistula* leaf extracts. Petri dishes containing 20 ml of Mueller Hinton Agar (MHA) were swabbed with the respective pathogens. Wells of 6mm diameter were cut into solidified agar media. 20 (mg/ml) and 50 (mg/ml) of each extract was poured in the respective wells and the plates were incubated at 37°C overnight. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period. Streptomycin was used as positive control. Sterile distilled water used as a negative control

RESULTS AND DISCUSSION

Phytochemical screening

The results of preliminary phytochemical analysis of aqueous leaf extracts revealed the presence of various bioactive compounds like alkaloids, phenols, protein, saponins, and flavonoids. (Table 1)

Table 1: Phytochemical screening of leaf extract of *Cassia fistula* Linn.

Phytoconstituents	Aqueous extract
Alkaloids	+
Phenol	+
Protein	+
Saponins	+
Steroids	-
Flavonoids	+
Cardiac glycosides	-
Terpenoids	-

+ indicates presence - indicates absence

Synthesis of Iron Nanoparticles

The reduction of ferric ions was confirmed through the development of black color after addition of ferric chloride. This indicated the presence of iron nanoparticles. The black color of the solution was due to surface Plasmon resonance (Figure 1). Control (without ferric chloride) showed no color change. Both solutions were incubated at room temperature for 24 hours. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Fe+ ions is complete within 4 hours after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, it is meant that there was no observable variation in the optical properties of the nanoparticle solutions with time.

Figure 1: Change in color from brown to black indicates the synthesis of Iron Nanoparticles.

A- Aqueous extract of *Cassia fistula* Linn.
B- 0.1M Ferric chloride solution.
C- Synthesized Iron Nanoparticles.

Characterization Studies

UV-Visible Spectrophotometer analysis

An UV Visible spectrum is the preliminary characterization of iron nanoparticles. The bio reduction of Fe ions in aqueous solutions was monitored by measuring UV-Vis spectra. The wavelength was scanned from 800 to 200nm giving the expected spectra. The absorption spectra of synthesized iron nanoparticle has absorbance peak 220nm (Figure 2). The broadening of the spectra indicated that the iron nanoparticles are polydispersed. The intensity of absorption peak increases with increasing time period. Similar results on visual observations and a peak around 220 nm have been reported for *Cuminum cyminum* seed extract synthesized iron nanoparticles.[22]

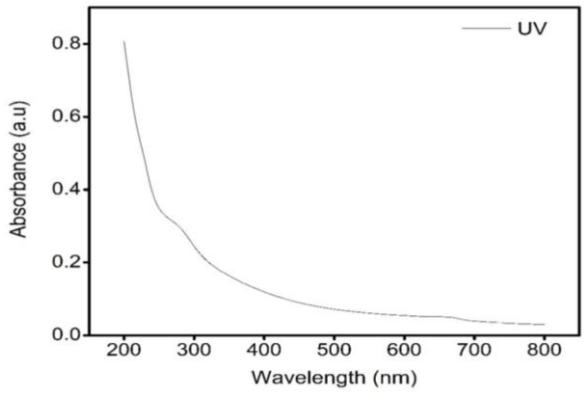


Figure 2: UV-Visible spectroscopy of Iron Nanoparticles synthesized from *Cassia fistula* Linn Leaf extract.

Scanning Electron Microscopy analysis

SEM analysis was used to determine the morphology and size of synthesized iron nanoparticles. A small volume of iron nanoparticles suspension was taken for SEM analysis on electron microscope stub. The stub was placed briefly in a drier and then coated with carbon coated copper. Pictures were taken by random scanning of the stub. Figure-3 showed the distribution of iron nanoparticles produced by the leaf extract of *Cassia fistula* Linn. The SEM micrograph showed individual iron nanoparticle as well as number of aggregates. The shape of iron nanoparticle was found to be almost spherical shape under various magnifications. The size of the nanoparticles ranged from 20-50 nm. Images at high resolution showed the iron nanoparticles are well dispersed. The SEM results were consistent with reported results of synthesized iron nanoparticles using *Camellia sinensis* extract.[23]

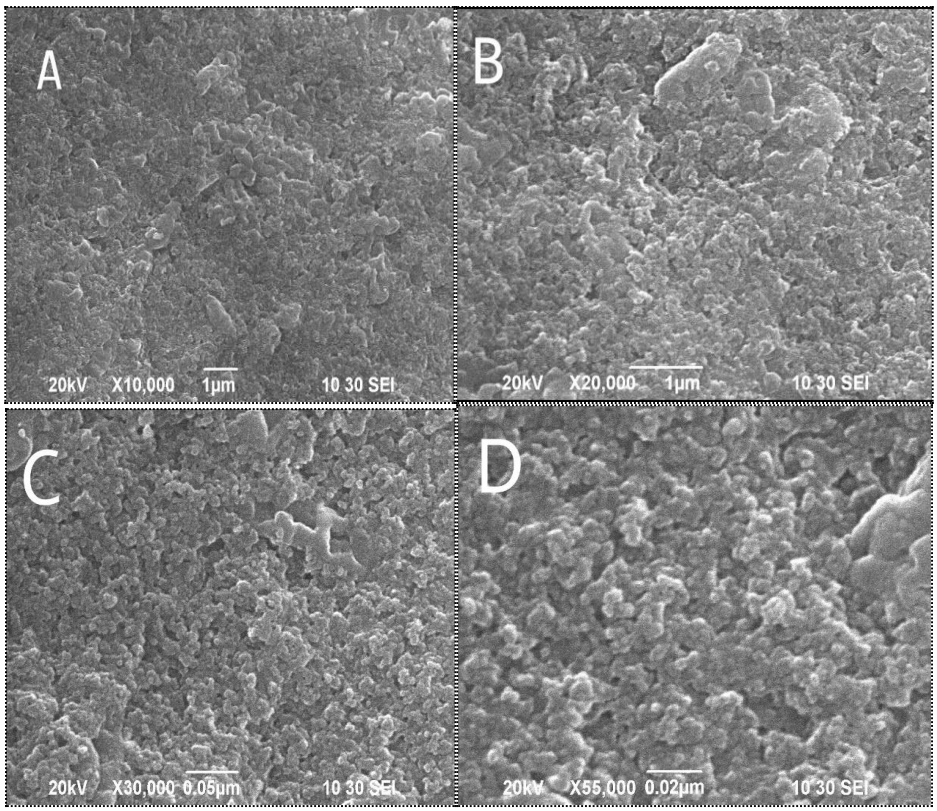


Figure 3: SEM analysis of synthesized Iron Nanoparticles from *Cassia fistula* Linn Leaf extract.
A) 10,000 X magnification, B) 20,000 X magnification,
C) 30,000 X magnification, D) 55,000 X magnification

FT-IR Spectroscopy analysis

FT-IR measurement was carried out to characterize the organic molecules which are responsible for the stabilization of iron nanoparticle synthesized from the plant. The frequency range is measured as wave numbers typically over the range 4000-400 cm^{-1} (Figure 4). FTIR measurement of iron nanoparticles showed the presence of peaks at 422.43, 497.66, 1069.57, 1545.05, 1641.49 and 3375 cm^{-1}. The peaks 3375.57 $cm^{-1}$and 1069.57 cm^{-1} signify the characteristic band of O-H group and sulphate group respectively. The peaks at 1545.05 cm^{-1} corresponded to C-C and C-N stretching respectively the presence of biopolymers. The peak at 1641.49 cm^{-1} showed the bond stretch for N-H group in Primary amines. The presence of alkenes in the range between 650-100 cm^{-1} represents O-H group in carboxylic acid.

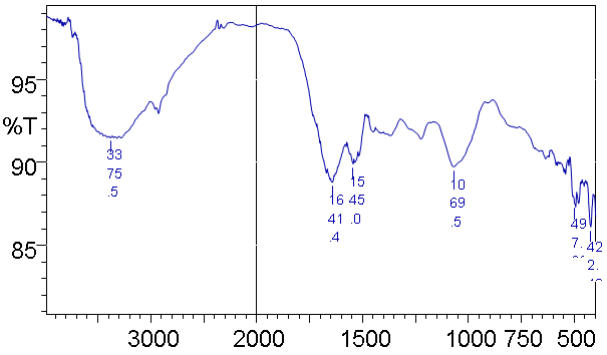


Figure 4: FT-IR analysis of synthesized iron nanoparticles from *Cassia fistula* Linn Leaf extract.

Antibacterial assay

In the present study antibacterial assay was performed against Gram positive and Gram negative bacterial pathogens using the synthesized iron nanoparticles from *Cassia fistula* Linn leaf extract. Streptomycin was used as a control. The iron nanoparticles showed inhibition against all the bacteria (Figure5, Table 2).Iron nanoparticles showed maximum zone of inhibition 16 mm and 15 mm against *Staphylococcus* sp and *Pseudomonas* sp respectively. The iron nanoparticles bind with cytoplasmic membrane and kill the bacterial cell. This is because of the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles. [19]

Tayyaba Naseem *et al.* (2015) [17] reported that iron nanoparticles were synthesized from five different plants, *Lawsonia inermis*, *Gardenia jasminoides*, *Azadirachta indica* and *Camellia sinensis* leaves extract and *Cinnamon zyelanicum* bark extract and it was found that all are susceptible to all bacterial strains.

Table 2: Antibacterial activity of iron nanoparticles of *Cassia fistula* Linn leaf extract.

S. no.	Bacteria	Zone of inhibition 20mg/ml (mm)	Zone of inhibition 50mg/ml (mm)
1	*Bacillus* sp	7mm	10mm
2	*Staphylococcus* sp	7mm	**16mm**
3	*Pseudomonas* sp	9mm	**15mm**
4	*Klebsiella* sp	7mm	9mm

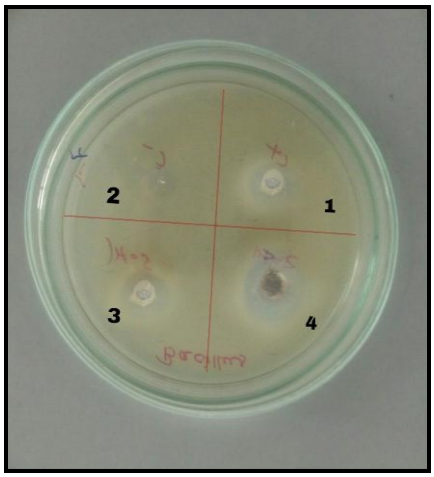
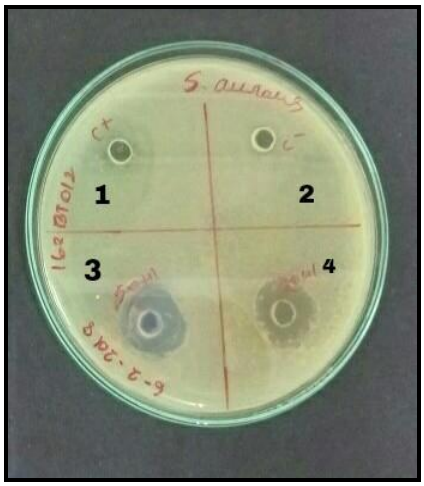
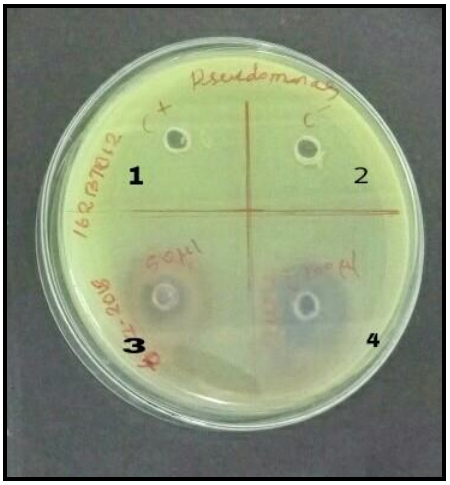


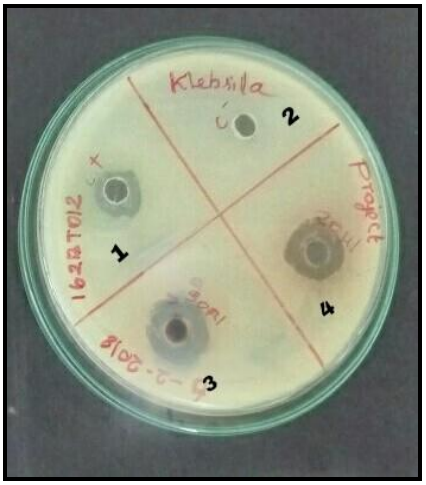
Figure 5: *Bacillus* sp



***Staphylococcus* sp**



***Pseudomonas* sp**



***Klebsiella* sp**

1-Positive control, 2- Negative control, 3 -20 µl of FeNPs, 4- 50 µl of FeNPs.

CONCLUSION

Medicinal plant species constitute important alternatives to conventional medicine in a large number of developing countries, especially within poor communities that inhabit rural areas and lack access to health services. The study concluded that *Cassia fistula* Linn leaf extract synthesized iron nanoparticles are being used for various applications in human beings. Green synthesis of nanoparticles has many advantages such as eco-friendly, low cost and large-scale synthesis. Biological synthesis of metal nanoparticles is a traditional method and the uses of plant extracts have a new awareness for the control of disease, besides being safe and no phytotoxic effects. From the overall results, it could be concluded that *Cassia fistula* Linn leaf extract is the best candidate species for the biosynthesis of iron nanoparticles. Further, the biogenic iron nanoparticles exhibited applicable antibacterial activity.

REFERENCES

1. Mohammed Rafiqkhan, Vijitha Viswambhara. Pharmacognostic profile and phytochemical investigation of *Syzyium cumini* bark extracts. International Journal of Pharma and Bio Sciences, 2015; 6(3): 359-365
2. Davidson Hunt I. Ecological ethnobotany: stumbling toward new practices and paradigms. MASA J, 2000; 16: 1–13
3. Mukherjee PW. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi, India: Business Horizons Publishers.
4. Bodeker C, Bodeker G, Ong CK, Grundy CK, Burford G, Shein K.WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Geneva, Switzerland: World Health Organization, 2005.
5. Bandaranayake WM. "Quality control, screening, toxicity, and regulation of herbal drugs,'' in Modern Phytomedicine. Turning Medicinal Plants into Drugs. Ahmad I, Aqil F, Owais M., editors. (Weinheim: Wiley-VCH GmbH & Co. KGa A;), 2006; 25–57.
6. Rasool Hassan BA. Medicinal Plants (Importance and Uses). Pharmaceut Anal Acta, 2012; 3: 10.
7. Singh R. Medicinal Plants: A Review. Journal of Plant Sciences. Special Issue: Medicinal Plants, 2015; 3(1): 50-55.
8. Savita Chewchinda, Mansuang Wuthi-udomlert, Wandee Gritsanapan, "HPLC Quantitative Analysis of Rhein and Antidermatophytic Activity of *Cassia fistula* Pod Pulp Extracts of Various Storage Conditions, BioMed Research International, 2013; 1-5.
9. Sepideh Miraj. *Cassia fistula* Linn: Evidence for pharmaceutical applications. Der Pharmacia Lettre, 2016; 8(14): 129-131
10. Perry F. Flowers of the World. Hamlyn, London, 1992; 320.
11. Rudd VE. Fabaceae, Subfamily Caesalpinioideae In: M.D. Dassanayake and F.R. Fosberg (Eds.), A Revised Handbook to the Flora of Ceylon, Volume 7, Amerind, New Delhi, 1991; 34-107.
12. Chou KS, Lu YC, Lee HH. Effect of alkaline ion on the mechanism and kinetics of chemical reduction of silver. Materials Chemistry and Physics, 2005; 94: 429-433.
13. Lin WC, Yang MC. Novel Silver/Poly (vinyl alcohol) Nanocomposites for surface-enhanced Raman Scattering-active substrates. Macromolecular Rapid Communications, 2005; 26(24): 1942-1947.
14. 14.Shin HS, Yang HJ, Kim SB, Lee MS. Mechanism of growth of colloid silver nanoparticles stabilized by polyvinyl pyrrolidonein gamma - irradiated silver nitrate solution, Journal of Colloid and Interface Science, 2004; 274: 89-94.
15. Asmita J.Gavhane., P. Padmanabhan, Suresh P. Kamble and Suresh N. Jangle. Synthesis of silver nanoparticles using extract of neem leaf and triphala and evaluation of their Antimicrobial activities. International Journal of Pharma and Bio Sciences, 2012; 0975-6299.
16. Rohit kumarBargah. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. Journal of Pharmacognosy and Phytochemistry, 2015; 4(1): 07-09.
17. Tayyaba Naseem and Muhammad Akhyar Farrukh. Antibacterial activity of green synthesis of Iron Nanoparticles using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract. Journal of Chemistry, 2015. 7.http://dx.doi.org/10.1155/2015/912342.
18. Saranya S, Vijayarani K, Pavithra S. Green synthesis of Iron Nanoparticles using aqueous extract of *Musa ornata* flower sheath against pathogenic bacteria. Indian Journal of Pharmaceutical Sciences, 2017; 79(5): 688-694.
19. 19.Indhumathy J, Gurupavithra S, Ravishankar K, Jayachitra J. Green synthesis of silver nanoparticles using *Cassia fistula* leaf extract and its applications. Mintage Journal of Pharmaceutical and Medical Sciences, 2014; 3(3): 20-22.
20. Laura Martinez Maestro, Emma Martin Rodriguez, Fiorenzo Vetrone, Rafik Naccache. Nanoparticles for high efficient multiphoton fluorescence bioimaging. Optics express, 2010; 18: 10.
21. Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. Acta Biol Med Exp. 1990; 15: 113-115.
22. Nandini T, Subhashini D. Antioxidant efficacy of Iron Nanoparticles from aqueous seed extract of *Cuminum cyminum*. International Journal of PharmTech Research, 2015; 8(7): 19-25.
23. Gottimukkala KSV. Green Synthesis of iron nanoparticles using green tea leaves extract. Journal of Nanomedicine & Biotherapeutic Discovery, 2017; 7:151.