CHARACTERSATION AND ANTIBACTERIAL STUDY OF GREEN SYNTHESISED IRON OXIDE NANOPARTICLES FROM PLANT LEAF EXTRACTS OF *Lawsonia inermis AND Gardenia jasminoides*

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BY

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"CHARACTERSATION AND ANTIBACTERIAL STUDY OF GREEN SYNTHESISED IRON OXIDE NANOPARTICLES FROM PLANT LEAF EXTRACTS OF *Lawsonia inermis AND Gardenia jasminoides*"** submitted to Mahatma Gandhi University in the partial fulfillment of the requirement of Bachelor of Science in Biotechnology, is a record of original work done by **ANJANA UNNIKRISHNAN** under the guidance of **FEBY K JOHN**, Lecturer, Department of Biotechnology, SreeSankara College, Kalady, during the academic year 2017-18.

Mrs. Feby K John
Department of Biotechnology

For
Mrs.Sujaya Lakshmi
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Examiners:

1. Reshya Rasim

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DECLARATION

I hereby declare the dissertation entitled **"CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIS OF IRON NANOPARTICLES USING LAWSONIA INERMIS AND GARDENIA JASMINOIDES LEAVES EXTRACT"** submitted to Mahatma Gandhi University in the partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology, is a record of original work done by me, under the guidance of Mrs. **FEBY K JOHN**, Lecturer, Department of Biotechnology, Sree Sankara College Kalady, and that it has not been previously included in thesis, dissertation or report submitted to this University or any other institution for a Degree, Diploma or any other qualification.

Place: Kalady

Date: 11/4/2018

ANJANA UNNIKRISHNAN

DEDICATED TO,

ALMIGHTY,

MY RESPECTED TEACHERS,

FAMILY & FRIENDS……..

ACKNOWLEDGEMENT

It is with profound sense of gratitude that I compile and present this project on posture before the medical world and esteemed public. First of all, I raise my voice of love and gratitude to GOD almighty, for the successful completion of this project.

It is my pleasure and privilege to record my deep sense of gratitude to my teachers. I am extremely grateful to Mrs. FEBY K JOHN (DEPARTMENT OF BIOTECHNOLOGY) for their valuable guidance all throughout the development of this project.

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Last but not the least; I take this opportunity to thank my parents, friends who helped for the successful completion of this project.

CONTENTS

INTRODUCTION

Nanotechnology is the study and application of extremely small things and can be used across all the other science fields such as chemistry, Biology, Physics, Materials science, and Engineering. Nanotechnology is the manipulation of matter on an atomic, molecular, and supramolecular scale.

Nanoparticles are considered as important structural masses of nanotechnology. Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. The unique and most important property of the nanoparticles is that they unveil superior activity. Scientific research on nanoparticles is intense as they have many potential applications in medicine, Physics, Optics and Electronics.

There are remarkable application of iron nanoparticles in the areas of diagnostic biological probes, Catalysis, Display devices, and Optoelectrons. Metal nanoparticles are widely synthesized using physical and chemical processes, which allow one to acquire particle with the preferred characteristics.

Green synthesis of metallic nanoparticles has accumulated an ultimate interest over the last decade due to the distinctive properties that make them applicable in various fields of science and technology. Green synthesis has advances over chemical and physical method because it is cost operative, atmosphere friendly, and easily scrabbled up for large scale synthesis.

In this method there is no need to use high energy, temperature, and toxic chemicals. Green synthesized nanoparticles are cheap and economical and have many applications in science.

As our lifestyle is getting techno-survey, we are moving away from nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are lots of herbs used for the aliments related to different seasons. There is a need to promote them to save human life. These herbal products are today are the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and environment.

From the dawn of civilization, human beings have used various medicinal plants to fight diseases. In this study, a conventional method was used to obtain iron nanoparticles (FeNPs) using the leaves extract of *Gardenia jasminoides* and *Lawsonia inermis* plants.

Lawsonia inermis is a dwarf herb, commonly known as "mehendi or henna '' in Pakistan. It is the source of the dye henna used to dye skin, hair and fingernails, as well as fabrics including silk, wool and leather. Today henna is cultivated commercially on a large scale, especially in India, but has also become naturalized in warmer parts of South

and North America, Australia and the West Indies, where it is often referred to as "West Indian Mignonette". Henna only survives in temperature of +13°C or higher. Today, henna is cultivated commercially on a large scale especially in India, Pakistan, Yemen, Iran, Afghanistan, Somalia etc...

Henna is often used as a hedge plant because of its beautiful appearance and the fragrant flowers. It is renowned worldwide due to its cosmetic use for the reason of exclusive active principles in the leaves. It contains different variety of molecules which are bioactive. It is believed to decrease body temperature in situation of high fever and give beautiful healthy hair.

Strong antimicrobial, anticancer, anti-inflammatory, analgesic, antiparasitic, and virucidal properties of this plant have been reported. *Lawsonia inermis* leaves were studied for their antimicrobial prospective and they exhibited notable antibacterial activity against Gram – negative bacterial strains.

Scientific classification

Kingdom: Plantae

Order : Myrtales

Family : Lyrthraceae

Subfamily : Lythroideae

Genus : *Lawsonia*

Species : *L.inermis*

Binomial name : *Lawsonia inermis*

Gardenia jasminoides belongs to Rubiaceae family and is native to the tropical and subtropical parts of Africa, South Asia, Australia, and Oceania. Gardenias are widely practiced as an ornamental flower in bouquets, as house plants, and as outside plants. It is widely used in gardens in warm temperate and subtropical climates, and as a houseplant in temperate regions.

This plant is known as one of the most valuable plant species in traditional Chinese medicine and is considered highly effective as a hemostatic agent; drains fair and are effective in treating injuries to the muscles, joints and tendons. Is has been in cultivation in china at least a thousand years. Many varieties have been bred for horticulture, with low growing, and large and long flowering forms.

In many Asian countries it has been used as a folk medicine. This plant has numerous medicinal uses for treating hemorrhage, jaundice, toothaches, hepatitis, sprains, wounds and skin conditions. "crocetin" is an extracted chemical compound from the *Gardenia berry*, from which a yellow-silk dye has been made for this treatment.

Scientific classification

Kingdom: Plantae

Oder: Gentian ales

Family: Rubiaceae

Genus: Gardenia

Species: G. *jasminoids*

Binomial name: *Gardenia jasminoids*

Both *Lowsonia inermis* and *Gardenia jasminoides* leaves were studied for their antimicrobial prospective and they exhibited notable antibacterial activity against gram-negative bacterial strains. Bacterial resistance to various antibiotics is a serious clinical dilemma, so different antimicrobial activities were performed using plant as a source.

The development in the field of green chemistry has delivered different nanomaterial as substitute antibacterial agents.

In this present study, an effort is made to synthesize iron nanoparticles using leaves extract of *Lawsonia inermis* and *Gardenia jasminoides* as reducing agent. The characterization of green synthesized iron nanoparticles was characterized by FTIR (Fourier transform infrared spectroscopy), DLS (Dynamic light scattering) and UV Visible spectra analysis. Also antibacterial activity was studied against three gram negative such as *Escherichia coli, Pseudomonas,* and *Klebseilla* and two gram positive such as *Bacillus* and *Staphylococcus aureus* bacterial strains.

REVIEW OF LITERATURE

Nano word is originated from Latin word, which means dwarf. Ideal size range offered by nanotechnology refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a meter. The branch nanotechnology in the science that particularly deals with the processes that occur at molecular level and of Nano length scale size. Nanotechnology is now become an allied science which is almost commonly used in other fields of science like electronics, physics and engineering since many decades. Nanotechnology has created potential impact in various fields like medicine including immunology, cardiology. Endocrinology, ophthalmology, tumor targeting and gene delivery.

Nanotechnology is an efficient in improving the bioavailability of water-soluble drugs. Nanoparticles are considered as important masses of nanotechnology. The unique and most important property of the nanoparticles is that they unveil superior activity. Metal nanoparticles are widely synthesized using physical and chemical processes, which allow one to acquire particles with the preferred characteristics.

An important area of research in nanotechnology deals with the biomimetic synthesis of nanoparticles by using biological sources like plant leaf, bacteria, fungi, etc., which offers numerous benefits of eco-friendliness & effective in various medicinal applications as they do not use any toxic chemicals in the synthesis protocol. In early days nanoparticles aroused primarily by either physical or chemical methods by using non-deleterious solvents or substances like hydrazine, sodium borohydride, hydrogen, heavy metals, etc. and radiation chemicals which causes great damage in the environment as well as side effects in the health(9 –12). To overcome this problem, bio-inspired synthesis of nanoparticles as a choice by targeting in wide range has been carried out

Green synthesis has advances over chemical and physical method, green synthesis of metallic nanoparticles has an ultimate interest over the last decade due to their distinctive properties.

Green synthesized nanoparticles are cheap and economical and it offer better influence, control over crystal growth and their steadiness.

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health. A large number of traditional plants are subjected to tissue culture and pharmacological studies for their plant based drugs and conservation.

From the dawn of civilization, human beings have used various medicinal plants to fight diseases. Metal nanoparticles that are synthesized by using plants have emerged as nontoxic and ecofriendly.

Medicinal plants have been a part of modern life style of a man and these plants are a source of important therapeutic aid for alienating human ailments. Most of the medicinal plants even today are collected from wild. Consequently, cultivation of these plants is urgently needed to ensure their availability to the industry as well as to people associated with traditional system of medicine.

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many diseases. The plant extracts have been developed and proposed for use as antimicrobial substances.

Lawsonia inermis popularly known as Mehendi or Henna is a cosmetically renowned plant of the oriental region possesses diverse pharmacological activity including anti carcinogenic, antimicrobial, anti-inflammatory, analgesic, antipyretic, hepatoprotective, anti tuberculostatic. In search of new anticancer drugs from natural sources many researchers have reported anticancer and chemo preventive properties of Henna extracts/compounds in their pre-clinical studies. Lawson, one of the major constituent of henna, is used as a starting material in the synthesis of a variety of clinically valuable anticancer drugs such as atovaquone, lapachol, and dichloroallyl Lawson.

L.inermis is a small shrub which has its unique bio-active principles like Sugars, Fraxetin, Tannin, Gallic acid, Lawson, Resins, Coumarins, etc. in their leaves. Among those Lawson is the major ingredient which gives its characteristic colour(lavhate,2007). This plant has been used in medicinal field since ancient times. This is the only plant known which possess healing attributes and now it is used in intense scientific study (4-9). The leaves of this plant are used in the treatments of wounds, ulcers, cough, bronchitis, lumbago, rheumatagia, inflammations, diarrhea, dysentery, leucoderma, scabies, boils, anemia, hemorrhages, fever, falling of hair and greyness hair (14-17).

In the present study, review and authentication of the various aspects of the plant Lawsonia inermis was carried out. This plant is mainly present in subtropical and tropical areas and is used all over the world.

Traditionally, in Asian countries like India and Pakistan, plant leaves are applied to hands, hairs and feet. Morphologically this plant is considered as medicine or shrub.

The medicinal properties exhibited by this plant mainly due to its wide range of phyto-chemical compounds present in them. These includes 1,4-Naphoquinone, 2-Hydroxy-1,4-Naphthoquinone, Aesculelin, β-Sitosterol, Esculetin, Cosmosiin, Laloiside, Quinone, Scopoletin, Tiliani, etc.

Gardenia is an evergreen shrub or small tree that can grow up to 12 meters tall, but usually it is much shorter. The fragment flower is used for scenting tea. They are also sometimes eaten raw as a delicacy, pickled or preserved in honey.

The whole plant is antispasmodic, antiperiodic, cathartic, anthelmintic and external-antiseptic. The roots are used to treat headache, dyspepsia, nervous disorders, and fever.

'Crocetin' is an extracted chemical compound from the Gardenia berry, from which a yellow- silk dye has been made for this treatment

The leaves are applied in febrifuges poultices. The fruits are used against jaundice and diseases of the kidneys and lungs.

Antibacterial activity of many plants has been reported by the researchers. Medicinal plants represent a rich source of antimicrobial agents and plants are used medicinally in different countries and are a source of many potent and powerful drugs.

In nanometer size metallic nanoparticles, iron has received special attention because of its physical and chemical properties which are determined by its size, shape, composition, crystallinity, and structure. Against the bacterial strains causing digestive problems, iron nanoparticles of corn flakes-like morphology gave excellent antibacterial activity.

Bacterial resistance to various antibiotics is a serious clinical dilemma, so different antimicrobial activities were performed using plant as a source. The development in the field of green chemistry has delivered different nanomaterial as substitute antibacterial agents

Many medicinal plants are considered to be potential antimicrobial crude drug as well as a source for novel compounds with antibacterial activity.

AIM AND OBJECTIVE

- To determine green synthesis of the iron nanoparticle from *Gardenia jasminoides* and *Lawsonia inermis* leaf extract
- To characterize iron oxide nanoparticles.
- To determine antibacterial study of synthesized nanoparticles against human pathogens.

MATERIALS AND METHODS

MATERIALS

SAMPLE COLLECTION

Fresh leaves of Gardenia jasminoides and Lawsonia inermis (Henna) was collected from various houses nearby college. Iron sulphate (FeSO4 were purchased from the Biochemistry laboratory Department of Bioscience, Sree Sankara College Kalady.

PREPARATION OF POWDER

Fresh leaves of Henna and Gardenia were washed in sterile distilled water to remove dust particles and plant material was then placed to dry under sunlight for seven days. After incubation, all of the dried leaves of plants were ground using grinder. After the process of grinding, the leaves powder went through sieving to get very fine particle of uniform size. Nanoparticles are synthesized using sieved powder.

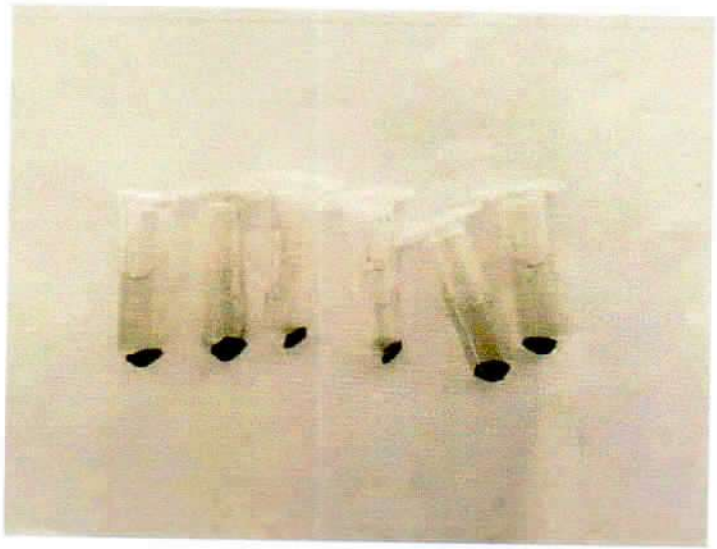
PREPARATION OF FeNPs

A simple conventional heating method was used in the synthesis of iron nanoparticles (FeNPs) by using plant extract. Plant extract is prepared by dissolving 2gm of sieved powder in 50ml of distilled water and the resulting mixture kept on stirring for 3 hours by using shaker. The resulting solution was placed for 1 hour to stable down and then filtered. 10ml of 0.001M FeSO4 solution was used in which plant extract (filtrate) was added after every interval of 5 minutes using 2ml in each interval until 50ml; resulting mixture was stirred at 70°C. The difference of temperature was noted after every interval of 5 minutes. Allowed to cool down and the solution was subjected to centrifugation at 10,000rpm for 2 minutes. Black pellet formed after centrifugation was taken. The plant extracts (filtrate) acts as reducing, capping, and stabilizing agent in iron particle synthesis.

Incubation

Preparation of powder

Centrifugation



Pellets

MICROORGANISM USED

GRAM POSITIVE	GRAM NEGATIVE
Staphylococcus aureus	*Escherichia coli*
Bacillus	*Klebsiella*
	Pseudomonas

MEDIAS AND CHEMICALS USED

NUTRIENT AGAR

Nutrient agar was used for maintaining cultures of bacteria.

NUTRIENT BROTH

Nutrient broth was used for preparing liquid cultures.

MULLER HINTON AGAR

Muller Hinton agar is used to check antibacterial activity by well diffusion method.

METHODS

CHARACTERIZATION TECHNIQUE

The characterization techniques for iron nanoparticles were determined by the help of Mahatma Gandhi University, Bioscience Department.

Three techniques were used to characterize the synthesis of iron nanoparticles.

FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)

FTIR is a very versatile tool for surface characterization of nanoparticles' surface provided a specific setup is attached to the spectrometer. Under specific conditions, the chemical composition of the nanoparticles surface can be determined and the surface reactive site responsible for the surface reactivity can be identified. In addition, the chemical reaction taking place at the nanoparticles surface can be monitored in situ as a function of various parameters, such as temperature and gaseous environment.

DLS (DYNAMIC LIGHT SCATTERING)

Current research demands the fastest and most popular method of determining particle size. DLS widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the Nano and submicron ranges. In this technique, solution of spherical particles in Brownian motion causes a Doppler shift when they are exposed against shining monochromatic light exposure hits the moving particle which results in changing the wavelength of the incoming light. Extent of this change in wavelength determines the size of the particle. This parameter assists in evaluation of the size distribution, particle's motion in the medium, which may further assists in measuring the diffusion coefficient of the particle and using the autocorrelation function. Dynamic light scattering (DLS)

offer the most frequently used technique for accurate estimation of the particle size and size distribution.

UV-VISIBLE SPECTROSCOPY

UV-Visible spectroscopy offer the possibility to characterize nanoparticles, have been used to compare aqueous colloidal suspension and also used UV spectroscopy to study the relationship between absorbance spectra and particle size distribution of quantum-sized Nano crystals.

ANTIBACTERIAL STUDY

AGAR WELL DIFFUSION METHOD

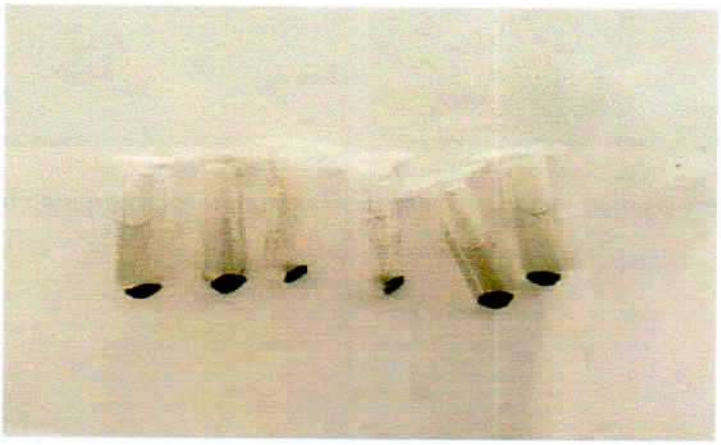
The antibacterial activity was carried out by agar well diffusion method using Muller Hinton agar plates. Three human pathogenic Gram negative (Escherichia coli, Pseudomonas and Klebsiella) and two Gram positive (Staphylococcus aureus, Bacillus) bacterial strains were used for antibacterial activity by this method. Lawn culture of organism was prepared using sterile swabs soaked in nutrient broth culture of organism over dry agar plates. Using well puncher, wells of 8mm diameter were punched on the solid agar and 30ul of sample suspension was loaded in each wells. The plates were incubated for 24 hours at 37°C.

The diameter of the zone of inhibition was measured in millimeter.

RESULTS

1. GREEN SYNTHESIS

The synthesis of iron oxide nanoparticles involves the following results:



Black pellets sediment during centrifugation showing synthesis of FeNPs

Pellets are oven dried at 50°C

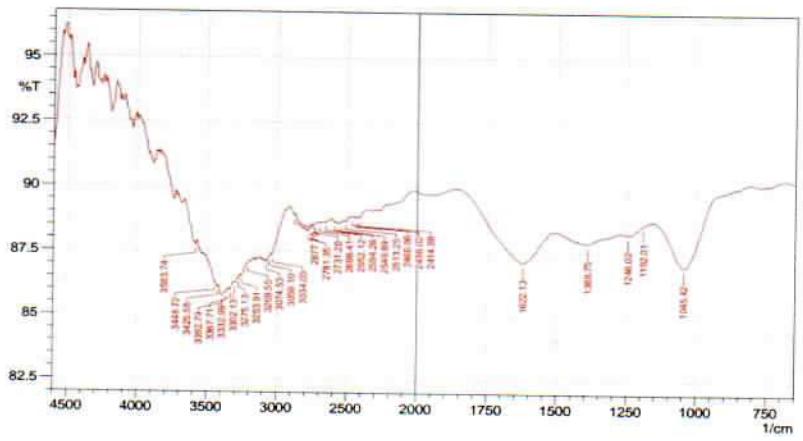
The so formed nanoparticles obtained from the dried plate using a sterile spatula

2. FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY) ANALYSIS OF FeNPs SYNTHESIZED USING HENNA AND GARDENIA LEAVES EXTRACT

The main constituent of Henna is Lawson (2-hydroxy-1, 4-naphthoquinone). It contains benzene unit, p-benzo-quinone unit, and phenolic group. The henna extract was evaporated to dryness to get a solid mass. The phenolic O-H stretch appeared at 3302.13-3275.13-1. The aromatic C=C stretching frequency which appeared at 1622.13cm-1 is due to p-coumaric acid present in the sample. Thus Lawson was characterized by IR spectroscopy. It was inferred that Lawson has coordinated with Feo through the phenolic oxygen, aromatic ring, and C=C group of the p-benzoquinone resulting in the formation of Feo which has Lawson as capping and stabilizing agent on the anodic sites of the metal surface. The hand at 1045.42cm-1 was due to Fe as shown in below figure. Thus FTIR spectral study leads to the conclusion that the fingermark film consists of Feo Lawson complex.

COMPOUNDS	PEAK RANGE (cm-1)
-OH group	3302.13 – 3275. 13
C=C group	1622.13
C=O group	1388. 75
Fe vibrations	1045.42

Table showing compounds and their peaks of FTIR of Henna leave extract

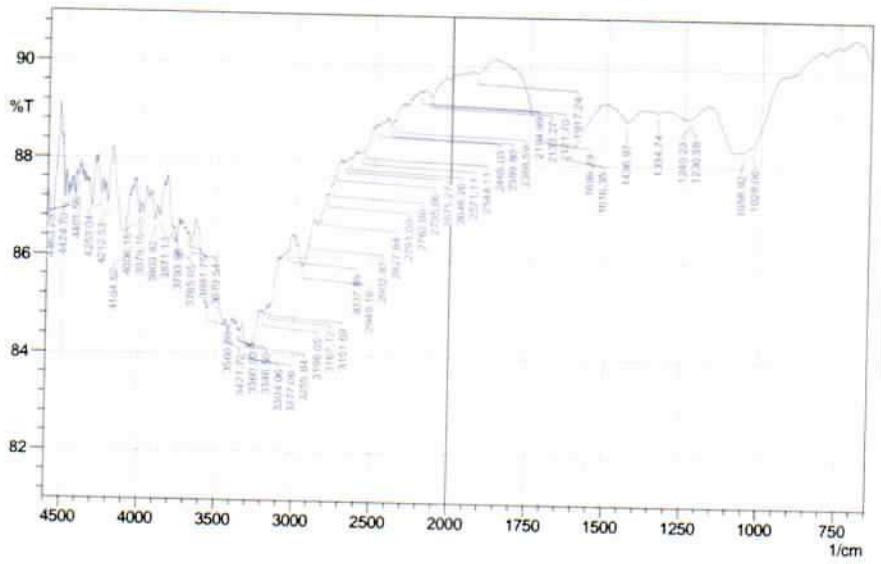


FTIR spectra of FeNPs synthesized using Henna leave extract

The FTIR spectra of Fe nanoparticles synthesized with Gardenia leave extract are given below. The broad absorption band in the region from 3421.72 – 3360.00cm-1 represents –OH group stretching and another peak at 2735.00cm-1 represents C-H stretching. The bands at 1616.35cm-1 and 1436.97cm-1 may be assigned, respectively, to a C=O stretching vibration band (C=O) and a coupled vibration involving the bending and the C-N stretching modes of the amino bond of the biomass. The peak at 1240.20cm-1 is corresponding to the vibrations of C-O in C-CCOOR. The band at 1028.06cm-1 was due to Fe vibrations.

COMPOUNDS	PEAKS RANGES (cm-1)
-OH group	3421.72-3360.00
C-H group	2735.00
C=O group	1616.35
C-N group	1436.97
C-O group	1240.20
Fe vibrations	1028.06

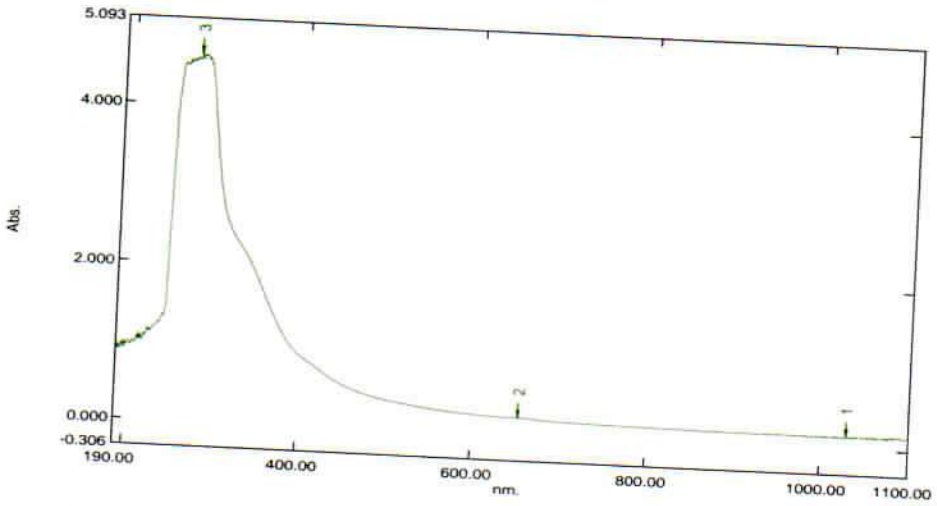
Table showing compounds and their peaks of FTIR of Gardenia leave extract.



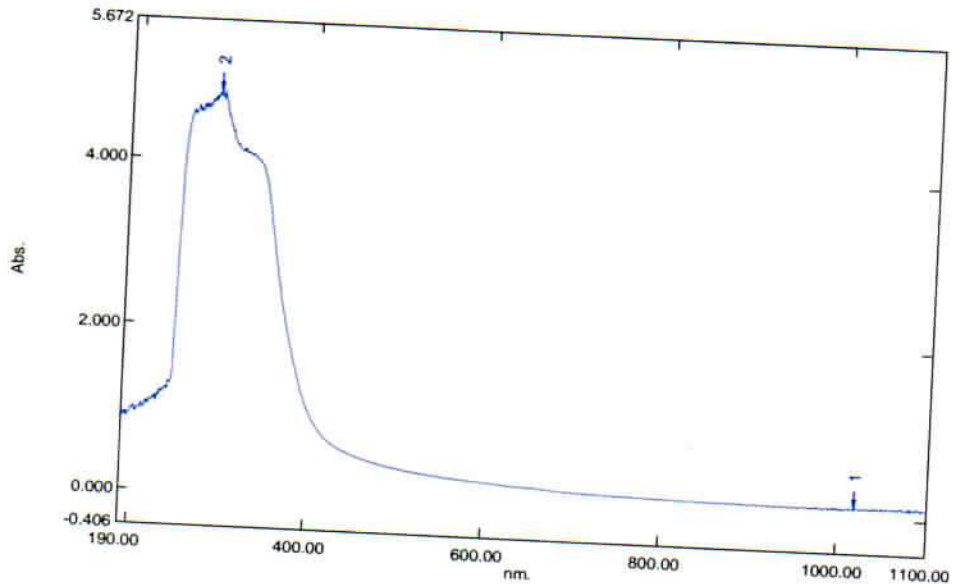
FTIR spectra of FeNPs synthesized using Gardenia leave extract

UV-VISIBLE SPECTROSCOPIC ANALYSIS OF FeNPs SYNTHESIZED USING HENNA AND GARDENIA LEAVE EXTRACT

The Green synthesized iron oxide nanoparticles using *Lawsonia inermis* and *Gardenia jasminoides* was validated by UV-Visible spectroscopic analysis and their scanning absorbance vs. wavelength (λ) has been established. The characteristics peaks of IO nanoparticles were observed at 370 nm, which is due to charge transfer spectra.



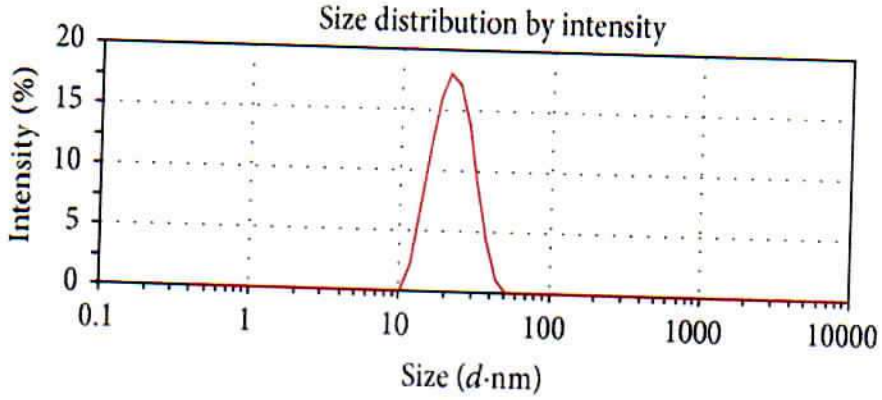
The UV-VIS spectrum spectra of FeNPs synthesized using Henna leave extract.



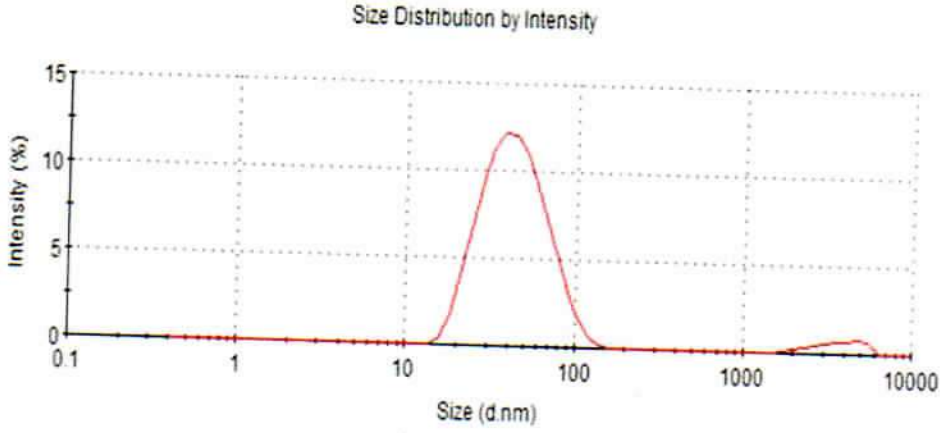
The UV-VIS spectrum spectra of FeNPs synthesized using Gardenia leave extract

DLS ANALYSIS

The particle size distribution of green synthesized iron oxide nanoparticles using Henna and *Gardenia* leave extract is shown in the figure below. The particle size distribution of FeNPs determined by laser diffraction method with a multiple scattering technique revealed that the particle size distribution of iron oxide nanoparticles ranges approximately from 10nm and 91 nm respectively. The distribution of oxide nanoparticle is more uniform with a narrow distribution range.



Particle size distribution of FeNPs synthesized using Henna leave extract



Particle size distribution of FeNPs synthesized using *Gardenia* leave extract

ANTIBACTERIAL RESULTS

Iron nanoparticles were synthesized using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract and it was found that certain concentration of the leaves extract are susceptible other being resistant to bacterial strains. The results iron oxide nanoparticles synthesized using *Lawsonia inermis* and *Gardenia jasmonoides* and the graph comparison is given respectively. FeNPs of *Gardenia jasminoides* were more potent at a concentration of 60µl against *Staphylococcus aureus* with zone of inhibition 30mm, whereas for *Lawsonia inermis* was 24mm at the same concentration. Against *Escherichia coli* and *Klebsiella sp*, the zone of inhibition of iron nanoparticles of *Lawsonia inermis* and *Gardenia jasminoides* leave extract was 19mm & 15mm and 17mm & 22mm respectively (60µl).

In the present study it shows a high zone of inhibition against both the Gram positive bacteria especially in the case of Gardenia sample. At lower concentrations (10µl) *Klebiella sp* and *E.coli* shows resistance to FeNPs synthesized from Henna extracts.

BACTERIAL PATHOGENS	ZONE OF INHIBITION AT DIFFERENT CONCENTRATIONS(mm)		
	10µL(A)	30µL(B)	60µL(C)
Staphylococcus aureus	13	19	24
Bacillus sp	10	17	25
Klebsiella sp	No zone	8	15
Escherichia coli	No zone	12	19

Table 3: Antibacterial activity of FeNPs synthesized from Henna leaves extract at different concentrations.

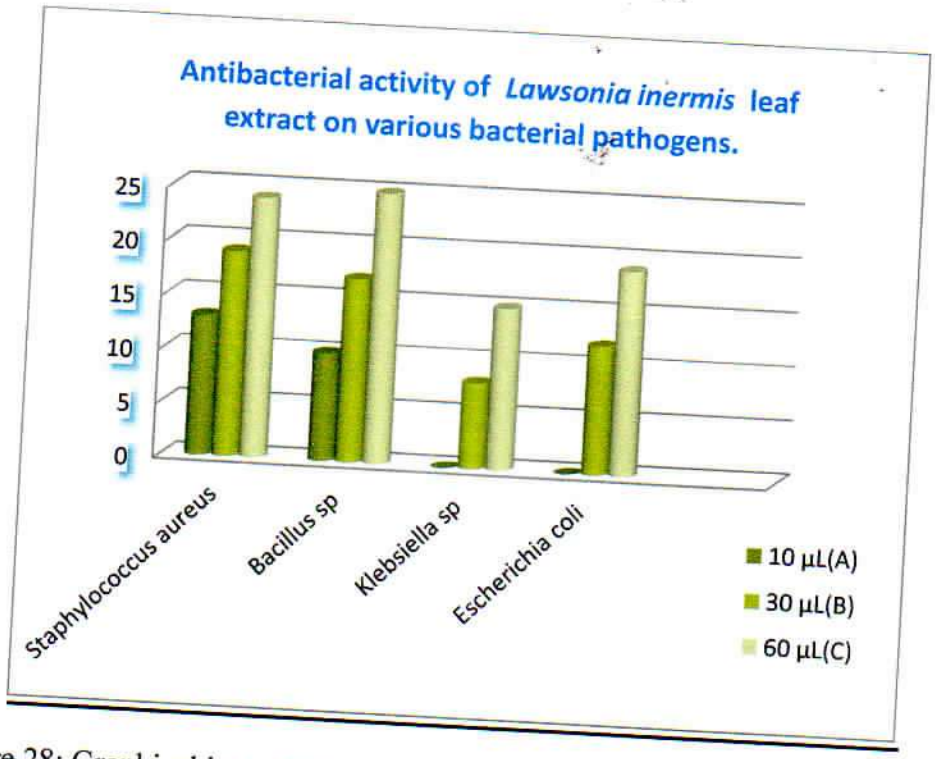


Figure 28: Graphical interpretation of antibacterial activity of FeNPs synthesized from Henna leaves extract.

BACTERIAL PATHOGENS	ZONE OF INHIBITION AT DIFFERENT CONCENTRATIONS(mm)		
	10µL(A)	30µL(B)	60µL(C)
Staphylococcus aureus	17	23	30
Bacillus sp	14	20	27
Klebsiella sp	10	15	22
Escherichia coli	9	11	17

Table 4: Antibacterial activity of FeNPs synthesized from *Gardenia* leaves extract at different concentrations.

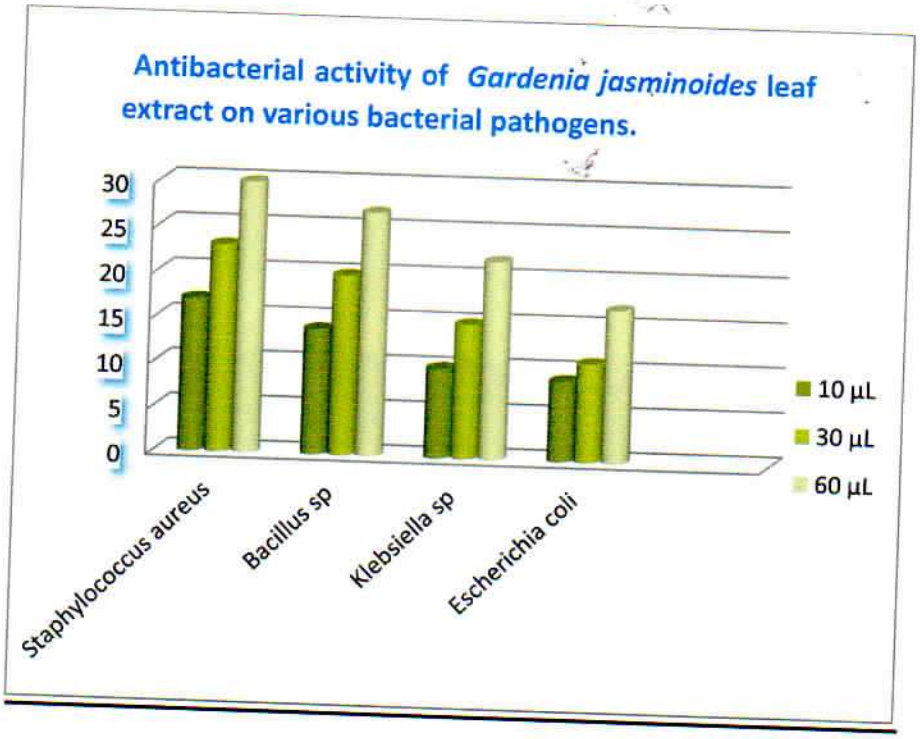
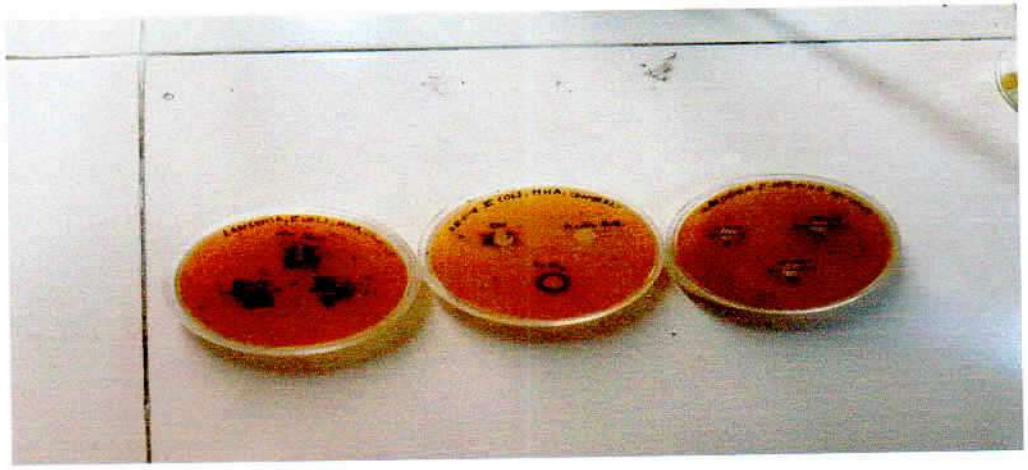


Figure 29: Graphical interpretation of antibacterial activity of FeNPs synthesized from *Gardenia* leaves extract.

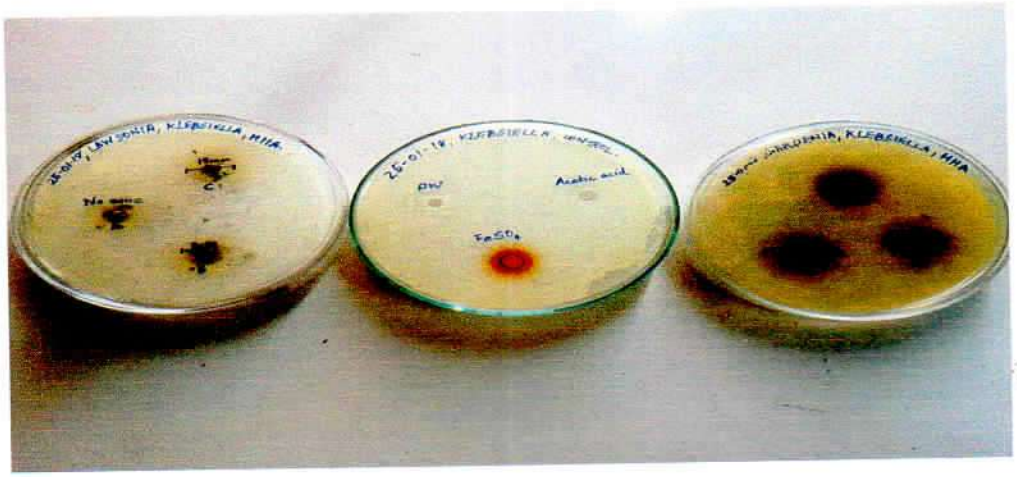
MULLER HINTON AGAR PLATES SHOWING ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED FeNPs

Plates showing zone of inhibition against *Staphylococcus aureus* at different concentrations for Lawsonia and Gardenia (left-right) with control in the center

Plates showing zone of inhibition against Bacillus sp at different concentrations for Lawsonia and Gardenia (left-right) with control in the center.



Plates showing zone of inhibition against *E.coli* at different concentrations for Lawsonia and Gardenia (left-right) with control plate in the center



Plates showing zone of inhibition against Klebsiella sp at different concentrations for Lawsonia and Gardenia (left-right) with control plate in the center.

DISCUSSION

The result of the study showed that, an ecofriendly green synthesis of iron nanoparticles using Gardenia jasminoides and Lawsonia inermis leaves extract was investigated.

FTIR analysis confirmed the presence of FeNPs in both samples. It also investigate the main constituent of Henna extract is Lawson (2 hydroxy 1, 4- naphthoquinone).

Validation by UV-Visible spectroscopy established that the peaks of IO nanoparticles were observed at 370nm.

DLS study determined that the particle size distribution ranges from 10 – 120nm with mean particle size of 66nm and 102nm.

The antibacterial study shows that zone of inhibition was formed to be high in Gram positive than Gram negative bacteria.

As a result, the green synthesis using Lawsonia inermis and Gardenia jasminoides leaves extracts can be efficient method for the synthesis of iron oxide nanoparticles.

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APPENDIX

1. MULLER-HINTON AGAR

INGREDIENTS	GRAMS/LITRE
Casein acid hydrolysate	17.50
Beef heart infusion	300.00
Starch	1.50
Agar	17.00
Distilled water	1000 ml
Final PH (at 25°C)	7.3± 0.2

Suspended 38 grams in 1000ml distilled water. Heated to boiling to dissolve the medium completely. Sterilized by autoclaving at 15lbs pressure (121°C) for 15 minutes.

2. NUTRIENT AGAR

INCREDIENTS	GRAMS/LITRE
Peptone	5.00
Sodium chloride	5.00
Beef extract	3.00
Yeast extract	3.00
Agar	15.00
Distilled water	1000ml
Final PH (at 21°C)	7.4±0

3. NUTRIENT BROTH

INCREDIENTS	GRAMS/LITRE
Beef extract	1
Yeast extract	2
Peptone	5
Sodium chloride	5
Final PH	6.8±0.2