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Comparative Analysis and Synthesis of Silver Nanoparticles from Selected Parts of Mimosa Pudica to Treat Urinary Tract Infection

Running title: Comparative analysis and synthesis of silver nanoparticles from selected parts of Mimosa pudica to treat urinary tract infection

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Abstract
Mimosa pudica L. is an annual herb that is known for its sensitive nature. This plant is now growing as a weed in mostly all the countries. It has been known to have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anti-asthmatic, analgesic and anti-depressant activities and thus is widely used in herbal medicine. In the present study, the antimicrobial activity of ethanol extract of parts of Mimosa pudica (leaves, stems and roots) was compared with the extract infused with the synthesis of silver nanoparticles against various pathogenic bacteria causing UTI (Urinary Tract Infection) such as Escherichia coli, Pseudomonas aeuroginosa, Enterobacter, Proteus, Staphylococcus saprophyticus, Citrobacter, Klebsiella, and Enterococcus aureus at different concentrations and was analyzed with the crude ethanol extract.. The surface topography and composition of the sample is detected by using SEM (Scanning Electron Microscope), Infrared spectrum of absorption, emission. The stability of colloidal dispersion is analyzed by Zeta potential and for the presence of interfering substance Ultraviolet-visible spectroscopic method is also used. At the end, silver nanoparticles showed a maximum zone of inhibition than normal ethanol extract against UTI pathogens which implied that synthesis of silver nanoparticles from the plant extract may be helpful in the treatment of UTIs.

Keywords: Mimosa pudica, Antibacterial, Urinary Tract infection, Zone of inhibition, SEM, Zeta potential, Ultraviolet-visible spectroscopy.

1. INTRODUCTION

1.1. URINARY TRACT INFECTIONS AND ITS' CAUSATIVE AGENTS

The Urinary tract system in the human body comprises of several organs such as Urethra, bladder, ureters etc. There are several types of bacteria that causes infection in this system and those infections that occurs in various parts of the urinary system are recognized as Urinary Tract Infections (UTI).

These UTIs are more common in women compared to men in their lifetime. There are different types of UTI based on the organ affected by the bacteria which can be differentiated through various symptoms that occurs in

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the body. The bacteria affect the urethra and multiplies in the UTI which is studied here.

This UTI causes various anomalies in women such as menopause, blockage of urinary tract, and abnormalities in birth control. The causative organism E. coli which is present in the large intestine follows the path through anus into the urethra thereby initiating and multiplying the infection. The organism can infest the urinary bladder and can also infect the kidneys if not treated at the proper time. Due to shorter urethras in women, the bacteria get easy access to the bladder making them prone to the UTI.

1.2. MEDICINAL PLANTS

Since time immemorial, various plants have been used as a medicine in healing various ailments. These types of plants having medicinal properties are mostly abundant in India. One such medicinal plant is the Mimosa pudica L which is derived from the word "mimic" meaning sensitivity of leaves and "pudica" meaning to shrink. It belongs to the family Fabaceae and the leaves of this plant are known to fold inwards and swirl when it senses touch and then reopens after some minutes. It acts like the sun drew drosera which is known to be sensitized by insects [1]. It can be recognized by many other local names such as sensitive plant, sleeping grass, touch-me-not, etc [2]. These plants have characteristic pinkish colored flowers with woody looking reddish brown stems. It is mainly rich in tannins, steroids, triterpenes, alkaloids, glycosides such as c-glycoside, flavonoids [3].
The plant has various names in different parts of the world. It is referred to as "Lajjalu" in Ayurveda and "Namaskari" in Sanskrit. In Hindi, this plant is called as chuimui [4] or lajwanti because of its sensitivity to touch. In tamil, it is known as Thottal Sinungi, which translates to "little cry when touched".

Fig.1.1 - Habitat (Elavamalai, Bhavani, Erode District)

Fig.1.2 - *Mimosa pudica*.

Fig.1.3 - *Mimosa pudica* with buds.

Scientific Classification

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Fabales
Family	: Fabaceae
Subfamily	: Mimosoideae
Genus	: Mimosa
Species	: M. pudica

This plant's native habitat is Brazil, South America, and Central America but now it grows tropically worldwide

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as a weed [5] In regions like South Asia, Pacific islands, Tanzania, etc., it is considered as an invasive species except for some countries like Nigeria, Mauritius and parts of East Asia though they got introduced to it [6]. The northern territory declared the plant as a weed [7].

The leaves and roots of Mimosa pudica are known to treat piles and fistula including some conditions such as sore gum and it is also known as a blood purifier [8].

The diseases caused by impure blood, bile, and conditions such as Jaundice, leprosy, fever, ulcers and smallpox can be treated by using this plant as a medicine according to Ayurvedic and Unani system of medicine [9]. In this present study, antimicrobial activity of Mimosa pudica against some microbes responsible for UTIs can be determined.

1.3. NANOPARTICLES

The foremost research in modern sciences are about nanoparticles. These nanoparticles are of one dimension and measures around 100nm or less. Nanoparticles can alter the properties of materials formed from it since the surface area per weight is greater in nanoparticles as compared to other large materials which makes them to be more reactive to other molecules.

Nanoparticles are usually not individual particles though they fit in the size range to that of the fine particles which ranges around 100nm to 2500nm. They usually act as a unit in terms of properties and other characteristics.

It is found out that the artisans of the ninth century used Nanoparticles to create a glitter effect on their pots. Nanoparticles as inhalable vaccines may also be a possibility according to research scientists. In this study, silver nanoparticles are synthesized because of its effectiveness against many bacteria, viruses, and other microbes even at low concentrations and its non-toxicity towards humans.

1.4. ANTIBACTERIAL ACTIVITY OF PLANT EXTRACT AND NANOPARTICLE IN MIMOSA PUDICA

It was researched that a toxic alkaloid called Mimosine [10] is present in Mimosa pudica. The plant extract has some amount of Crocetin dimethyl ester and consists of 17 percent of green yellow fixed oil. The plant also reportedly some amount of tubulin and a phytohormone called turgorins. The leaves extract has been found out to have adrenalin like substance. Tannin of about 10 percent is present in the roots and the mucilage in the seeds has D-xylose and D-glucuronic acid.

It is found out that the movement of the leaves when touched is because of the derivatives of 4-α-(b-D-glucopyranosyl-6-sulphate) acid. The leaf extract was found to contain quinines, terpenoids, phenols, coumarins and saponins using photochemical screening [11].

Nanoparticles can attach themselves onto bacterial membrane by electrostatic interaction and thus causes disturbance in the integrity of bacterial membrane. This is how the Nanoparticles exhibit toxicity to pathogens. This induces the oxidative stress thus making it obtain antimicrobial effect.

2. LITERATURE REVIEW

2.1. NANOTECHNOLOGY USED IN HERBAL MEDICINE

One of the most actively researched and emerging areas in today's world of modern sciences is Nanotechnology. It basically involves the application, production, and manipulation of materials of size ranging from a micron to that of individual atoms. These nanoparticles play a very crucial role in diagnostics, sensing, imaging, artificial implants, tissue engineering, drug delivery and gene delivery, etc [12].

A sustainable and reliable green process of synthesizing nanoparticles is the major research and development ongoing in the field of nanotechnology. Nanomaterials basically are synthesized chemically but biological methods of synthesis using biomaterials are also becoming viable. Since the nanoparticle synthesis involves biological methods using microorganisms, plants, or plant enzymes, these are considered as an eco-friendly alternative to physical and chemical methods of synthesis [13].

These biologically synthesized nanomaterials are widely popular because of their abnormal properties and

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activities and hence are now being used as drug carriers, pesticides, etc. [14]. Nanomaterials synthesized by plants offer various advantages such as compatibility in pharmaceutical and bio-medical fields, cost effectiveness and these can also be scaled up easily in case of large scale synthesis.

The particles of Silver have been proved to provide an inhibitory effect on microbial action in medical processes and conditions. Silver nanoparticles can also be used as a topical ointment on open and burnt wounds to prevent infection and in culinary items [15].

2.2. PLANT EXTRACTS USED FOR URINARY TRACT INFECTION

The secondary metabolite produced by plants acts as a direct precursor or as a lead compound when employed in the pharmaceuticals. If the plant extracts show different target sites to the ones shown by antibiotics, it is expected to be active against the drug resistant microbial activities [16].

The preparation of medicine from several herbal plants and its medicinal properties have been passed on through generation from the ancient Indian Literature and these were scientifically proven to be effective against many such diseases [17].

Six medicinal plants namely Coriander sativum, Syzgium aromaticum, Cinnamomum cassia, Zingiber officinale, Terminalia chebula and Azadirachta indica and their parts were used to evaluate the antibacterial activity against common UTI microbes [18].

Antimicrobial activity of Mimosa pudica was tested with various extracts such as petroleum ether, ethyl acetate, acetone and aqueous against various human pathogenic bacteria such as Escherichia coli, Pseudomonas aeuroginosa, Lactobacillus, Salmonella typhi and Staphylococcus aureus also with plant pathogenic fungus such as Pestalotia foedians, Fusarium oxysporum and Paecilo mycesvariotii at different concentrations [19].

2.3. PLANT BASED NANOPARTICLES

The chemical synthesis of nanoparticles is done by using many toxic solvents, and high energy is involved. Synthesis of microbe mediated nanoparticles is not industrially feasible since it requires high laboratory maintenance. Thus, the green synthesis i.e the plant-based synthesis of nanoparticles is the most efficient and environmentally friendly method. Therefore, the study used Moringa oleifera extract for the nanoparticle synthesis with Silver metal ions because of its useful properties [20].

With each passing day, new uses and benefits of metal nanoparticles are being identified in the nanotechnology field. These metal nanoparticles use noble metals such as platinum, gold, silver, palladium. The AgNPs (silver nanoparticles) are the most used since they have a wide range of advantages [21].
The leaves of Mimosa pudica had shown to exhibit various medicinal properties such as anti-diabetic [22], anti-allergic [23], anti-inflammatory, antibacterial, antioxidant and anticancer activity [24]. However, silver nanoparticles synthesized from Mimosa pudica have been determined to have anti-parasitic effect.

3. MATERIALS AND METHODS

3.1. COLLECTION OF PLANT MATERIALS

The plant Mimosa pudica was found and collected freshly from the village of Elavamalai in Bhavani situated in the Erode District, Tamilnadu, India. The plant was identified at the Botanical survey of India, Coimbatore, Tamilnadu, India (AccessionNo: BSI/SRC/5/23/2015/Tech/906).

The leaves, stems and roots of the plants were washed and rinsed with water and shade dried. The dried parts of the plants were then ground into coarse powders separately and was stored in different airtight containers at room temperature until extraction.

3.2. MICROORGANISMS

The microorganisms which were considered for this study included UTI causing pathogenic bacteria such as Streptococcus saphrophyticus, Escherichia coli, Enterobacter, Proteus, Pseudomonas aeuroginosa, Klebsiella, Citrobacter, Acetobacter and Enterococcus. These organisms were collected from the Microserv laboratory situated in Coimbatore, Tamilnadu, India.

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3.3. PREPARATION OF PLANT EXTRACT

The powdered parts of the plant were first weighed for 12.5g each and packed in filter bags separately. The extracts of various parts of the Mimosa pudica plant were prepared by the method of solvent extraction using ethanol as the solvent using Soxhlet apparatus for 24h.

The extracts thus obtained were concentrated using a rotary evaporator and the solvents were recovered. The yield of the extraction was determined, and the extracts were stored in separate airtight containers until further use.

Fig.3.1 - Process of plant extraction.

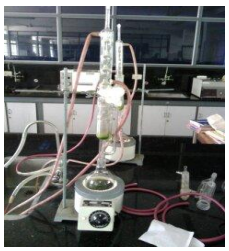

Fig.3.2 - Experimental setup

3.4. PREPARATION OF NANOPARTICLES

The extracts of the selected parts (leaves, stem and root) of the plant were taken 10ml each and mixed with 90 ml of silver nitrate (1mM con.) [25]. This apparatus was stored at room temperature for 10 minutes. After some time, a brown yellow color appeared on all three solutions which indicated that the silver nanoparticles were synthesized from plant extracts with the help of ethanol solution. These solutions were than taken in centrifuge tubes and it was centrifuged at 10,000 rpm for 20 min. The pellets thus obtained were separated and dried. These were collected in separate micro centrifuge tubes and were thus used for further SEM testing and antimicrobial activity [26].

3.4. CHARACTERIZATION OF NANOPARTICLES

3.4.1. MORPHOLOGICAL ANALYSIS BY SEM

The surface topography and the composition of the samples were analyzed using Scanning Electron Microscopy (SEM).

3.4.2. FTIR MEASUREMENT

Infrared spectrum of absorption, emission, photoconductivity of all three samples were obtained by using Fourier Transform Infrared Spectroscopy (FTIR).

3.4.3. ZETA POTENTIAL MEASUREMENT

The stability of the colloidal dispersion of the samples were found out by measuring its Zeta Potential.

3.4.4. UV-SPECTROPHOTOMETRIC ANALYSIS

The presence of any interfering substance was found out using Ultraviolet- visible spectrophotometric analysis.

4. RESULTS AND DISCUSSION

4.1. CHARACTERIZATION OF SILVER NANOPARTICLES

4.1.1. MORPHOLOGICAL ANALYSIS BY SEM:

The particle size analysis was done using SEM at different magnifications and the average size of the leaf, stem and root-based silver nanoparticles was found to be approximately 353.4 nm, 203nm and 323.4 nm respectively.

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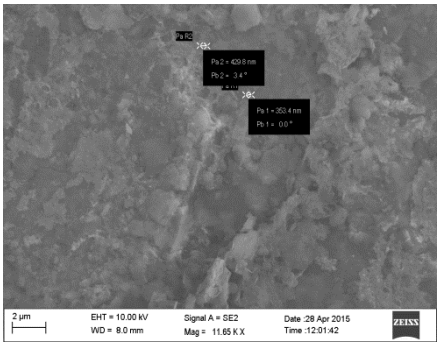

Fig. 4.1 - SEM image of silver nanoparticles from leaf extract

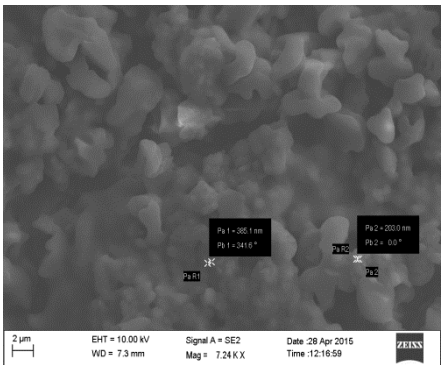

Fig. 4.2 - SEM image of silver nanoparticles from stem extract

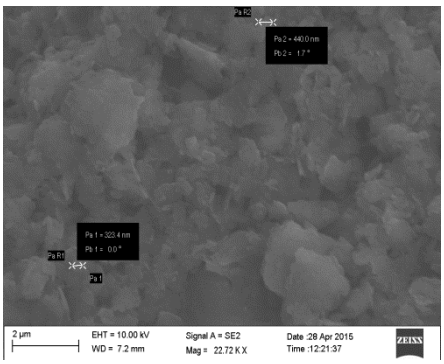

Fig. 4.3 - SEM image of silver nanoparticles from root extract

4.1.2. FTIR ANALYSIS

The lyophilized leaf, stem and root samples were mixed with dry potassium bromide pellet (KBr) and were subjected to a pressure of about 5x106 Pa in an evacuated die and thus obtained a clear transparent disc of diameter 13 mm and thickness 1mm. IR spectra region 4000-400 cm-1 were recorded at temperature on Perkin-Elmer fourier transform spectrometer equipped an air cooled DTGs (deuterated triglycine sulfate) detector.
For each spectrum, 100 scans were done at a spectral resolution of 4cm-1. The frequencies for all sharp bands were accurate to 0.01cm-1.

4.1.3. ZETA POTENTIAL ANALYSIS

The magnitude of the zeta potential corresponds to the potential stability of the colloidal system. An outsized negative or positive zeta potential of all the particles in the suspension implies that they will tend to repel one another and thus the particles will have no tendency to come together and flocculate. The general line between stable and unstable suspensions is typically taken at either +30 or -30 mV.

Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable (Refer Graph.4.1).

Here, the nanoparticles obtained from leaves have a zeta potential of -6mV. The obtained result is nearer to the range 0 to ±5 mV. We conclude from the result that the nanoparticle has the property of flocculating together (Refer Graph 4.2).

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Nanoparticles from roots have a zeta potential of -2.82 mV. This again tells that the particles will flocculate easily in the solution (Refer Graph 4.3).

Nanoparticle from stem has the zeta potential value that lies in this region with -27.5mV and the incipient instability is marked by the range from ±10 to ±30mV.

4.1.4. UV-SPECTROPHOTOMETRIC ANALYSIS

An absorption peak between 380nm-460nm confirms the presence of silver nanoparticles. The similar peak was obtained in all the extracts from Mimosa pudica using ethanol as a solvent (Refer Graph 4.4, Graph 4.5, & Graph 4.6).

4.2. ANTIBACTERIAL ACTIVITY

4.2. ANTIBACTERIAL ACTIVITY OF MIMOSA PUDICA'S LEAF, STEM AND ROOT

The crude form of botanical extracts of Mimosa pudica have been used as medicines. The activity of crude plant extracts is usually attributed to the complex mixture of active compounds.

In the present study, the antibacterial activity of crude extracts from leaf, stem and root of Mimosa pudica was tested against bacteria which are responsible for UTIs such as Streptococcus saphrophyticus, Escherichia coli, Enterobacter, proteus, Klebsiella, Acetobacter, Citrobacter, Enterococcus and Pseudomonas aeuroginosa (Refer Table 4.1 & Table 4.2).

4.2.2. COMPARATIVE ANALYSIS

By comparing the antibacterial activity of both crude plant extract and extract-based nanoparticle, the zone of inhibition was increased up to approximately 0.2-1.0 cm while using extract-based nanoparticle.

This is because the silver nanoparticle naturally has an antibacterial activity and thus when it is integrated into a plant extract, the activity is increased.

In Klebsiella species, the crude extracts didn't show any effect but in the case of extract-based nanoparticles, the inhibition takes place.

From this study, it is concluded that the antibacterial activity may increase by synthesizing silver nanoparticles.

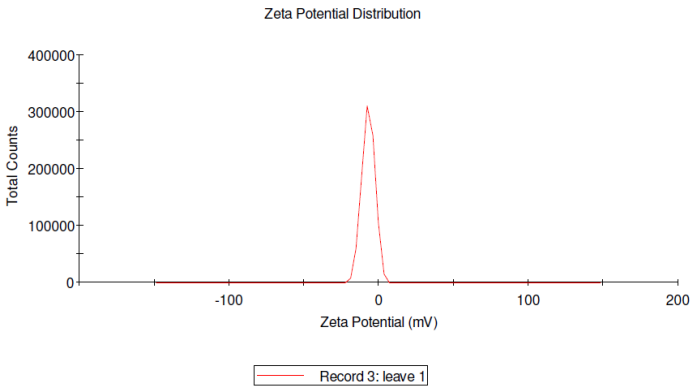
5. CONCLUSION

In conclusion, an attempt has been made to compare the antibacterial activity of crude extracts and extract-based nanoparticles of Mimosa pudica.

The efficacy of antibacterial activity of nanoparticle formulated using plant extracts were found to be higher than that of plant extracts alone.

Nanoparticle based extracts may be a good alternative to other synthetic antibiotics for the control of Urinary Tract Infections.

These results could encourage the search for novel formulation of plant-based nanoparticle for UTIs which offers an alternative to synthetic antibiotic from other plants.



Graph 4.1: Zeta potential analysis of Mimosa leaves

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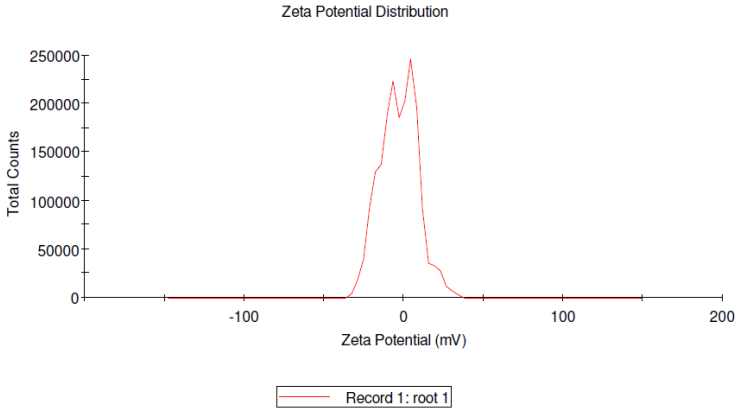
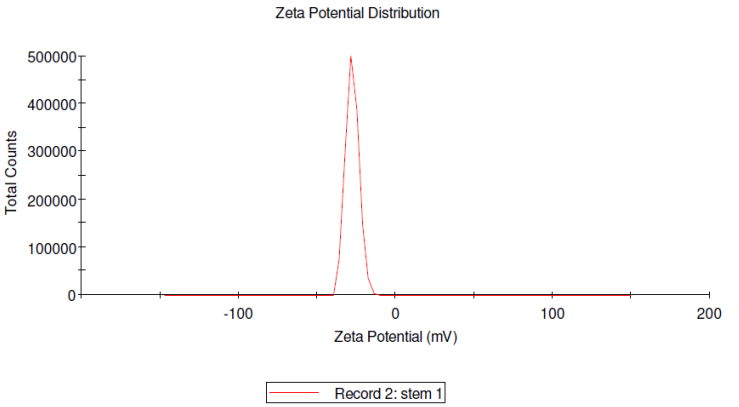
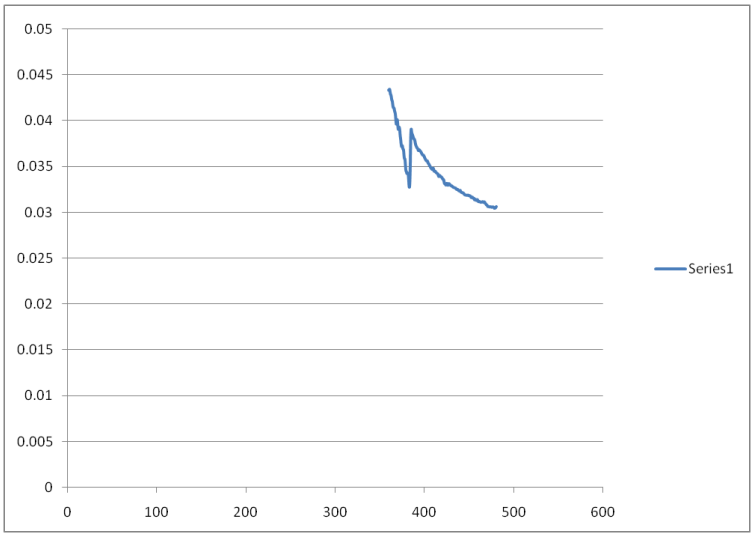


Graph 4.2: Zeta potential analysis of Mimosa Root



Graph 4.3: Zeta potential analysis of Mimosa stem



Graph 4.5: UV- Spectrophotometric analysis of Stem extract

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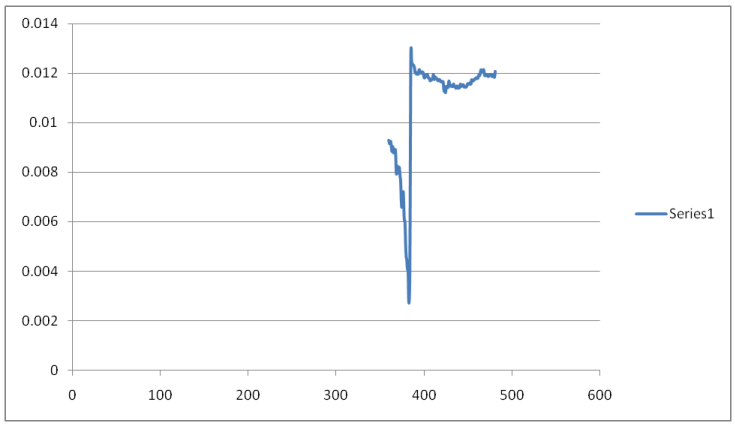

Graph 4.6: UV- Spectrophotometric analysis of Leaf extract

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Table 4.1: Antibacterial activity of crude extract

Bacteria species	Leaves			stem			root			Ethanol (control)			Streptomycin (control)		
	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl
	Obtained Zone of inhibition with respect to the mentioned extract in mm														
Enterobacter	0.5	0.8	1	1	1.5	2.5	0.5	2	2.5	1	1.8	2.5	1.8	2.2	3
Pseudomonas aeuroginosa	0.5	0.8	1	0.8	1	2	0.5	1	2	0.2	0.5	1	1.5	1.8	2
Staphylococcus saprophyticus	1.8	2	2.5	0.5	1	1.2	0.5	0.8	1.3	0.4	0.6	1	1.8	2.5	3
Proteus	0.5	1	1.2	1	2	2.5	0.5	1.1	1.5	2.5	3	3.2	3	4	5
Klebsiella	-	-	-	-	-	-	1	1.5	2.5	0.5	0.8	1	2	2.5	3
Citrobacter	0.5	1.8	2.5	0.5	1	1.5	-	1	1.5	0.5	0.5	1	2	3	4
E.coli	0.8	1	1.2	0.5	1	1.2	1	2	2	1.8	2	2.5	2	3	4
Enterococcus	1	1.5	2	1	1.5	2	0.5	1.2	2	2	2.5	3	1.5	2	2.5

Table 4.2: Antibacterial activity of extract-based nanoparticle

Bacteria species	Leaves			stem			root			Ethanol (control)			Streptomycin (control)		
	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl
	Obtained Zone of inhibition with respect to the mentioned extract in mm														
Enterobacter	0.8	1	1.2	1.3	1.8	2.5	0.7	1.2	1.8	1	1.8	2.5	1.8	2.2	3
Pseudomonas aeuroginosa	0.7	1.1	1.3	1	1.4	2.1	0.8	1.2	2.1	0.2	0.5	1	1.5	1.8	2
Proteus	0.8	1	1.2	1.2	2.3	2.6	0.7	1.2	1.6	2.5	3	3.2	3	4	5
Staphylococus saprophyticus	2.1	2.3	2.5	0.7	1.2	1.5	0.6	1	1.4	0.4	0.6	1	1.8	2.5	3

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Klebsiella	0.5	0.8	1.2	0.6	1	1.1	1.2	1.8	2.4	0.5	0.8	1	2	2.5	3
Citrobacter	1.3	2	2.2	0.8	1.1	1.6	1.2	1.6	2	0.5	0.5	1	2	3	4
E.coli	1	1.2	1.5	0.6	1	1.3	1.4	2.2	2.4	1.8	2	2.5	2	3	4
Enterococcus	1.2	1.7	2.1	1.2	1.6	2	0.8	1.3	2.1	2	2.5	3	1.5	2	2.5