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. (Mimosaceae) is the one of the herbs that shows the activity of sensation while touch and it grow as a weed in all the country. In major it has an antibacterial, antifungal, antioxidant, anti-inflammatory, anti-asthmatic, analgesic and anti-depressant activities. In the present study, antimicrobial activity of ethanol extract of *Mimosa pudica* compared with the extract infused with the synthesis of silver nano particles against various pathogenic bacteria causes UTI (Urinary Tract Infection) such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter*, *Proteus*, *Staphylococcus saprophyticus*, *Klebsiella*, and *Enterococcus aureus* at different concentrations analyzed with the crude ethanol extract. At the end Silver nano particles showed a maximum zone of inhibition than normal ethanol extract against UTI pathogens. The surface topography and composition of the sample is detected by using SEM (Scanning Electron Microscope), Infrared spectrum of absorption, emission and the stability of colloidal dispersion is analyzed by Zeta potential and to for the presence of interfering substance Ultraviolet-visible spectroscopic method is also used.

1. Introduction

1.1 Urinary Tract infections and its agents:

Several different parts such as Urethra, bladder, ureters etc., together comprise the Urinary system. Infection in any part of the Urinary system is considered as Urinary tract infections (UTIs) and it is mainly caused by the several different types of bacteria.

Compared to men, women are having the higher possibility rate to have UTI in their lifetime. The signs and symptoms of UTI may vary based on the particular part of the organ where the microorganism affected. This UTI is commonly occurs through the urethra where the bacteria enters and starts multiplies.

There are number are risk factors are related to this infection especially in women such as urinary tract blockage, menopause, birth control abnormalities. The bacteria *E.coli* from the humans large intestine enters in to urethra from the anus which initiates the infection by multiplying the numbers from there, the microorganisms can travel up to the bladder, and if the infection isn't treated, continue on to infect the kidneys. Women maybe especially susceptible to UTIs because they need shorter urethras, which permit bacteria quick access to the bladder

1.2 Medicinal Plants:

Plants have been an exemplary source of medicine for thousands of years. India is that the largest producer of medicinal plants and is rightly called the "Botanical garden of the world".

Mimosa pudica L. belongs to the family Mimosaceae. *Mimosa pudica* may be a creeping annual or perennial herb often grown for its curiosity value, because the compound leaves fold inward and droop when touches and reopen within a minutes. *Mimosa pudica* is native to Brazil, but is now a pan tropical weed [1]. It is derived from the word "mimic" means to sensitivity of leaves and "pudica" means bashful, retiring or shrinking. *Mimosa* mimics the animal sensitivity that is sensitivity to light; time of day, gravity or like sun drew *drosera* which reacts to the contact of insects [2]. So *Mimosa* is understood as sensitive plant, sensitive plant, sensitive plant, sleeping grass, touch me not, *Lajjalu* in Ayurveda and *Namaskari* in Sanskrit [3]. It has reddish brown woody stems and pinkish flowers. It mainly contains tannins, steroids, triterpenes, alkaloids, glycosides, flavonoids, c-glycoside [4]. This plant leaves and roots are used in the treatment of piles and fistula. This plant is also used in the treatment of sore gum and is used as blood purifier [5].

In Ayurvedic and Unani system of medicine, this plant has been used in disease arising from corrupted blood, bile, fever, piles, jaundice, leprosy, ulcers, and small pox.[6]. In this present study, antimicrobial activity of *Mimosa pudica* against some microbes responsible for UTIs can be determined. *Mimosa pudica* is additionally referred to as chuimui [7] or lajwanti in hindi due to its unique property to droop or collapse when touched and exposes a couple of minutes later. Its other names are Betguen Sosa (Guam), Memege (Niue), Mechiuaiu (Palau), Limemeihr (Pohnpei), Ra Kau Pikikaa (Cook Islands).

The Chinese name for this plant translates to "shyness grass". [8] Its Sinhala name is Nidikumba, where 'nidi' means 'sleep'. Its Tamil name is Thottal Sinungi, where 'Thottal' means 'touched' and 'Sinungi' means 'little cry'. Other common names include Makahiya (Philippines, with maka- meaning "quite" or "tendency to be", and -hiya meaning "shy", or "shyness"), Mori Vivi (West Indies).

In Urdu it is known as Chui-Mui. In Bengali, this is often referred to as 'Lojjaboti', the shy virgin. In Indonesia, it's referred to as Putri Malu (Shy Princess). In Myanmar (Burma) it's called 'Hti Ka Yoan' which suggests "crumbles when touched".

It has been described as “sparshaat sankochataam yaati punashcha prasruta bhavet” -a plant which folds itself when touched and spreads its leaves once more after a short time.

Scientific Classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Subfamily : Mimosoideae

Genus : *Mimosa*

Species : *M.pudica*

Mimosa pudica is native to South America and Central America. It is considered an invasive species in Tanzania, South Asia, South East Asia and lots of Pacific Islands. [8] It is a declared weed in the Northern Territory. [9] Control is recommended in Queensland. [10]

It has also been introduced to Nigeria, Seychelles, Mauritius and East Asia but is not regarded as invasive in those places [8].

1.3 Nano-particles

In general nanoparticles are considering a discovery of modern science. Nanoparticles were employed by artisans as far back because the ninth century for generating glittering effect on the surface of pots.

Nanoparticles have one dimension that measures 100 nanometers or less. The properties of the many conventional materials changed when formed from nanoparticles. This is typically because nanoparticles have greater surface area as per weight than larger particles which causes them to be more reactive to some other molecules.

A nanoparticle may be a small object that behaves as an entire unit in terms of its transport and properties. In terms of diameter, fine particles covers a range between 100 and 2500 nanometers. Nanoparticles may or might not exhibit size related properties that differs significantly from those observed in fine particle or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually not mentioned as nanoparticles.

The surface change of protein filled nanoparticles has been shown to affect the ability of the nanoparticle to stimulate immune response. Researchers are thinking that these nanoparticles could also be utilized in inhalable vaccines.

Nanoparticle research is currently the foremost studied branch of science with the quantity of uses of nanoparticles in various fields. The particles have wide variety of applications in biomedical, optical and electronic fields.

In this study silver nanoparticles are synthesized because it has various and important applications. Silver has been known to possess a disinfectant effect and has been found in applications starting from traditional medicines to culinary items. It has been reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, viruses and other eukaryotic micro-organisms at low concentrations without side effects.

1.4 Antibacterial activity of plant extract and Nanoparticle in *Mimosa pudica*:

M. pudica contains Mimosine [10], which is a toxic alkaloid. Adrenalin like substance has been identified within the extract of its leaves. Some workers have reported the presence of Crocetin dimethyl Ester within the extract of the plant. Roots contain tannin up to 10 per cent. Seeds contain a mucilage which consists of D-xylose and d-glucuronic acid.

The plant extract contains green yellow fixed oil up to 17 per cent. The plant is reported to contain tubuline and a replacement class phytohormone turgorines is found to move within the plant.

The periodic leaf movement factors are reportedly the derivatives of 4- α -(b-D-glucopyranosyl-6-sulphate) acid. The preliminary photochemical screening of the *M.pudica* leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins. [11]

Ayurveda has declared that its root is bitter, acrid, cooling, vulnerary, alexipharmic, and utilized in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, and fatigue and blood diseases.

Nanoparticles are toxic to pathogens, the mechanism is nanoparticles are able to attach to the membrane of the bacteria by electrostatic interaction and disturbs the integrity of bacterial membrane. Nano-toxicity is generally triggered by the induction of oxidative stress, through this the nanoparticle obtain antibacterial effect.

2. LITERATURE REVIEW

2.1 NANOTECHNOLOGY USED IN HERBAL MEDICINE

Nanotechnology is one of the most active emerging research areas in modern sciences. Nanotechnology is that the production, manipulation and application of materials with size starting from but a micron thereto of individual atoms.

Nanomaterials are synthesized chemically but now it is possible by using biological materials. Nanoparticles of biological origin are of great interest due to their unusual properties and activities. Bio-based nanoparticles can be used as novel pesticides, drug carriers, etc [12]

The plant based nanoparticles have following advantages: Offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other bio-medical applications.

Cost effective, environment friendly, easily scaled up for giant scale synthesis. Silver has been recognized as having inhibitory effect on microbes present in medical and process. Silver nanoparticle can be used as a topical ointment to prevent infection against burn and open wounds.

The development of reliable green process for the synthesis of nanoparticles is a crucial aspect of current nanotechnology research. Nanoparticle plays an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering [13].

Biological methods for nanoparticle synthesis using microorganism, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods [14].

2.2 PLANT EXTRACTS USED FOR URINARY TRACT INFECTION:

Plant produce a good sort of secondary metabolite which is employed either directly as precursors or as lead compounds within the pharmaceutical industry and it's expected that extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial activities.

The medicinal properties of several herbal plants and their preparation have been documented in ancient Indian literature and found to be effective in treatment of numerous diseases [15].

Six plants (Coriander sativum, Syzgium aromaticum, cassia, common ginger, Terminalia chebula and Azadirachta indica) and their parts were wont to evaluate the antibacterial activity [16].

The antibacterial activity of aqueous, ethanol, ethyl acetate, methanol, Petroleum ether, Chloroform, Extracts of Biophythm sensitivum or mukkutty (whole plant was used), Nutmeg (nut of jathikai was used), Aerva Lanata or cheroola (whole plant was used) and Boerrhavia Difffusa or thazhuthama (leaves were used) was determined against 5 UTI isolates. i.e. Escherechia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, streptococci viridians, by disc diffusion method.

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 [17].

2.3 PLANT BASED NANOPARTICLES:

Synthesis of nanoparticles is straight forward, efficient, and eco-friendly as compared to chemical-mediated or microbe- mediated synthesis. The chemical synthesis involves toxic solvents, high, energy and heat conversion and

microbe involved synthesis isn't feasible industrially thanks to its lab maintenance. Since, green synthesis is that the best choice to choose the synthesis of nanoparticles, therefore the nanoparticles was synthesized by using aqueous extract of *Moringa oleifera* and metal ions (such as silver). Silver was of particular interest due to its distinctive physical and chemical properties [18].

Researchers within the field of nanotechnology are finding that metal nanoparticles have all types of previously unexpected benefits. They are usually prepared from noble metals, that is, silver, gold, platinum and palladium while silver nanoparticles (AgNPs) being most exploited [19], because of its wider range of applications.

Though biological method is usually adopted for the synthesis of silver nanoparticles, use of plant extracts is widely studied thanks to its advantages over others. Among different plants, the leaves of *Mimosa pudica* had shown to exhibit various medicinal properties such as anti-diabetic [20], anti- allergic [21], anti-inflammatory, antibacterial, antioxidant and anticancer activity [22]. However, silver nanoparticles synthesized from *M. pudica* have been assayed for anti-parasitic effect.

3. Materials And Methods

3.1 COLLECTION OF PLANT MATERIALS

Mimosa pudica plant was collected freshly from the Elavamalai village in Bhavani of 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 plant were washed with water then rinsed with water. They were shade dried, ground into coarse powder and stored in airtight container and stored in room temperature until used for extraction.

3.2 MICROORGANISMS

The microorganisms used for the study includes human pathogenic bacteria such as *Streptococcus Saphrophyticus*, *Escherichia coli*, *Enterobacter*, *proteus*, *Klebsiella*, *Acetobacter*, *Enterococcus*, *Pseudomonas aeruginosa* are collected from Microserv laboratory in Coimbatore.

3.3 PREPARATION OF PLANT EXTRACT

The coarsely grounded powdered various parts of plants were separately extracted in a Soxhlet apparatus with ethanol. For preparation of various parts of plant extract, 12.5g of powdered plant parts was weighed and packed in the filter bag.

It was extracted with ethanol separately for 24 h using Soxhlet apparatus. The obtained extracts were evaporated using Rotary Evaporator and the solvents were recovered. The yield was determined and stored in an airtight container until used for further studies

3.4 PREPARATION OF NANOPARTICLES

Take 10 ml of the selective parts of plant extracts was (leaves, stem and root) mixed with 90 ml of silver nitrate (1mM con.) [23] and it is Stored at room temperature for 10 mins, then after sometimes brown yellow color appeared. This indicates that the silver nanoparticles were synthesized from plant extracts with the help of ethanol solution. Then this solution was taken in centrifuge tube and it was centrifuged at 10,000 rpm for 20 min. The pellets

were taken after centrifugation and dried, dried pellets were collected in a micro centrifuge tube and pellets were used for testing SEM and antimicrobial activity. [24]

3.4 CHARACTERIZATION OF NANOPARTICLES

3.4.1 MORPHOLOGICAL ANALYSIS BY SEM

The sample's surface topography and composition was detected by using SEM (Scanning Electron Microscope).

3.4.2 FTIR MEASUREMENT

Infrared spectrum of absorption, emission, photoconductivity of sample was obtained by using FTIR (Fourier Transform Infrared Spectroscopy)

3.4.3 ZETA POTENTIAL MEASUREMENT

Stability of colloidal dispersion was found out by using Zeta potential.

3.4.4 UV-SPECTROPHOTOMETRIC ANALYSIS

To find out the presence of interfering substance Ultraviolet- visible spectroscopic analysis is also used.

4. Results And Discussion

4.1 CHARACTERIZATION OF SILVER NANOPARTICLES

4.1.1 MORPHOLOGICAL ANALYSIS BY SEM

Particle size was analyzed using SEM at different magnification 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.

4.1.2 FTIR ANALYSIS

The lyophilized leaf, stem and root samples were mixed with dry potassium bromide pellet (KBr) and subjected to a pressure of about 5×10^6 Pa in an evacuated die to produce a clear transparent disc of diameter 13 mm and thickness 1mm. IR spectra region $4000 - 400 \text{ cm}^{-1}$ were recorded at temperature on a 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 4 cm^{-1} . The frequencies for all sharp bands were accurate to 0.01 cm^{-1} .

4.1.3 ZETA POTENTIAL ANALYSIS

The magnitude of the zeta potential gives a sign of the potential stability of the colloidal system. If all the particles in suspension have an outsized negative or positive zeta potential then they're going to tend to repel one another and there'll be no tendency for the particles 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, for our 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)

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)

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

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 shows the peak obtained between 380–460 nm, it confirms the presence of silver nanoparticles.

4.2 ANTIBACTERIAL ACTIVITY

4.2.1 Antibacterial activity of *Mimosa pudica* leaf, stem and root

The botanical extracts from the plants *mimosa pudica* in their crude form 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 is responsible for urinary tract infection such as *Streptococcus Saphrophyticus*, *Escherichia coli*, *Enterobacter*, *proteus*, *Klebsiella*, *Acetobacter*, *Enterococcus* and *Pseudomonas aeruginosa* (Refer Table 4.1 & 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 upto approximately 0.2-1.0 cm when using extract based nanoparticle.

This is because the silver nanoparticle naturally have an antibacterial activity when it is integrated into an plant extract the activity gets increased.

In *klebsiella* species the crude extracts doesn't shows 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 antibiotic for the control of Urinary Tract Infections.

These results could encourage the search for novel formulation of plant based nanoparticle for UTI offering an alternative to synthetic antibiotic from other plants.

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Tables

Table 4.1
Antibacterial activity of crude extract

Bacteria species	Leaves			stem			root			Ethanol (control)			Streptomycin (control)		
	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l
	Obtained Zone of inhibition with respect to the mentioned extract in mm														
<i>Enterobacter</i>	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
<i>Pseudomonas aeruginosa</i>	0.5	0.8	1	0.8	1	2	0.5	1	2	0.2	0.5	1	1.5	1.8	2
<i>Staphylococcus saprophyticus</i>	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
<i>Proteus</i>	0.5	1	1.2	1	2	2.5	0.5	1.1	1.5	2.5	3	3.2	3	4	5
<i>Klebsiella</i>	-	-	-	-	-	-	1	1.5	2.5	0.5	0.8	1	2	2.5	3
<i>Citrobacter</i>	0.5	1.8	2.5	0.5	1	1.5	-	1	1.5	0.5	0.5	1	2	3	4
<i>E.coli</i>	0.8	1	1.2	0.5	1	1.2	1	2	2	1.8	2	2.5	2	3	4
<i>Enterococcus</i>	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 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l	20 μ l	40 μ l	60 μ l
Obtained Zone of inhibition with respect to the mentioned extract in mm															
<i>Enterobacter</i>	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
<i>Pseudomonas aeruginosa</i>	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
<i>Proteus</i>	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
<i>Staphylococcus saprophyticus</i>	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
<i>Klebsiella</i>	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
<i>Citrobacter</i>	1.3	2	2.2	0.8	1.1	1.6	1.2	1.6	2	0.5	0.5	1	2	3	4
<i>E.coli</i>	1	1.2	1.5	0.6	1	1.3	1.4	2.2	2.4	1.8	2	2.5	2	3	4
<i>Enterococcus</i>	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

Figures

Figure 1

Habitat (Elavamalai, Bhavani, Erode District)

Figure 2

Mimosa pudica.

Figure 3

Mimosa pudica with buds.

Figure 4

Process of plant extraction.

Figure 5

Experimental setup

Figure 6

SEM image of silver nanoparticles from leaf extract

Figure 7

SEM image of silver nanoparticles from stem extract

Figure 8

SEM image of silver nanoparticles from root extract

Figure 9

Zeta potential analysis of Mimosa leaves

Figure 10

Figure 11

Zeta potential analysis of Mimosa stem

Figure 12

UV- Spectrophotometric analysis of Root extract

Figure 13

UV- Spectrophotometric analysis of Stem extract

Figure 14

UV- Spectrophotometric analysis of Leaf extract