Green Synthesis Of Silver Nanoparticles From Madhuca Longifolia Towards The Control Of Human Pathogens

[1]M.Ramesh , [2]Dr. C. Dhanalakshmi
[1,2] Prist university,Puducherry campus

***Abstract*:** Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds. Many of these indigenous medicinal plant are used as species and plant. In the present study, silver nano particles were synthesized from *Madhuca longifolia* . It's phytochemical and antibacterial effectswere investigated.

Index Terms : **Alkalnoids, Tannis, Flavonoids , Phenolic compounds and *Madhuca longifolia***

Introduction

Nanoparticles are little groups of molecules around 1 to 100 nanometers in length. 'Nano' gets from the Greek word "nanos", which implies predominate or to a great degree little. It can be used as a prefix for any unit like a second or a liter to mean a billionth of that unit. A nanosecond is a billionth of a second. A nanoliter is a billionth of a liter. And therefore a nanometer is a billionth of a meter or 10^{-9} m. A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100nm, due to a wide variety of potential applications in biomedical, optical and electronic fields.

2. Materials and Methods Used

2.1 Accumulation Of Plant Material And Preparation Of Extracts

Matured leaves of *Madhuca longifolia* werecollected from the local field of Aalavanthan Nallur, Kuzhumani road, Trichy, India. The leaves were shade dried, ground into a coarse powder. Powder was first defatted with Acetone and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract. Aqueous extract were obtained by maceration for 24 hrs **(Song and Kim 2009)**

A. Authentication of plants

The leaves of *Madhuca longifolia* was identified and authenticated by The Director, Department of Rapinant Herbarium and Center for Molecular Systematic, St. Joseph's College, Trichy-2

B. Extraction Procedure

Extraction was carried out using the above mentioned leaves of *Madhuca longifolia*.

C. Aqueous Extraction

100g of fine powder of leaves of *Madhuca longifolia* was dissolved in 500ml of distilled water with intermittent shaking for 4 days.Then the extracts was filtered into solid form at 55° C using hot air oven. The extracts were weighed, and stored in a sterile container for further use.

D. Alcohol Extraction

100g of coarse powder of leaves of *Madhuca longifolia* was taken and dissolved in 300ml of alcohol. It is allowed to stand for 3 days at 37°C under occasional shaking. Then the extracts were filtered through three layered muslin cloth and condensed in to solid form at 40o C using hot air oven. The extracts were weighed to find out the extract value, and stored in a sterile container for further use.

3. PHYTOCHEMICAL TEST (Paulkumar*Et Al* ., 2014)

A. Alkaloids (Paulkumar*Et Al* ., 2014)

To 2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

B. Flavonoids(Bar *Et Al.*, 2009)

To 5 ml of methanolic remove, 1 ml of 10% NaOH arrangement was included. From the side of the measuring glass 2 drops of concentrated HCl was included. Yellow shading swinging to dry means that nearness of flavonoids.

C. Glycosides (Bar *Et Al.*, 2009)

To 25ml of dilute sulphuric acid was added 5ml extract and boiled for 15 minutes. It was then cooled and neutralized with 10%NaOH with 5ml of Fehling solution was then added. Brick red precipitate indicated the presence of glycosides.

D. Preparation Of Silver Nitrate Solution (Narayanan *Et Al.*, 2008)

Commercially purchased silver nitrate (molecular weight-169.87) was used to prepare 1mM concentrations. Appropriate amount of silver nitrate was weighed and dissolved in distilled water.

E. Preparation Of Silver Nanoparticles (Sathishkumar *Et Al.*, 2009)

To 750 ml of each millimolar grouping of silver nitrate. The conelike jars were then presented to the daylight (while being persistently shaken) for the amalgamation of the nanoparticles to start. The colors of the mixture turns fromen to brown when exposed to sunlight and once it turns to colorless the particles were settled at the bottom of the flasks. The particles were then centrifuged (high speed centrifuge) and the supernatant was removed. To the particles now settled at the bottom of the centrifuge tubes, about 1ml acetone was added for the removal of the moisture content from the nanoparticles. The nanoparticle suspension were transferred to a watch glass, air dried, collected, weighed and stored in a sterile container.

F. Uv-Vis Spectra Analysis (Sathishkumar *Et Al.*, 2009)

The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and 700 nm ranges.

G. Antibacterial Assay

Sterile Petri plates containing 20 ml of Nutrient agar or Muller Hinton agar were seeded with 0.01ml of 18 hours old test bacterial culture with calibrated loop (Hi-media) and lawned evenly using sterile cotton swabs. Appropriate quantity of different extracts were dissolved in Dimethyl sulfoxide (DMSO) and sterilized by using syringe filter. 200µg, 400µg, 600µg, and 800µg/ well concentration of plant samples, positive controls and negative controls were added into the 6mm diameter well. Incubation was made at 37º C for 24 hours. The assessment of antibacterial activity was based on the measurement of diameter of the inhibition zone formed around the well, using Himedia scale. Streptomycin sulphates 100µg were used as a positive control. DMSO was used as a negative control. (Perez *et al* .,1990).

4. Results and Discussion

4.1 Confirmation And Of Phyto Silver Nanoparticles (Snps) Synthesis

I) Visual Observation

In the present study, SNPs was synthesized using *Madhuca longifolia* leaves extract and yellowish brown colour was developed by addition of Silver Nitrate. The time duration for colour change and its thickness varies from plant to plant. The time taken for the reaction mixture to change colour was shown and the pH was changed from 4.0 to 4.60. (Table: 3; Plate:2). The reason for changing color may due to quantitative variation in the formation of SNPs or may be due to the availability of H^+ ions to reduce the silver. Sometimes, SNPs exhibit yellowish brown colour in aqueous solution as there is surface Plasmon vibrations in silver nanoparticles. Silver nitrate with its reducing property act as good conductivity, catalytic and chemical stability. The aqueous silver ions when mixed with herbal extracts can reduced inside solution and forms silver hydrosol. The reduction of silver ions into silver particles during its contact with plant extract was followed by colour change from colourless or pale yellow to brown **(Putheti *et al.*, 2008).**

ii) UV- VIS Spectroscopy

The reduction of silver metal ions to silver nanoparticles was preliminarily analysed using UV-Vis Spectrophotometer between 300-700nm (Table 2 and fig 1). This analysis showed an absorbance peak at 420 nm which was specific for Ag

nanoparticles. UV– obvious spectroscopy is a critical procedure to decide the arrangement and strength of metal. Nanoparticle in watery arrangement. The response blend changes the shading by including different centralizations of metal particles. These colour changes arise because of the excitation of surface Plasmon vibrations in the silver Nanoparticle **(Kelly *et al* ., 2003)**. It shows yellowish to dark brown in colour. The dim dark colored shade of silver colloid is acknowledged to surface Plasmon reverberation (SPR) emerging because of the gathering of free conduction electrons initiated by an interfacing electromagnetic field.

iii) SEM

The SEM image showing the high intensity of silver nanoparticles synthesized by *Madhuca longifolia* extract further confirmed the development of silver nanostructures. Plate: 4. shows the SEM image of SNPs. It has further provided further insight into the morphology and size details of the silver nanoparticles. SEM investigation demonstrated the molecule size of around 10 µm also the precious stone structure of the nanoparticles. The silver nanoparticles incorporated through green course are profoundly lethal to multidrug safe microscopic organisms thus has an extraordinary potential in Biomedical applications. The present study showed a simple, rapid, economical route to synthesized silver nanoparticles. Application of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications makes this method potentially exciting for the large scale synthesis of other inorganic materials (nano-materials).

Our results are supported by the following findings. Todaynonmaterials are at the primary stage of fast developing nanotechnology phase. Nanomaterials are facilitating modern technology to deal with nano-sized objects, their unique properties especially size-dependent one makes them superior materials and essential in human activities. At present, nanomaterials are as of now being utilized in medicinal applications, for example, tranquilize bearers, solid antibacterial, identification for pathogens/proteins and tissue designing and so on. There is some new improvement towards controlling the properties of nanomaterials, e.g. new strategy has been accounted for in which attractive nanoparticles are headed to the tumor for medication discharge or simply warming with a specific end goal to wreck the encompassing tissues. In wound recuperating administration, another helpful reaction has been produced in which tranquilize is discharged as needs be to the kind of wound, open or shut, extensive or little and medication is discharged at specificrates. In tissue designing, a fleece keratin/Ha nano-composite has been accounted for in which cells indicated enhanced plausibility proportion of organics and inorganics comparative with those of normal bones.For antibacterial properties different applications has been reported such as silver nanoparticles loaded surgical masks and surface moiled cotton by nano titanium dioxide with great antibacterial properties etc .(**Santhoshkumar *et al2* .011).**

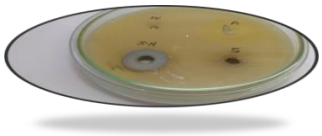
iv) Antibacterial Activity

Antibacterial activity of ethanol extractof *Madhuca longifolia* leaves was tested against human pathogens *Viz. Strptococcus pneumonia, Enterobacter sp* and *Proteus sp* . *Streptococcus pneumoniae* was highly sensitive to Ethanol extract,the zone of inhibition was 19mm followed by *Enterobacter sp* (17mm), *Proteus sp* (15mm). It is more or less related to positive control Chloromphenical which exerted the zone of inhibition in the range of 21 mm to 25mm. Table 5 ; Plate: 5-10 shows the antibacterial activity of *Madhuca longifolia* leaf extracton different pathogens .Plate :11-13 shows the antibacterial activity of SNPs of *Madhuca longifolia* leaf extracton different pathogens .With the current investigation, it is lime lighted that, when compared to water extract alcohol extract of *Madhuca longifolia* was effective in controlling *Sterptococcus pneumoniae, Enterobacter sp* and *Proteus sp.*.In addition, if the extracts are converted into SNPs by green synthesis the penetration of SNPs might be high inside the pathogens; hence their antibacterial activity was observed in quiet high range. Moreover the phytochemicals of *Madhuca longifolia* was also played a major role after the SNPs penetration inside the cells . On further analysis, from this *Madhuca longifolia* we could find a safe, natural antibacterial agent to combate the diseases caused by*Sterptococcus pneumoniae, Enterobacter sp* and *Proteus sp.*

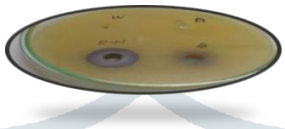
Table-1: Preliminary Phytochemical Screening Of *Madhuca Longifolia*

S.NO	TEST	AQUEOUS	ETHANOL
1	Alkaloids	+	+
2	Anthaquinone	-	-
3	Coumarin	+	+
4	Flavonoids	-	+
5	Glycosides	-	+
6	Phenols	+	+
7	Saponin	+	+
8	Steroids	-	+
9	Tannins	+	+
10	Terpenoids	-	-

Antibacterial Activity Of Silver Nano Particles



Proteus.sp



Enterobacter sp

S.pneumoniae

Fig 5.2 Antibacterial activity of silver nano particles

Phytochemical analysis of *Madhuca longifolia*

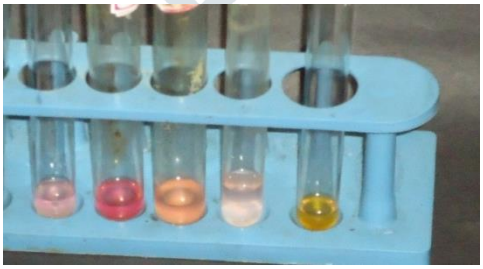


Fig 5.3 Phytochemical Analysis of water and alcohol extract of *Madhuca longifolia* leaves

Table 5. 2 UV-Vis Analysis Of *Madhuca Longifolia*

S.no	Wave Length	Absorbancence
1	276.15	1.7600
2	570.20	0.2963
3	735.40	0.2896
4	756.55	0.2901
5	901.15	0.2892

Table 5.3: Indication Of Color Change In The Synthesis Of Silver Nano Particle (Snps)

S.No	Plantleafextract+AgNo3	Color change		pH change		Color intensity	Time	Result
	Scientific name	Before	After	Before	After			
1	*Madhuca longifolia*	Light yellow	Brown	4.0	4.60	+++	20 min	Positive

Table`5.4 Ft-Ir Analysis Of *Madhuca Longifolia*

S.NO	FREQUENCY RANGE	WAVE LENGTH RANGE	TYPE AND GROUP
1	3907.14	3900-3950	Oximes
2	3464.79	3500-3200	alcohols, phenols
3	2967.03	3300–2500	carboxylic acids
4	2728.77	2830-2695	Aldehydes
5	2196.56	2260-2100	Alkynes
6	1673.79	1760-1665	carbonyls (general)
7	1365.86	1370-1350	Alkenes
8	1155.21	1250-1020	aliphatic amines
9	840.24	850-550	alkyl halides
10	597.80	690-515	alkyl halides

Table 5.5: Antibacterial Activity Of Ethanol Extracts Of *Madhuca Longifolia Leaves*against Pathogens

S.No	Organisms	Concentration of extract in µl/ zone of inhibition in mm					
		200 µg	400 µg	600 µg	800 µg	Positive control	Negative control
1	*Proteus.sp*	11.0	12.3	12.7	15.0	21.0	-
2	*Enterobacter sp*	10.0	10.3	10.6	17.0	23.0	-
3	*S.pneumoniae*	10.6	11.0	11. 7	19.0	25.0	-

Table 5.6: Antibacterial Activity Of Aqueous Extracts Of *Madhuca Longifolia Leaves*against Pathogens

S.No	Oraganisms	Concentration of extract in µl/ zone of inhibition in mm					
		200 µg	400 µg	600 µg	800 µg	Positive control	Negative control
1	*Proteus.sp*	11.0	11.3	11.7	12.0	21.0	-
2	*Enterobacter sp*	13.0	13.5	14.0	**14.4**	23.0	-
3	*S.pneumoniae*	10.9	11.7	12. 7	13.0	25.0	-

Table 5.7: Antibacterial Activity Of Silver Nano Particles (Agnps) Of *Madhuca Longifolia Leaves*against Pathogens

S.No	Oraganisms	Concentration of extract in µl/ zone of inhibition in mm					
		200 µg	400 µg	600 µg	800 µg	Positive control	Negative control
1	*Proteus.sp*	11.7	12.3	12.7	**15.6**	21.0	-
2	*Enterobacter sp*	13.3	13.9	14.4	**18.3**	23.0	-
3	*S.pneumoniae*	11.4	12.3	13.2	**21.8**	25.0	-

5 .CONCLUSION

In conclusion, this green chemistry approach toward the synthesis of AgNPs or SNPs possesses several advantages *viz*, easy process by which this may be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing, other medical and electronic applications makes this method potentially stimulating for the large-scale synthesis of nanomaterials. The present examination incorporated the bio-decrease of silver particles through therapeutic plants concentrates and testing for their antimicrobial movement.On further analysis, from this *Madhuca longifolia* , we could find a safe, natural antibacterial agent to combat the diseases caused by *Sterptococcus pneumoniae, Enterobacter sp* and *Proteus sp.* In addition to that, with the assistance of biotechnology, clinical research and bioinformatics, we would definitely find an alternate safe drug candidate from this *Madhuca longifolia* unripe fruit.

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