

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MORUS ALBA EXTRACT AND THEIR ANTIBACTERIAL PROPERTIES

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Abstract

Background: Metal nanoparticles can be used to control a range of resistant and pathogenic microorganisms. Nanoparticles are used in many different fields, including medicine, diagnostics, drug and gene delivery, electronics, cosmetics, coatings, biosensors, imaging, and environmental remediation. Of the many metal nanoparticles, silver nanoparticles have been the focus of much investigation because of their distinct optical properties, conductivity, chemical stability, and catalytic activity. This method allowed the synthesis of silver nanoparticles in an environmentally friendly way, which was confirmed by (UV-Vis) spectrophotometry and FTIR. Morus alba leaf extract was utilized as the reducing, stabilizing, and capping agent in the aqueous phase.

Aim: This work presents an investigation on the biosynthesis of silver nanoparticles (AgNPs) using an aqueous extract of Morus alba (mulberry) leaves and an evaluation of its antibacterial qualities.

Method: The antibacterial properties of synthesized AgNP are evaluated using agar disk and agar well diffusion. The production of silver nanoparticles is verified by the development of a characteristic color (yellow brown), which stands for the reduction of Ag⁺ ions.

Results: The produced AgNPs shown antibacterial efficacy against Pseudomonas aeruginosa and Escherichia coli. The biosynthesized silver nanoparticles had better antibacterial capabilities against the studied microbes, as evidenced by the formation of zones of inhibition.

Conclusion: In order to fight human pathogens (Escherichia coli and Pseudomonas aeruginosa), Morus alba and AgNPs may be employed as safe, efficient, and environmentally friendly antibacterial agents.

Keywords:

Silver Nanoparticles, Morus Alba, Green Synthesis, Ethnopharmacology, Antibacterial properties.

1. Introduction

Because of their non-toxic, optical, catalytic, bio-sensing, drug transport, antioxidant, cytotoxic, and antibacterial properties, silver nanoparticles are becoming more and more popular and are in high demand, with an annual demand of 500 tons [1, 2]. Silver nitrate was bio-reduced in the presence of plant extract to create AgNPs [3]. There are several benefits of bio-reducing AgNO₃ with plant extract for the synthesis of AgNPs, including reduced waste generation, quick crystallization, economic effectiveness, and environmental friendliness [4, 5]. In order to defend themselves against various diseases, plants generate a vast diversity of secondary metabolites. Secondary metabolites are helpful in the green synthesis of AgNPs by acting as stabilizing agents. Flavonoids convert silver ions into AgNPs also control its size. For the stabilization of AgNPs tannins play an important role [6, 7] [6,7]. Polyphenols increase the antibacterial activity of AgNPs by increasing the membrane permeability of *P.aeruginosa* and *E.coli* [8-10].

We have chosen a leaf extract from the plant *Morus alba*, generally known as mulberry, which has both medical and commercial value. *Morus alba* is widely available and reducing synthesis cost. The biological activities of these leaves are believed to be influenced by the high concentration of bioactive compounds e.g. flavonoids, polyphenols and tannins which have potent antioxidant and natural stabilizing properties. Additionally, mulberry don't require hydrazine which is a hazardous reducing agent [11, 12].

The anti-diabetic, antibacterial, antioxidant, neuroprotective, anti-cancerous, and hepatoprotective properties of mulberry are also utilized. The present work aimed to assess the antibacterial activity of mulberry aqueous leaf extract against human pathogens and to employ it for the environmentally friendly production of AgNPs. By examining the zone of inhibition, the antibacterial activity of the produced nanoparticles will be evaluated [11-13].

2. Materials and methods

Table 1.1 Materials

Material	Quantity
Broth	20ml for each organism
Distilled water	150ml
Muller Hinton Agar	80ml
AgNO ₃ (Rapped in Aluminum Foil)	10mg (0.01g)
Plant Leaves	10g
Test organism	2 Bacterial culture plates (<i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i> isolated from wound swabs)

2.2 Methods

2.2.1 Plant material collection and extract preparation

In February, sterile polythene bags were used to gather *Morus alba* leaves from MNSAUM Multan, Pakistan. After being cleaned with tap water, the leaves were thinly cut. The sliced leaves were boiled in 100 milliliters of double-distilled water for one hour, maintaining the water bath's temperature at 80 degrees Celsius, to create an extract. Whatman filter paper No. 1 in a funnel was used to filter the extract (Fig 1.1A) [14].

2.2.2 Biosynthesis of AgNPs and optimization

In the biosynthesis of AgNPs, about 20 g leaves in 100 ml dis.H₂O were boiled (80°C) at water bath for half an hour. Whatman filter paper No. 1 in a funnel was used to filter the extract. One mM aqueous AgNO₃ solution is prepared at room temperature. Sodium hydroxide used to adjust the pH at 10 for good nanoparticle formation. AgNO₃ solution was titrated against *Morus alba* extract for green synthesis, maintaining the water bath's temperature at 80°C. In the titration process, 5 milliliters of extract were employed until a yellow colour appeared. After 2 hours, a yellowish-brown solution emerged, confirming the green production of AgNPs. Stir the solution continuously for better synthesis of AgNPs. Fuhrer, (UV-Vis) spectroscopy used to analyse the solution which show peaks at 400 nm [15, 16].

3. Biological and catalytic activity of AgNPs

3.1 Antibacterial assay by disc diffusion and Well Diffusion Method

The disc diffusion and well diffusion assays were used to evaluate the antibacterial activity of silver nanoparticles. Two separate test tubes were used to create the nutrient broth solution, which was then autoclaved. The test tubes holding the nutrient broth were filled with the strains of *P. aeruginosa* and *E. coli*. Overnight, the injected bacterial cultures were maintained at 37°C in an incubator. Eighty milliliters of Mueller Hinton medium were produced and autoclaved to investigate the antibacterial activity of silver nanoparticles. After that, it was transferred into four Petri dishes, two of which were employed to investigate the antibacterial activity of silver nanoparticles (AgNPs) against *P. aeruginosa* and the other against *E. coli* (Fig 1.1 A). Using sterile cotton swabs, 50 µl of fresh overnight cultures of *P. aeruginosa* and *E. coli* were applied to the medium on four separate plates. Two Petri dishes were filled with sterile Whatman filter paper discs, each 4 mm in diameter, and dipped in a sample solution of nanoparticles. The remaining two Petri dishes were utilized for the Well Diffusion Method (Fig 1.1C).

A sterile Pasteur pipette was used to prepare the wells on Mueller Hinton agar plate (Fig 1.1D). 50 µl of biosynthesized AgNPs were added to each well (Fig 1.1 E). For twenty-four hours, the plates were incubated at 37°C. The zone of inhibition (measured in millimeters) was used to assess the antibacterial activity [17, 18].

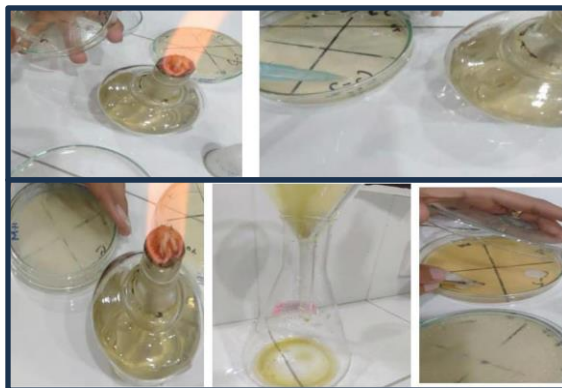


Figure 1.1 (A) Illustrating Agar Plate Solidification (B) Whatman filter paper No. 1 was used to filter the boiling leaf extract of Morus Alba (C). A sterile paper disc was set on the agar surface (D). Using a sterile Pasteur pipette to make a well (E). Fifty microliters of biosynthesized AgNPs were added to wells.

4. Results:

Zones of inhibition showed that the biosynthesized silver nanoparticles were more effective against the studied bacteria in terms of their antimicrobial capabilities. Silver nanoparticles (AgNP) at 50µL demonstrated the highest zone of inhibition (2.2 cm) against *Pseudomonas aeruginosa* in the well diffusion assay, whereas the lowest zone of inhibition (1.2 cm) was demonstrated against *Pseudomonas aeruginosa* in the disk diffusion assay. The findings show that, in comparison to the comparable disc diffusion experiment, the biosynthesized AgNPs utilized in the well diffusion assay had greater antibacterial activity.

Table I.2: Zones of inhibition diameters (cm) for 50 µL of silver nanoparticle reaction mixture following incubation with different microbes

Test organisms	Sr.no	Zone of Inhibition(cm)	
		Reaction Mixture (AgNP) 50µL	
		Well Diffusion method	Disc Diffusion method
Escherichia coli	1	1.1	1.5
	2	1.7	1.7
	3	1.2	1.4
	4	1.2	1.5
Average		1.3	1.5
Pseudomonas aeruginosa	1	2	1.2
	2	2.1	1.5
	3	2.2	1.3
	4	2.1	1.1
Average		8.4	1.2

Escherichia coli			
Well diffusion method		Disc Diffusion method	
Mean	1.3	Mean	1.525
Standard Error	0.135401	Standard Error	0.062915
Median	1.2	Median	1.5
Mode	1.2	Mode	1.5
Standard Deviation	0.270801	Standard Deviation	0.125831
Sample Variance	0.073333	Sample Variance	0.015833
Kurtosis	3.483471	Kurtosis	2.227147
Skewness	1.812802	Skewness	1.129338
Range	0.6	Range	0.3
Minimum	1.1	Minimum	1.4
Maximum	1.7	Maximum	1.7
Sum	5.2	Sum	6.1
Count	4	Count	4
Confidence Level(95.0%)	0.430905	Confidence Level(95.0%)	0.200225
Lower Ci	0.869095	Lower Ci	1.324775
Upper Ci	1.730905	Upper Ci	1.725225

Pseudomonas aeruginosa			
Well diffusion method		Disc Diffusion method	
Mean	2.1	Mean	1.275
Standard Error	0.040825	Standard Error	0.085391

Median	2.1	Median	1.25
Mode	2.1	Mode	#N/A
Standard Deviation	0.08165	Standard Deviation	0.170783
Sample Variance	0.006667	Sample Variance	0.029167
Kurtosis	1.5	Kurtosis	0.342857
Skewness	0	Skewness	0.752837
Range	0.2	Range	0.4
Minimum	2	Minimum	1.1
Maximum	2.2	Maximum	1.5
Sum	8.4	Sum	5.1
Count	4	Count	4
Confidence Level(95.0%)	0.129923	Confidence Level(95.0%)	0.271753
Lower Ci	1.970077	Lower Ci	1.003247
Upper Ci	2.229923	Upper Ci	1.546753

t-Test: Two-Sample Assuming Equal Variances		
Escherichia coli		
	Variable 1	Variable 2
Mean	1.3	1.525
Variance	0.073333	0.015833
Observations	4	4
Pooled Variance	0.044583	
Hypothesized Mean Difference	0	
Df	6	
t Stat	-1.50699	

P(T<=t) one-tail	0.091266	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.182533	
t Critical two-tail	2.446912	
Pseudomonas aeruginosa		
	Variable 1	Variable 2
Mean	2.5	2.1
Variance	1.666667	0.006667
Observations	4	4
Pooled Variance	0.836667	
Hypothesized Mean Difference	0	
Df	6	
t Stat	0.618442	
P(T<=t) one-tail	0.279507	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.559015	
t Critical two-tail	2.446912	

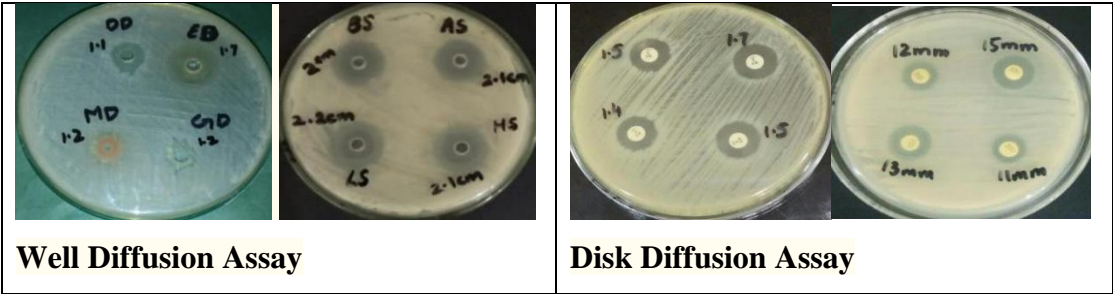


Figure 1.2: A zone where synthetic AgNPs inhibit *P. aeruginosa*. B AgNPs' inhibition zones against *E. coli*. C AgNP-induced inhibition zones against *E. coli* D AgNP-induced inhibition zones against *P. aeruginosa*

4.1: UV-visible spectroscopy

The mulberry leaves extracts that were made in different solvents were analysed by UV-Visible spectroscopy. This was done to determine the peaks characteristics of prepared extracts. The mulberry extract was analysed in the range of 200-800nm wavelength. Two distinct peaks were found in the mulberry leaf ethanolic extract's absorbance. The first absorbance peak of aqueous leaves extract was 3.5 at 280 nm, second was 3.9 at 300nm and third was 3.6 at 320 nm. The first absorbance peak at 280 nm shows that Morus alba contains polyphenols which is responsible for its medicinal properties. Morus alba leaves extract absorbance gradually decreases from 350 to 740 nm and in the visible region absorbs less light (Figure 1.3) [19-23].

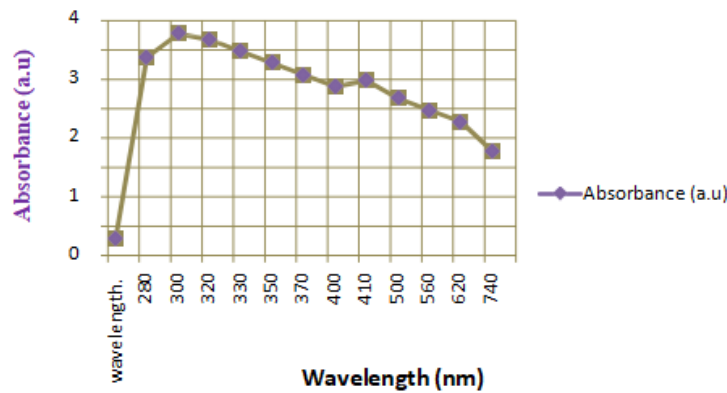


Figure 1.3: UV-visible absorbance spectrum of Morus alba leaves ethanolic extract: The absorbance of plant extract was 3.8 at 300 nm, and 3.6 at 320 nm.

4.2: FTIR Analysis:

FTIR analysis provides information about phenolic compounds of plant extracts. For RTIR analysis supernatant was discarded and pellet was dried to make it powder. The FTIR spectrum was recorded in the range of 600-4000cm-1 for extracts of Morus alba.

The result of FTIR analysis of ethanolic plant extracts recorded showed prominent peaks at wavelength between 1084-1009, 1646 and between 2929-2975 which indicated the presence of saturated primary alcohol, aromatic rings and C-H stretching of aromatic aldehyde and these compounds are responsible for its anti-inflammatory and antibacterial properties(Figure 1.4) [22, 23].

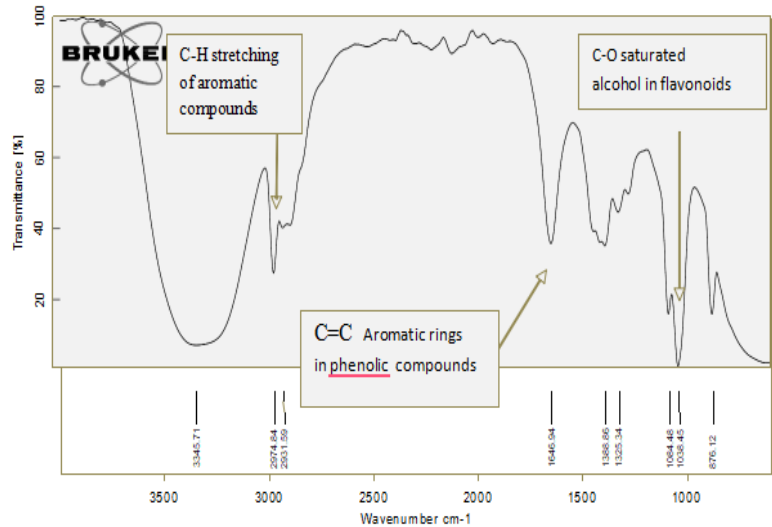


Figure 1.4: FTIR spectrum of Morus alba leaves ethanolic extract from 600-4000cm-1

5. Discussion

Recent years have seen a rise in antibiotic resistance, which has become a serious global health concern. Silver nanoparticles have recently been implicated in antibacterial properties in a different way. AgNP production typically calls for hazardous chemicals and a lot of energy, which can have serious negative effects on the environment. These days, we use biological resources, such as plant extracts of various herbs, microbes, and different enzymes, to create these silver nanoparticles in an environmentally friendly manner. A variety of photochemical, such as flavonoids and polyphenol, are crucial for stabilizing the nanoparticles [6, 7].

The development of a distinctive color (yellow brown), which represents the reduction of Ag^+ ions, verifies the creation of silver nanoparticles. The antibacterial activity of manufactured AgNPs can be shown in two ways: the well diffusion method and the agar disk approach. A distinct zone of inhibition against the tested microorganism forms during the course of treatment. Our findings showed that AgNPs' zone of inhibition against *P. aeruginosa* (2.2 cm) in the Well diffusion experiment was larger than that of the disk diffusion assay [8-10]. In previous studies AgNPs are synthesized chemically that is toxic for environment. In this research, we synthesized NPs by using *Morus alba* that is ecofriendly and inhibit the bacterial growth [11].

Our research on the antibacterial activity of silver nanoparticles at least suggests that the antimicrobial activity against test organisms was influenced by the concentration and amount of AgNPs. As anticipated, a larger amount of AgNPs solution was used in the well diffusion test than in the disk diffusion assay. In this AgNPs synthesized from *Morus alba* shows larger zone of inhibition (2.2cm) at the concentration of only 50 ul which shows that *Morus alba* contains more polyphenols compounds which make them more antibacterial [24].

AgNPs' synthesized from *Morus alba* bactericidal activity can be attributed to a number of variables when discussing their mode of action as an antimicrobial agent. AgNPs causes the bacterial cell lysis by destroying the cell membrane. They may result in the development of an electron-light zone in the cell's core, where DNA molecules condense. It is thought to be a defense mechanism of the bacterial cell to shield the DNA from AgNP damage. However, the bacterial cell loses its capacity to replicate as a result of DNA condensation, which inhibits or suppresses growth. AgNPs' synthesized from *Morus alba* generate Reactive oxygen species that cause the bacterial cell death by damaging their DNA. Gram negative bacteria use defense mechanism to remove harmful compounds from body surfaces but AgNPs disable these defense mechanism and these harmful compounds accumulate in body and cause its cell death [25, 26].

Various studies have also reported a similar mechanism in which oxygen and zero valent silver (Ag^0) combined to form hydrogen peroxide (H_2O_2), which has the potential to harm bacterial cell walls. Depending on whether the bacteria are gram-positive or gram-negative, the antibacterial activity can change. Because of their strong peptidoglycan layer, which protects them from AgNPs, gram-positive bacteria, for example, exhibit fewer alterations. It demonstrates that whereas a small amount of AgNPs (25 $\mu\text{g}/\text{ml}$) is sufficient for the optimum performance against gram positive bacteria, a high concentration (50 $\mu\text{g}/\text{ml}$) of AgNPs provides the strongest activity against gram-negative bacteria.

According to our findings, silver nanoparticles synthesized from *Morus alba* have antibacterial efficacy against a wide range of pathogenic and multidrug-resistant microbes, which is consistent with earlier research. Flavonoids, alkaloids, and polyphenols are among the phytochemicals it contains [27]. These compounds have reducing and stabilizing properties that prevent aggregation and ensure uniform sample size distribution in the production of silver nanoparticles as well as in the reduction of inflammation and

oxidative stress in medical conditions. Consequently, the generation of harmful substances is decreased. Because mulberries are inexpensive, this approach of synthesizing NPs is also cost-effective because it lowers the total cost of production. Additionally, the antibacterial efficacy of commercial medications against harmful microbes was improved by the silver nanoparticles [25, 26].

Optimize the concentration of mulberry extract by lowering reaction time, temperature, and pH in order to standardize or create a transparent, sequential manufacturing process that will increase the efficiency of silver nanoparticle production. To confirm the stable synthesis of AgNPs, advanced techniques such as FTIR, and (UV-Vis) spectroscopy are employed [1, 3, 7, 28].

In future examine the advantages of AgNPs in a number of additional domains, such as manufacturing antimicrobial creams, patches, and sprays for consumer convenience, biomedical applications, agriculture, and the environment. Assess the toxicity of AgNPs generated by the mulberry plant to human cells to guarantee safe biomedical applications and environmental impact, including NPs' biodegradability and potential future consequence [1, 4, 6, 13, 21, 25].

We acknowledge that there may be two limitations to our research. Initially, we examined antibacterial activity while maintaining the same level of silver nitrate (AgNO_3) in each well. The second is that we haven't used control. These restrictions demonstrate how challenging it is to gather information on antibacterial action.

6. Conclusion

Biosynthesized silver nanoparticles from *Morus alba* demonstrated strong antimicrobial activities against gram negative bacteria isolated from wound sample. Advanced techniques such as FTIR and (UV-Vis) spectroscopy are used to characterize and determine the mechanism of action of AgNPs. This study confirms that AgNPs synthesized from *Morus alba* were more effective against *Pseudomonas aeruginosa* by showing larger zones of inhibition. In future examine the advantages of AgNPs by using metagenomics and apply as an antimicrobial creams, and further study on Multidrug resistant bacteria..

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