

BIOGENIC SYNTHESIS OF NANOSILVER PARTICLES FROM ROOT EXTRACT OF *MORUS ALBA* L. AND THEIR BIOLOGICAL ACTIVITIES

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Abstract

Morus alba (Moraceae), mulberry, is considered a great source of therapeutic agents for the treatment of chronic diseases like cancer, high blood pressure, high cholesterol and ulcer. The present study reported the simple and successful synthesis of silver nanoparticles by using aqueous, ethanol and methanol root extracts of *M. alba* and the analysis of their efficacy against different biological activities. UV-Vis spectroscopy, X-Ray diffraction (XRD) and scanning electron microscopy (SEM) techniques were used to characterize the morphology of silver nanoparticles. Brine shrimp cytotoxicity, antioxidant, antibacterial, antifungal, antitumour assays were performed and free radical scavenging activity was measured using the DPPH free radical assay. The nanoparticles were characterized by UV and XRD, UV spectra showed the characteristic peaks ranging from 400 - 800 nm. With the help of SEM analysis, the size of silver nanoparticle was found to be 297 nm and their shape was irregular and had a rough surface. Among all the extracts, methanolic extracts showed strong antibacterial activity (MIC = 0.5), antifungal activity (MIC = 0.5), with MIC value of 0.5 as compared to ethanolic extract (MIC = 5) and aqueous extracts (MIC = 10). Methanolic extracts also showed the highest antioxidant activity (IC₅₀ = 158.68), antitumour activity (IC₅₀ = 105.03), and cytotoxicity activities (IC₅₀ = 24.83) as compared to other extracts. These results suggest that secondary metabolites in the root extracts of *M. alba* may be responsible for enhanced biological activities. Nanoparticles synthesized by employing plants can have advantages over other biological entities which can overcome time consuming processes and be environmentally friendly.

Rezumat

Morus alba (Moraceae), este considerat o sursă de compuși biologic activi pentru bolile cronice precum cancerul, hipertensiunea arterială, și ulcerul. Studiul prezintă sinteza nanoparticulelor de argint prin utilizarea extractelor apoase, etanolice și metanolice din rădăcină de *M. alba* și analiza activității biologice a acestora. Pentru a caracteriza morfologia nanoparticulelor de argint s-au folosit tehnici de spectroscopie UV-Vis, difracție cu raze X (XRD) și microscopie electronică cu scanare (SEM). S-au efectuat teste de citotoxicitate, teste privind acțiunea antioxidantă, antibacteriană, antifungică, antitumorală și de *scavenger*. Spectrele UV au arătat variații ale nanoparticulelor între 400 și 800 nm. Cu ajutorul analizei SEM, s-a constatat că dimensiunea nanoparticulelor de argint este de 297 nm, cu formă neregulată și suprafață rugoasă. Extractele metanolice au prezentat activitate antibacteriană și antifungică puternică. De asemenea extractele metanolice au prezentat, cea mai potentă activitate antioxidantă, antitumorală și citotoxică.

Keywords: *Morus alba*, silver nanoparticle, biological activities, cancer, secondary metabolites

Introduction

Plants have always been a part of our lives being used for various medicinal purposes since ancient times. Plants are nature's "chemical factories". They are cost efficient and require low maintenance. Researchers have recognized different plant based compounds that are extensively used in various medicines [1]. In developing countries like Pakistan, around 3.3 billion people are using medicinal plants on a regular basis to cure various ailments [2]. Root bark of *Morus alba* (mulberry) is widely used as a medicine in China since 659 A.D. and Chinese pharmacopoeia listed its

root bark, leaves, fruits, and stem as a constituent in drugs preparations [3], and it is an official drug of the British Herbal pharmacopoeia [4]. The root bark has anti-helminthic properties and root juice has coagulation effects. Albanol A isolated from the root bark has been proven as an effective medicine to cure leukaemia [5]. Root bark has also been used in herbal medicine as anti-chloristic, anti-cough, liver and kidney protective agent, diuretic, hypotensive and analgesic. Roots are a rich source of amino acids, carbohydrates, triterpenes, proteins and vitamins C [6].

Plants provide a great area for synthesis of nanoparticles, free from toxic chemicals and have the ability of coping naturally. Silver nanoparticles are using silver in range of 1 nm and 100 nm in size. The desired structure and size is achieved by controlling parameters [8]. In commercial products silver nanoparticles are in the range of 5 to 50 nm [1].

Nanotechnology is a common word these days, but many of us do not realize its amazing impact on our daily lives. The implications of nanotechnology are wide-ranging and could include medicine, military applications, computing and astronomy [9]. The science of nanotechnology is developing new commercial applications in the pharmaceutical industry including advanced drug delivery systems, new therapies, optoelectronics and *in-vivo* imaging. Nanotechnology is designed to provide a novel and improved approach to cancer diagnosis and treatment [10, 11].

Silver nanoparticles exhibit new and improved properties according to their distribution, size and morphology [12]. Silver nanoparticles are mostly used due to their unique properties in biosensing, photonics, electronics, chemical sensing, catalysis, optics, antimicrobials, biomaterial and pharmaceutical sector [1], which can be incorporated into composite fibres, cosmetic products, cryogenic superconducting materials, electronic components and food industry [13, 14]. Silver nanoparticles can be synthesized by different methods such as electro-chemicals [12], biological techniques [15], radiation [16], physical and phytochemicals method [17]. Physicochemical methods of silver nanoparticles synthesis use high temperature, pressure, toxic chemicals and produces hazardous byproducts. Therefore it is important to explore safe processes for the synthesis of silver nanoparticles as biodegradable polymers, micro-organisms or plant extracts [18, 19].

The green synthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Plant-mediated synthesis of nanoparticles is a green chemical approach that connects nanotechnology with plants [19]. Novel methods of ideally synthesizing nanoparticles (NPs) are formed at ambient temperatures, neutral pH, low costs and being environmentally friendly. Keeping these goals in view, nanomaterials have been synthesized using various routes. Among the biological alternatives, plants and plant extracts seem to be the best option [20].

Biosynthesis of silver nanoparticles from several plants like *Solanum torvum* [20], *Euphorbia hitra* [17] and *Coriandrum sativum* [21] has been reported.

Plants have advantages over other biological entities for the synthesis of nanoparticles because overcome the time consuming method of employing microbes and also maintaining their culture which can lose their potential towards synthesis of nanoparticles [13].

The roots of *M. alba* have been widely used in health-care for curing depression, dementia, nephrotoxicity, Parkinson's disease and as a skin whitening agent [22], and are cheap and simple source for the treatment of cancer by reducing the invasiveness of cancerous cells [23]. Therefore in this context, the present study was designed to synthesize silver nanoparticles (AgNPs) from the root bark of *M. alba* and their efficacy was analysed against different biological activities.

Materials and Methods

Study Area

The present research work was carried out in the Department of Biotechnology, Women University of Azad Kashmir, Bagh, Pakistan, in collaboration with Nano-Science and Technology Department, National Centre for Physics Quaid-i-Azam University Islamabad, Pakistan. The plant material (*Morus alba*) was collected from different areas of Bagh Azad Kashmir, Pakistan, and was confirmed by an expert taxonomist from the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan, the plant being registered with voucher number WUB-T-174 in the herbarium.

Chemicals

All the chemicals used in the experimental work were of the highest grade of purity. Chemicals were purchased from Sigma Chemical Co, USA, and E. Merck, Germany.

Preparation of plant extracts

Extracts of shade dried roots of *M. alba* were prepared by simple maceration. The plant material i.e. roots (100 g) was grounded and soaked in 200 mL of methanol, ethanol and aqueous solvent separately for 7 days at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) by occasional soaking and filtered through Whatman filter paper. The filtrate was concentrated in a rotary evaporator (Rotavapor R-200, Buchi) and finally dried at 45°C in vacuum oven (Vacucell, Einrichtungen GmbH) to obtain the crude extract. The extract weight was calculated and stored at 4°C for further analysis (Table I).

Green synthesis of silver nanoparticles

Silver nitrate (AgNO_3) (Sigma-Aldrich) was used as a precursor salt for the synthesis of the silver nanoparticles.

Table I

Extracts of roots of *Morus alba* using three different solvents

Sample name	Solvent	Weight of sample (g)	Solvent added (mL)	Extract weight (mg)
Roots	Methanol	100	1250	1250
	Ethanol	100	1460	1460
	Aqueous	100	1070	1070

For the synthesis of AgNPs 50 mM stock solution of AgNO₃ was used. 100 mL aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Then 1.0, 2.0, 3.0, 4.0 and 5.0 mL of root extract prepared in methanol, ethanol and aqueous was added separately to 10 mL aqueous silver nitrate solution and stirred continuously. The solution was kept in a dark chamber for 3 days until the solution colour changed to yellow or dark yellow. After three days, the solution turns yellow to yellow-red or dark brown indicating the formation of silver nanoparticles. The solution was filtered and monitored by periodic sampling by the UV spectrophotometer.

UV-Vis spectroscopy

The bio-reduction of silver nitrate AgNO₃ to silver nanoparticles (AgNPs) was monitored periodically by UV-Vis spectroscopy (Perkin-Elmer Lambda-25 spectrophotometer) after the dilution of the samples. The UV-Vis spectrometric determinations were made between 200 and 800 nm against water as reference [4, 21].

X-ray diffraction

Freeze dried powders of the synthesized silver nanoparticles from the root extracts of *M. alba* (methanol, ethanol and aqueous) were used for x-ray diffraction analysis. Crystalline metallic silver nanoparticles were examined using XRD (Bruker D8 advance).

Scanning Electron Microscopy Analysis (SEM)

SEM analysis was used to check the morphology of nanoparticles as well as their size from the nanometer to micrometer scale. Silver nanoparticles were analysed by placing a drop of sample suspension over carbon coated grid and after drying it was examined and photographed in SEM at CAEPE (Centre for Advanced Electronics and Photovoltaic Engineering), International Islamic University Islamabad, Pakistan.

Biological activities

Following, biological activities assessments were performed on the nanoparticles of root extracts of *M. alba*.

Brine shrimp cytotoxicity assay. Brine shrimp cytotoxicity assay was performed according to the methodology presented in literature [24]. Brine shrimp (*Artemia salina*) larvae, used as test organisms, hatched at 37°C in artificial sea water. To a 20 mL glass vial, 0.5 mL sample of extracts was added to have final concentration of 1000, 100 and 10 µg/mL. The solvent from each vial was evaporated, followed by the addition of 2 mL of artificial seawater. 10 shrimps were transferred into each vial and the final volume was adjusted to 5 mL and kept under fluorescence light at 37°C for 24 h. The test was performed in triplicate and survivors were counted by using a magnifying glass and a Pasteur pipette. Percentage death was calculated (by Abbott's formula). LD₅₀ values were determined by Finney computer program [25].

Antibacterial assay. The antibacterial assay was performed by disc diffusion method by following the

methodology of Chan YSS *et al.* [26]. The antibacterial activity was tested against five bacterial strains; three were gram positive i.e. *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) and *Micrococcus luteus* (ATCC 10240) and two were gram negative i.e. *Escherichia coli* (ATCC 15224), and *Enterobacter aerogenes* (ATCC 13048). 100 µL of 24 hours old bacterial cultures in nutrient broth were spread over Petri dishes (7 cm) containing 25 mL solidified nutrient agar. Sterile filter paper discs (diameter 6 mm) impregnated with 100 µL of extract dilutions (1, 5 and 10 mg/mL DMSO) were placed over each of the culture plates. The plates were then incubated at 37°C for 24 hours. The anti-bacterial activity was determined by measuring zones of inhibition around the discs in each plate. Paper discs impregnated with 100 µL of a solution of 2 mg/mL of Cefixime (Cef) were used as positive control while DMSO was used as negative control. The test was performed in triplicate and the mean standard deviation was calculated.

Antifungal assay. The antifungal assay was performed according to the methodology of [27, 28]. The antifungal activity was studied against five fungal strains namely *Mucor species* (FFBP 0300), *Aspergillus niger* (FFBP 0198), *Aspergillus flavus* (FFBP 0064), *Aspergillus fumigatus* (FFBP 66) and *Fusarium solani* (FFBP 0291). All fungal strains were grown on 6.5% SDA (Sabouraud dextrose agar, pH 5.7) at 28°C and preserved at 4°C in refrigerator. Terbinafine (Ter) (200 µg/mL) was used as standard drug while DMSO was used as negative control. Three concentrations of each of the extracts i.e. 1, 5 and 10 mg/mL DMSO were tested. SDA plates containing 25 mL medium were inoculated with 100 µL fungal spores suspensions which was prepared by harvesting fungal spores' suspension in 2% w/v Tween 20 solution in distilled water and turbidity was adjusted to 0.5 McFarland turbidity standards. Sterile filter paper discs (diameter 6mm) impregnated with 100 µL of extract solution was placed over each of the culture plates. Inoculated plates were then incubated at 28°C for 24 h and zones of inhibition were measured in millimetres (mm) around the each disc.

Antitumour Assay. Antitumour potato disc assay was performed by following the standard procedure as reported by [29]. A 48 h old culture of *Agrobacterium tumefaciens* strain was used to test the plant extracts. An inoculum of 1.5 mL containing six different concentrations of plant extracts (10,000, 1000, 100, 10 and 1 µg/mL), bacterial cultures and distilled water was prepared. DMSO was used as negative control and vinblastine sulphate was used as positive control. Red skinned potatoes were surface sterilized by using 0.1% HgCl₂ solution followed by washing with distilled water three times. A borer of 8 mm diameter was used to dug out potato cylinders and cut into 4 mm discs. Autoclaved agar solution (1.5% w/v) was poured in Petri dishes (20 mL) and allowed to solidify. Ten discs

were placed on agar surface of each Petri dishes and 50 μ L of inoculum was poured on the top of each disc. The plates were sealed with parafilm to avoid moisture loss and incubated at 28°C in dark. After 21 days of incubation, potato discs were stained with Lugol's solution (10% KI, 5% I₂), and tumours were counted under dissecting microscope. More than 20% tumour inhibition was considered significant. Tumour inhibition was calculated by using following formula:

$$\% \text{ of tumour inhibition} = ((1 - T_s)/T_c) \times 100,$$

where; "Ts" is Number of tumours in the sample and "Tc" is number of tumours in control.

Free radical scavenging activity. Free radical scavenging activity was measured by using 2,2, diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH free radical assay was performed according to the procedure described by [24]. The DPPH solution was prepared by dissolving 3.92 mg in 100 mL of methanol. A volume of 2800 μ L of DPPH solution was added to glass vials followed by the addition of 200 μ L of plant extraction solution, leading to the final concentration of 1000, 500 and 100 μ g/mL. Mixtures were shaken well and incubated

in dark at 37°C for 1 h. Absorbance was measured at 517 nm by using a spectrophotometer (PDA 8453, Agilent). DMSO was used as negative control and ascorbic acid (AsA) was used as positive control. Each test was performed in triplicate and percentage inhibition was measured according to the following formula:

$$\% \text{ scavenging effect} = ((1 - A_s)/A_c) \times 100,$$

where; "Ac" is Absorbance of negative control and "As" is Absorbance of test sample.

Results and Discussion

The present study focused on the green synthesis of silver nanoparticles (AgNPs) from the root methanol, ethanol and aqueous extracts of *M. alba*.

Characterization of silver nanoparticles

Silver nanoparticles were successfully synthesized within three days from different extracts. After synthesis of silver nanoparticles, they were characterized by UV-Vis spectroscopy, X-ray diffraction analysis and SEM analysis.

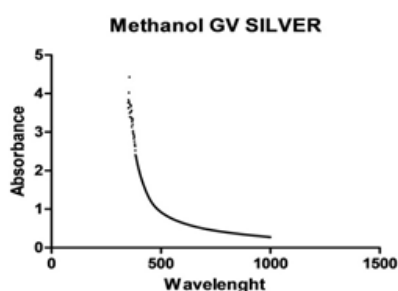


Figure: 2A

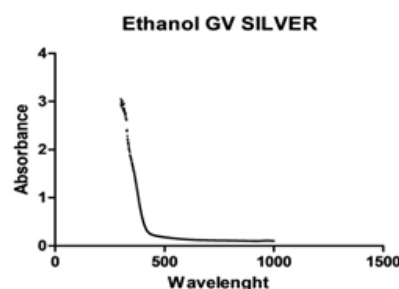


Figure: 2B

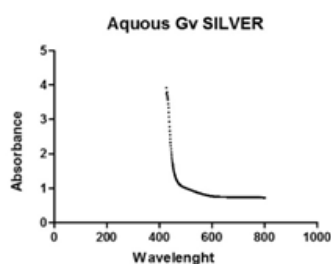


Figure: 2C

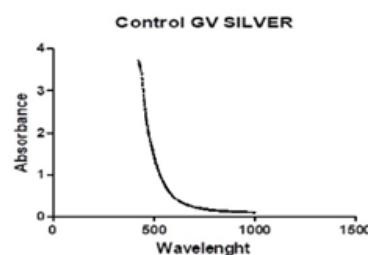


Figure: 2D

Figure 1.

UV peaks of silver nanoparticles obtained from different *Morus alba* extracts or control

(A) = methanol; (B) = ethanol; (C) = aqueous; (D) = control

UV spectroscopy

The reduction of silver ions to AgNPs was observed when the plant extract was treated with AgNO₃ (Figure 1 A-D). The peaks for silver nanoparticles were observed between 400 nm to 450 nm which are characteristic for silver nanoparticles. Absorption peaks of mulberry leaf extract were in the range of 380 nm to 800 nm. The peaks of methanol, ethanol,

aqueous and control extracts of silver nanoparticles were observed between 500 - 800 nm, 480 - 800 nm, 450 - 800 nm, 500 - 800 nm respectively observed by UV spectroscopy.

XRD diffraction

The crystalline nature of the silver nanoparticles was confirmed through the XRD method. The characteristic peak of silver nanoparticles in accordance to Miller

Indices was obtained by the methanol extract (Figures 2 A-D). On the basis of the face-centered cubic structure of silver the XRD pattern showed numbers of Bragg reflections. Peaks of XRD values were found at 20, 25, 30, 35, 40 were observed to

121, 200, 210, 220 and 311 planes. X-ray diffraction results clearly showed that the silver nanoparticles formed by the reduction of Ag^+ ions by the *Morus* root extracts were crystalline in nature.

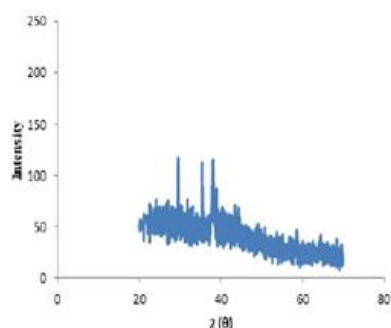


Figure: 3A

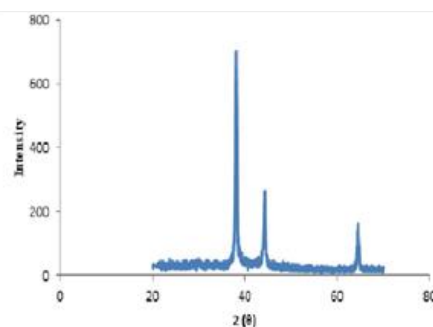


Figure: 3B

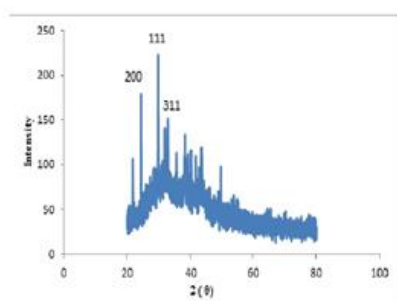


Figure: 3C

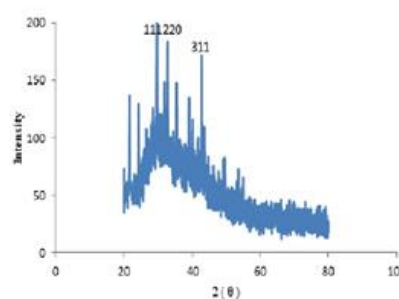
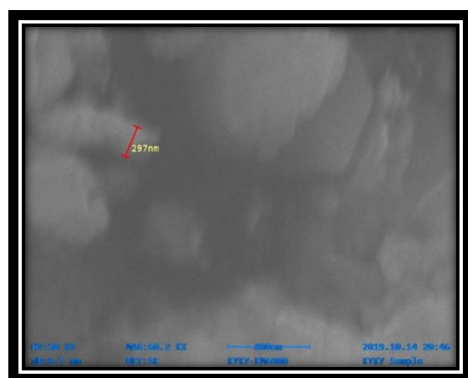


Figure: 3D

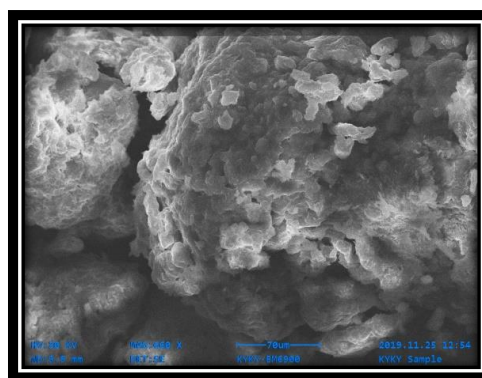
Figure 2.

UV peaks of silver nanoparticles obtained from different *Morus alba* extracts or control

(A) = methanol; (B) = ethanol; (C) = aqueous; (D) = control



(A)



(B)

Figure 3.

SEM images of silver nanoparticles: (A) = size of silver nanoparticles and (B) = shape of silver nanoparticles

SEM Analysis

SEM has been used to assess the morphology and size of the silver nanoparticles synthesized. The nanoparticles were irregular in shape and their surface was rough. The size of silver nanoparticles was found to be 297 nm (Figure 3A and Figure 3B).

Biological Activities

Antibacterial activity. Nanoparticles synthesized from root extracts of *M. alba* showed significant antibacterial activities against the tested microorganisms. Among all extracts (methanol, ethanol and aqueous), the methanolic extract showed the strongest antibacterial activity with MIC value of 0.5 as compared

to ethanolic extract (MIC = 5) and aqueous extracts (MIC = 10). Control fractions of all the extracts showed lower antibacterial activity i.e. methanol (MIC = 10),

ethanol (MIC = 10) and aqueous (MIC = 10) as compared to nanoparticles (Table II).

Table II

Antibacterial activity of *M. alba* against bacterial strains

Plant	Extracts type	Samples	Antibacterial Activity MIC (µg/mL)				
			<i>S. aureus</i>	<i>B. subtilis</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>E. aerogenes</i>
<i>Morus alba</i>	methanol	NP	0.5	0.5	1	0.5	1
		C	10	10	10	10	10
	ethanol	NP	5	5	5	5	5
		C	10	10	10	10	10
	aqueous	NP	10	10	15	10	10
		C	15	15	-	10	15
	Post. Cont.	Cef.	6	6	12	12	12
	Post. Cont.	Ter.	-	-	-	-	-

Cef = cefixime; Ter = terbinafine; C = control extract; Post. Cont. = positive control

Antifungal activity. All the nanoparticles synthesized from different extracts of *Morus alba* root showed strong antifungal activity against all fungal strains as compared to control fractions. Among all the extracts (methanol, ethanol and aqueous), the methanolic extract showed the strongest antifungal activity with MIC

value of 0.5 as compared to ethanolic extract (MIC = 5) and aqueous extracts (MIC = 10). Control fractions of all the extracts showed lower antifungal activity i.e. methanol (MIC = 10), ethanol (MIC = 10) and aqueous (MIC = 15) as compared to nanoparticles (Table III).

Table III

Antifungal activity of root extracts of *M. alba* against fungal strains

Plant	Extracts type	Samples	Antifungal Activity MIC (µg/mL)				
			<i>Mucor sp.</i>	<i>A. niger</i>	<i>A. flavis</i>	<i>A. fumigatus</i>	<i>F. solani</i>
<i>Morus alba</i>	methanol	NP	0.5	0.5	0.5	0.5	1
		C	10	10	10	10	10
	ethanol	NP	1	1	1	5	5
		C	10	10	10	10	10
	aqueous	NP	10	10	10	10	5
		C	15	15	15	15	15
	Post. Cont.	Cef.	-	-	-	-	-
	Post. Cont.	Ter.	3	5	3	3	5

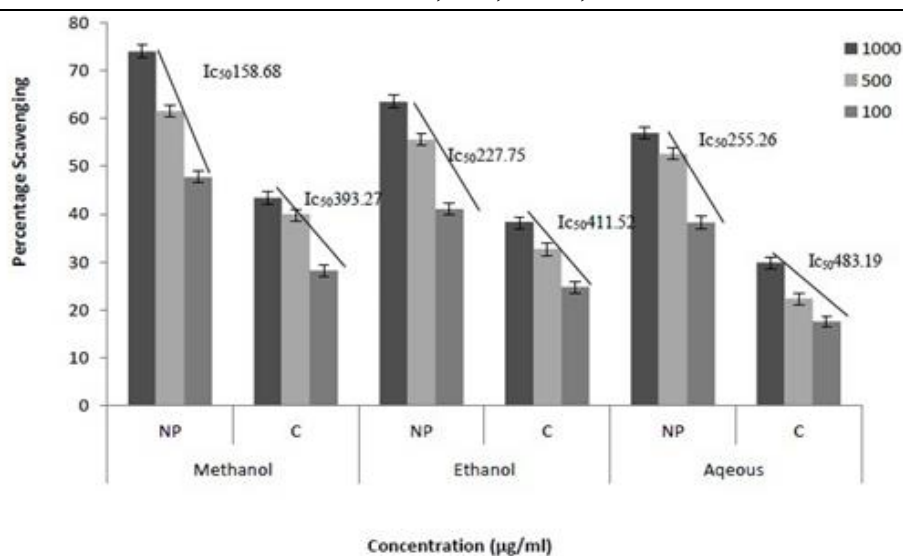
Cef = cefixime; Ter = terbinafine; C = control extract; Post. Cont. = positive control

Antioxidant activity. The synthesized nanoparticles from aqueous roots extracts of *M. alba* showed highest antioxidant activity. The synthesized nanoparticles showed the strongest antioxidant activity as compared to the control extract. From all the extracts (methanol, ethanol and aqueous), the methanolic extract showed the strongest antioxidant activity with IC₅₀ value of 158.68 as compared to the ethanolic extract (IC₅₀ = 227.75) and aqueous extract (IC₅₀ = 255.26). Control fractions of all the extracts showed lower antifungal activity i.e. methanol (IC₅₀ = 393.27), ethanol (IC₅₀ = 411.52) and aqueous (IC₅₀ = 483.19) as compared to nanoparticles (Figure 4).

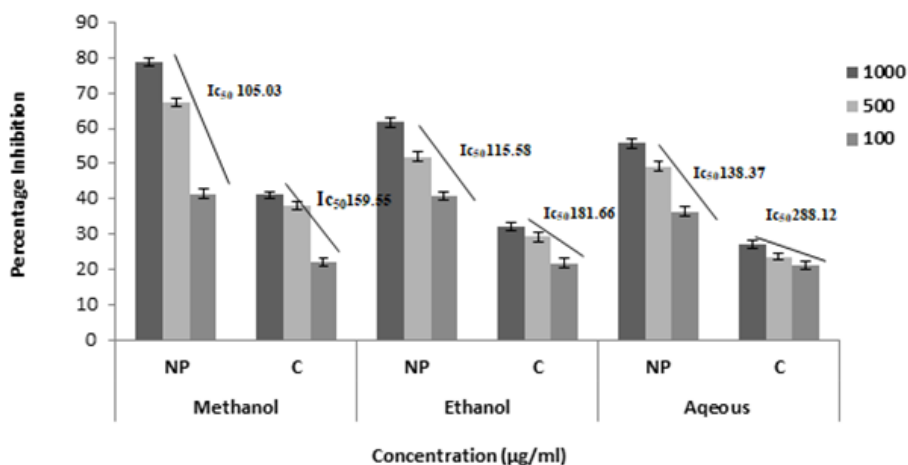
Antitumour activity. Nanoparticles synthesized from different roots extracts of *M. alba* (ethanol, methanol and aqueous) showed strong antitumour activity. From all the extracts (methanol, ethanol and aqueous), the methanolic extract showed the strongest antitumour activity with a IC₅₀ value of 105.03 as compared to the

ethanolic extract (IC₅₀ = 115.58) and aqueous extract (IC₅₀ = 138.37). Control fractions of all the extracts showed lower antitumour activity i.e. methanol (IC₅₀ = 159.55), ethanol (IC₅₀ = 181.66) and aqueous (IC₅₀ = 288.12) as compared to nanoparticles (Figure 5).

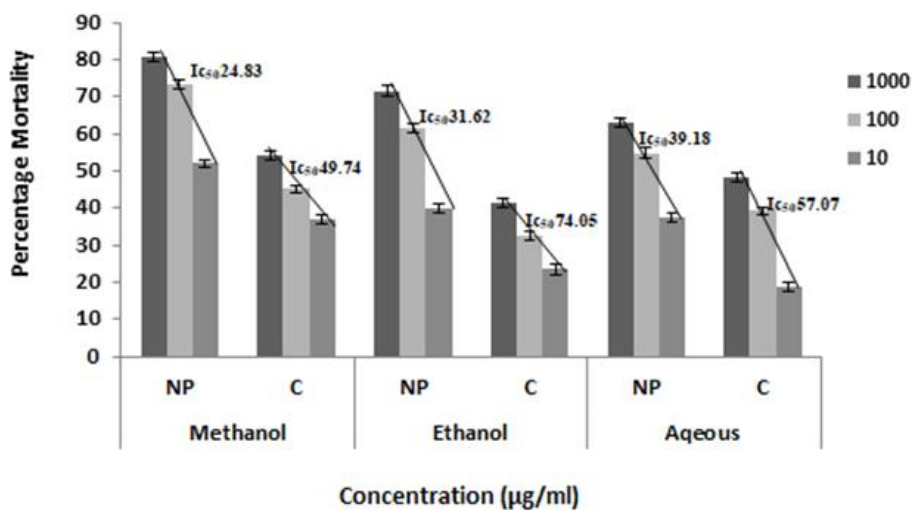
Cytotoxicity activity. Nanoparticles synthesized from different roots extracts of *M. alba* (ethanol, methanol and aqueous) showed strong cytotoxic activity. Methanolic extract of *M. alba* showed a more significant cytotoxic effect. From all the extracts (methanol, ethanol and aqueous), the methanolic extract showed the strongest cytotoxic activity with IC₅₀ value of 24.83 as compared to ethanolic extract (IC₅₀ = 31.62) and aqueous extracts (IC₅₀ = 39.18). Control fractions of all the extracts showed lower cytotoxicity activity i.e. methanol (IC₅₀ = 49.74), ethanol (IC₅₀ = 74.05) and aqueous (IC₅₀ = 57.07) as compared to nanoparticles as shown in (Figure 6).

**Figure 4.**

IC₅₀ values of antioxidant activity of nanoparticle from the methanol, ethanol and aqueous root extracts
The data represents the mean values of three replicates together with the standard deviations shown as error bars

**Figure 5.**

IC₅₀ values of antitumour activity of nanoparticle from the methanol, ethanol and aqueous root extracts
The data represents the mean values of three replicates together with the standard deviations shown as error bars

**Figure 6.**

IC₅₀ values of cytotoxic activity of nanoparticle from the methanol, ethanol and aqueous root extracts
The data represents the mean values of three replicates together with standard deviations shown as error bars

Plants are a valuable source of natural products for maintaining human health. In history of all civilizations the use of medicinal plants for curing diseases has been recognized. For the medicinal property of the drugs the compounds that are responsible are usually secondary metabolites [30].

In recent years herbal medicines have gained importance due to their efficacy and low cost [31, 32]. Pakistan has a varied climatic condition supporting the growth of nearly 6000 higher plant species of which nearly 12 percent plant species are reported for their medicinal value and the number is increasing constantly due to current interest of local researchers in natural products [6]. Medicinal compounds derived from plants have potential value and proposed as a tangible benefit of biodiversity and therefore a basis for promoting its conservation [33].

Different species of *Morus* are famous for their medicinal properties such as *Morus alba* (Mulberry) and *Morus rubra*. *M. alba* have strong antifungal, antioxidant, antitumour, nephroprotective, anti-HIV, anti-hyperglycemic, skin tonic and neuroprotective activities [9]. Mulberry shows a resistance to pests and disease, and comparatively requires low costs for cultivation as compared to other plants. Plantations of mulberry provide a low utilization of degraded or productive agricultural land, and can be used to produce electricity, heat, gas and liquid fuel production, and are one of the most important sources of fibres [34].

The present work focused on green synthesis of silver nanoparticles from root extract of *M. alba*, due to its cost effectiveness, eco-friendly and medicinal properties. Green synthesis of nanoparticles is the current market demand due to environmentally friendly process and reduced production of hazardous matter [35]. Silver nanoparticles synthesis was indicated by colour changed from yellow to grey due to reduction of silver nanoparticles within 7 days. UV, XRD and SEM were used to characterize the silver nanoparticles. Characterization of synthesized silver nanoparticles was performed with slight modifications in the methodology of [2].

Stability and formation of AgNPs in methanol, ethanol and aqueous solution were confirmed by using UV-Vis spectroscopy (Figure 1 A-D). It is usually documented that UV-Vis spectroscopy could be used to examine the shape and size of nanoparticles [36]. The crystalline nature of silver nanoparticle was confirmed by X-ray diffraction dimension (Figure 2 A-D). Comparison of XRD spectrum with the standard confirmed that the silver particles formed in the form of nanocrystals, as evidenced by the peaks at 20, 25, 30, 40 values corresponding to 111, 220, 311 and these results are in accordance with the findings of literature [37]. Further confirmation of the silver nanoparticles synthesis was proved by the Scanning Electron Microscopy (SEM) which has shown the size as well as shape

of the silver nanoparticles. The shape was found to be rough and the size was 297 nm.

Bioassays have proved to be good tools for the biological evaluation of plant extracts and their nanoparticles. Crude botanical extracts comprised of very effective mixtures of bioactive compounds which can be analysed by simple bioassay procedures and various separation techniques [38]. In the present study, nanoparticles from different extracts (methanol, ethanol, aqueous) were prepared and evaluated for their biological activities like antimicrobial, cytotoxic, antioxidant and antitumour activities.

Brine shrimp cytotoxicity assay was a useful probe for the pharmacological activities in plant extracts [39]. In this study different fractions of *M. alba* roots extract were analysed as compared to control roots. All the nanoparticles from different extracts showed higher cytotoxic activities as compared to controls (Figure 5). Many prenylated flavonoids have been isolated from *Morus indica* root bark [40] and silver nanoparticles synthesized reported the strong cytotoxic activity.

In the antimicrobial assay, nanoparticles from all root extracts showed strong activities against all bacterial strains tested whereas control plant extract showed less antibacterial activities (Table II). Adavallan K *et al.* reported antibacterial activity of gold nanoparticles of *M. alba*, but there are no reported studies on antibacterial activity of silver nanoparticles so far [37]. The synthesized AgNPs from *M. alba* showed strong antifungal activity against all the tested fungal strains (Table III). This is the first report of antifungal activity of silver nanoparticles from *M. alba*. However, Hafez RA *et al.* reported antifungal activity of silver nanoparticles synthesized from *Morus nigra* [7]. The result suggests that silver nanoparticles from root extracts of *M. alba* can be a good source of antifungal products. It can serve as an increased plant antimicrobial defence and increased synthesis medicinally important secondary metabolites.

Antitumour potato disc assay is a valuable tool, which indicates the antitumour activity of test compounds. The present study revealed moderate to high degree of inhibition of tumour formation in case of all extracts tested (Figure 4). Silver nanoparticles presented significant inhibition of tumour formation especially the methanolic extracts showed the highest activity as compared to all other extracts (ethanol, aqueous). This is the first report of antitumour activity of silver nanoparticles of *M. alba* as there are no reported studies on antitumour activity.

The DPPH free radical scavenging assay was used to evaluate the antioxidant potential of the samples. This assay has been used for investigating antioxidant properties of wheat, vegetables, herbs, edible seed oils, conjugated linoleic acids, and flours by using different organic solvent systems including ethanol, aqueous acetone, methanol, aqueous alcohol

and benzene [41-45]. It is a non-enzymatic method currently used to provide basic information about the ability of compounds to scavenge free radicals. Reduction of DPPH by an antioxidant results in the loss of absorbance at 517 nm [46]. Significant DPPH scavenging activity was observed in all synthesized silver nanoparticles from different extracts of *M. alba* (Figure 3). Silver nanoparticles synthesized from methanolic extracts showed more pronounced antioxidant activity compared to other extracts. Our results are in accordance with other published data [47]. These results suggest that secondary metabolites in the root extracts of *M. alba* may be responsible for this enhanced antioxidant activity.

Conclusions

The present study reported the simple and successful method for the synthesis of silver nanoparticles by using *M. alba* roots extract. Advantage of the synthesis of silver nanoparticles using *M. alba* root extracts is that it is efficient, economical, cost effective and environmentally friendly. The present study and data showed that silver nanoparticles from the root extract of *M. alba* was found to have best biological activities as compared to control extracts and secondary metabolites can be the cause for all these activities. The insight of this study has made possible in conclusion that nanoparticles synthesized from *Morus alba* plants can be used to treat multiple disease and meet the increasing demand of drug manufacturers for its multi pharmacological importance.

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Conflict of interest

The authors declare no conflict of interest.

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