(REFEREED RESEARCH)

LIQUIDAMBAR ORIENTALIS MILL. LEAF AQUEOUS EXTRACT FOR THE SYNTHESIS OF SILVER NANOPARTICLES AND IMMOBILIZATION ON TEXTILE FABRICS FOR BIOMEDICAL APPLICATIONS

LIQUIDAMBAR ORIENTALIS MILL. YAPRAĞI SULU EKSTRAKTI İLE GÜMÜŞ NANOPARTIKÜL SENTEZI VE BİYOMEDİKAL UYGULAMALAR İÇİN TEKSTİL LİFLERİNE İMMOBİLİZASYONU

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ABSTRACT

Biosynthesis of silver nanoparticles (AgNPs) are often applied to textiles for their strong antimicrobial activity and have potential use in various application. In this paper, a study has been performed to identify the bioreduction of silver nitrate ($AgNO_3$) by the leaf of *Liquidambar orientalis* Mill. plant in Turkey and its application on cotton (Co) and cotton/polyester (Co/PES) fabrics. Characterization of silver AgNPs was made by using UV-visible spectroscopy, Fourier Transform Infrared (FTIR) spectrums and Transmission Electron Microscope (TEM) images while Scanning Electron Microcopy (SEM) was used to characterize the surface of textile fabrics. The inhibition zones were studied by using *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* bacteria to determine antimicrobial activity of both AgNPs and AgNPs loaded fabrics. The UV–Vis spectra gave surface plasmon resonance at 450 nm for synthesized AgNPs and nanoparticle synthesis was completed within 1 min. *In vitro* antimicrobial activities against gram (+) (*Staphylococcus aureus)* and gram (-) (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae)* bacteria of AgNPs and AgNP immobilized cotton and cotton/PES fabrics were demonstrated. SEM images of treated fabrics indicated silver nanoparticles were well dispersed on the surfaces of specimens.

Keywords: *Liquidambar orientalis* Mill., Plant leaf aqueous extract, Silver nanoparticle, Antimicrobial textiles, Biomedical application.

ÖZET

Gümüş nanoparçacıkların (AgNPler) biyosentezi farklı uygulamalarda kullanma potansiyeline sahiptir ve gümüş nanopartiküller güçlü antimikrobiyal aktiviteleri nedeniyle tekstil mamüllerine sıkça uygulanmaktadır. Bu yayında, Türkiye'de bulunan *Liquidambar orientalis* Mill. bitkisinin yaprağı tarafından gümüş nitrat biyoindirgenme performansı ve onun pamuk (Co) ve pamuk/poliester (Co/PES) kumaşlardaki uygulanabilirliği araştırılmıştır. Gümüş nanoparçacıkların karakterizasyonunun belirlenmesinde UV-Görünür Spektroskopi, Fourier Transform Infrared (FTIR) Spektroskopi ve Geçirimli Elektron Mikroskobu (TEM) kullanılırken; tekstil kumaş yüzeylerinin karakterizasyonunda ise Taramalı Elektron Mikroskobu (SEM) kullanılmıştır. AgNPler ve AgNP yüklü kumaşların antimikrobiyal özelliklerinin belirlenmesinde *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* bakterileri kullanılarak inhibisyon çapları araştırılmıştır. UV-Görünür Bölge Spekrumu sentezlenen AgNPlar için 450 nm'de yüzey plazmon rezonansı vermiştir ve nanoparçacık sentezi 1 dakika içinde tamamlanmıştır. AgNPler ve AgNP immobilize pamuk ve pamuk/PES kumaşların gram (+) (*Staphylococcus aureus)* and gram (-) (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae)* bakterilere karşı *in vitro* antimikrobiyal aktiviteleri gösterilmiştir. İşlem görmüş kumaşların SEM görüntüleri, gümüş nanoparçacıkların örneklerin yüzeylerine dağılımlarının iyi olduğunu göstermektedir.

Anahtar Kelimeler: *Liquidambar orientalis* Mill. , Bitki yaprağı sulu özütü, Gümüş nanoparçacık, Antimikrobiyal tekstiller, Biyomedikal uygulama.

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INTRODUCTION

Liquidambar orientalis Mill. tree is commonly known as 'Sığla Tree' or 'Storax Tree' in Turkey and has a local distribution in the south-western coastal district of Turkey [1]. The plant (*Liquidambar orientalis* Mill.) is an endemic species in Turkey belonging to *Hamamelidaceae* family. The most productive sites for *L.orientalis* is soils rich in surface waters during summer months [2-3]. *Liquidambar orientalis* Mill. is found in Southwest Anatolia mainly Marmaris, Köyceğiz, Fethiye, Ula and Cine-Bucak lying between Aydın, Denizli, Burdur, Antalya and Isparta and storax which is the gum like exudate of this plant was used for pharmaceutical effects in Turkish folk medicine. A literature survey reveales that many researches were conducted for its traditional medicinal purposes [4], antibacterial activity of storax [2,5,6], heartwood [7,8], and antimicrobial, antioxidant, mutagenic, and antimutagenic activities and the phenolic compounds of the ethanolic extract of the leaves of Liquidambar orientalis Mill. [9-11]. Biomedical applications of nanotechnology has become one of the most important field in the past decades. Inert, functionalized, and biocompatible nanomaterials, AgNPs have attracted considerable attention due to their potential in biomedical analysis and detection [12-14], tissue engineering applications [15-17]. Another important application of silver nanoparticles is in hygiene textiles to protect the consumers againts the diseases or odor causing microorganisms [18-20]. Most importantly, hygiene textile is needed in places like health care settings, schools, hotels or any other crowded public areas where protection from diseases is a growing concern. Hence, the use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. and posses low toxicity to human cells, high thermal stability and low volatility [21]. The antimicrobials have different modes of action. Silver cause bacterial cell death by multiple mechanisms. Two of them are reported as the strong binding of Ag+ with disulfide (S–S) and sulfhydryl (−SH) groups found in the proteins of microbial cell walls, and Ag+ ions disrupting normal metabolic processes by displacement of essential metal ions such as Ca^{2+} or Zn^{2+} [18, 22]. The antimicrobial property of silver nanoparticles is efficient due to their extremely large surface area. Additionally, their action occurs possible by three physical and chemical properties. These can be counted as follows: first, attachment to the cell membrane and disturb its proper function, like permeability and respiration, second, penetration inside the bacteria and causing further damage by possibly interacting with sulfur- and phosphorus containing compounds such as DNA, and third, nanoparticles release silver ions, which will have an additional contribution to the bactericidal effect of the silver nanoparticles [18, 21, 23]. Production of nanoparticles through chemical methods may contain toxic chemicals in the synthesis protocol. There is a growing need to develop environmentally friendly process approaches of nanoparticle synthesis that do not use toxic materials [24]. Biological methods of silver nanoparticle synthesis using plant extract have been studied as a possible eco-friendly "green" methods alternative to chemical and physical methods [21, 24-27]. Various plant extracts have been reported as mediator in ion reducing process to form AgNPs [28]. The leaves of Pine (*Pinus desiflora*), Persimmon (*Diopyros kaki*), Ginkgo (*Ginko biloba*), Magnolia (*Magnolia kobus*) and Platanus (*Platanus orientalis*) [24], *Mentha piperita* (Lamiaceae) [25], *Aloe* V*era* [26], *Coleus amboinicus* Lour [27], parsley (*Petroselinum crispum*) [28], carob (*Ceratonia siliqua*) [29], *Clitoria ternatea* and *Solanum nigrum* [30], *Ficus microcarpa* [31], banana *(Musa balbisiana)*, neem *(Azadirachta indica*) and black tulsi *(Ocimum tenuiflorum)* [32], Tulsi (*Ocimum sanctum*) [33], *Aerva lanata* [34], fig (*Ficus carica*) [35], *Asiatic Pennywort* and *Bryophyllum* [36].

In this paper, the results of AgNPs synthesis by a "green process" using *Liquidambar orientalis* Mill. leaf aqueous extract as reducing agent were reported for the first time [37, 38]. UV-Visible spektrophotometer, FTIR, and TEM measurements were performed to characterize the nanoparticles. Cotton and Cotton/PES fabrics were coated with AgNPs. Their antimicrobial activity against *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumoniae), Staphylococcus aureus (S. Aureus),* and *Candida albicans (C. albican*s) were evaluated by both hole and disk diffusion methods for AgNPs while only disc diffusion method was used for textile fabrics. SEM analysis were used to detect the changes on the surface of the fabrics.

MATERIALS AND METHODS

Materials

Silver nitrate ($AgNO_3$) was purchased from Merck and used as received without further purification. Scoured and bleached woven 100 % cotton and 50 % cotton/ 50 % Polyester (PES) fabrics used in this work have the weights of 135 g/m^2 and 153 g/m^2, respectively. Sığla tree (*Liquidambar orientalis* Mill. Tree) leaves were obtained from Marmaris, Turkey. *E. coli* ATCC 25922; *S. aureus* ATCC25923; *P. aeruginosa* ATCC 27853 *K. pneumoniae* ATCC13883; *C. albican*s ATCC 10231; methicillin resistant *S. aureus* ATCC 29213 microbial strains were tested for their antimicrobial activities. Müller Hinton Agar and Brain Heart Infision broth were purchased from Oxoid and Merck, respectively. Cotton and cotton/Polyester (PES) fabrics were obtained from a local producer in Turkey. Distilled water was used throughout the research.

Liquidambar orientalis Mill. Leaf Extract Preparation

Plant leaves were collected, thoroughly washed, and dried for one week at room temperature. The broth solution was prepared by boiling an 18 gr of squashed dry leaves in 240 mL of distilled water for 15 minutes before decanting. The resulting extract was filtered and daily prepared solutions was used in the experiments throughout the research.

Synthesis of Silver Nanoparticles

A known amount of leaf extract (10 mL, 7.5 % w/v) was added to the mixture of 10 mL of 10^{-2} M aqueous $AgNO_3$ and 5 mL 25 % Ammonia solution and made up to 100 mL of distilled water. The formation of silver nanoparticles was followed by UV-Vis absorbance measurements in the range of 250-800 nm and Uv-Vis absorption spectra of silver nanoparticle formation were taken every 30 min until no absorbance change was observed.

UV-Vis Absorbance Spectroscopy Studies

The reduction of silver nitrate to silver manoparticle was followed by absorbance measurements on a Labomed Inc. UV-VIS Double Beam PC& 8 Scanning Auto Cell spectrophotometer at a resolution of 1 nm.

Fourier Transform Infrared Spectroscopy (FTIR) Measurements.

FTIR measurements of the dried *L. orientalis* leaf extract and synthesized silver nanoparticles using potassium bromide (KBr) substrates were carried out on a Thermo-Scientific iS10 Fourier transform infrared spectrometer (FTIR) at a resolution of 4 cm^{-1}.

TEM Measurements

TEM measurements of AgNP's were performed on a JEOL model FEG TEM instrument operated at an accelerating voltage of 200 kV.

Antimicrobial activity measurements

Antimicrobial activity of AgNP's were tested against four different microorganisms (i) *E. coli*; (ii) *S. Aureus*; (iii) *P. aeruginosa*; and (iv) *K. pneumoniae*. Agar-well and disc diffusion methods were used [39-41]. The pathogenic cultures were inoculated in Brain Heart Infusion broth at 37°C for 24 hours and then aliquot of 1 ml bacterial cultures was evenly spreed at 1 % ratio (10^6 CFU/ mL) on petri dishes containing Müller Hinton Agar at 45-50°C. Sterile empty antibiogram dicsc of 6 ± 0.5 mm diameter (Whatman No:1) are impregnated with thorougly vortexed 30 μL AgNPs solution and placed on the culture medium. In the case of hole diffusion method, a 6 mm core of agar was removed from the surface of the seeded agar with the help of a gel puncture and thorougly vortexed 30 μL of AgNPs solution was applied. All petri dishes were incubated at 37°C for 24 h after maintained 2 h at 4°C [41-43]. The diameters of the inhibition zones were measured by vernier caliper [42-44].

Immobilization of silver NPs on textiles and their antimicrobial properties were examined with 100 % and 50 % cotton/ 50 % polyester fabrics. Each cotton fabric was put into an Erlenmeyer flask containing 30 ml leaf extract and 30 ml solutions of 10^{-2} M aqueous $AgNO_3$ separately and kept at room temperature on water bath shaker for 24 hour. After 24 hour, the samples were rinsed and dried at 25 ºC temperature [45]. Antimicrobial properties of the silver nanoparticles (AgNP) loaded cotton and cotton/PES fabrics were tested with five different microorganisms (clinical strains), *E. coli, P. aeruginosa, K. pneumoniae, S. aureus* and *C. Albican*s, according to the disc diffusion method with some modifications [46]. Untreated, treated cotton fabric samples and gentamycin control discs shaped with 7 ±1 mm diameter were placed on the surface of the inoculated plates. The plates were incubated at the 37°C temperature for 24 hours and the diameter of inhibition zone (mm) including the disc diameter was measured for each sample [45]. The structure of the treated and untreated cotton fabrics were studied by scanning electron microscopy (SEM) using Phillips XL-30S FEG device.

RESULTS AND DISCUSSION

UV-Visible spectral studies

The present investigation demostrates the formation of silver nanoparticles by the exposure of silver nitrate to the plant leaf, *L. orientalis* Mill. water extract. Silver nanoparticles have been prepared at room temperature by mixing silver nitrate solutions and leaf extracts collected from plant. Evolution of the silver nanoparticles starts a minute after adding extract to the $AgNO_3$ solution and is indicated by the beginning evolvement of a characteristic surface plasmon extinction band. The reduction of silver nitrate and nanoparticle formation was monitored by UV-Visible Spectroscopy. The surface plasmon absorption of these Ag(0) particles is presented in Figure 1. As Fig. 1 shows, the UV–vis spectra was recorded from the aqueous silver nitrate – *L. orientalis* Mill. broth reaction medium as a function of time of reaction. The kinetics of silver nanoparticle formation was given for 2, 30, 60, 180, and 480 minutes and the plasmon absorption band is in the range of 380-470 nm. It is observed that the silver surface plasmon resonance band peak was centered at 450 nm and intensity do not increase too much as a function of reaction time. The colour of plant leaf extract, brownish yellow changed to dark brown which indicates the formation of silver nanoparticles (not shown). It is well known that silver nanoparticles exhibit yellowish-brown in water due to excitation of surface plasmon vibrations in the metal nanoparticles [47].

It was observed that the reduction of silver ions into silver nanoparticles started and completed immediately at room temperature. This behaviour greatly indicates the rapid biosynthesis of silver nanoparticles [29]. The spectrum also indicates that the nanoparticle formation was rapidly completed without nanoparticle aggregation since background increases over 450 nm did not observed in the UV-Visible spectrum [48]. Additionally, the spectrum greatly indicates that all the $AgNO_3$ was converted to metallic silver in the presence of 7.5 % w/v *L. orientalis* Mill. leaf broth.

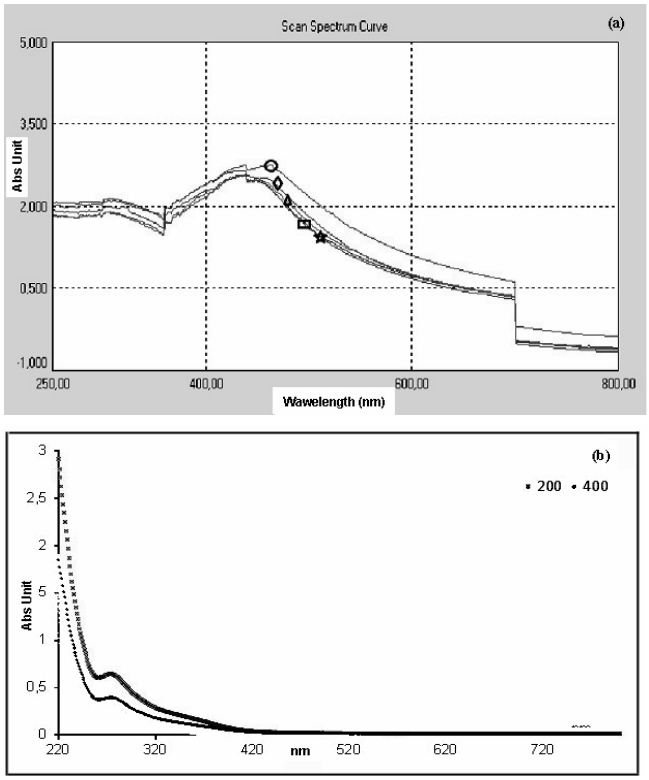


Figure 1. **(a)** UV-Visible spectrum analysis, Plasmon resonance of silver nanoparticles reduced by *L. orientalis* Mill. at 430 – 450 nm. The time intervals are: 1 min; 30 min; 60 min; 180 min; 480 min ; **(b)** UV-Vis spectra of *L. orientalis* Mill. leaf broth a) 200 times, b) 400 times diluted.

The initial broth concentrations were also tested from 0.25 % up to 30% w/v broth concentrations by various researches and biosynthesis was conducted at various time intervals [25,26,47-52].

Figure 1b shows the UV-Vis spectra of of *L. orientalis* Mill. leaf broth which showed maximum at 274 and 225 nm wavelenghts. The maximum peak absorbance indicates the presence of polyphenolic content or glycosidically bound aromatic compounds in the water extract [53-55].

FT-IR spectra of *L. orientalis* Mill. extract and AgNPs

FTIR analysis was used for the characterization of the leaf extract and the resulting silver nanoparticles. Figure 2a shows the FTIR spectrum of the plain *L. orientalis* leaf water extract which have peaks at 3291, 2918, 2846, 1698, 1626, 1390, 1318, 1225, 1036, and 821 cm^{-1}. In the characterization of the tannins, the vibrational frequencies at 3300–2500 cm-1 were attributed to OH stretching of H-bonded OH groups in ATR-FTIR spectrum [56]. The distinctive spectral frequencies for the C—H and C—C stretching of aromatic compounds of tannins were reported as 2800-3000 cm^{-1} and 1619-1630 cm^{-1}, respectively. A distinctive frequency at 1626 cm^{-1} was detected for a catechol moiety of a condensed tannin, catechin [56]. In a research, it was reported that the vibrational spectral frequency at 1036 cm^{-1} indicated the presence of C — O stretching ether groups. It was also claimed that the band at 1315 and 1037 cm^{-1} in the spectrum of tannin belongs to phenol groups. FT-IR peak values of *Semecarpus anacardium* chloroform leaf extract were found at 1222.85 cm^{-1} for C-N bound of amines and amides which has the group frequency between 1180-1360 cm^{-1} [57]. The deformation vibrations of the C—H bond in the benzene rings also give small absorption bands in the 906-748 cm^{-1} range [58]. The FTIR spectrum displayed absorption band at 1698 cm^{-1} for ester carbonyl functionality [59].

FTIR spectra of AgNPs solution was shown in Figure 2b. The peaks for silver nanoparticles stabilized by *L. Orientalis* was observed at 3172, 2078, and 1030 cm^{-1}. The signal at 1225 cm^{-1} of asymmetric stretching vibration of sulfate group and at 821 cm^{-1} of sulfate groups attached to a primary hydroxyl group disappeared after synthesis of AgNPs. The broad peak at 1030 cm^{-1} suggests that sulfate groups play role in reducing and stabilizing AgNP [60]. Additionally, it was reported that this broad peak also denotes the ether linkage (-C-O-C-), germinal methyls (-C-O-), –C=C- groups from aromatic rings and alkyne bonds, respectively. Structures like flavonoids and terpenoids compounds have stretching vibrational bands at indicated wavelenghts [49, 61-64].

TEM analysis results

Figure 3a-b shows representative transmission electron microscopy (TEM) images of the nanoparticles synthesized using the defined amount of *L. Orientalis* Mill. leaf extract for 6 h and it can be seen that the products are predominantly nearly spherical nanostructures. Cylindrical shaped nanoparticules were also estimated. Most of the nanoparticles were aggregates with only a few of them were scattered, as observed under TEM. The size rage of the synthesized AgNPs was between 50-150 nm as can be seen in the figure. The product was analyzed by an Energy Dispersive Spectroscope (EDS) analysis and the presence of elemental silver was confirmed at 3 keV in the silver region (Figure not given). It was indicated that silver was the major product and that carbon could be detected in the particle. To the best of our knowledge, this is the first report on the synthesis of silver nanoparticles using the plant, *L. orientalis* Mill. leaf extract.

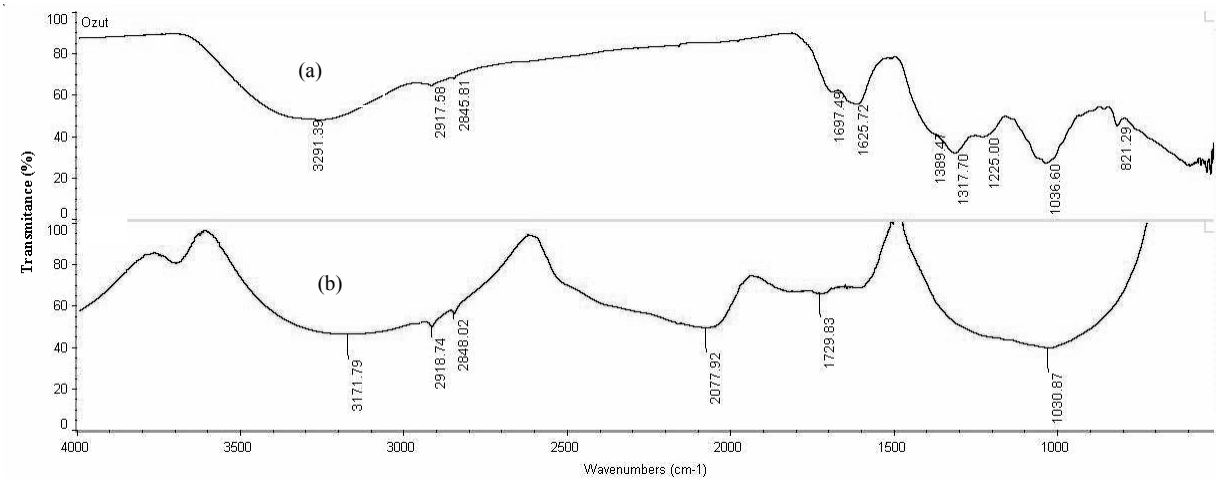
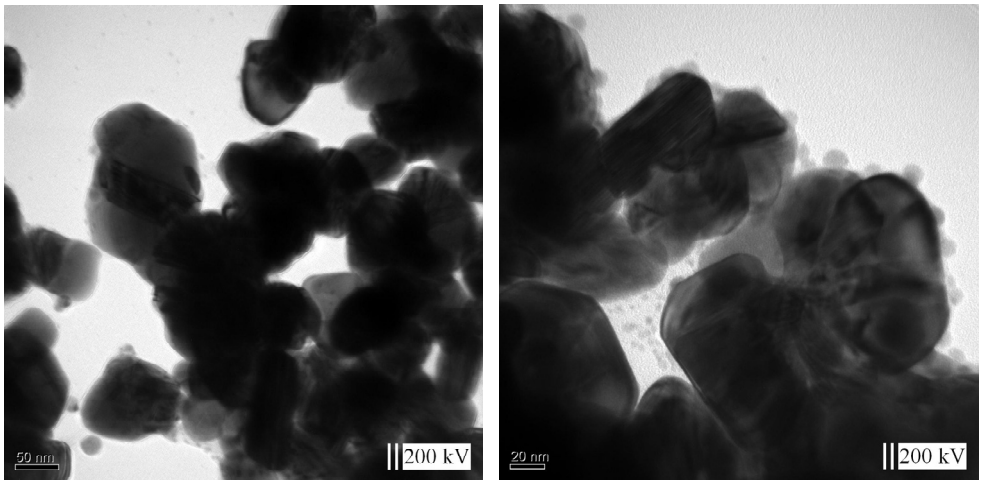


Figure 2. FTIR spectrum of (a) Plain *L. orientalis* Mill. leaf extract and (b) silver nanoparticles synthesized using Plain *L. orientalis* Mill. leaf extract



(a) (b)

Figure 3. HR-TEM EDS analysis data of AgNPs a,b) TEM image of AgNPs

In-vitro antimicrobial activity measurements

The effect of AgNps on microbial growth was studied by incorporating AgNps either as disc or well-diffusion method in Müller Hinton agar plates that were inoculated with 10^6 CFU/mL from the strains, *E. coli, S. Aureus, P. aeruginosa,* and *K. Pneumoniae*. The antibacterial activity of silver nanoparticles against *S. Aureus* was highest than that of other bacterial strains as shown in Table 1. The variation in cell wall composition between Gram positive (*S. Aureus*) and Gram negative (*E. coli*) bacteria may cause this antimicrobial difference. The antibacterial activities varied for other tested Gram negative bacteria and also showed lesser antibacterial activity both in the case of *P. aeruginosa* and *K. Pneumoniae*, respectivelly compared to that of *E. coli*. This observation may be due to morphological differences of bacteria which lead to surface area variations available to interact with or high lipid content of outer membrane of gram (-) bacteria [65]. It was identified by Morones that nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its functions like permeability and respiration. Additionally, they are able to penetrate inside the bacteria and cause further damage by possibly interacting with biological molecules such as DNA through sulfur and phosphorus groups [23,66]. Antibacterial activity of silver nanoparticles by leaf and callus extracts from Sesuvium portulacastrum, with and without PVA was tested against clinical bacterial strains of PA-*Pseudomanas aeruginosa*, KP-*Klebsiella pneumoniae*, SA-*Staphylococcus aureus*, LM-*Listeria monocytogenes*, ML-*Micrococcus luteu*. It was observed that inhibition zone formation varied with test microbes and type of extracts [61].

The antimicrobial activities of the untreated and treated cotton and cotton/PES fabrics tested against gram (+) (*S. Aureus),* gram (-) (*E. coli, P. aeruginosa, K. pneumoniae)* bacteria and fungus (*C. Albicans*). The results of the quantitative antimicrobial assessment by disc diffusion method of the fabric samples loaded with silver nanoparticles indicated an inhibitory effect against microorganisms which inhibition zones were shown in Table 1. Inhibition zones show higher antimicrobial activity for cotton/PES fabrics while antifungal activity was smaller when tested against *C. Albicans*. The gram (+) bacterial strain of *S. Aureus* produced the minimum inhibition zone diameters when treated with AgNPs loaded cotton and cotton/PES fabrics on the contrary to free AgNPs. The cocci shape of this bacterial strain may exhibit less contacting area with the AgNPs on the surface of the fabrics compared with that of the rod shape bacterial strains.

Table 1. Antimicrobial assessment of the AgNPs and AgNPs loaded fabrics.

Microorganisms	Inhibition zones (mm)					
	AgNPs	Gentamycin (Control)	AgNPs loaded cotton fabrics !	Gentamycin (Control)	AgNPs loaded 50 % cotton/50 % PES fabrics !	Gentamycin (Control)
E. coli	7.4 ± 0.2 ! 7.0 ± 0.4 !!	13.8 ± 1.2	8.3 ± 1.3	15.3 ± 1.7	8.6 ± 1.6	15.3 ± 1.3
S. aureus	7.3 ± 0.2 ! 7.9 ± 0.3 !!	13.6 ± 1.4	7.5 ± 1.3	14.5 ± 1. 5	8.5 ± 2.0	14.5 ± 2. 5
P. auroginosa	6.9 ± 0.2 ! 6.9 ± 0.3 !!	14.2 ± 1.8	9.8 ± 0.8	14.5 ± 2. 5	9.3 ± 2.5	14.5 ± 2. 5
K. pneumoniae	6.8 ± 0.3 ! 6.9 ± 0.3 !!	15.4 ± 0.4	8.3 ± 1.5	14.2 ± 1. 2	9.3 ± 2.5	14.2 ± 0. 8
C. albicans			7.5 ± 1.5		9.0 ± 1.0	
Weight differance %			5.42 ± 0.50		3.80 ± 0.71	

The values were given as the average of minimum three experimental results. !: Hole method, and !!: Disc diffusion method results

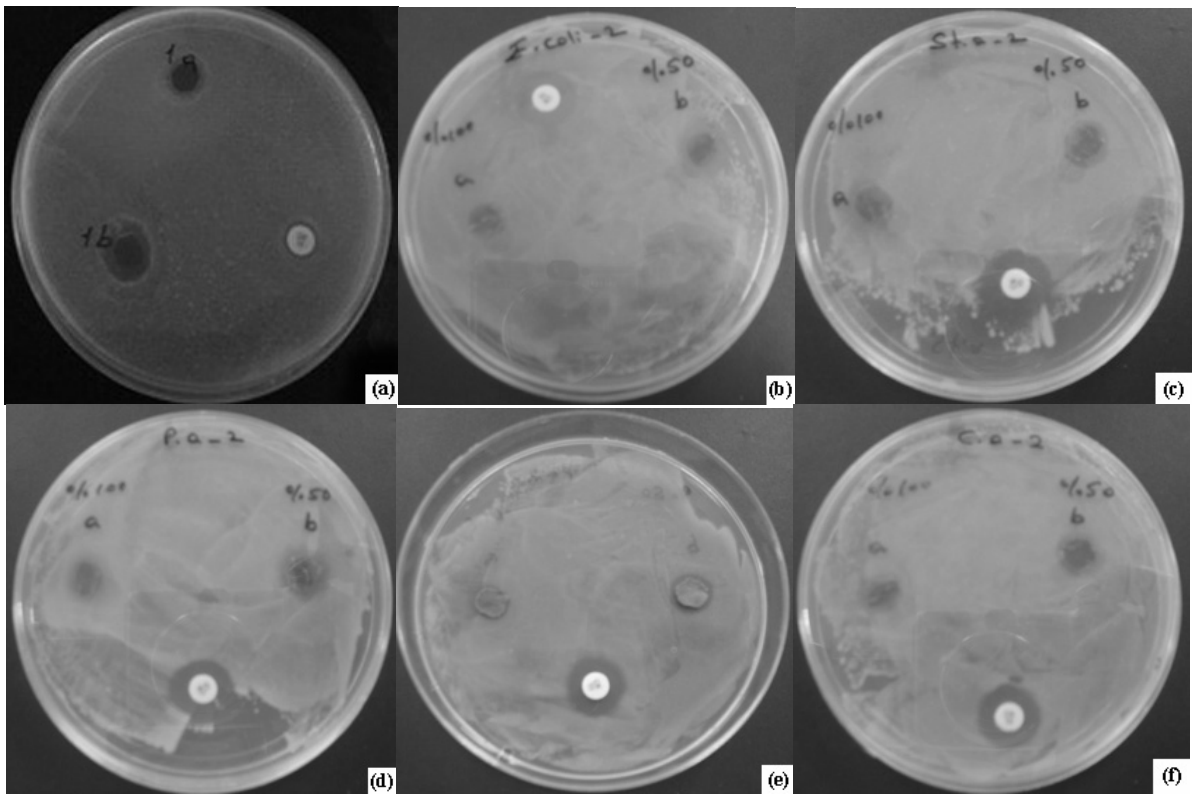


Figure 4. Antibacterial activity of **(a)** AgNPs against E. Coli, and AgNPs loaded cotton and Cotton/PES fabric samples and gentamycin disc (white disc) against **(b)** E.coli, **(c)** S. Aureus, **(d)** P. Auroginosa, **(e)** K. Pneumoniae, **(f)** C. Albicans. 100 % cotton and 50/50 % Cotton/PES fabrics were indicated by (a) and (b) notations.

The photograph of inhibition zone formation of AgNPs was only given against *E.coli* as in Fig 4A. Figure 4B-F shows the inhibition zone tests carried out on petri plates both for untreated and treated cotton and cotton/PES fabrics. There were a significant colour change in cotton and cotton /PES fabric samples after AgNP impregnation. The cotton/PES containing fabrics immobilized with silver nanoparticles showed larger inhibition zone than fabrics containing only cotton. This may be attributed to the surface availability due to PES or hydrophilicity difference in fabrics due to the presence of hydrophobic polyester structure.

The morphologies of untreated cotton and cotton/PES fabrics as well as cotton and cotton/PES fabrics loaded with AgNPs were shown in Figures 5. Scanning electron microscopy (SEM) micrograph analysis confirmed the Ag-NPs embedded on to the fabrics. The fiber surface of the cotton fabrics has covered by nearly evenly distributed AgNPs but a kind of agglomeration occured on cotton/polyester fabrics. The persistency of antimicrobial activity of the cotton and cotton/PES fabrics was tested after one and ten washing process and the results were reported elsewhere [67]. The treating of cotton and polyester fabrics with silver nitrate aqueous solution imparts antibacterial activity against Gram-positive, *S. aureus* and Gram-negative bacteria, *E. Coli* [68]. It was observed that the zone of inhibition of Gram-positive bacteria was greater compared to that for Gram-negative bacteria by Wasif and Laga [69,70]. The loading of fabrics with silver nanoparticles needs to be optimized due to significant colour change in fabrics after treatment and research experiments continued in the scope of this purpose [67].

CONCLUSIONS

The present study reports the synthesis of AgNPs using the leaf of *Liquidambar orientalis* Mill. plant. High reduction rate to AgNPs was estimated and conversion was completed within one minute in various sizes and shapes. UV-vis spectroscopy, TEM, EDS and SEM analysis were performed to characterize the free and immobilized AgNPs. FTIR analysis depicted the capping and stabilization properties of nanoparticles coming from bioresource. Both free and immobilized AgNPs on cotton and cotton/PES fabrics showed bactericidal activity againts both gram (-) and gram (+) nozocomial four bacterial strains. So, it can be concluded that *L. orientalis* Mill. leaf water extract can be applied as cheap and environment-friendly bioresource/biomaterial for the synthesis of silver nanoparticles with antimicrobial activity. However, more detailed research studies for plant material structure effect on reduction of silver nitrate and particle size variation besides activities of AgNPs on cellular level may help to elucidate related scientific areas.

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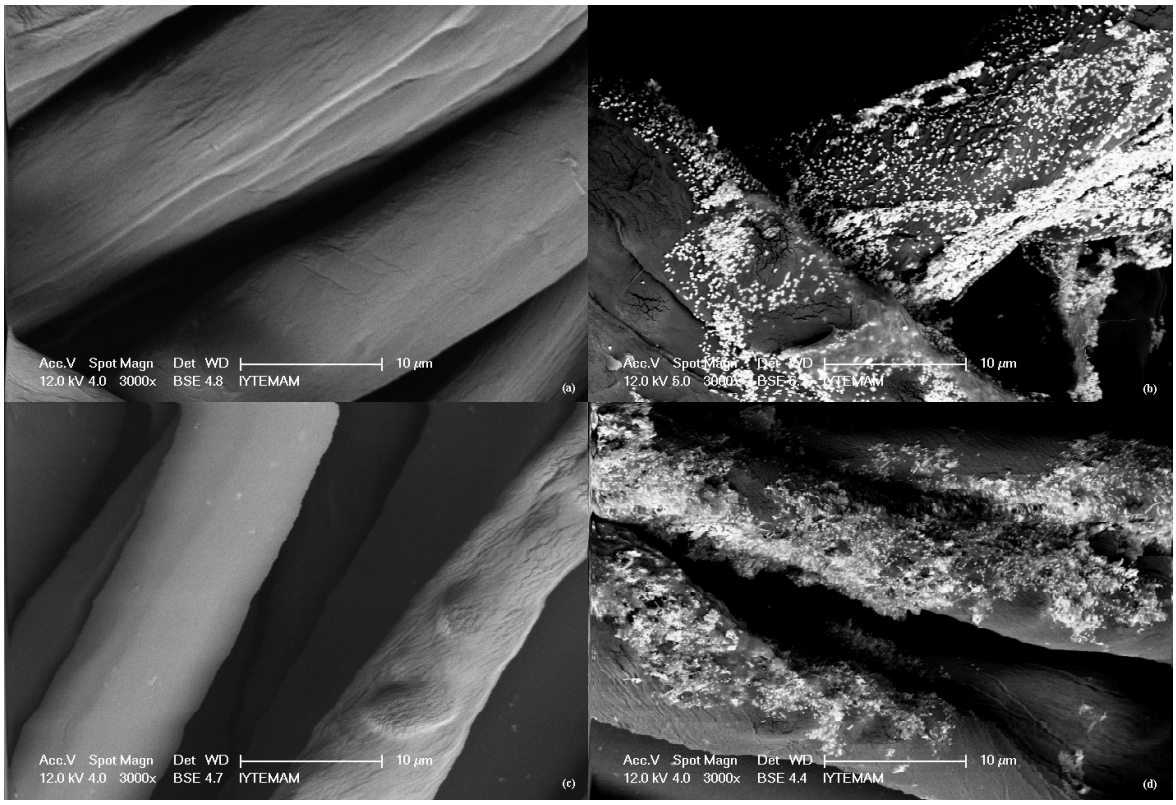

Figure 5. SEM micrographs of a) untreated cotton fabrics, b) treated cotton fabrics. c) untreated cotton/PES fabrics, d) treated cotton/PES fabrics.

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