Antimicrobial effect of Silver nanoparticales produced by bark of *salix alba* on growth of two species of humans pathogenic bacteria

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Abstract

In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO3 solution through the extract of *Salix alba* leaf extract as reducing agent as well as capping agent. Nanoparticles were characterized using uv-vis absorption spectroscopy . These biologically synthesized nanoparticles were found to be highly toxic against different bacterial spp.The effect of ethanolic and nanoparticales extract of *salix alba* on growth of two clinical isolates and they are *staphylococcus aureus* (Gram positive*) ,Pseudomonas aeroginosa*(Gram negative).The hieghest effect of the ethanolic extract observed on *Staphylococcus aureus* by inhibition zone(16mm), then *Pseudomonas aeroginosa* by inhibition zone(10mm) , but when nanoparticales extract was used the antimicrobial effect inhibition zone was increased of both pathogenic species by inhibition zone (27mm) in *Pseudomonas aeuroginosa* and (22mm*)* in *staphylococcus aureus*.

Introduction

The development of new resistant strains of bacteria to current antibiotics [1] has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [2].

Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes[3]. In the present study, reducing silver ions present in the aqueous solution of silver nitrate by the help of *Salix alba* extract and their antibacterial assessment was performed to produce novel drugs to overcome drug resistance and adverse reaction

The noble metals especially gold and silver due to their in numerable applications in different branches such as catalysis, photonics, photography and more importantly in the field of medicine as antimicrobial factors have drawn much attentions to themselves[4].In addition colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical

stability,catalytic and antibacterial activity [5].Silver nano-particles have many important applications which include, spectrally selective coating for solar energy absorption and intercalation material for electrical batteries, as optical receptors, polarizing filters, catalysts in chemical reaction , biolabelling and as antimicrobial agents[6].

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [7]. Nanotechnology concerns with the development of experimental processes for the synthesis of nanoparticles of different sizes,shapes and controlled dispersity[8]. This provides an efficient control over many of the physical and chemical properties[9] sensingdevices[10],catalysis[11] and medicine[12]. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols.[13].Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, enegy,temperature and toxic chemicals[14]. Medicinal plants have been a source of wide variety of biologically active compoundsfor many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. Relatively 1-10 % of plants are used by humans out of estimated 250,000 to 500,000 species of plants on Earth [15]. The plants are relatively cheap source of biological material having a vast variety of metabolites, primary or secondary, available in them for selecting the molecule of desired biological activity.

Aim of study

The aim of this study was to determine the differences between the effect of ethanolic extract and the effect nanoextract leaf of *Salix alba* on the growth of two pathogenic bacteria ,one is Gram positive and the other is Gram nigative bacteria by using silver nitrate.

Materials and method UV-Vis Spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the uv-vis spectrum of the reaction medium at 3 hours after diluting a small aliquot of the sample into distilled water. uv-vis spectral analysis was done by using uv-vis spectrophotometer

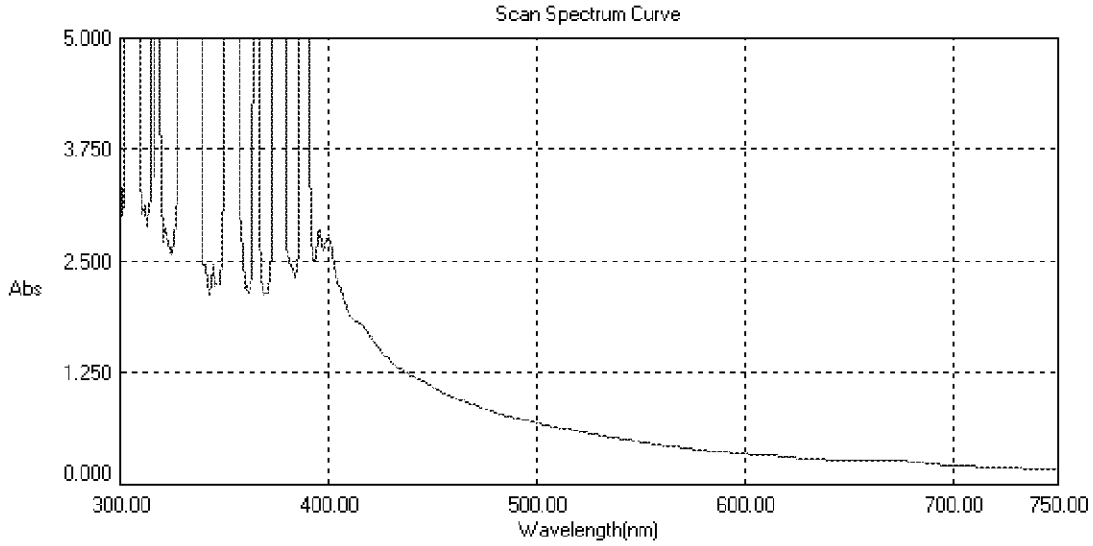

Fig. 1. UV-Vis absorption spectra of silver nanoparticles synthesized by Salix alba leaf extract after 2 hrs (Black) and 4 hrs (Red)

Extraction Methods

Plant barks commonly used in herbal medicine were dried and pulverized with motor and pestle or electric mill. The fine powder of plant parts were extracted with boiled ethanol by soxholet for 7 h. The solution was filtered through Whatman filter paper using a Buchner funnel under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 40°C to obtain the bark extract. Then, the resulting extract was stored, protected from light in a refrigerator at 4°C in a glass container until use.

Antimicrobial Assay

Antimicrobial activity of *S. alba* extract was determined by the agar-well diffusion method. two bacterial strains were used, *Staphylococcus aureus* (Gram positive), and *Pseudomonas aeruginosa*(Gram negative) .The antimicrobial activity was performed using nutrient agar for bacteria. The cell culture suspension was adjusted by comparing against 0.4-0.5 McFarland scale standard.

These suspensions (100 mL) of target strain were spread on the plates. For the investigation of the antimicrobial activity, the extract of *S. alba* were weighed and dissolved with distilled water to obtain 10, 20, 40, 60 or 80 mg mL^{-1} extract concentration (Table 1). Each sample (100 mL) was filled into the wells of agar plates directly. The diameter of the inhibition zone (mm) was measured after overnight incubation. All samples were tested in triplicate..

Synthesis of silver nanoparticles

5mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Salix alba* bark extract was added into 90 ml of aqueous solution of 5 mM Silver nitrate for reduction into Ag+ ions and kept at room temperature for 4 hour.

Results and discussion:

UV-Vis Spectra analysis

Reduction of silver ions present in the aqueous solutionof silver complex during the reaction with the ingredients present in the *Cleome viscosa* plant leaf extract have been seen by the uv-vis spectroscopy and found that uv-vis spectrograph of the colloidal solution of silver nanoparticleshas been recorded as a function of time by using quartz curette with water as reference. Maximum absorbance wasseen at 455nm,indicating that the formation of spherical silver nanoparticles in majority or anisotropic particles whose appearance and ratio increases with timebut the uv-vis spectra for the leaf extract alone showed noabsorption in the spectral window between400-700nm, similar to the work of [16], The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups [17].

The antibacterial activity of ethanolic extract

Ethanolic extractof bark of *Salix alba* which were investigated against various pathogenic bacteria of Gram positive bacteria *S. aureus* and Gram nigative strains *P.auroginosa* , using well diffusion tecnique..The highest antimicrobial activity to the extract was shown by inhibition zone in *S.aureus* (16mm)(fig. A1) and *P.aeuroginosa* (10mm)(fig. B1) .The obtained results have shown that the ethanolic extract of *salix alba* had important antimicrobial activity . This could be explained by the presence of flavonoide in this extract ,although the cell wall of gram positive bacteria is thick in comparision with the cell wall of gram negative bacteria ,it is more susceptible to external effect ,has been attributed to the external lipopolysaccharide wall that surrounds the peptidoglycan cell wall of the former or the susceptibility may be due to changes occur in mix systems which are responsible for the multi-resistance in bacteria ,or to changes occur in o-antigen which is one of the component of Lipopoly saccharides in the bacteria cell wall.or may be because of glucosides content in *Salix alba* bark extract especially sulicylic acid compound as well as salicinase and salicicoside which show antibacterial activity against *S.aureus* [18].

The antibacterial activity of silver nanoparticales(agNPs) extract

The reduction of the metal ions through leaf extracts of *Salix alba* leading to the formation of silver nanoparticles of fairly well-defined dimensions. In the present study we found that Salix can be also good source for synthesis of silver nanoparticles.The reaction started with in first hour of the incubation with silver nitrate (1 mM).This extract which were investigated against various pathogenic bacteria of gram positive (*Staph.aureus*) and gram nigative strains

P.auroginosa,.using well diffusion technique.The dimeter of inhibition zones around each well with AgNPs is represented in.The highest antimicrobial activity was observed against and *P.aeruginosa* (25mm)(fig.B2) *and S.aureus* (22mm)(fig. A2,).Silver has been used for its well known antimicrobial properties . This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications 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 (nanomaterials). The reduction of silver ions and stabilization of the silver NPs was thought to occur through the participation of Leaf proteins and metabolites. Most importantly, the reaction was simple and convenient to handle, and it is The nanosilver believed that it has advantages over other biological syntheses [19].The effect of nano-extract leaf may be because the phosphorylation of bacterials trypsine protein. As tyrosine phosphorylation in bacteria would lead to activation of various protein substrates like RNA polymerase sigma factors and UDP glucose dehydrogenases[20], decreased phosphorylation may reflect inhibition of activity of these enzymes with critical implications on bacterial growth. A recent report has described tyrosine phosphorylation of bacterial single-stranded DNA-binding proteins (BsSSB), ubiquitous molecules binding DNA invarious functional stages like replication and recombination [21]. Aphospho-signalling pathwayhas also been shown to be critical for bacterial cell cycle progression [22]. The identity of the substrate peptides and that of the putative tyrosine phosphatases responsible for observed dephosphorylation in gram-negative bacteria. The present findings, along with reported interactions of silver nanoparticles with thiol rich enzymes and bacterial genomic DNA [23] can explain the inhibitory effect of the nanoparticles on growth of gram negative bacteria.Interestingly, phosphorylation of protein tyrosine kinases involved in exo-polysaccharide and capsular polysaccharide biosynthesis and transport has been reported in a number of Gram negative and Gram positive bacteria [24].Silver nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets. The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. Silver nanoparticles act primarily in three ways against Gram-negative bacteria: ones nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration; seconed they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA; third nanoparticles release

silver ions, which have an additional contribution to the bactericidal effect of the silver nanoparticles [25]. Although bacterial cell lysis could be one of the reasons for the observed antibacterial property, nanoparticles also modulate the phosphotyrosine profile of putative bacterial peptides, which could thus affect bacterial signal transduction and inhibit the growth of the organisms. The effect is dose dependent and is more pronounced against gram negative organisms than gram-positive ones. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity [26] of nanoparticles towards human cells before proposing their therapeutic use .The nanosilver was found to have wider antimicrobial activity in *S.aureas* and *P.aeroginosa*. We believe that the silver nanoparticle has great potential for applications in catalysis, biomedical, and pharmaceutical industries.

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Table 1-Effect of ethanolic and nano-extract of leaf of *Salix alba* on humans pathogen.

Highest inhibition zone of nano-extrac in 80mg mL^{-1}	Highest inhibition zone of ethanolic extract in 80mg mL^{-1}	Disease	Name of pathogens
27 mm	10 mm	Urinary tract infection	*Pseudomonas aeruginosa*
22mm	16 mm	Wound Infections and abscesses	*Staphylococcus aureus*

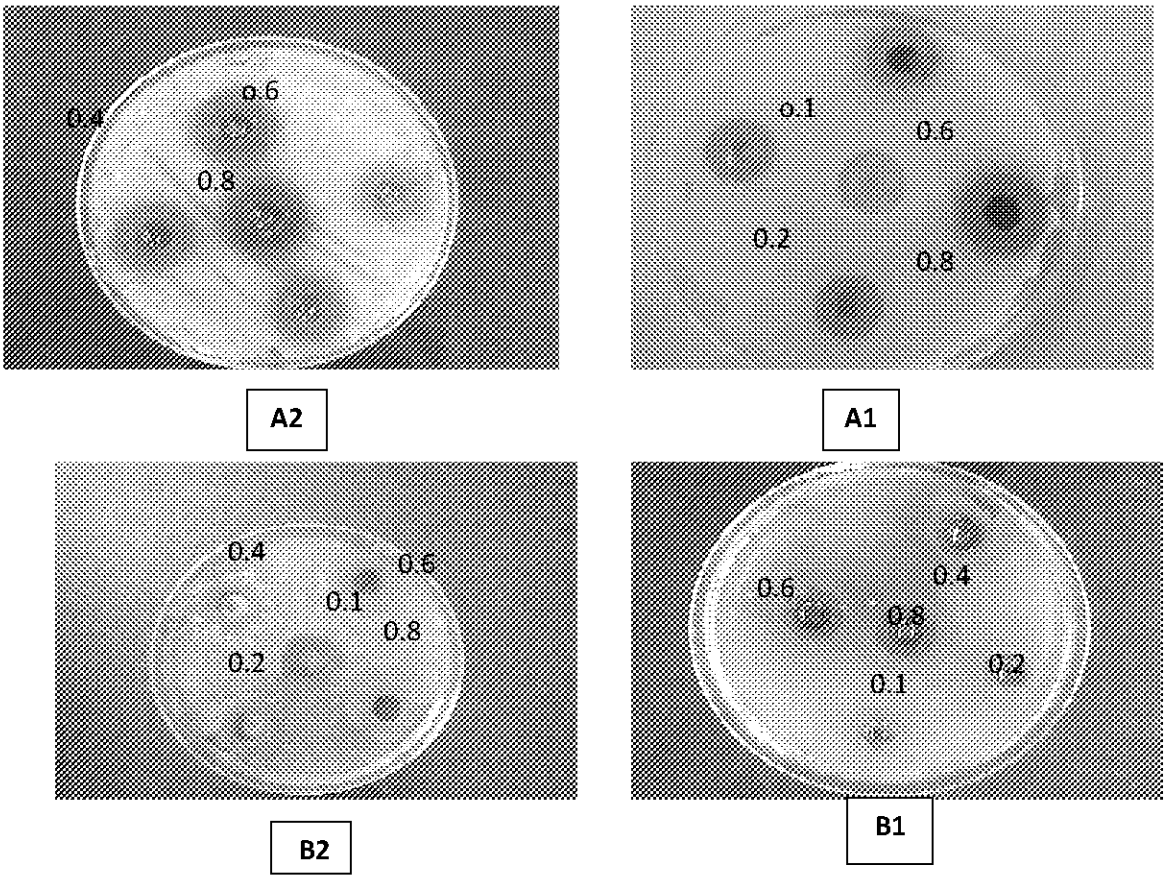

Figure 1. Inhibition zone effects of *S. alba* bark extract. Numbers 10, 20, 40, 60 and 80 mg mL^{-1}, respectively. (A1)*Staphylococcus aureus* with ethanolic extract only (A2)*Saphylococcus aureus* with nanoextract. (B1)*Pseudomonas aeruginosa* with ethanolic extract only.(B2*) Pseudomonas aeroginosa* with nanoextract.

الفعالية التثبيطية لمستخلص الفضة النانوي باستخدام لحاء الصفصاف الابيض في نمو نوعين من البكتريا الممرضة للا نسان.

في هذه الدراسة تم استخدام طريقة صديقة للبيئة وهي طريقة Green synthesis في تحضير المستخلص النانوي من 1ملي مايكرون نترات الفضة من مستخلص نبات الصفصاف الذي يعتبر كعامل مختزل .لوحظ من خلال استخدام هذه الطريقة بان المستخلص النانوي له سمية عالية على نوعي البكتريا قيد الدراسة وهي بكتريا المكورات العنقودية *Staphylococcus aureas*الموجبة لصبغة كرام وبكتريا الزوائف الزنجارية *Psuodomonase aeroginosa* . اعلى تثبيط للمستخلص النباتي لوحظ في نمو بكتريا المكورات العنقودية الموجبة لصبغة كرام بمنطقة تثبيط بلغت (16 ملمتر) ،تليها بكتريا الزوائف الزنجارية بمنطقة تبيط بلغت (10ملمتر) ، ولكن عند استخدام المستخلص النانوي لوحظ زيادة في منطقة التبيط بالنسبة لكلا النوعين فقد بلغت اعلى منطقة تثبيط في بكتريا *Psudomonas aeroginosa* وهي (37ملمتر) تليها في ذلك بكتريا *Staphylococcus aureas* بمنطقة تثبيط بلغت (23 ملمتر).