

Antimicrobial effects of ethanolic and nano extract produced by bark of *Salix alba* on growth of some pathogenic microbes.

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ABSTRACT

In this study, the comparison between the effectiveness biological of alcoholic and nano extract used by green synthesis method of *Salix alba*(willows) plant on the growth of two types of pathogenic microbes isolates which have been obtained from AL-alywia hospital for children that's *Proteus vulgaris*(Gram-ve) ,and yeast *Candida albicans*(Gram +ve) . Alcoholic extract that gave the highest effect on growth of *Proteus vulgaris* bacteria by inhibition zone (8mm) , but not effected on the growth of the yeast *Candida albicans*. While the nano extract has been shown that the highest effect on the growth of *Proteus vulgaris* bacteria by inhibition zone reached to (21mm), followed by *Candida albicans* by inhibition zone reached to (11mm).

الفعالية الميكروبية للمستخلص الكحولي والنانوي للحاء نبات الصفصاف الابيض في نمو بعض الاحياء المجهرية المرضية.

الخلاصة

تم في هذه الدراسة المقارنة بين الفعالية البيولوجية للمستخلص الكحولي و النانوي المحضر بطريقة green synthesis لنبات الصفصاف الابيض في نمو بعض الاحياء المجهرية المرضية للانسان والتي تم الحصول عليها من مستشفى العلوية التعليمي للاطفال والمتمثلة ببكتريا *Proteus vulgaris* وخميرة *Candida albicans* . اعطى المستخلص الكحولي اعلى منطقة تثبيط بلغت (8 mm) في بكتريا *Proteus vulgaris* في حين لم يؤثر في نمو الخميرة *Candida albicans* , اما بالنسبة الى المستخلص النانوي فقد اعطى اعلى منطقة تثبيط (21mm) في بكتريا *Proteus vulgaris* تليها الخميرة *Candida albicans* بمنطقة تثبيط بلغت (11mm)

Key words:-plants extract ,green synthesis ,Antimicrobiale activities.

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INTRODUCTION

Since ancient times, medicinal plants as alternatives remedies for the treatment of many diseases because they contain a lot of effective material that's antimicrobial or antioxidants potentials like phenols, tannins, alkaloids and many others, that's have a synergistic effects of the secondary products of these plants [1,2].

Recently have been relying on medicinal plants as alternatives therapeutic for the emergence of prescription antibiotic resistance by pathogens to chemical materials that's used for treatment of various infections [3]. The bark of the willow plant was used in the treatment of many conditions, including arthritis, menstrual, dental and back pain, reduce fevers. and it is used as an anti-inflammatory drug. [4].

Biological methods are used to synthesis of nanoparticles either using microorganisms[5], enzymes[6], and plant or plant extract an possible ecofriendly (green synthesis) method by using silver ions, and that alternating with chemical and physical methods.

Several suggestions or explanations have been developed about the effect of silver ion/silver on growth of bacteria and all point to the heavy metal interaction with proteins and, especially the interaction with thiol (-SH) groups that which leads to inactivation of the protein[7].

The objective of the present study was to investigate the antimicrobial activity of ethanol and nano-extracts extract of *S. alba* bark.

Materials and methods

Extraction Methods

Plant barks commonly used in herbal medicine were dried and pulverized with mortar and pestle or electric mill. The fine powder of plant parts were extracted with boiled ethanol by Soxhlet 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.

Bacterial strains and growth conditions

The investigated microbial strains are identified strains and were obtained from AL-Alweia children's hospital in Baghdad.

Assay for antibacterial activity Preparation of inoculums

The test bacterial strains were inoculated into nutrient broth and were incubated at 37°C on shaker. The inoculum size was maintained as per 0.5 McFarland standard (1×10^8 cfu/ml). The activated inoculum was used for antibacterial assay.

Antibacterial susceptibility testing

Agar well diffusion method

The screening of alcoholic extracts of different plant species for antibacterial activity was determined by agar well diffusion method. The nutrient Agar No. 2 media (Hi-Media) was inoculated with 200 μ l of the inoculum (1×10^8 cfu/ml) when the temperature of media reached 40-42°C and then poured into the Petri plate. After the media was

solidified, a well was prepared in the plates with the help of a cup-borer (6 mm). The well was filled with 100 μ l of the extract. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The plates were incubated at 37°C for 24 h. The result of antibacterial activity was obtained by measuring the diameter of the zone of inhibition. All samples were tested in triplicate. Controls included solvent without ethanolic and nano plant extract.

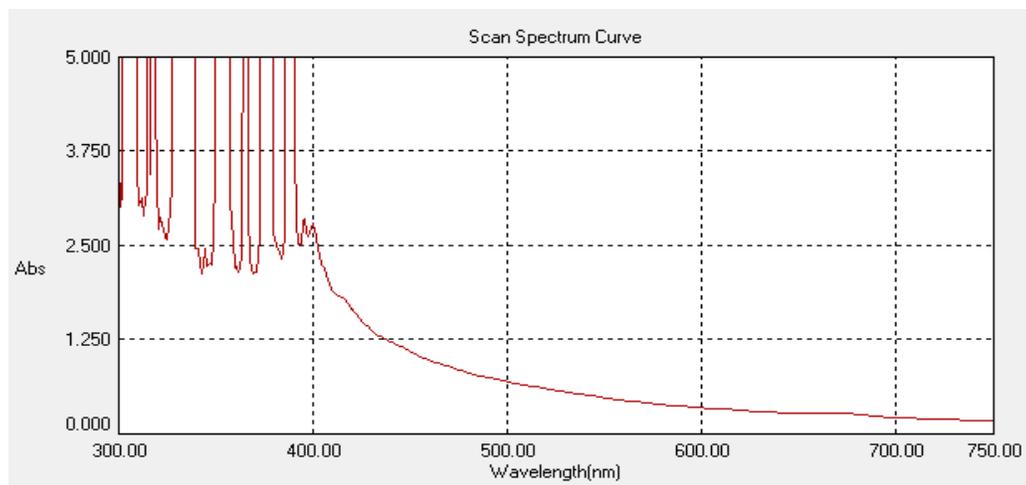
UV-Vis Spectra analysis

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 (TechcompUV2300) spectrophotometer.

Result

UV-Vis Spectra analysis

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles[2].



Figure(10):- UV-Vis absorption spectra of silver nanoparticles synthesized by *Salix alba* leaf extract

Antimicrobial test of an ethanolic and nano extract leaves of *S. alba* :-

Antimicrobial activity of *S. alba* extract was determined by the agar-well diffusion method. two strains used in the present study.

(Table1) shows the affect of ethanolic and nano extract of the leaves of *S. alba* plant on the growth oftwo types of pathogenic bacteria in this study. The ethanolic extract gave the highest effect on the growth of *P.vulgaris* bacteria by(8mm)(Fig.A1), but did not affect on the growth of yeast *C. albicans*(Fig.A2).While the nanoextract showed the highest effect on the growth of *P. vulgaris* bacteria by inhibition zone(21mm)(Fig.B1)compared to (8 mm) when used the ethanolic extract only. As for the yeast, the inhibition zone was and (11mm)(Fig.B2) on the growth of *C. albicans* .

Table 1. Antimicrobial activity of the ethanolic and nano leaf extract of *S. alba* Concentration mg mL⁻¹

Microbes name	Ethanolic extract mg mL ⁻¹	Nanoextract mg mL ⁻¹
Proteus vulgaris (G-ve)	8mm	21mm
Candida albicans (G+ve)	-ve	11mm

-ve= Not effect

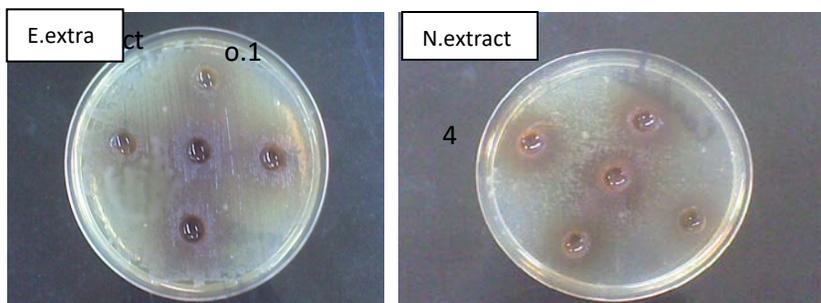
Discussion

According to the (table 1) we see that the effects of both alcoholic and nano extract was the largest effect on the growth of Gram negative than the Gram positive bacteria, that's may be because the installation of the cell wall of Gram negative bacteria, who works to facilitate the passage of the silver ion to the plasma membrane and this leads to be much affected to Ag⁺ than Gram positive bacteria. The aqueous silver nitrate solution when exposed to plant extract was reduced in solution, there by leads to silver hydrosol formation, Silver has good disinfecting character; this was enhanced with the *S. alba* ethanolic and nano extract reduction, or because the bioactive compounds that's present in the plants are responsible for the reduction of the silver ions like polyphenols, flavonoids and tannins. Recent microbiological and chemical experiments have revealed that the interaction of silver ions with thiol groups plays an essential role in inactivation [8].

Silver ion and silver based compounds are highly toxic to micro organisms, showing strong biocidal effect against microbial species. The silver nano particles produced by microbes and plant extracts are known to exhibit potent antimicrobial activity.. Despite their extensive use, the antibacterial mechanism of the silver nanoparticles is still unclear. However it has been reported [9] that when silver nanoparticles are attached to the surface of the cell membrane, the respiratory function and permeability of the bacterial cells become unstable. Other studies suggest that when bacteria are treated with silver ions, DNA tends to lose its ability to replicate [10].

Conclusion

We used a fast, eco-friendly, and convenient green method for the synthesis of silver nanoparticles from silver nitrate using *S. alba* extract. colour changes occur due to surface Plasmon resonance during the reaction with the ingredients present in the *S. alba* leaf extract resulting in the formation of silver nanoparticles. The present study also proved to have potential antibacterial activities with the *S. alba* extract synthesised silver nanoparticles and this might be due to denaturation of bacterial cell wall.



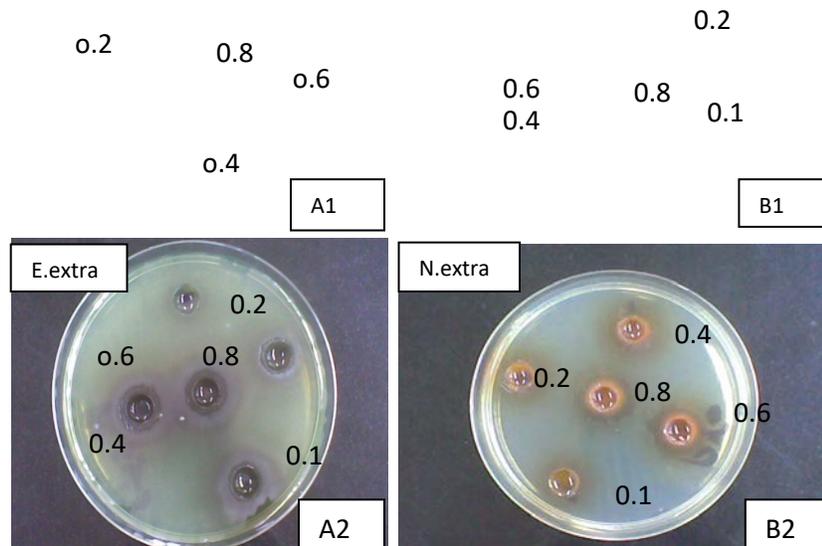


Figure (2). Inhibition zone effects of *S. alba* bark of ethanolic extract and nanoextract. (10, 20, 40, 60, 80, and 80 mg mL⁻¹, concentration respectively. (A1, B1) *Proteus vulgaris*, (A2, B2) *Candida albicans*.

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