

The Antibacterial Activity of *Angelica glauca* Edgew (Apiaceae) in The Form of Silver Nanoparticles

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Abstract

Angelica glauca (Apiaceae) is traditionally an over-the-counter herbal preparation; although nowadays, nanotechnology formulates silver nanoparticles (AgNPs) from *Angelica glauca*. Utilization of green chemistry through nanoparticle formulations contributed to pharmaceutical properties enhancements in plants' crude extracts. The present study aims to evaluate the antibacterial activity of the synthesized silver nanoparticles (AgNPs) from *Angelica glauca* crude methanolic extract and perform phytochemicals assay screening *Angelica glauca* AgNPs by scan electron microscope (SEM), and finally *in vitro* antibacterial assay of the methanolic extract and silver nanoparticles. After soxhlet extraction, the recovered crude methanolic extract of dried *Angelica glauca* roots was subjected to nanoparticles derivatization, additionally, the AgNPs were analyzed with SEM. Five metabolites were detected, the *in vitro* antibacterial assay was performed using disc diffusion method against four selected microbial species. The extract yield was 13.3g, and the phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, steroids, and coumarins. The detected particle size was 70.90 nm ± 13.60 (mean ± SD). The crude extract's antibacterial potential was enhanced by the nanoparticulation. The results of this study display the AgNPs semisynthetic product as an approach to improve the crude extracts' bioactivities. In conclusion, *Angelica glauca* contains several bioactive phytochemicals which can be used in curing health-related problems.

Keywords: Antimicrobial activity, *Angelica glauca*, Methanolic extract, Silver nanoparticles.

النشاط المضاد للبكتريا لحشيشة الملاك ايدجو (الفصيلة الخيمية) في شكل حبيبات فضة نانوية

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الخلاصة

حشيشة الملاك (الفصيلة الخيمية) مستحضر طبي عشبي تقليدي بدون وصفة طبية، اما في الوقت الحاضر فان تقنية النانو صنعت حبيبات نانوية فضية من حشيشة الملاك باستخدام طريقة التخليق الأخضر من خلال تخليق الحبيبات النانوية والتي يعزى لها زيادة الصفة الصيدلانية في المستخلصات الخام النباتية. هدفت هذه الدراسة لتقييم النشاط المضاد للبكتريا لحبيبات الفضة النانوية المصنعة من المستخلص الخام الميثانولي لحشيشة الملاك لتخليق حبيبات الفضة النانوية لحشيشة الملاك من مستخلصها الميثانولي، كشف المجاميع الكيميائية النباتية، التحري عن الحبيبات الفضة النانوية بالميكروسكوب الإلكتروني. أخيراً تقييم النشاط المضاد للبكتريا خارج الجسم الحي لحشيشة الملاك لأشكال المستخلص الميثانولي و حبيبات الفضة النانوية الاستخلاص ب الساكسوليت هو الأسلوب المستخدم في تحضير خلاصة حشيشة الملاك الميثانولية، لاستخدامه في تحضير حبيبات الفضة النانوية لحشيشة الملاك والتي تم التحري عنها بالمجهر الإلكتروني، تم الكشف عن ٥ مجاميع كيميائية نباتية، تقييم النشاط المضاد للبكتريا خارج الجسم الحي ضد أربعة أنواع مختارة من اجناس المايكروبات. المستخلص الميثانولي انتج ١٣,٣ غرام من ٢٥٠ غرام من حشيشة الملاك، بالإضافة إلى أن اختبار الكشف المجاميع الكيميائية النباتية كان موجباً للفلافونويد والعفص والستيرويدات والكومارين، حجم الحبيبات التي اكتشفها المجهر الإلكتروني كان ٧٠,٩ نانومتر ± ١٣,٦٠. بمتوسط حجم ± انحراف معياري. ان النشاط المضاد للبكتريا للمستخلص الميثانولي تعززت بواسطة حبيبات الفضة النانوية. عرضت نتائج هذه الدراسة ان لحبيبات الفضة النانوية شبه المصنعة تستخدم كنهج لتحسين النشاط الحيوي للمستخلصات الخام. نستخلص من هذه الدراسة ان حشيشة الملاك تحتوي على العديد من المجاميع الكيميائية النباتية النشطة حيويًا والتي يمكن استخدامها في علاج العديد من الامراض الصعبة المتعلقة بالصحة.

الكلمات المفتاحية: نشاط مضاد للميكروبات، حشيشة الملاك، خلاصة ميثانولية، حبيبات الفضة النانوية.

Introduction

The rise in the multi-drug resistant bacteria numbers due to wrong diagnosis and antibiotics' abuse is a global concern, and the pharmaceutical platform is always in need of new antibiotics to overcome the resistant species, in this manner, the plants contribute to this field with several entities and formulation such as in forms of extracts or nanoparticles^(1,2).

A traditional herb native to Indian subcontinent and applied widely in traditional medicine is the Himalayan *Angelica* family (Apiaceae) with more than 60 discovered species; the three most common species of *Angelica*, namely *Angelica glauca*, *Angelica cyclocarpa*, and *Angelica archangelica*⁽³⁾.

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For centuries *Angelica glauca*, called Chora, has been used as an over-the-counter herbal preparation owing to its anti-inflammatory, diuretic, antispasmodic, laxative, and expectorant properties, and in the treating with respiratory and urinary tract infections⁽⁴⁾.

The nanotechnology has many procedures in nanoparticles synthesis; however the most popular one is the use of eco-friendly renewable medicinal plant extracts in green nanoparticle synthesis owing to its content of phytochemicals that act as reducing agents in capping and stabilizing nanoparticles. *Angelica* species contain several active metabolites like coumarins, flavonoids, tannins, alkaloids, and carbohydrates which possess antimicrobial and antioxidant properties⁽³⁾. Silver nanoparticles are the most common nanoparticles type in use due to the antiseptic activity owing to their antimicrobial and antifungal effects, adds an additive action to nanoparticles⁽⁵⁾.

The present work aims to synthesize *Angelica glauca* silver nanoparticles from its methanolic extract, detect phytochemicals, investigate the morphology in *Angelica glauca* AgNPs by using SEM, and finally in vitro antibacterial activity assessment of *Angelica glauca* of crude root extract and compare it with the silver nanoparticles forms.

Materials and Methods

Extraction of the crude plant material.

The roots of *Angelica glauca* was purchased from India and manually pulverized to obtain 250 gram of dried fine powder. The dried root powder was subjected to soxhlation with one liter of 70% methanol for 24 h, sieved through folded gauze, and filtered using Whatman filter paper No.1. The crude extract was recovered from the solvent by a rotary evaporator under vacuum and reduced temperature at 45°C and yielded yellow residue of 13.3g and kept at refrigeration. The preparing *Angelica glauca* methanolic extract was used as a reducing agent in preparation *Angelica glauca* AgNPs, in the upcoming phytochemical screening and antibacterial activity assessments⁽⁶⁾.

Angelica glauca mediated green silver nanoparticles synthesis

To prepare *Angelica glauca* silver nanoparticles, 2 ml of silver nitrate solution (10 mmol/L) was added to an equal quantity of the crude extract in a drop-by-drop manner, and the mixture was heated to 55 °C for 60 min and vigorous stirring, the mixture color change was objectively observed⁽¹⁾. *Angelica glauca* extract (yellow solution) changed to a brown colloidal solution after adding the AgNO₃ solution indicating *Angelica glauca* silver nanoparticles formation. Then, the formed solution was centrifuged at 8000 rpm for 10 min then, sediment was preserved in refrigeration^(7,8).

Screening the phytochemical components

Phytochemical screening tests were carried out to confirm the presence of alkaloids, tannins, flavonoids, saponin, steroids, coumarins, terpenoids, quinine, proteins, and carbohydrates in *Angelica glauca* methanolic crude extract^(9,10,11).

Alkaloids detected by the addition of one ml of Mayer's reagent mixed with 1ml of the extract. A yellow color formation confirms the presence of alkaloids. Terpenoids detected by the addition of two drops of chloroform was mixed with 1ml of 0.1N hydrochloric acid then 1ml of the extract was added to the mixture and heated for 2 minutes. A red-brown color formation confirms the presence of terpenoids. Tannins detected by the addition of three drops of 10% lead acetate were added to 1ml of the extract. Yellow precipitate formation is a positive reaction of tannins. Carbohydrate detected by the addition of one ml of sulfuric acid was added to 1ml of the extract. A red ring formation is an indication of carbohydrate. Saponin detected by the addition of three drops of distilled water was added to 1ml of the extract with shaking. A foam formation indicates the presence of saponin. Coumarins detected by the addition of one ml of diluted 0.1N nitric acid was added to 1ml of the extract. A yellow precipitate confirms the formation of coumarins. Flavonoid detected by the addition of one ml of ferric chloride was added to 1ml of extract, brown color confirmed the presence of flavonoids. Quinine detected by the addition of one ml of 10% sodium hydroxide, was added to 1ml of the extract. A red color formation confirms the presence of quinine. Protein detected by the addition of one drop of 0.1N nitric acid was added to 1ml of the extract. A red color confirms the presence of proteins. Steroids detected by the addition of one ml of chloroform mixed with one ml 0.1N sulfuric acid were added to 1ml of the extract with shaking. The red color confirms the presence of steroids.

The Angelica glauca AgNPs morphology detection

Scan electron microscope (SEM)

One drop of *Angelica glauca* AgNPs was placed on an SEM sample holder and air-dried. A SEM was performed by (TESCAN-MIRA 3, Czech Republic) to observe the morphology of *Angelica glauca* AgNPs⁽¹²⁾.

Antibacterial activity

The antibacterial assay for both the crude methanolic extract and the previously synthesized AgNPs derivative were carried out via disc diffusion method against the selected bacterial isolates of *Staphylococcus aureus* ATcc 3100, *Bacillus subtilis* ATcc 1100, *Escherichia coli* ATcc 7312, *Klebsiella pneumonia* ATcc 7012 obtained from microbiology laboratory, department of Clinical Laboratory Science. Subcultures were obtained after inoculating the isolated bacteria on a selective agar medium for each bacterial strain, the streaked fresh cultures were maintained in incubated for 24 h. at 37°C; on the other hand, activity of 1% dimethyl sulphoxide DMSO had been excluded by testing the absence of antibacterial activity against all isolated bacteria used in this study (negative control via disc diffusion method)⁽¹³⁾.

Disc diffusion method

Disc diffusion protocol was carried to determine the antibacterial activity of *Angelica glauca* crude methanolic fraction and the AgNPs derivative against *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumonia*. The susceptibility test was carried out by taking standardized inoculums of the bacterial cultures after adjusting its turbidity to achieve 0.5 McFarland then a swab from the bacterial culture was streaked on a Muller-Hinton agar medium plates. Six millimeters discs of Whatman filter paper No.1 were placed on inoculated agar medium after sterilization and soaking in *Angelica glauca* methanolic extract and *Angelica glauca* AgNPs separately in the following concentrations of 0.5, 1, 2, 4, 8, and 16 mg/ml after dissolving with negative control of 1% DMSO⁽¹⁴⁾.

Chloramphenicol 10 mg/disc was used as a positive control against all bacterial isolates; then, the inoculated plates were incubated at 37°C overnight. The inhibition zones were measured in millimeters against all the tested bacteria, susceptibility test carried out in triplicate, then mean and stander deviation were calculated⁽¹⁾.

Results and Discussion

The yield of the total crude extract after drying was 13.3g (5.3%) yielded from 250 g. However *Angelica glauca* AgNPs yield from methanolic extract was 1.5 g(0.6%).

Throughout history medicine utilized herbs which were considered safe, less toxic, and economic. The pharmacological activities displayed by medicinal plants are attributed to their phytochemicals used to cure several health conditions, and were the source of many medications in clinical practice⁽¹³⁾.

The plant extract yield in this work is dependent on several factors such as time of harvesting, geographical location, and extraction methods⁽¹⁵⁾.

On the other hand, the color change of the methanolic crude extract from yellow to dark brown color with colloidal dispersion after adding AgNO₃ which indicates the formation of *Angelica glauca* AgNPs. Nevertheless, the successful procedure in formations of AgNPs colloidal particles and color change to brown due to the reduction of Ag⁺ to Ag⁰ as a result of surface plasmon trembling in the methanolic extract⁽¹⁶⁾.

Phytochemical tests

The phytochemical qualitative test results based on color change and attributed to the interaction between the reagent and the various types of the phytochemicals. However phytochemical reactions of *Angelica glauca* roots were positive for alkaloids, flavonoids, tannins, steroids and coumarins. Furthermore, detection of terpenoids, carbohydrate, saponin, quinine, and proteins showed negative result.

Previously, phytochemicals obtained from *Angelica glauca* methanolic root which showed positive results for alkaloids, flavonoids, glycosides, steroids, saponin, and tannins, additionally, variation in the phytochemical profile may occur depending on the *Angelica* species, and the type of the diluent used for the extraction.^(17,18)

Table 1. The results of phytochemical screening tests of *Angelica glauca* methanolic extract.

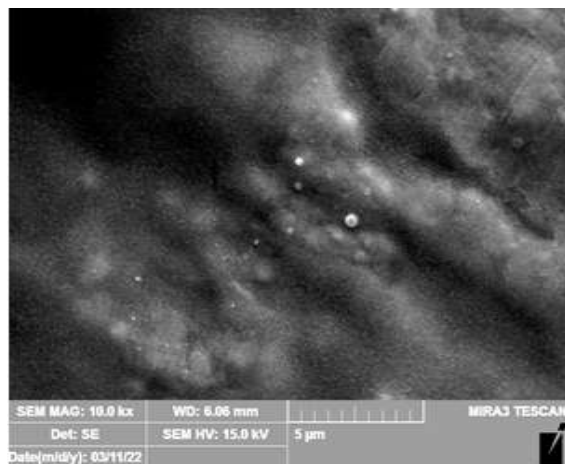
Type of phytochemicals	Result
Alkaloids	+
Terpenoids	-
Flavonoids	+
Tannins	+
Carbohydrates	-
Saponin	-
Quinines	-
Proteins	-
Steroids	+
Coumarins	+

The detection test of *Angelica glauca* AgNPs Scan Electron Microscopy (SEM)

The size and morphology of derivatized *Angelica glauca* AgNPs were determined by SEM. Based on the SEM analysis, the *Angelica glauca* AgNPs size was 70.9 ± 13.60 (mean \pm SD) in all measurements, Figure 1 (A, B).



A - *Angelica glauca* AgNPs size



B- *Angelica glauca* AgNPs shape

Figure 1. (A, B). Morphology (size and shape) of *Angelica glauca* AgNPs by SEM.

Nanoscience was globally used to produce a wide range of products. Although green synthesis of AgNPs using plant extracts to avoid toxicities, nowadays plant extracts are used widely in nanoparticles synthesis due to the presence of large quantities of phytochemicals which act as reducing agents to produce nanoparticles from metal salts without having any toxic by-product⁽¹⁹⁾.

The latter test was SEM which determined *Angelica glauca* AgNPs morphology in spherical particles, well dispersed, and their size was 70.9 ± 13.60 (mean size in nm \pm SD), although previously, the AgNPs expressed spherical shape, with an average size of 21 nm in diameter. Data obtained from SEM with spherical particles and sizes less than 100 nm indicate a suitable nanoparticle formulation procedure⁽²⁰⁾.

Antibacterial activity

In vitro antibacterial activity of *Angelica glauca* methanolic extract and AgNPs were assessed via the disc diffusion method, and their antibacterial activity was increased in a dose-dependent manner. Table 2 and Figure 2 demonstrate the antibacterial activity by measuring the inhibition zones expressed by *Angelica glauca* methanolic extract against G+ bacteria as *S. aureus* and *B. subtilis* were ranged from (0 \pm 0.00-0 \pm 0.00) and (0 \pm 0.00, 21 \pm 1.00) however, the inhibition zone against G- bacteria as *E.coli*, and *K. pneumonia* were ranged from (0 \pm 0.0-10.6 \pm 1.15), and (0 \pm 0.00-0 \pm 0.00). The observed antibacterial activities of *Angelica glauca* AgNPs are illustrated in Table 3 and Figure 3; the inhibition zones against G+ such as *S. aureus*, and *B. subtilis* ranged (0 \pm 0.0-14.6 \pm 0.57) and (14.6 \pm 0.57-21.3 \pm 1.15) respectively. Furthermore, the inhibition zones against G- bacteria as *E. coli*, and *K. pneumonia* ranged (10 \pm 1.00-16.3 \pm 1.15) and (0 \pm 0.00-10.3 \pm 0.57), respectively.

Table 2. The inhibition zones' diameters in millimeters expressed by *Angelica glauca* crude methanolic extract against selected pathogens.

Conc.	<i>S. aureus</i>	<i>B.subtilis</i>	<i>E .coli</i>	<i>K .pneumonia</i>
	Zone of inhibition (mean diameter in nm \pm SD)			
0.5	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00
1	0 \pm 0.00	0 \pm 0.00	10 \pm 0.00	0 \pm 0.00
2	0 \pm 0.00	0 \pm 0.00	10 \pm 0.00	0 \pm 0.00
4	0 \pm 0.00	13.3 \pm 1.52	10 \pm 1.00	0 \pm 0.00
8	0 \pm 0.00	15.3 \pm 0.57	10 \pm 1.00	0 \pm 0.00
16	0 \pm 0.00	21 \pm 1.00	10.6 \pm 1.15	0 \pm 0.00

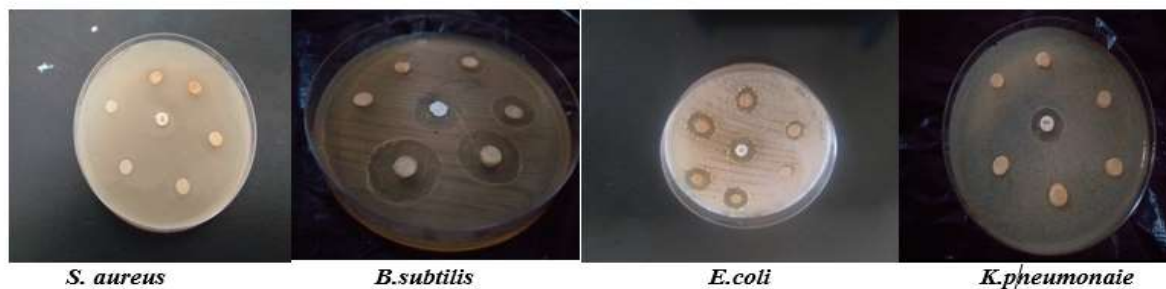


Figure 2. Inhibition zones displayed by testing the antibacterial activity of *Angelica glauca* crude methanolic extract against selected bacterial strains using disc diffusion assay.

Table 3. The inhibition zones' diameters in millimeters expressed by *Angelica glauca* methanolic extract derived-AgNPs against selected pathogens.

Conc.	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
	Zone of inhibition (mean diameter in mm±SD)			
0.5	0±0.00	14.6±0.57	10±1.00	0±0.00
1	0±0.00	14.6±1.52	11±1.0	0±0.00
2	0±0.00	15±1.00	14.6±0.57	0±0.00
4	13±1.00	15±0.00	15.3±0.57	0±0.00
8	14±1.00	17±1.00	15.3±0.57	10.3±0.57
16	14.6±0.57	21.3±1.15	16.3±1.15	10.3±0.57

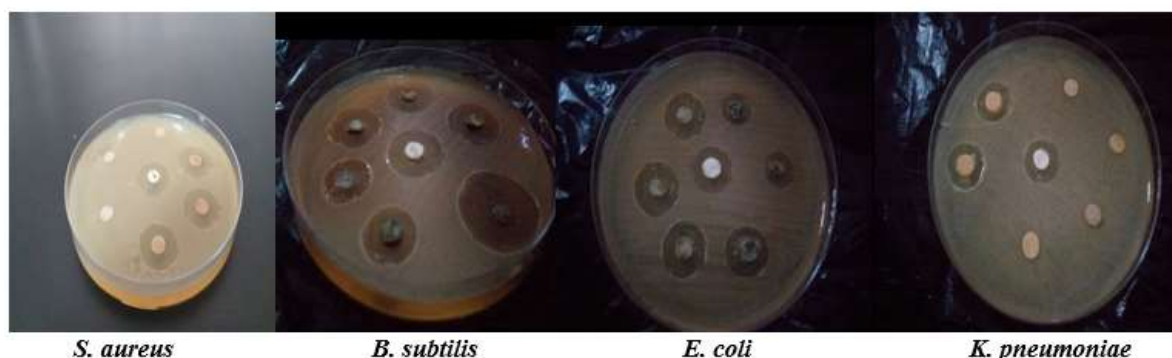


Figure 3. Inhibition zones displayed by testing the antibacterial activity of *Angelica glauca* methanolic extract derived-AgNPs against selected bacterial strains using disc diffusion assay.

Notably, the antibacterial activity of *Angelica glauca* in two formulations of methanolic and AgNPs showed that *Angelica glauca* AgNPs had higher antibacterial activity compared to the crude methanolic extract against all the tested bacteria. However several mechanisms of antibacterial action of phytochemicals have been reported, for instance they may act by inhibiting microbial growth, inducing cellular membrane perturbations, interference with certain microbial metabolic processes and modulation of signal transduction. Molecular basis for the modes of action in phytochemicals-based *in vitro* antimicrobial activity was the most accepted theory⁽²¹⁾.

Angelica glauca crude methanolic extract inhibited *B. subtilis* > *E. coli* > *S. aureus* and *K. pneumoniae*, while the last two bacteria showed resistance.

Furthermore, AgNPs demonstrated more activity compared to its extract which were in the following manner *B. subtilis* > *E. coli* > *S. aureus* > *K. pneumoniae*. The antibacterial effect of *Angelica glauca* AgNPs compared to its non-derivatized extract form is attributed to the small particle size and larger surface-to-volume ratios, in addition to coating phytochemicals with AgNO₃ will enhance the binding to bacterial cell membrane, which verifies bacterial cell death^(1,22,23).

Conclusion

The two *Angelica glauca* formulas exhibited various degrees of antibacterial properties; *Angelica glauca* contain several health-related bioactive phytochemical compounds which can be used in curing health-related problems, however reducing phytochemicals into nanoparticles have several properties in pharmaceutical applications as well as our results.

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Conflict of Interest

None

Funding

None

Ethics Statements

No animal or human include in the study.

Author Contribution

The authors contribute in result analysis, writing and reviewing the manuscript.

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