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GREEN SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF SILVER NANOPARTICLES MEDIATED BY WATER INFUSION OF *SONCHUS OLERACEUS* L. FLOWERS

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ABSTRACT

Phytochemical analysis revealed that the infusion of Sonchus oleraceus L. flower is rich in flavonoids, phenolics, reducing sugars and total sugars. The aqueous extract of this flower has been used as a green reducing and stabilizing agent in addition to its desirable biological activity. The biosynthesis of reduced silver nanoparticles was very fast, and silver nanoparticles were synthesized by exposing a mixture of $AgNO_3$ and Sonchus oleraceus L. flower extract to UV-irradiation at different time intervals. Parameters such as reactants ratios and reaction time were varied and investigated. The developed nanoparticles were characterized with the aid of many spectroscopic and analytical techniques such as Ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). On contrast to the aqueous extract of the flowers, the resulting silver nanoparticles found to possess broad antimicrobial spectrum increasing proportionally with the increase in concentration of the silver nanoparticles against wide variety of bacterial and fungal pathogenic strains.

Keywords: *Sonchus oleraceus, antimicrobial spectrum, phenolics, flavonoids, silver nanoparticles.*

INTRODUCTION

Nanoparticles are described as particulate dispersions or solid particles with a size in the range of 10-1000nm (Mohanraj and Chen, 2006; Langer, 2000; Kommareddy *et al.*, 2005), that can drastically modify their physico-chemical properties compared to the bulk material (El-Shahaby *et al.*, 2013). Nanoparti-

cles can be made from a variety of bulk materials and can expli¬cate their actions depending on the chemical constituents and on the size and/or shape of the particles (El-Shahaby *et al.,* 2013; Brunner *et al.,* 2006). Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields, sensors, information and communication technology, transparent sunscreen lotions, stain-resistant fabrics, scratch free paints for cars, products labeling and textiles antibacterial dressings (Abhilash, 2010; Samadi–Maybodi *et al.,* 2011).

Metallic nanoparticles exhibit size and shape-dependent properties that are of interest for applications ranging from data storage, antibacterial activity, catalysts and sensing to optics (Sudrik *et al.,* 2006; Choi *et al.,* 2007; Yu *et al.,* 2013; El-Nouri *et al.,* 2013; Hutter and Fendler, 2004; Johnston *et al,* 2010). For example, the antibacterial activity of different metal nanoparticles as silver colloids is closely related to their size; i.e., the smaller the silver nuclei, the higher the antibacterial activity. Also, the catalytic activity of these nanoparticles is also dependent on their size, their structure, shape and chemical–physical environment. Generally, specific control of shape, size, and size distribution is often attained by using different synthesis methods, stabilizers and reducing agents (Paul *et al.,* 2004; Redhead *et al.,* 2001; Kholoud *et al.,* 2010; Zhang *et al.,* 2006; He *et al.,* 2004).

The noble metal nanoparticles such as gold (Au), platinum (Pt) and silver (Ag) nanoparticles have gained a considerable interest over the last decade owing to their important applications (Okuda *et al.,* 2005).

A number of methods have been used for the synthesis of silver-based nanoparticles involving physical, chemical and biochemical techniques (Amin *et al.,* 2012).

Green chemistry is an important branch of biosynthesis of nanoparticles. Natural compounds like glucose (Raveendran *et al.,* 2003), chitosan (Li *et al.,* 2011), soluble starch (El-Rafie *et al.,* 2011), some microorganisms (Vigneshwaran *et al.,* 2007; Vigneshwaran *et al.,* 2011)... etc., have attracted considerable research interest as safer alternative, reducing and stabilizing agents to synthesize the silver nanoparticles.

Synthesis of nanoparticles through biochemical routes, using plant extracts as reducing and capping agents, has received special attention, due to the aseptic environment maintained during these processes (Vigneshwaran *et al.,* 2007; Vigneshwaran *et al.,* 2011; Prathna *et al.,* 2011). Therefore, the medicinal plants of therapeutic importance were being widely used for the size- and shape-controlled synthesis of silver nanoparticles (El-Shahaby *et al.,* 2013; Amin *et al.,* 2012; Zhan *et al.,* 2011; Dubey *et al.,* 2010).

***Sonchus oleraceus* L.** (Asteracea) is an annual weedy plant known as Sow Thistle (Whyte *et al.,* 2001). It is broadly distributed in many countries including Egypt. It prefers growing in waste places, shores and cultivated fields (Cambie and Ferguson, 2003b). It has been used traditionally as a leafy vegeta-

ble in the Mediterranean diet, young leaves are edible as a salad or pot herb, but are bitter (Zeghichi *et al.,* 2003). The Sow Thistle has various uses in folk medicine as anticancer, digestive, purgative, emollient, blood purifier and also as liver tonic (Jain and Singh, 2014).

It is an erect herb with simple branches that are smooth and glabrous without any hair or bristles. The stems are hollowed and have a milky, latex-type sap and its lower part usually gets a purple brown color later in spring. The leaves differ with age, the old leaves are stalked elongated and deeply lobed with color varies from pale green to green blue and may have a serrated outline but on prickles or hair. The fruits are simple achene, brownish in color and oval/oblong in shape. The shape of involucral fruit is vase like round bottomed with tapering apes (Jain and Singh, 2014).

MATERIALS AND METHODS

Plant material and preparation of the infusion :

The flowers of the wild *Sonchus oleraceus* plants were collected from their original habitats at Mansoura, Egypt. The plant was taxonomically identified and authenticated according to (Boulos, 2005).

About 30 gm of the chopped fresh *Sonchus oleraceus* L. flowers were mixed with 200 ml deionized water and boiled for three hours in water bath at 80 ^{o}C. The extract obtained was filtered through Whatman no. 1 filter paper, and the filtrate was collected and stored at 4 ^{o}C for any further use.

A portion of the flowers was air dried in shadow for 15-20 days and then grinded into fine powder for phytochemical analysis.

Phytochemical analysis:

Total phenolic compounds:

The determination of total phenolic compounds in the plant extract was carried out using Folin Ciocalteu assay developed by Wolfe *et al.* (2003). Gallic acid was used as a standard.

Total flavonoids content:

The total flavonoids were determined using aluminum chloride colorimetric assay described by Zhishen *et al.* (1999). Catechin was used as a standard.

Total soluble sugars and total carbohydrates content:

Carbohydrates content and total soluble sugars were estimated using the method described by Thayumanavan and Sadasivam, (1984). Glucose was used as a standard.

Synthesis of silver nanoparticles:

Aqueous silver nitrate solution (4 mM) was prepared and used for the synthesis of a series of silver nanoparticles in the presence of aqueous extract of *Sonchus oleraceus* L. flower upon exposure of the mixture to UV irradiation as a reducing agent for the Ag^{+} ions. Different v/v ratios of the extract and the aqueous $AgNO_3$ solution were mixed thoroughly (15:60, 25:50, 37.5:37.5, 50:25, and 60:15) and the mixtures were subjected to UV-irradiation at different time intervals. After 20 seconds, the solution turned into reddish brown color indicating the formation of silver nanoparticles.

Instrumental analysis:

ATI Unicom UV-Vis. Spectrophotometer:

The reduction of Ag^+ ions into silver nanoparticles was controlled by recording the UV-Vis spectra of the reaction mixture at different time intervals. The UV-Vis spectra of the synthesized silver nanoparticles were recorded in the range of 200-800 nm using ATI Unicom UV-Vis. spectrophotometer with the aid of ATI Unicom UV-Vis. vision software V 3.20. The analysis was done at room temperature using quartz cuvettes (1 cm optical path), and the blank was the corresponding aqueous solution of *Sonchus oleraceus* L. flower.

Fourier Transform Infrared (FTIR) spectroscopy:

FTIR measurements were carried out for both the aqueous extract and the developed Ag nanoparticles to identify the biomolecules that can share in the reduction of the Ag^+ ions and capping of the resulting silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed using Mattson 5000 FTIR spectrometer in the range of 400–4000 cm^{-1} with a resolution of 8 cm^{-1} at room temperature.

Transmission electron microscope (TEM) measurement:

The morphology of the developed Ag nanoparticles was investigated by transmission electron microscopy (TEM) (JEOL TEM-1230, Japan) attached to a CCD camera at an accelerating voltage of 120 kV. The samples were prepared by dropping of the silver nanoparticles suspension on carbon coated copper grids and allowing the solvent to slowly evaporate overnight at room temperature and under vaccum before recording the TEM images.

Microbial susceptibility testing:

Filter paper disc assay:

The antimicrobial activity of the aqueous plant extract was estimated by the filter paper disc assay (Murray *et al.,* 1998) using inoculums of 10^6 bacterial and fungal cells or10^8 yeast cells / ml to spread on nutrient agar, Czapek Dox agar and Sabouraud agar plates, respectively.

The sterilized filter paper discs (Whatman no.1, 6mm in diameter) were immersed in the aqueous plant extract, solutions of synthesized nanoparticles and in water served as control. The discs were placed on the surface of the agar plates seeded with the tested pathogenic strains. The plates were incubated for 4-7 days at 28 ^oC for fungi, for 18-24 hours at 37 ^oC for bacteria and for 24-48 hours at 30 ^oC for yeast (Sardari *et al.,* 1998).

Tested organisms:

Bacteria: "*Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigells spp., E. coli, Proteus vulgaris*".

Fungi: "*Aspergillus niger, Erwenia carotovora, Fusarium solani, Asperigillus flaviries, Asperigillus ochraceous* and *Trichothecium spp*" .

Stock cultures of the tested pathogenic strains were obtained from the microbiology lab at Faculty of Medicine in Mansoura University.

RESULTS

Phytochemical analysis:

Total phenolics content:

The total phenolic compounds in the aqueous extract of Sonchus oleraceous flower was reported as gram of gallic acid equivalent/100 gram of the dried flowers with reference to the standard curve (y = 0.0063x, r^2 = 0.987). The total phenolics content was 0.90 ± 0.04 gram of gallic acid equivalent/100 gram of the dried flowers.

The total Flavonoids content:

The total flavonoids were expressed as gram catechine equivalent per 100 gram of the dried flowers with reference to the standard curve (y = 0.003 x, r^2= 0.994). The total flavonoids content was 0.168 ± 0.012 gram catechine equivalent/100 gram of the dried flowers.

Total soluble sugars and total carbohydrates content:

The total soluble sugars and total carbohydrates were 0.81 ± 0.05 and 0.95 ± 0.02 gram glucose equivalent / 100 gram of the dried flowers, respectively.

The synthesis of the silver nanoparticles has been approved by measuring the UV-Vis spectra of the reaction mixture Figure (1). The formation of silver nanoparticles was observed upon changing the color of the Sonchus oleraceus L. flower extract from yellow into reddish brown due to the coherent oscillation of electrons at the surface of nanoparticles resulting in surface plasmon resonance (SPR). As apparent from Figure (1), the absorption peak appeared at about 460 nm is corresponding to the characteristic surface plasmon resonance of the resulting Ag nanoparticles. The formed silver nanoparticles using *Sonchus oleraceus* L. flower aqueous solution were found to be very stable and polydispersed.

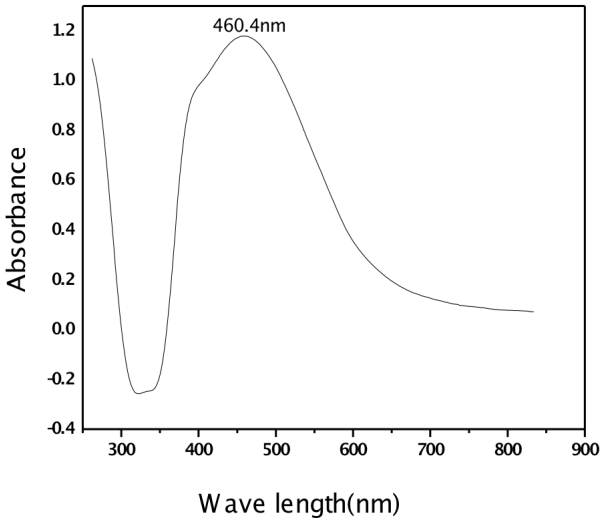


Fig. (1) : UV–vis spectra of Ag nanoparticles recorded as a function of reaction time.

FTIR spectroscopy :

FTIR analysis was carried out in order to identify the possible reducing and stabilizing biomolecules in the aqueous extract of *Sonchus oleraceus* L. flower as in figure (2). FTIR absorption spectra of plant extract before and after the development of Ag nanoparticles are shown in Figure (2). FTIR spectrum of *Sonchus oleraceus* L. flower extract showed bands around 1068, 1254, 1404, 1639, 2932, 2925 and 3408 cm^{-1}. The band around 1025-1200 cm^{-1} corresponds to the C–O stretching, the band around 1620-1650cm^{-1} corresponds to the aromatic rings. The strong broad band appearing at about 3440 cm^{-1} in both FTIR spectra can be assigned to the stretching vibrations of O–H groups present in phenolics and flavonoids.

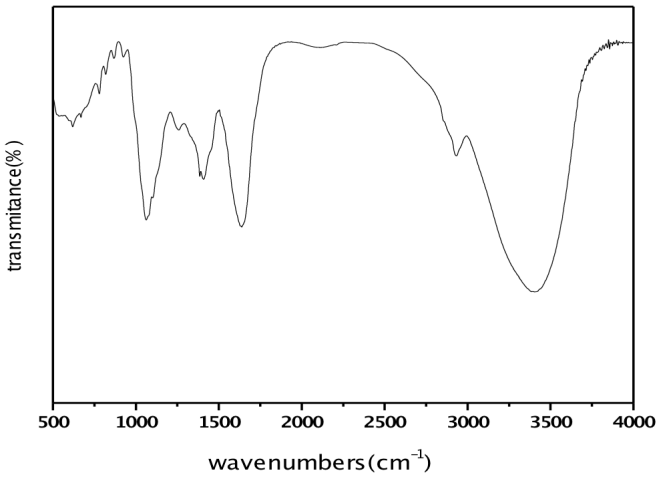


Fig. (2) : FTIR spectrum of *Sonchus oleraceus* L. flower extract.

Transmission electron microscopy:

Transmission electron microscopy (TEM) has been used for characterization of the size, shape and morphology of the formed Ag nanoparticles. The typical TEM micrographs and the corresponding particle size distribution of the *Sonchus oleraceus* L. flower -Ag nanoparticles formed after UV-irradiation reduction is presented in Figure (3). The obtained *Sonchus oleraceus* L. flower -Ag particles were in the nano range with uniform and spherical shapes with average particle diameters from 12 to 28 nm.

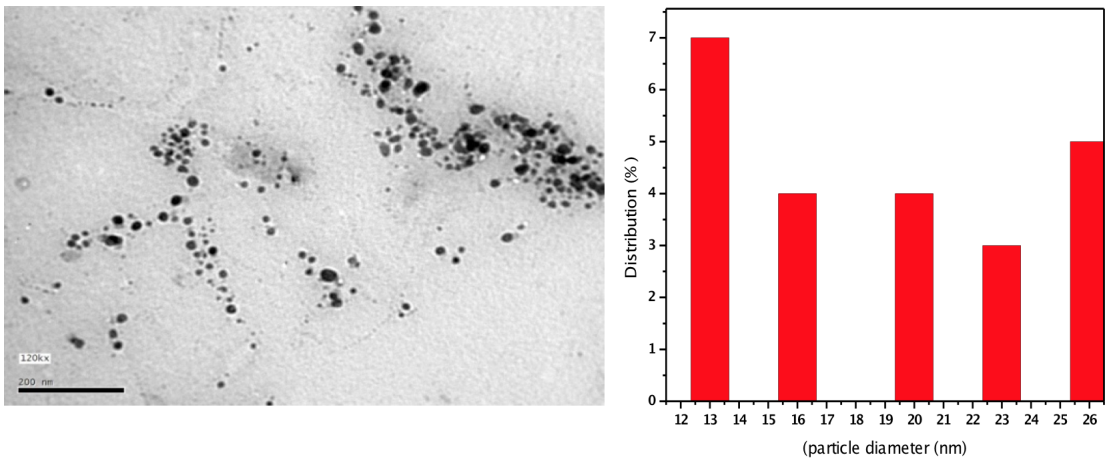


Fig. (3) : Transmission electron microscopy images of silver nanoparticles derived from Sonchus oleraceus L. flower extract using UV irradiation.

Evaluation of the antimicrobial activity:

Microbial Susceptibility Testing (Disc diffusion assay):

Silver is well known for its broad antimicrobial spectrum. The results of the antimicrobial activity of the aqueous extract of *Sonchus oleraceus* L. flower and the prepared silver nanoparticles using different v/v ratios of the water extract and the aqueous $AgNO_3$ solution (15:60, 25:50, 37.5:37.5, 50:25, and 60:15) that were mixed thoroughly and subjected to UV-irradiation, tested against several pathogenic microbial strains using disc diffusion assay are summarized in table1.

The aqueous extract of *Sonchus oleraceus* L. flower was found to possess broad antimicrobial spectrum against almost 75% of the tested organisms.

As the percent of synthesized silver nanoparticles increased, the antimicrobial spectrum increased. The silver nanoparticles synthesized from the mixture with the ratio $4AgNO_3$: 1 aqueous extract showed the broadest antimicrobial spectrum against all of the tested pathogenic strains among all the other prepared extracts as shown in table 1 and figure 4.

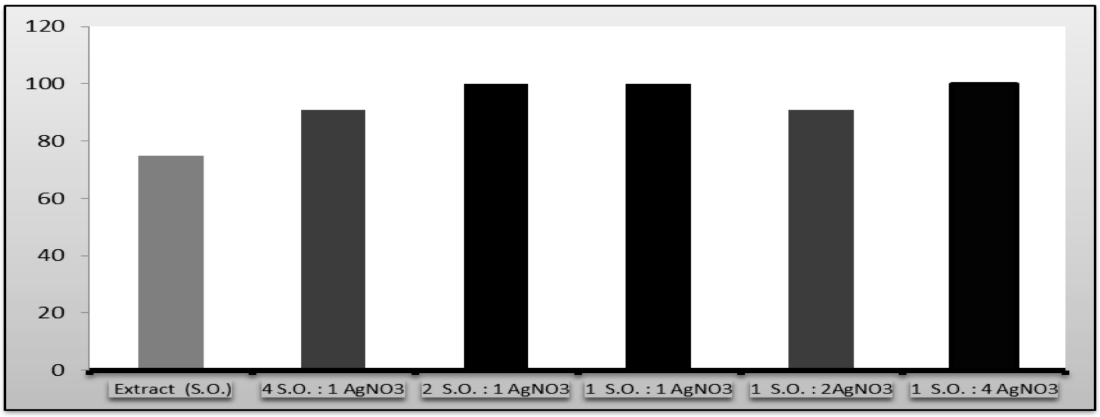
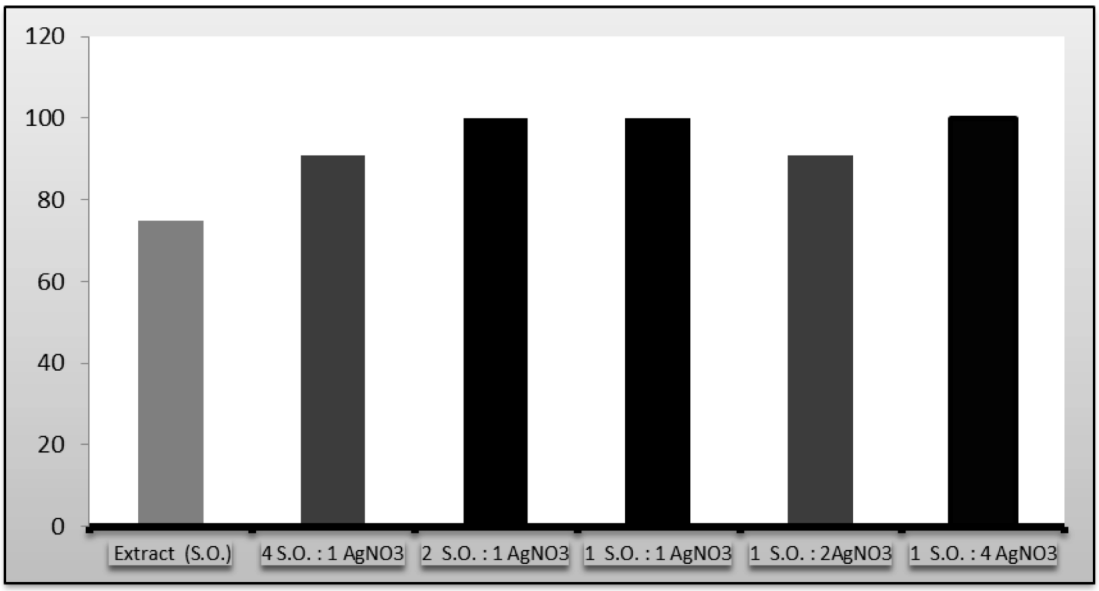


Fig. (3) : Transmission electron microscopy images of silver nanoparticles derived from Sonchus oleraceus L. flower extract using UV irradiation.



S.O. = *Sonchus oleraceus*

Fig. (4) : % of the antimicrobial spectrum of the aqueous extract of *Sonchus oleraceus* L. flower alone and in combination with different concentrations of $AgNO_3$ from which nanoparticles synthesized.

Table (1) : Antimicrobial activity of the wild Sonchus oleraceus water extract and the synthesized silver nanoparticles using mixtures of different ratios of the plant extract and silver nitrate solution using disc diffusion assay.

Tested Pathogenic Microbial strains	Zone of Inhibition (ZOI)					
	Extract (S. O.)	4 S. O. : 1 $AgNO_3$	2 S. O. : 1 $AgNO_3$	1 S. O. : 1 $AgNO_3$	1 S. O. : 2$AgNO_3$	1 S. O. : 4 $AgNO_3$
Staphylococcus epidermis	13	12	12	13	13	41
Klebsiella pneumonia	9	10	10	10	19	27
Pseudomonas aurignosa	13	12	11	11	12	19
Staphylococcus aureus	12	11	12	15	20	17
Shigella spp.	8	9	8	9	15	20
E. coli	8	9	9	7	-	15
Staphylococcus biogenesis	6	8	8	15	21	20
Erwenia carotovora		8	10	20	20	20
Proteus vulgaris	9	8	8	11	10	18
Asperigillus flaviries		-	9	8	9	15
Asperigillus ochraceous		10	12	12	26	45
Penicillium purpurgenium	8	10	11	10	10	18

Zone of inhibition, including the diameter of the filter disc (6.0 mm) .

DISCUSSION

An efficient way for green synthesis of silver nanoparticles using *Sonchus oleraceus* L. flower extract was used. The development of yellowish brown color owing to the surface Plasmon resonance with absorption maxima at 460 nm ensured the formation of silver nanoparticles (Aberoumand and Deokule, 2008; Maestri *et al.,* 2006). Sonchus oleraceus extracts are rich with active secondary metabolites like flavonoids and phenolics. Flavonoids is well known to play a vital role as reductant in synthesis of silver nanoparticles (Ghosh *et al.,* 2012; Egorova and Revina, 2000). Thus the estimated flavonoids and phenolics content in the water extract of *Sonchus oleraceus* flower strongly support the potential of *Sonchus oleraceus* in bioreduction of Ag^+ to Ago. Similarly the predominant reducing sugars in the extract play a vital role in bioreduction (Ghosh *et al.,* 2012; Batarseh, (2004). Likewise the non soluble carbohydrate content like starch reflects the capping properties of the extract (Sharma *et al.,* 2009).

The FTIR absorption spectra afforded information about the chemical changes in the functional groups involved in the bioreduction of precursors and evolution of the shape in nanoparticles (Ghosh *et al.,* 2012; Socrates, 2001).

Silver ion and silver based compounds are highly toxic to microorganisms, showing strong biocidal effect against microbial species as they are highly reactive species with large surface area. Silver nanoparticles produced using plant extracts as reductant are known to exhibit broad antimicrobial spectrum (El-Shahaby *et al.,* 2013). Antimicrobial activity determined using disc diffusion method assured that synthesis of nanoparticles using *Sonchus oleraceus* flower extract and UV-irradiation lead to a greater bactericidal and fungicidal effect with increasing the concentration of the synthesized nano particles on the pathogenic tested microorganisms than either the water extract alone or the silver nanoparticles with lower concentration. This experiment demonstrated the synergy between water extract of *Sonchus oleraceus* L. flower and silver nanoparticles.

The results of our study revealed that the combination of silver nanoparticles and water extract of *Sonchus oleraceus* L. flower had improved the antimicrobial potential against the tested pathogenic strains. This approach seems to be one of the best models to be used in therapeutic management of infectious diseases.

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الملخص العربى

توصيف وفحص جسيمات الفضة النانونية المحضرة بطريقة آمنة بيئيا بإستخدام مستخلص زهرة نبات الجعضيض ودراسة كفائتها كمضادات ميكروبية

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يهدف هذا البحث الى اختيار إستخدام نبات الجعضيض كنبات بري يمتلك خصائص حيويه معروفه وإستخدام مستخلص من هذا النبات في تحضير جسيمات الفضة النانونية بطريقة آمنة بيئيا. بالإضافة إلى دراسة التطبيقات الحيوية لجسيمات الفضة النانونية المحضرة وكذلك دراسة خواصها الفيزيائية.

وقد أظهرت التحاليل الكيميائية للمستخلص المائي لأزهار نبات الجعضيض *Sonchus oleraceus* L. إحتوائه على وفرة من المركبات الفينولية، الفلافونيدات، السكريات المختزلة والسكريات الكلية. وقد إستخدم هذا المستخلص كعامل مختزل طبيعي في تكوين جسيمات الفضة النانونية إلى جانب إستخداماته البيولوجية. وكانت عملية تحضير جسيمات الفضة النانونية سريعة جدا عن طريق تعريض خليط من نترات الفضة والمستخلص المائي لأزهار نبات الجعضيض إلى الأشعة فوق البنفسجية على مدار فترات زمنية متباينة. وقد تم دراسة تأثير العديد من العوامل على تكوين الجسيمات النانونية مثل نسب المواد المتفاعلة والمدى الزمني لحدوث التفاعل. وقد تم وصف وتمييز الجسيمات النانومترية المتكونة بإستخدام عدة تقنيات مثل مطياف الأشعة فوق البنفسجية UV-Spectroscopy ، مطياف تحويل فورييه بالأشعة تحت الحمراء (FTIR) و الميكروسكوب الإلكتروني النافذ (TEM).

وقد أظهرت النتائج كذلك مدى القدرة الضد ميكروبية للجسيمات النانونية المتكونة ضد مجموعة من الكائنات المرضة البكتيرية والفطرية تزداد بزيادة تركيز جسيمات الفضة النانونية والتي كانت أعلى في كفائتها بكثير عن الستخلص المائي لأزهار نبات الجعضيض.

JOESE 5

GREEN SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF SILVER NANOPARTICLES MEDIATED BY WATER INFUSION OF *SONCHUS OLERACEUS* L. FLOWERS

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