



Synthesis of herb silver nanoparticle and study the effect against some bacterial infection

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Abstract

Antibacterial activity of synthesized silver nanoparticles was done by agar well diffusion method against three pathogenic bacteria *Proteus vulgaris* , *E.coli* . *Staphylococcus aureus* . The green synthesized silver nanoparticles can be used in the field of medicine, due to their high antibacterial activity. In this present investigation we report the green synthesis of silver nanoparticle (AgNPs) by using the leaf extract of three herb ex,*Rosemary officinalis* , *Punica granatum*, *Artemisia vulgaris*

Keywords: Nanoparticle , green herb, pathogenic bacteria , antibacterioial

INTRODUCTION

Nanotechnology provides essential oil have been known good plant form to modify and develop the important properties of metal in the form of nanoparticles having promising application in diagnostic , biomarkers, cell labelling(1, 2), contrast agents for biological imaging, antimicrobial agent, drug delivery systems and nanodrug for treatment of various disease(1, 2). Nanotechnology eco-friendly ways (3). Nanoparticle deal with the synthesis of nanoparticles with controlled size ,shape nanoparticle one and two and diversity and increase specific surface of materials of the nanometer scale length (3, 4). The silver nanoparticle are vigorously involved in the antimicrobial activity against a lot of disease causing food born and water borne pathogenic bacteria and fungus(5). *Artemisia* is also shrub plant that growth in several countries. The aerial parts of this plant activity the silver. *Rosemary* its herb and oil are coarsely rich source mainly used as spice and flavoring in food processing its desirable for against both in food processing for its desirable flavor, high antioxidant activity and natural antimicrobial agents (6,7,8,9) reported that *rosemary* plant parts are rich source compound with high antimicrobial activity so against both Gram positive and negative bacteria. High percent of the antimicrobial activity that attributed to carnosic acid and carnosol. Each plant essential oil have been known of centuries, but their strong flower limited their use in food(10). The antibacterial properties of nanoparticle having size between one and 100 nanoparticle are ascribed to their small size and increase specific surface area.(11,12). Ecofriendly synthesis of palladium nanoparticle(7dNPS) were achieved using *Phyllanthus emblica*(*P.emblica*) seeds as reducing agent . The aim of the present study was to investigate the medically important plant is used in the synthesis of medically silver nanoparticle and study the effect of herbs ,nanoparticle against *S.aureus*, *E.coli*, *Proteus.vulgaris*.

MATERIALS AND METHODS

Collection of plant materials

Aerial parts of *R.officinalis* and *A.vulgare* were collected from medicinal plants garden of pharmacognosy and medicinal plants department of college of pharmacy of Al Mustansiriya university, while the fruits cover and peel of *P. granatum* plant was obtained from locally market of Baghdad. The plant samples were identified authentically by national herbarium of Iraq .

Preparation of Extracts

All plant sample extracts were washed, dried under room temperature and converted to powder using mechanical grinder, 150 g of each plant powder was individually packed in thimble of Soxhlet apparatus and extracted with 1000 ml of aqueous ethanol(ethanol water 80-20 v/v) for 12 each extract was filtered

and concentrated under vacuum using rotary evaporator to get dry residue.

Synthesis of Silver Nanoparticle

10 ml of extract of the each herb was treated with 90 ml of 1mM silver nitrate solution and kept in room temperature. Subsequently the synthesis of silver nanoparticle was initially identified by brown colour formation and further monitored by measuring UV-vis spectra of the reaction mixture.

Antibacterial Activity of Synthesized silver Nanoparticles

The antibacterial activity of synthesized silver nanoparticle was performed by agar well diffusion method against pathogenic bacteria . *S.aureus* , *E.coli*,*P.vulgaris* fresh overnight culture of each strain was swabbed uniformly onto the individual plate containing sterile agar and 5 wells were made with the diameter of 6mm. Then 25 microliter of purified silver nanoparticle , extract of three herbs , and silver nitrate solution were poured into each well and antibiotic disc Gentamicin placed as control and incubated for 24 hour at 37 c . After incubation the different levels of zonation formed around the well and its was measured. This experiment was repeated for three times. (13).

Determination of MIC(minimal inhibitory extract)

The MIC value of Ag-NPs and extract were determined by broth microdilution assay. The Ag-NPs were serially diluted two fold with deionised water concentration ranging for 50-7.8 mg/ml . The extract were serially diluted to fold with 10 % (DMS) containing 1-1.56 mg ml after shaking 100 ml of dilute AgNPs . 0.1 , and extract was added to each well of 96 well microliter plates. Microbial suspension were adjusted to 00.5 MacFarland and diluted to 1x10⁶ CFU/ ml then 100 ml of the suspension was added to each well and incubated at 35 -42 c for 24 hour MIC value were determined as the lowest concentration compound that incubated bacteria after 24 hours(14).

RESULTS

Table(1) have shown zone of inhibition mm of clinical isolation and concentration of *A.vulgaris* and nanoparticle. Table (2) have shown antibacterial activity of silver nanoparticle and *A. vulgaris*. Table (3) have shown zone of inhibition zone mm of clinical isolation of concentration *R. officinalis* extract nanoparticle Table (4) have shown antibacterial activity of silver nanoparticle of *R.officinalis* of Ag

NPs to the plant species mg /ml. Table (5) have shown zone of inhibition of clinical isolation concentration of *Punica granatum* peel extract nanoparticle Table (6) have shown antibacterial activity of silver nanoparticle of *Punica granatum* peel AgNPs mg / ml.

Table (1): Zone of inhibition in mm of clinical isolation and concentration of *A. vulgaris* and nanoparticle (extract + AgNPs).

Type of M-O	Herb + nanoparticle			Herb only	Antibiotic(control)
	1%	3%	5%		
1. <i>S.aureus</i>	17	18	20	6	10
2. <i>Proteus.vulgaris</i>	11	12	13	7	8
3. <i>E.coli</i>	12	13	14	6	7

Table (2): Antimicrobial activity of silver nanoparticle and *A. vulgaris* AgNPs mg/ml.

Strain	MIC	MBC	MIC	MBC
1. <i>S.aureus</i>	60	120	4.30	6.10
2. <i>proteus.vulgaris</i>	10.3	20.6	2.5	2.5
3. <i>E.coli</i>	120	460	3.2	3.2

Table (3) : Zone of inhibition zone mm of clinical isolation of concentration *R. officinalis* extract nanoparticle (extract + AgNP).

Type of M.O	1%	3%	5%	Rosmary only	Antibiotic(control)
1- <i>S-aureus</i>	8	9	10	7	10
2- <i>Proteus.vulgaris</i>	11	12	13	8	10
3- <i>E.coli</i>	10	11	12	7	11

Table (4): Antimicrobial activity of silver nanoparticle of *R. officinalis* of AgNPs mg/ml.

Strain	MIC	MBC	MIC	MBC
1- <i>S-aureus</i>	60	120	4-30	
2- <i>Porteus.vulgaris</i>	10-3	20-6	2-5	5-10
3- <i>E.coli</i>	120	459	3-20	3-10

Table (5): Zone of inhibition of clinical mm of clinical isolation of concentration *p.granatum* peel extract nanoparticle (extract + AgNPs).

Type of M.O	1%	3%	5%	Peels only	Antibiotic(control)
1- <i>S-aureus</i>	13	14	15	10	12
2- <i>Proteus.vulgaris</i>	14	15	16	9	11
3- <i>E.coli</i>	5	6	7	4	6

Table (6): Antimicrobial activity of silver nanoparticles of *p.granatum* peel AgNPs mg/ml.

Strain	MIC	MBC	MIC	MBC
1- <i>S-aureus</i>	30	60	3-29	5-10
2- <i>proteus.vulgaris</i>	10-3	20-5	2-2	2-4
3- <i>E.coli</i>	60	120	2-20	2-30

DISCUSSION

The *A.vulgaris*, *Rozemary*, peel extract of *P. granatum* quickly reduce Ag to Ago and enhance synthesis of silver nanoparticle with highly antibacterial activity. (15). Addition of the aqueous herbal extract to the 1mM aqueous AgNO₃ solution resulted in change of colour within 5-10 minutes which can be varied according to the plant species chosen. The reason could be the qualitative variation in the formation of SNPs or availability of H⁺ or to reduce the silver. The change in colour was obtained which resulted due to the excitation of the surface plasmon resonance (SPR) variation of the silver nanoparticle formed. The green herb are the site of photosynthesis and accessibility of more the ion to reduce the silver nitrate into silver nanoparticle.

We have been synthesizing nanoparticle from the three herbs *A.officinalis*, *R. officinalis* and peel of *p. granatum* extract which acts as an excellent source for the reducing agent (15). This result is in agreement with M.Vanagumet al (16) 2014 who reported the green synthesized silver nanoparticle can be used in the field of medicine, due to their high antibacterial activity. The present study included the bioreduction of silver ions through medicinal plants extracts and testing for their antibacterial activity. The aqueous silver ion exposed to the extract, the synthesis of silver nanoparticle were confirmed by the change of colour of plant extract. The mechanisms of inhibitory effect of silver ion on microorganisms is somewhat known. Some studies have reported that positive changes in the silver ion concentration for their antibacterial. The mechanisms of the reaction involve the reduction of aqueous metal ion with plant leaf extract. Plant extract colour change after complete of the reaction. And it is well known that the silver nanoparticle exhibit yellowish brown bacteria on dark brown based on their size. This result is in agreement with Y. Hua et al 2017(17) who reported all strains especially the resistant strain

were significantly inhibited by herb Ag Nps at comparatively low concentration

(MIC) ranging from 0.032 to 0.063 mg/ml. Therefore, proposed the AgNps will have agreement potential in anti MR bacteria (18). The result showed the synthesized nanoparticle showing good antibacterial effect against *E.coli*, *S.aureus*, *p.vulgaris* so the present study accent the use of herb for the synthesis of silver nanoparticle with potent antibacterial effect (19). Both the component of metallic silver antibacterial and metabolic of plant has antibacterial well known nanoparticle development mechanism. Nanoparticle adheres to the cell wall and increases the pore size of cell membrane which ultimately facilitates the plant metabolite to enter the cell or interrupt the bacterial colonization. The green synthesized nanoparticle could be used in the medical field against disease due to their high efficiency as antibacterial agent. The silver nanoparticle exhibit a high antibacterial effect due to their developed surface which provides the maximum contact with environment. Furthermore, their toxicity is presumed to be size and shape dependent because small size of nanoparticle may pass through cell membrane. Inside bacteria, nanoparticle can interact with DNA, thus losing its ability to replicate which may lead to cell death. Green synthesized nanoparticles also have the more effective antimicrobial zone inhibition of the pathogen.

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