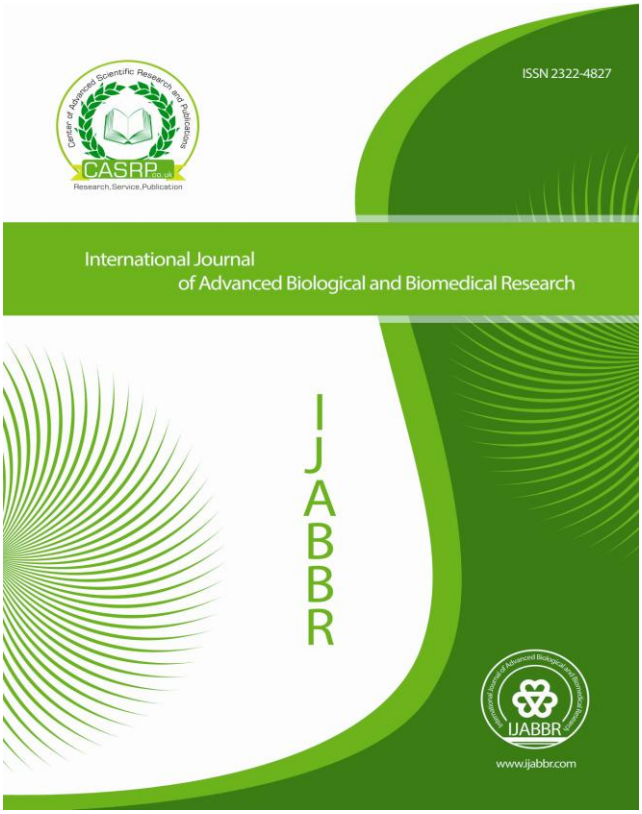

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Antibacterial activity of silver Nanoparticles produced by *Rosmarinus officinalis* L leaf extract against some human pathogenic bacteria

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Abstract

The synthesis of nanoparticles from biological processes is evolving a new era of research interests in nanotechnology. The aim of this study was to determined antibacterial activity of silver nanoparticles produced by *Rosmarinus officinalis* L leaf extract against some human pathogenic bacteria. The formation and characterisation of AgNPs were confirmed by UV-Vis spectroscopy, energy-dispersive spectroscopy (EDX), X-ray diraction (XRD) and transmission electron microscope (TEM). All strains were obtained from standard laboratory and the minimum inhibitory concentrations were investigated by microdulition method.The result show that, the levels of MIC was observed ranges from 1.25 to 2.5 mg/ml. The highest MIC value was observed against *S.pneumoniae, Hafnia alvei, S. saprophyticus.*

Keywords: Silver Nanoparticles, Human pathogens, Rosmarinus *officinalis*.

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1. Introduction

Human beings are often infected by microorganisms such as bacteria, molds, yeasts, and viruses present in their living environments. Because of the emergence and increase in the number of multiple antibiotic-resistant microorganisms and the continuing emphasis on health-care costs, many scientists have researched methods to develop new effective antimicrobial agents that overcome the resistances of these microorganisms and are also cost-effective.In order to prevent or reduce infection a new generation of dressing incorporating antimicrobial agents like silver was developed (Yin et al., 1999). Recently, metal nanoparticles have gained a lot of attention due to their unique chemical, optical, magnetic, mechanical, and electric magnetic properties. Thus metallic nanoparticles are used in different applications such as electronics, catalysis and photonic (Vaidyanathan et al., 2009). It is well known that silver ions and silver-based compounds are highly toxic to microorganisms. Thus silver ions have been used in many kinds of formulations (Sondi and Salopek-Sondi, 2004). Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non- toxic to environmental routes. Green synthesis offers improvement over synthetic, chemical or micro-organisms methods as it is cost effective, environmentally friendly and can easily be scaled up for large scale synthesis.Rosemary (*Rosmarinus officinalis* L.) originally growsin southern Europe. Its herb and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent (Lo et al., 2002; Ouattara et al., 1997). The aim of this study was to determined antibacterial activity of silver nanoparticles produced by *Rosmarinus officinalis* L leafextract against some human pathogenic bacteria.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Bacterial strains were obtained from standard laboratory. Evaluate the antibacterial activity of the plant extracts were investigated using strain of bacteria Streptococcus pyogenes ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619, S. saprophyticus ATCC®15305, Hafnia alvei ATCC 51873, Acinetobacter. baumannii ATCC 19606, Enterococcus faecalis ATCC 29212, Proteus mirabilis ATCC 35659, Serratia marcescens ATCC 274, Staphylococcus aureus ATCC® 25923. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4^{o}C until required for study.

2.2. Agar disk diffusion assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (CLSI, 2002). The procedure followed is briefly described here. Streptococcus pyogenes ATCC® 19615™, *Streptococcus pneumoniae* ATCC 49619,S. saprophyticus ATCC®15305, Hafnia alvei ATCC 51873, Acinetobacter**.** baumannii ATCC 19606, Enterococcus faecalis ATCC 29212, Proteus mirabilis ATCC 35659, Serratia marcescens ATCC 274, Staphylococcus aureus ATCC® 25923 plates were grown overnight on blood agar, Nutrient agar and colony suspension was prepared using the sterile salin water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ceftazidim, erythromycin, ceftazidime, ampicillin, amikacin and tetracyclin.

2.3. Plant materials and MIC determination of silver nanoparticles

The leaf of *Rosmarinus officinalis* L were collected in the region of Iran. The seeds were dried at room temperature and transferred into glass containers and preserved until extraction procedure was performed in the laboratory and Silver nitrate ($AgNO_3$) was used as the source for silver synthesis. The biosynthesis of silver nanoparticles using the extract of *R.officinalis L* was preliminary confirmed by the change in the color of the solution from yellow to brown and the MIC (Minimum Inhibitory Concentration) is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The broth micro-dilution method was used to define MIC. Briefly, serial doubling dilutions of the silver nanoparticles produced in the plant *R.officinalis* L leaf extract were prepared in a 96-well micro-titer plate ranged from 12.5ppm to 200ppm. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates

were wrapped loosely with cling film to ensure that the bacteria would not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37ºC for 18-24 hours. The color change was then assessed visually. The microorganism growth was indicated by turbidity.

3. Results and discussion

The silver nanoparticles revealed Gaussian distributions with average diameter of 20 nm with somedeviations. In this study, the minimum inhibitory concentration (MIC) was used. The levels of MIC was observed ranges from 1.25 to 2.5 mg/ml. The highest MIC value was observed against *S. pneumoniae*, *Hafnia alvei, S. saprophyticus* (Table1).

Table1
Antimicrobial susceptibility, MIC nanoparticles produced in the plant *R.officinalis L* leaf extract for Standard bacteria.

Bacterial	MIC extract plant (mg/ml)	Antibiotic resistance
Staphylococcus aureus	1.25	E, CE,TE, AM
Streptococcus pyogenes	1.25	-
Streptococcus pneumoniae	2.5	E, CE, CF, AM
Hafnia alvei	2.5	E, TE, AM
S. saprophyticus	2.5	E, CF, TE, AM
Acinetobacter. baumannii	1.25	CE, TE
Enterococcus faecalis	1.25	E, CE, AM
Proteus mirabilis	1.25	E, TE, AM
Serratiamarcescens	1.25	CE

E= Erythromycin, CE= Cefixime, CF= Ceftazidime, TE= Tetracyclin, AM=Ampicillin, AN=Amikacin.

Silver nanoparticles (Ag-NPs) have been known for its inhibitory and bactericidal effects in the past decades (Cho et al., 2005). Antibacterial activity of silver containing materials can be applied in medicine for reduction of infections on the burn treatment (Ulkur et al., 2005; Parikh et al., 2005), prevention of bacteria colonization on catheters (Rupp et al., 2004; Samuel and Guggenbichler, 2004) and elimination of microorganisms on textile fabrics (Yuranova et al., 2003; Jeong et al., 2005) as well as disinfection in water treatment (Chou et al., 2005). The study of Sondi and Salopek-Sondi, the result shows that silver nanoparticles haveexcellent antibacterial activity against *E. coli* (Sondi and Salopek-Sondi, 2004). Ag+ inhibits phosphate uptake and exchange in Escherichia coli and causes efflux of accumulated phosphate as well as of mannitol, succinate, glutamine, and proline (Schreurs and Rosenberg, 1982). The study of G. Guzman, the nanoparticles of silver showed high antimicrobial and bactericidal activity againstgram positive bacteria such as *Escherichia Coli*, *Pseudimonasaureginosa* and *staphylococcus aureus* which is a highly methicillin resistant strain (G.Guzman et al., 2009). The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag+ treatment (Feng et al., 2000). In addition, it was also shown that Ag+ binds to functional groups of proteins, resulting in protein denaturation (Spadaro et al., 1974). Moreover, Ag+ can lead to enzyme inactivation via formatting silver complexes with electron donors containing sulfur, oxygen, or nitrogen (thiols, carboxylates, phosphates, hydroxyl, amines, imidazoles, indoles; Ahearn et al. 1995). Ag+ may displace native metal cations fromtheir usual binding sites in enzymes (Ghandour et al., 1988).

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