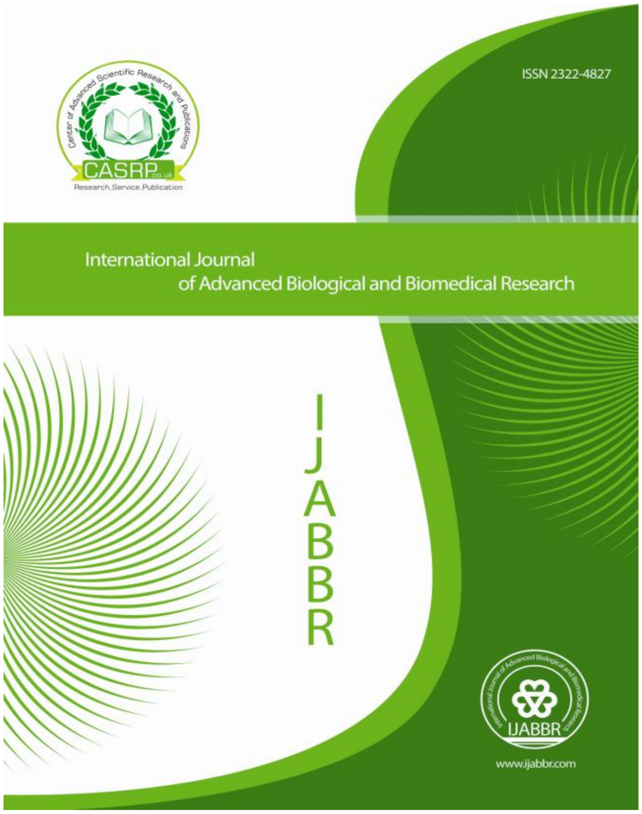
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Antibacterial effects of silver nanoparticlesagainst resistant strains of *E.coli* bacteria

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Abstract

The aim study antibacterial effects of silver nanoparticles against resistant strains of *E.coli* bacteria.12 strains of E. coli strains arising urinary infection were isolated from hospitalized patient in zabolhospitals. Minimum inhibitory concentration of winter cherry were determined by dilution method in various concentration on bacteria. The highest MIC values was found to be 100ppm against one *E.coli* and the least MIC values was observed in 12.5 ppm. This work, integrates nanotechnology and bacteriology, leading to possible advances in the formulation of new types of bactericides.

Keywords: Antibacterial activity, Nano silver, E. coli.

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1. Introduction

The ongoing emergence of multi drug resistant bacteria and the infections caused by them is on the rise very steeply.This is alarming and a global threat.Recently, many such plants have been gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development.Metal nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties (Alivisators, 1996; Brunchez et al., 1998). Various techniques, including chemical and physical meanshave been developed to prepare metal nanopartieles, such as chemical reduction (Yu, 2007; Vorobyova, 1999), heat evaporation (Bae, 2002; Smetana et al., 2005), electrochemical reduction (Liu and Lin, 2004; Sandmann et al., 2000), photochemical reduction (Malic et al., 2005; Keki et al., 2000), so on. Agreat deal of effort has been put into the bio synthesis of inorganic materials, especially metalnanoparticles using microorganisms (Mandal et al., 2006). Silver nanoparticles have more applications in many areas, including biomedical, materials science, and catalysis. A single silver nanoparticle interacts with light more efficiently than a particle of the same dimension composed of any known organic or inorganic chromophore.The antibacterial activity exhibited by silver nano particles depends on AgNO3 concentration. Mubarak Ali. et al. (Mubarak Ali et al., 2011) stated that once silver nano particles enter the bacterial cell, they would interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrates critical for cell viability and cell division. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Sondi and Salopek-Sondi, 2004; Morones et al., 2005). The aim study antibacterial effects of silver nanoparticlesagainst resistant strains of *E.coli* bacteria.

2. Materials and methods

2.1. Isolation of E. coli

All 12 strains of *E. coli* isolated from urine culture of hospitalized patients (Zabol, south-eastern Iran) suffered from urinary tract infections were evaluated. The samples were examined microscopically by Gram's stain. Samples with Gram negative results were inoculated on plates of nutrient agar, clede agar, MacConkey's and blood agar then incubated at 37°C for 24 hour. The colony showed fermenting of lactose on MacConkey agar and cled agar media were purified and identified according to their morphology as circular, rose - pink to red colonies on MacConkey agar medium and yellow colonies on cled agar. The isolates were identified by biochemical reactions e.g. catalase enzyme, potassium hydroxide test, Indole and methyl red test, vogesproskaur reaction, urease and citrate, H_2S and oxidase test.

2.2. Minimum inhibitory concentration (MIC)

The silver nanoparticles (Ag-NPs) powder used in this study was manufactured by Thermolon Korea, Inc. The broth microdilution method was used. Briefly, serial doubling dilutions of the silver nanoparticles produced in the *Saturejahortensis*seed extract were prepared in a 96-well microtiterplate 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 did 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 lowest concentration at which the color change occurred was taken as the MIC value. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

2.3. Statistical analysis

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

3. Results and discussion

The highest MIC values were found to be 100ppm against one *E.coli* and the least MIC values was observed in 12.5 ppm (Table1).

Table1
Antimicrobial susceptibility and MIC of silver nanoparticles against E. coli.

Bacterial cod	MIC(ppm)	Bacterial cod	MIC(ppm)
1	Growth	7	12.5
2	25	8	25
3	25	9	25
4	Growth	10	12.5
5	Growth	11	25
6	12.5	12	100

In the studythe highest MIC values was found to be 100 ppm against one *E.coli* and the least MIC values was observed in 12.5 ppm. The study of Banerjee, AgNPs obtained showed significantly higher antimicrobial activities against Escherichia coli (E. coli)(and Bacillus sp. in comparison to both AgNO3 and raw plant extracts. Additionally, a toxicity evaluation of these AgNP containing solutions was carried out on seeds of Moong Bean (Vignaradiata) and Chickpea (Cicer arietinum). Results showed that seeds treated with AgNP solutions exhibited better rates of germination and oxidative stress enzyme activity nearing control levels, though detailed mechanism of uptake and translocation are yet to be analyzed (Banerjee et al., 2014). The study of Duran the result show that the cotton fabrics incorporated with these silver nanoparticles exhibited antibacterial activity against *S. aureus*. The effluents obtained from the cotton fabric wash process were efficientlytreated by *C. violaceum* (Duran et al., 2007). The study of Yasin, AgNPs biosynthesized from bamboo leaves also exhibits greatantimicrobial activities against S. aureus and E.coli cultures (Yasin et al., 2013). Finally, this study shows that silver nanoparticles haveexcellent antibacterial activity against *E. coli.* This work, integrates nanotechnology and bacteriology, leading to possible advances in the formulation of new types of bactericides. However, future studies on the biocidal influence of this nanomaterial on other Gram positive and Gram-negative bacteria are necessary in order to fully evaluate its possible use as a new bactericidal material.

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