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Synergistic Effects of *Taxus baccata* Extract Mixtures with Silver Nanoparticles against Bacteria and Fungal

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ABSTRACT

Plants are rich source of natural products used for centuries to cure various the plant-derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness. Silver NPs represent material, which can be used as a potential antibacterial agent in medical applications and different commercial products due to its biological activity. Recently, silver NPs as well as various silver-based compounds containing ionic silver (Ag^+), exhibiting high antimicrobial activity, have been synthesized. Due to its biological activity. This study aimed to determine the effect of silver nanoparticles combined with *Taxus baccata L.* In this study the use with Hydroalcohlic extract *Taxus baccata L.* of method maceration (soaking) was prepared. Also antibacterial/antifungal activities of Compounds (extract, AgNPs, mixture of extract with AgNPs) were tested against three Gram-negative bacteria *Escherichia coli* (ATCC33218), *Acinetobacter baumannii* (ATCC150504) and *Klebsiella pneumoniae* (ATCC1827) and Gram-positive bacteria *Staphylococcus aureus* (ATCC 25293) and also fungi *Aspergillus oryzae* (ATCC20423). This research combines the inherent antimicrobial activity of silver metals with the *Taxus baccata* extract, yielding antibacterial activity-enhanced AgNPs.

Keyword: Silver nanoparticles; Nanotechnology; *Taxus baccata* extract; Anti-bacterial; Anti-fungal; Synergistic effects.

1. INTRODUCTION

Fundamental and applied physico-chemical research in the field of nano materials has witnessed rather great boom in the last few years. Nano materials attract attention due to their unique physico-chemical properties that are rooted in their diameter, eventually in their large surface area. These unique properties cannot be additionally found for the chemically identical material in its bulk form. The nanoparticles (NPs) are not nowadays only the target of scientific research but they can be continuously more and more frequently found not only in scientific laboratories, industrial applications, and chemical technologies but also as a part of common life due to their usage in commercially available products [1, 2]. Silver ions and silver-based compounds are highly toxic to microorganisms. Thus, silver ions have been used in many kinds of formulations [3], and

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recently it was shown that hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibit effective antimicrobial surface coating [4]. Silver NPs can effectively eliminate bacteria and yeasts even at rather low concentrations in units of mg/L [5, 6]. These low concentrations are not additionally toxic against higher organisms [6, 7]. The silver NPs can be, due to its high antibacterial activity, low toxicity against higher organisms and unproved bacterial resistance, considered one of the greatest antibacterial agents for the treatment of burns [8] or for the prevention of bacterial colonization on catheters, prosthetics and dental materials [9-15].

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems [16, 17]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [18]. *Taxus baccata* or the European yew is distributed throughout the temperate zones of the northern hemisphere. It is a small to medium-sized evergreen tree that historically has been used for weapon-making and medicine, and is poisonous except for the fruit [19]. The genus Taxus belongs to the Class Pinopsida, the Order Taxales and the Family Taxaceae. As the species are highly similar, they are often easier to separate geographically than morphologically. Typically, eight species are recognized [20]. The genus Taxus has generated considerable interest due to its content of diterpene alkaloids, particularly taxol (known also as the generic drug paclitaxel and by the registered trade name Taxol® BMS [Bristol-Myers Squibb]). The anticancer properties of taxol were discovered in *T. brevifolia* extracts in 1971 [21]. This plant is used traditionally for the treatment of high fever and painful inflammatory conditions. The leaves of this plant are used to make herbal tea for indigestion and epilepsy. Previously published literatures on *T. wallichiana* have reported immunomodulatory, anti-bacterial, anti-fungal, analgesic, anti-pyretic and anti-convulsant activities [22, 23].

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of the forests of northern Iran were collected. Then plant was washed thoroughly with tap water followed with sterilized distilled water for the removal of dust and sand particles. The flowers were shade dried in the dark at room temperature for few days and then homogenized to fine powder by a mechanical grinder; the powdered materials were passed through sieve number 40, and stored.

2.2. Preparation of plant extracts

Plant extracts were prepared according to a standard protocol. Prepared plant material (50 g) was transferred to dark-coloured flasks and mixed with different solvent water and ethanol (80% ethanol and 20% distilled water) respectively and stored at room temperature and in the dark. After 48 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40°C and with 15 rpm using Rotary (N-S 2005 HS, South Korea) evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4°C. After evaporation of the solvent, the crude extract was subjected to subsequent analysis. Silver nanoparticles were synthesized in the Laboratory of Physics, University Yasouj and size of nanoparticles was confirmed by SEM and XRD measurements.

2.3. Antibacterial activity (in vitro)

The Compounds (extract, AgNPs, mixture of extract

Table 1: *Antibacterial activities of constructed disks soaked in 80, 40 and 20 mg/mL of compounds (extract, AgNPs, mixture of extract with AgNPs) in diameter zone (mm) on various bacterial strains.*

Compounds	Gram negative bacteria mg/mL									Gram positive bacteria mg/mL		
	Escherichia coli			*Klebsiella pneumoniae*			*Acinetobacter baumannii*			*Staphylococcus aureus*		
	80	40	20	80	40	20	80	40	20	80	40	20
Extract	13.00	11.73	10.33	11.83	10.22	9.00	11.75	10.34	9.33	12.15	9.72	8.63
AgNPs	15.74	13.72	10.43	18.51	13.34	10.33	15.45	13.43	11.23	16.12	13.10	11.42
Mixture	14.73	12.62	11.00	16.82	14.62	9.22	15.00	12.33	10.28	14.72	13.32	10.62

with AgNPs) were appraised for their antibacterial activities by disk diffusion method at Muller Hinton agar medium (Merck, Germany) against three Gram-negative bacteria such as *E. coli* (ATCC33218), and *Acinetobacter baumannii* (ATCC150504), *Klebsiella pneumoniae* (ATCC1827) and also Gram-positive bacteria including *S. aureus* (ATCC 25293) [24]. For this mean, 15 mL of the agar medium was poured into a series of sterile petri plates. Then 0.1 mL of the specific bacterium including nearly 0.5 - 10^6 colony-forming units (CFU/mL) (equal to 0.5 McFarland standards) was inoculated for 24 h (old) on the surface of the plates and then swabbed and kept for adsorption about 10 min [25]. Sterile paper disks (6 mm in diameter) were loaded with trial samples which had been prepared on different concentrations (80, 40, 20 mg/mL in DMSO), placed on the agar medium. All the plates were incubated at 37°C for 24 h. Antibacterial activities of compounds were evaluated based on diameter of zone of inhibition (mm) and tabulated in Table 1. According to previous reports [25], there is no notable effect for DMSO on the biological environment. Gentamicin and for Gram-positive bacteria, Cephalexin were used as reference bactericidal drugs (positive controls).

2.4. Minimum inhibitory concentration (MIC)

The lowest concentration that prevents the growth of bacteria is considered as MIC. The MIC of the compounds was assessed against a specified bacterium based on a broth dilution method (Table 2). In this method, various concentrations of compounds (12.5–0.024 mg/mL) were prepared in the sterile test tubes using the serial dilution method. Then, 0.65 mL of sterile Muller Hinton broth medium and 0.1 mL of bacterium were added to test tubes and in final, the sets were incubated at 37°C for 24 h.

2.5. Measured minimum bactericidal concentration (MBC)

MBC of Compounds (extract, AgNPs, mixture of extract with AgNPs) were investigated by sub culturing a loop full of broth dilution MIC tests to Muller Hinton Agar medium on a plate and then incubated at 37°C for 24 h [25]. In this method, could observe bacterial growth on the surface of agar medium (Table 2).

2.6. Antifungal effects

Antifungal activities of Compounds (extract, AgNPs, mixture of extract with AgNPs) were checked against two fungal strains such as *A. oryzae.* For activity measurement, the prepared discs (that had been soaked in the various concentration of compounds 80, 40, 20 mg/mL in DMSO) were placed at different positions on a surface of petri plates covered by Sabouraud dextrose agar(SDA) medium (Oxoid, Hampshire, England) which have been fecundated with 100 mL (10^5 CFU/ mL) of fungal spore suspensions. The plates were incubated at 32°C for 7 days for *A. oryzae* (Table 3) [25]. A commercial antibiotic to control the Amphotericin B was chosen.

3. RESULTS AND DISCUSSION

3.1. Antibacterial bioassay (in vitro)

The antimicrobial activity of Compounds (extract, AgNPs, mixture of extract with AgNPs) were stud-

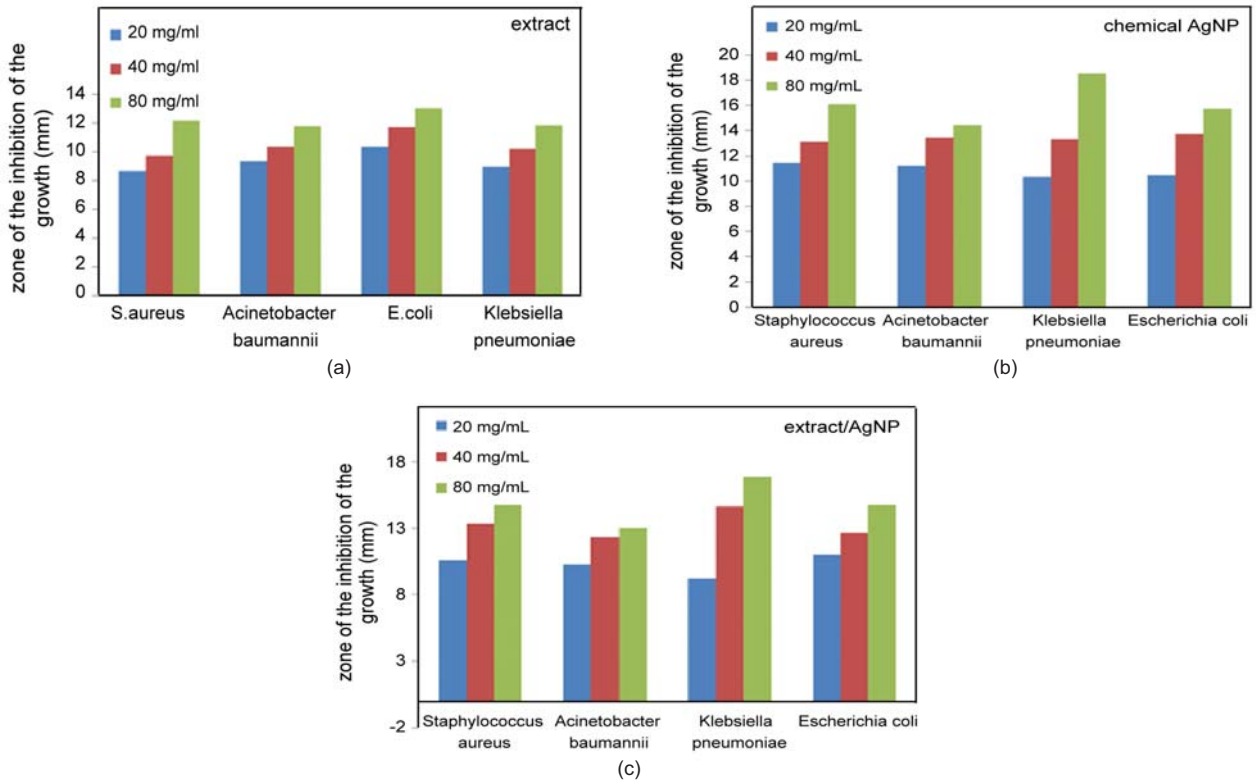

Figure 1: *Zone of the inhibition of the growth of constructed disks (soaked in 80, 40 and 20 mg/mL) of compounds against four bacteria.*

ied in different concentrations (80,40,20 mg/mL) against four pathogenic bacterial strains Gram positive *S. aureus* (ATCC 25293), three Gram- negative *E. coli* (ATCC33218), *Acinetobacter baumannii* (ATCC150504), *Klebsiella pneumoniae* (ATCC1827). The results of the antibacterial activities are presented in Table 1 and Figure 1. The acquired results revealed that the efficiency of these compounds as compared to standard antibiotics (Gentamicin and Cephalexin) in our conditions the antibacterial activity of the Compounds (extract, AgNPs, mixture of extract with AgNPs) increased linearly with increase in concentration of Compounds (mg/mL). The extract exhibits the highest activity at the lowest concentration of 20mg/mL showed which is equal to 10.33 mm against *E. coli*, while extract had lowest effect against *S. aureus.* For

Table 2: *Antibacterial activity (MBC and MIC in mg/mL) Taxus baccata L. extract and silver nanoparticles and their combination microdilution method.*

Extract and synthesized silver nanoparticles		Bio Silver nanoparticles		extract *Taxus baccata*		Bacteria
MIC	MBC	MIC	MBC	MIC	MBC	
0.195	0.195	0.78	1.56	3.125	3.125	*S. aureus*
0.78	0.78	0.195	0.195	1.56	3.125	*E.coli*
0.78	1.56	0.78	1.56	0.195	0.39	*Acinetobacter baumannii*
0.78	3.125	1.56	3.125	0.78	3.125	*Klebsiella pneumoniae*

Table 3: *Antifungal activities of constructed disks (soaked in 80, 40 and 20 mg/mL of compounds (extract, extract with AgNP, AgNP) based on diameter zone (mm) against fungal Aspergillus oryzae.*

Compounds	*Aspergillus oryzae* mg/mL		
	20	40	80
Extract	6.23	8.12	9.00
AgNP	9.56	9.87	10.34
Mixture	6.55	7.43	9.21

AgNPs exhibits the highest activity in this concentration showed which is equal to 13.22 mm against *Klebsiella pneumoniae* and for the bacterial extract when mixed with silver nanoparticle inhibition zone diameter 12.24 mm, respectively. These results demonstrate that the synergistic combination of the antibacterial activity of the extract with AgNPs enhances the antimicrobial effects. As it was noted above, the determination of the bactericidal effect of the Compounds (extract, AgNPs, mixture of extract with AgNPs) to the reference strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* was achieved by means of the dilution method MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration). MIC of *Taxus baccata L.* extracts was the least (0.195 mg/mL) for inhibition of growth of *Acinetobacter baumannii*, MIC and MBC for AgNPs 0.195 mg/mL against *Escherichia coli*. MIC and MBC mix *Taxus baccata L.* extract with AgNPs was 0.195 mg/mL against *Staphylococcus aureus*. In the case of *Acinetobacter baumannii* bacterium, the lowest MBC was obtained 0.39 mg/mL belonged to *Taxus baccata L.* activity of the medicinal plant with AgNPs enhances the antimicrobial effects.

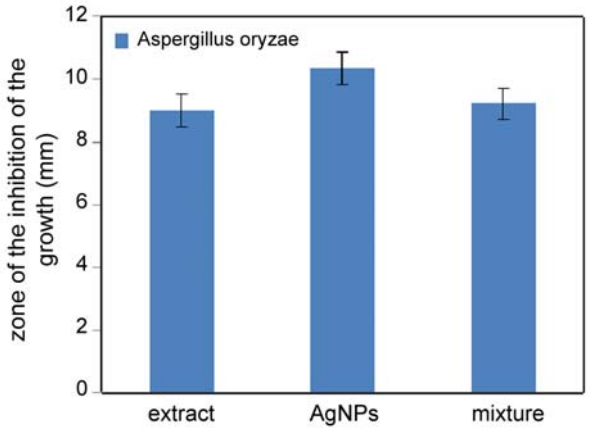

Figure 2: *Zone of the inhibition of the growth of constructed disks (soaked in 80 mg/mL) of compounds against fungal Aspergillus oryzae.*

3.2. Antifungal bioassay (in vitro)

In addition to antibacterial activities, compounds (extract, AgNPs, mixture of extract with AgNPs) were subjected to antifungal activities against A. oryzae fungal strain and the zone diameters of inhibition (mm) have been summarized in Table 3. Also for a clear comparison, the zone of the inhibition of the growth for disks constructed by soaking in 80 mg/mL of compounds (extract, AgNPs, mixture of extract with AgNPs) in DMSO has been depicted as column chart in Figure 2 investigations revealed that AgNPs has excellent antifungal activity on used funguses with respect to extract and mixture extract with AgNPs. Finally it is to be noted that the results in antimicrobial bioassay indicate that the mixture of AgNPs to extract improves significantly its antibacterial and antifungal activity.

4. CONCLUSIONS

Based on these findings, the silver NPs do not represent any risk for human beings, when used in medical applications and commercially available products, but only under the condition that the silver concentration is retained at units of mg/L, which is sufficient for the suppression of bacterial and yeast growth. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine

in traditional therapies of 80% of the words population. In the present work, extracts obtained from *Taxus baccata L.* shows strong activity against most of the tested bacteria and fungal strains. The results were compared with standard antibiotic drugs. From the above results the activities of hydroalcohol extracts of *Taxus baccata L.* shows significant antibacterial and antifungal activity. The results showed that the *Taxus baccata L.* extract against bacteria and fungus inhibiting effect was tested. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

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