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Enhancement of antimicrobial effect of some spices extract by using biosynthesized silver nanoparticles

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Abstract

Spices and silver nanoparticles are well recognized for its antimicrobial effect against food borne pathogens. The target of this research was to estimate the antimicrobial activity of four spices (clove, rosemary, thyme and black pepper) and the biosynthesized silver nanoparticles alone and in the combination of each spice with the biosynthesized silver nanoparticles. Broth microdilution method and subsequent checkerboard assay were used for evaluation of the antimicrobial activity. The antimicrobial activity was assessed against bacterial strains (*Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633) and yeasts (*Candida parapsilosis* AUMC 8909 and *Trichosporon domesticum* AUMC 8918). Silver nanoparticles showed good antimicrobial effect and clove exhibited the best antimicrobial effect within the examined spices. The combination demonstrated 8 synergistic and 8 additive effects for inhibition of growth of the tested microorganisms. The microbicidal effects were 7 synergistic and 10 additive effects. The results recommended the addition of spice in combination with silver nanoparticles for enhancement of antimicrobial activity, even for meat packaging in nontoxic concentrations.

Introduction

Meat and other food items are contaminated by bacteria and fungi. Food-borne pathogens as *Salmonella, Shigella, Escherichia, Listeria, Clostridium,* and *Vibrio* and their toxins have been health problems. Spices are necessary for most sectors of the food industry, particularly in the processing of meat. If spices were added to eradicate these microorganisms or hinder their growth before toxin production, so the use of spices strengths reduction of food-borne illnesses and food poisoning (Agaoglu *et al*., 2007). Spices are known to be added to the flavor and color of the food. Additionally, some spices are documented to have bactericidal or bacteriostatic activities so can be used as a preservative (Abd El-Aziz and Ali, 2013).

Spices have been applicable as food additives. They improve the flavor, taste and color of food, as well as they extend the shelf life of food by inhibiting the growth or decrease the food born pathogens. Plant known as natural antimicrobials, which have long been applied for the preservation of foods. Spices were used by the former Egyptians and have been previously used in China and India. Spices for example clove, cinnamon, mustard, garlic, ginger, and mint can substitute the health remedies. Most of spices show antimicrobial activity against bacteria, yeasts, and molds. The biological activity of spices based on the phenolic compounds, so can be effectively applied as food preservatives. Spices can be classified according to their antimicrobial activities into three categories; the first classified as strong (cinnamon, clove, mustard), the second as medium (allspice, sage, bay leaf, caraway, coriander, cumin, rosemary, thyme, oregano), and the the third as weak (black pepper, red pepper, ginger) (Tarik *et al*., 2016).

Rosemary (*Rosmarinus officinalis* L.) is a plant that is applied for numerous purposes, it is recognized as a culinary spice. Rosemary has high antioxidant activity and also rosemary essential oil is used as an antibacterial, and used for control of mould, anticancer mediator , inhibit osteoclast activity and increase bone density (Hendel *et al*., 2016). Silver nanoparticles (AgNPs) have been applied as antibacterial, antifungal, antiviral, and anti-inflammatory and catalytic activity due to its distinguishable physical, chemical, and biological. Thus, Silver nanoparticles can be used for prevention of biofilm formation in the meat industry (Gurunathan *et al*., 2014).

The conventional chemical method is recognized as being dangerous, energy and wealth exhaustive

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removing the conventional techniques to be environmentally friendly. Also, chemical synthesis of silver nanoparticles mostly ended in aggregation as the storage time extends while biosynthesis of nanoparticles using plant extracts which also known as green synthesis is low-cost, environmentally kind and produce stable nanoparticles (Sharma *et al.*, 2014).

Silver nanoparticles may be added in nontoxic concentrations to meat products as several studies carried on the toxicity of silver nanoparticles. Ivask *et al*. (2014) verified that silver nanoparticles had no cytotoxicity to mammalian cells at 26.7 mg/L. In another investigation recorded that AgNPs couldn't affect the mammalian cells morphology up to 6,500 ng/mL concentration. It has also been documented that AgNP didn't cause DNA damage to the mammalian fibroblast cells up to 25,000 ng/mL of concentration (Arora *et al*., 2008).

Nanomaterials have the ability to discover food spoilage and food pathogens through nanosensors. Also, nanoparticles used in food packaging consisting of polymers in combination with nanodevices is known as smart packaging. Smart packaging can be used to check the food or the enclosed environment throughout storage. Nanocomposite materials improve the packaging properties by preventing oxygen, moisture and carbon dioxide. Natural, edible Nanolaminates can also carry antioxidants and antimicrobials. for extension of shelf-life (Ramachandraiah *et al.*, 2015).

Packaging materials are coated with antimicrobial agents to be slowy released in the food to preserve food quality and safety. This mechanism of slow releasing is more efficient, as direct additives in the food which may react with other food components and results in loss of its activity. For example, protein rich food negatively affect the antibacterial activity of silver ion. So far, the most hopeful use of nanotechnology in meat industry seems to be meat packaging, but there were needs for more studies on the migration of the nanomaterials from the packaging and long term effects on human health (Makwana *et al*., 2015).

Antimicrobial synergism between the antimicrobial agents and bioactive plant extracts is a new concept and could be helpful or harmful. Silver has been believed as an effective antimicrobial. However, a toxicity troubles with silver have limited the use of silver nanoparticles, especially for food preservation. So the goal of the study was to explore the antimicrobial activity of silver nanoparticles and spices each alone against the tested microorganisms and the combination (synergistic effect) between silver nanoparticles and each spice. This could raise the antimicrobial strength of biosynthesized silver nanoparticles with spices and so decrease the effective concentration in particular when silver nanoparticles added in meat packaging.

Material and methods

Preparation of aqueous extract of Rosmarinus officinalis

Five grams of dry ground rosemary was boiled (100°C) in 100 ml distilled water for 5 minutes. The extract was filtered After cooling (1hr) with Whatman number 1 filter paper and different concentrations (1, 1:2, 1: 10 and 1:20) were prepared from the 5% aqueous extract.

Biosynthesis of silver nanoparticles

Aqueous extracts *Rosmarinus officinalis* (rosemary) is used for the preparation of the silver nanoparticles. The silver nitrate ($AgNO_3$) (AR grade ≥99.9% purity, Sigma-Aldrich) as a source for silver nanoparticles was purchased from Sigma-Aldrich Chemicals. The extract incubated with silver ion (1mg) in a dark chamber to diminish photo-activation of silver nitrate for 24 hours of incubation at room temperature. Control without the $AgNO_3$ was also kept in the same conditions.

Characterization of silver nanoparticles

UV-visible spectroscopy analysis

The optical properties of silver nanoparticles were carried out on Ultraviolet-Visible Spectroscopy (UV-Vis) (Evolution 300- UV-Visible spectrophotometer-England) at a resolution of 1 nm for verification of synthesis of silver nanoparticles and scanning the spectra between 300-900 nm. Metal nanoparticles exhibited a strong absorption of electromagnetic waves in the visible range due to the surface plasmon resonance (SPR) (Yousef and Nafady, 2014).

Atomic absorption spectroscopy

The Atomic absorption spectroscopy (AAS) was used for determining the concentration of silver nanoparticles (in the solution) (Buck model 210 VGP Atomic Absorption Spectrometer-USA).

Transmission electron microscope (TEM)

The morphology and sizes of silver nanoparticles were determined in the 3rd concentration by TEM micrographs using the JEOL TEM 100 CXII (Electron Microscope Unit, Assiut University, Egypt). The sample was prepared by locating a drop of synthesized silver nanoparticles on a negative carbon coated copper grids and dried in air (Ali *et al.*, 2015).

Aqueous preparation of spices extract

Aqueous extracts of four types of spices used in meat and meat products; cloves *(Syzygium aromaticum)*, black pepper *(Piper nigrum)*, rosemary *(Rosmarinus officinalis)*, thyme *(Thymus vulgaris)*, were prepared by mixing 15 grams of dry ground spices with 150 ml sterile distilled water for 24 hours at room temperature. Then the extracts were filtered and evaporated at 50°C till leave pasty material which weighed and stored at 4°C till used (Abd El-Aziz and Ali, 2013).

Microorganisms and inoculums preparation

The following bacterial strains and yeast previously isolated from meat products, which are known to cause food poisoning or food spoilage were used as test strains: *Pseudomonas aeruginosa* and *Salmonella typhimurium* previously isolated from chicken carcass meat and molecularly identified (Abd El-Aziz, 2013, 2015) , while *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Candida parapsilosis* AUMC 8909 (Assiut University Mycology Center) and *Trichosporon domesticum* AUMC 8918 were purchased. Nutrient agar (NA) was used for growing and purification of *Pseudomonas aeruginosa* and *Salmonella typhimurium, Escherichia coli* ATCC 8739 and *Bacillus subtilis* ATCC 6633. A single colony was inoculated into nutrient broth, overnight incubated at 35ºC and diluted to $5x10^5$CFU/ ml, while Sabouraud Dextrose Agar (SDA) media was used for purification of *Candida parapsilosis* AUMC 8909 and Trichosporon domesticum AUMC 8918. A single colony was inoculated into sabouraud dextrose broth, overnight incubated at 25 to 30ºC and diluted to $5x10^5$CFU/ml.

Antimicrobial activity of the biosynthesized silver nanoparticles and spices extracts

Detection of minimum inhibitory concentration (MIC)

The antimicrobial effect was checked by double fold micro-dilution method on some common food borne pathogens. Ten concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, 1.953, 0.976mg/ ml) using two fold serial dilution of each of plant extracts and biosynthesised silver nanoparticles (100 µl in each well) in the 96-well microtitre plate (one plate for each plant extract and one plate for silver nanoparticles) were prepared to begin with 500mg/ ml for plant extract and 11.6 µg/mL for silver nanoparticles. To each concentration 100 µl of 5 × 10^5CFU/ ml of the tested microorganism (*E. coli* ATCC 8739, B. subtilis ATCC 6633 *S. typhimurium, Ps. aeruginosa, C. parapsitasis* AUMC 8909 and *T. domesticum* AUMC 8918) were added in a final volume of 200 µl., respectively). Pure bacterial inoculum in broth was taken as positive control and nanoparticles and plant extract but without bacterial inoculum was taken as a negative control. The plates were incubated at 30ºC for 24hours and examined for the lowest concentration show no detectable growth (MIC) (Wiegand *et al.*, 2008).

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Detection of minimum bactericidal concentration (MBC)

After MIC determination of silver nanoparticles and plant extracts, aliquots of 10 µl from all wells with no noticeable bacterial growth were seeded in NA and SDA and the plates were incubated for overnight at 30°C. The plates examined for the lowest concentration of antimicrobial agent showed no colony growth of the tested microorganism on the NA or SDA plates (MBC) (Wiegand *et al.*, 2008).

Enhancement effect of silver nanoparticles and plant extracts

Silver nanoparticles were mixed with each plant extract (50 µl each) and different concentrations were prepared with the microorganisms (100 µl of 5 × 10^5 CFU/ ml) in a final volume of 200 µl as previously using the double fold serial dilution in the 96- well microtitre plates. The plates were incubated and tested for MIC and MBC. The assessment was calculated using the checkerboard assay method.

Checkerboard assay

To calculate the effect of the combinations, the fractional inhibitory concentration index (FICI) and fractional bactericidal concentration index (FBCI) are the sum of the FICs and/or FBCs of each of the antimicrobial agents, which were defined as the MIC and/or MBC of each drug when used in combination divided by the MIC and/or MBC of each drug when used alone. It was considered to be synergistic, additive, indifferent or antagonistic when the FIC or FBC indices were ≤0.5, >0.5 to ≤1, >1 to ≤2 and >2, respectively (Smekalova *et al.*, 2015).

Results and Discussion

Silver ions are used as antimicrobial agents (Yousef, 2014). However, higher concentrations of silver salts limit their use to avoid mammalian toxicity (Ivask *et al.*, 2014) . The antimicrobial effect

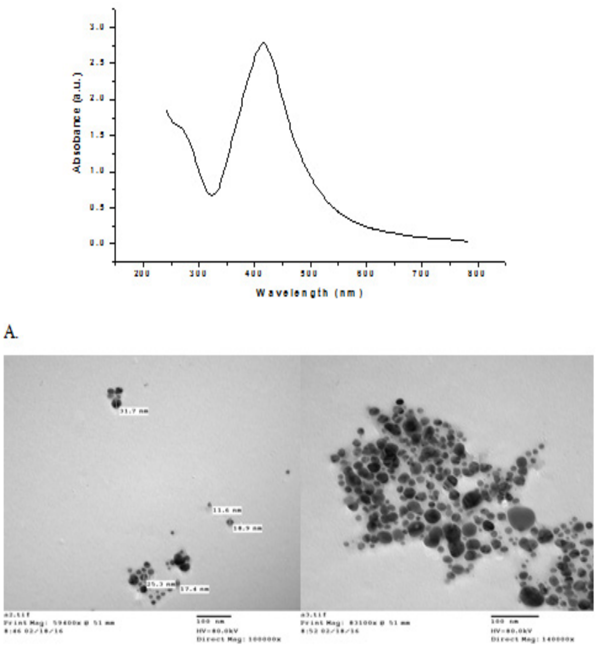

Figure 1. UV-Visible absorption spectrum of the silver nanoparticles shows a broad absorption band at 414 nm for silver nanopraticles after 24 hours incubation (A). TEM images of biosynthesized silver nanoparticles using rosemary extract. The particles are spherical with varying size (AgNPs in the range from 10-31.7 nm) (B).

of metal nanoparticles powered to their small size and surface to volume ratio, which permits them to interact directly with the microbial membrane (Duran *et al.*, 2005). Metallic nanoparticles can be synthesized by several physical and chemical methods (Mohamed *et al.*, 2012). However, the simple and eco-friendly biological systems would be more helpful in the application of metallic nanoparticles. Some microorganisms can reacte with metal ions causing reduction of them into metallic nanoparticles (Mukherjee *et al.*, 2008).

The extract of rosemary (phytochemical principle act as reducing and capping agent) was used for the biosynthesis of silver nanoparticles. Appearance of a yellowish –brown colour within 10 minutes in the reaction mixture indicated the creation of colloidal silver nanoparticles (Gurunathan *et al.*, 2009). Visual observation after 24 hours (due saturation of the capping agents after 24 hours) of incubation showed the change in intensity with appearance of brown colour. It can be supposed that the plant extract components reduce nitrate into nitrite and silver ion by reductase enzyme (protein) which leading to creation of silver nanoparticles that stabilized by capping peptide. This raise in intensity could be due to the development of extra nanoparticles, the colour relies on the particle's size, shape and the presence of adsorption layers and their structure (Pal *et al.*, 2007).

Figure1 shows a clear absorption band for silver

Table 1. Antibacterial activity of Silver nanoparticles alone

Microorganisms	MIC (µg/ml)	MBC (µg/ml)
Ps. aeruginosa	2.9	5.8
S. typhimurium	2.9	5.8
E.coli ATCC 8739	2.9	5.8
B. subtilis ATCC 6633	2.9	2.9
C. parapsilosis AUMC 8909	0.725	2.9
T. domesticum AUMC 8918	2.9	5.8

nanoparticles at 414 nm to confirm the reduction of silver nitrate after 24 hours incubation. A characteristic nanoparticles absorption band in the visible area between 350 and 550 nm, Plasmon resonance peak acquired at 420 nm observed in the third concentration (1:10) which is well-recognized for a variety of metal nanoparticles with sizes from 2-100 nm (Prasad and Jha, 2010). The silver nanoparticles band stayed around 420 nm demonstrating that the particles were well diffused without aggregation and stable. The exposed concentration of silver nanoparticles in the obtained solution was 11.6 µg/ml by atomic absorption spectrometer.

Transmission electron microscope (TEM)

The TEM images as shown in Figure1 spherical particles of the biosynthesized silver nanoparticles using rosemary extract with a varying size range from 10 to 31.7 nm (in the third concentration (1:10)), that can be used in several applications (Gade *et al.*, 2007).

Antimicrobial activity of silver nanoparticles alone

It has been well reported and experimentally confirmed that silver nanoparticle has the uppermost antimicrobial effect among the synthesized metal nanoparticles (Barapatre *et al.*, 2016). In the present study, the antimicrobial activity (MIC and MBC) of the biosynthesized silver nanoparticle was studied against against tested microorganisms; gram negative bacteria (*Ps. aeruginosa, S. typhimurium* and *E.coli* ATCC 8739), gram positive (*B. subtilis* ATCC 6633) and yeast (*C.parapsilosis* AUMC 8909 and *T. domesticum* AUMC 8918).

The biosynthesized silver nanoparticles

Table 2. Antibacterial activity of clove and rosemary each alone and in combination with silver nanoparticles

Microorganisms	Clove	MIC in combination		FICI	Effect	Clove	MBC in combination		FBCI	Effect
	(mg/ml)	(µg/ml)	(mg/ml)			(mg/ml)	(µg/ml)	(mg/ml)		
Ps.aeruginosa	125	0.725	31.25	0.5	(S)	250	1.45	62.5	0.5	(S)
S. typhimurium	125	0.725	31.25	0.5	(S)	250	0.725	31.25	0.25	(S)
E. coli ATCC 8739	62.5	0.3625	15.625	0.375	(S)	62.5	0.725	31.25	0.625	(A)
B. subtilis ATCC 6633	31.25	0.3625	15.625	0.625	(A)	62.5	0.725	31.25	0.75	(A)
C.parapsilosis AUMC 8909	62.5	0.3625	15.625	0.75	(A)	125	0.725	31.25	0.5	(S)
T. domesticum AUMC 8918	125	0.725	31.25	0.5	(S)	125	1.45	62.5	0.75	(A)
Microorganisms	**Rosemary**	**MIC in combination**		**FICI**	**Effect**	**Rosemary**	**MBC in combination**		**FBCI**	**Effect**
	(mg/ml)	(µg/ml)	(mg/ml)			(mg/ml)	(µg/ml)	(mg/ml)		
Ps. aeruginosa	500	1.45	62.5	0.625	(A)	-	2.9	125	0.5+ <0.25	(A)
S. typhimurium	500	1.45	62.5	0.625	(A)	-	1.45	62.5	0.25 + < 0.125	(S)
E. coli ATCC 8739	500	0.3625	15.625	0.15625	(S)	-	1.45	62.5	0.25+ <0.25	(S)
B. subtilis ATCC 6633	500	0.725	31.25	0.3125	(S)	-	1.45	62.5	0.5 + < 0.125	(A)
C. parapsilosis AUMC 8909	500	0.725	31.25	1.0625	(I)	-	2.9	125	1+ < 0.25	(I)
T. domesticum AUMC 8918	250	2.9	125	1.5	(I)	500	5.8	250	= 1 + 0.5 =1.5	(I)

S: Synergistic. A: Additive. I: Indifferent.
Clove :FICI were 4 Synergistic and 2 Additive , while FBCI were 3 Synergistic and 3 Additive .
Rosemary: FICI were 2 Synergistic, 2 Additive, and 2 Indifferent, while FBCI were 2 Synergistic, 2 Additive, and 2 Indifferent.

showed antimicrobial activity against the examined microorganisms. As shown in Table 1 the MIC values against the tested microorganisms were in the range of 0.725-2.9 µg/ml. However, the MBC values were in the range of 2.9- 5.8 µg/ml. Sharma *et al*. (2014) found that MIC and MBC for *E.coli* ATCC 8739 were 0.628 and1.25 µg/ml, respectively. This also confirmed by Prabhu *et al*. (2015) who observed that even least concentration of silver nanoparticles still have a high antimicrobial effect. The mechanisms by which AgNPs affect bacteria cannot be explained in a single and specific manner, as with antibiotics. AgNPs harm the bacterial cell wall, modify membrane permeability and cause a fall in plasma membrane potential. Furthermore, AgNPs harm the DNA, suppress enzymes, affect metabolic processes, modify protein expression and break the respiratory chain. Silver ions are liberated from the nanoparticle surface and come into the bacterial cell to produce reactive oxygen species (ROS), which damage bio-macromolecules (Smekalova *et al*., 2015).

Antibacterial activity of clove alone and in combination with silver nanoparticles

The MIC assay of the aqueous extract of clove showed that it had the highest antimicrobial effect among the examined spices, in particular against *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *C. parapsilosis* AUMC 8909 (Table 2). Similar results obtained by Sethi *et al*. (2013) and Amrita *et al*. (2009) who found that clove has effective inhibition against *E. coli*. Also, it showed bactericidal effects against *E. coli* ATCC 8739 and *B. subtilis* ATCC 6633. The effects of clove combined with the biosynthesized silver nanoparticles were synergistic, defined by FICI of $\le$0.5 against *Ps. aeruginosa, S.typhimurium, E. coli* ATCC 8739, and *T. domesticum* AUMC 8918, and by FBCI of $\le$0.5 against *Ps. aeruginosa, S. typhimurium,* and *C. parapsilosis* AUMC 8909. Additive interactions were observed, identified by FICI of >0.5 to $\le$1 against *B.subtilis* ATCC 6633 and *C. parapsilosis* AUMC 8909, and by FBCI of >0.5 to $\le$1against *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *T. domesticum* AUMC 8918. This substantiates the result found by Prabhu *et al.* (2015) who found

Table 3. Antibacterial activity of thyme and black pepper each alone and in combination with silver nanoparticles

Microorganisms	Thyme	MIC in combination		FICI	Effect	Thyme	MBC in combination		FBCI	Effect
	(mg/ml)	(µg/ml)	(mg/ml)			(mg/ml)	(µg/ml)	(mg/ml)		
Ps. aeruginosa	500	1.45	62.5	0.625	(A)	-	2.9	125	0.5+ <0.25	(A)
S. typhimurium	500	1.45	62.5	0.625	(A)	-	1.45	62.5	0.25+ <0.25	(S)
E. coli ATCC 8739	500	0.3625	15.625	0.15625	(S)	-	0.725	31.25	0.125+ < 0.0625	(S)
B. subtilis ATCC 6633	500	2.9	125	1.25	(I)	-	2.9	125	1 + < 0.25	(I)
C. parapsilosis AUMC 8909	500	2.9	125	4.25	(AN)	-	5.8	250	2 + <0.5	(AN)
T.domesticum AUMC 8918	500	1.45	62.5	0.625	(I)	500	2.9	125	0.5 + 0.25 = 0.75	(A)
Microorganisms	Black pepper	MIC in combination		FICI	Effect	Black pepper	MBC in combination		FBCI	Effect
	(mg/ml)	(µg/ml)	(mg/ml)			(mg/ml)	(µg/ml)	(mg/ml)		
Ps.aeruginosa	500	2.9	125	1.25	(I)	-	2.9	125	0.5+ <0.25	(A)
S. typhimurium	500	1.45	62.5	0.625	(A)	-	2.9	125	0.5+ <0.25	(A)
E.coli ATCC 8739	500	0.725	31.25	0.3125	(S)	-	2.9	125	0.5+ <0.25	(A)
B.subtilis ATCC 6633	500	1.45	62.5	0.625	(A)	-	2.9	125	1 + < 0.25	(I)
C.parapsilosis AUMC 8909	250	2.9	125	4.5	(AN)	-	-	-	-	(ND)
T. domesticum AUMC 8918	500	2.9	125	1.25	(I)	-	-	-	-	(ND)

S: Synergistic. A: Additive. I: Indifferent. AN : Antagonist. ND: Not detected.
Thyme: FICI were 1Synergistic, 2 Additive, 2 indifferent, and 1 antagonist, while FBCI were 2Synergistic, 2 Additive, 1 indifferent, and 1 antagonist.
Black pepper: FICI were 1 Synergistic, 2 Additive, 2 indifferent, and 1 antagonist , while FBCI were 3 Additive, 1 indifferent, and 2 not detected.

that clove nanoparticles have the highest activity against the tested organisms.

Antibacterial activity of rosemary alone and in combination with silver nanoparticles

As shown in Table 2 there are better antibacterial effects, when the combination between silver nanoparticles and rosemary, as rosemary alone gives mild antimicrobial effect. The combination between rosemary and silver nanoparticles resulted in synergistic effects against *E. coli* ATCC 8739 and *B. Subtilis* ATCC 6633 defined by FICI ≤ 0.5 and against *S.typhimurium* and *E.coli* ATCC 8739 defined by FBCI ≤ 0.5. Also additive were effects shown against *Ps. aeruginosa* and *S. typhimurium* defined by FICI >0.5 to ≤1 and against *Ps. aeruginosa* and *B. subtilis* ATCC 6633 defined by FBCI >0.5 to ≤1. The combinational antimicrobial effects against the tested yeast (*C. parapsilosis* AUMC 8909 and *T.domesticum* AUMC 8918) were indifferent. Also Genena *et al.* (2008) confirmed that rosemary has demonstrated antibacterial effects against *Ps. aeruginosa* and *E. coli* ATCC 8739.

Antibacterial activity of thyme alone and in combination with silver nanoparticles

Thyme alone gave weak inhibitory effects against the tested microorganisms. In combination with silver nanoparticles it showed synergistic effects against *E.coli* ATCC 8739 defined by FBCI ≤ 0.5 and FICI ≤ 0.5 and against *S.typhimurium* defined by FBCI ≤ 0.5. Additive interactions were shown against *Ps. aeruginosa* and *S. typhimurium* defined by FICI >0.5 to ≤1 and against *Ps. aeruginosa* and *T. domesticum* AUMC 8918 defined by FBCI >0.5 to ≤1. Indifferent and antagonistic effects against *B. Subtilis* ATCC 6633 and *C.parapsilosis* AUMC 8909 were investigated, respectively (Table 3). In contrast to Amrita *et al.* (2009) who detected thyme the most effective herbs against *E.coli* ATCC 8739.

Antibacterial activity of black pepper alone and in combination with silver nanoparticles

As shown in Table 3 black pepper alone exhibited

Table 4. Statistics for MIC and MBC of spices and silver nanoparticles for the examined microorganisms by using T test

Treatment	N	Mean	Std. Deviation	Std. Error Mean
Statistics for MIC				
Clove	6	88.5417	41.53625	16.95710
Rosemary	6	458.3333	102.06207	41.66667
Thyme	6	500.0000	.00000(a)	.00000
Black pepper	6	458.3333	102.06207	41.66667
Silver nanoparticles	6	2.5375	.88794	.36250
Statistics for MBC				
Clove	6	145.8333	85.39126	34.86083
Rosemary	6	83.3333	204.12415	83.33333
Thyme	6	83.3333	204.12415	83.33333
Black pepper	6	.0000	.00000(a)	.00000
Silver nanoparticles	6	4.8333	1.49755	.61137

a: cannot be computed because the standard deviation is 0.

antibacterial effects against tested microorganisms less than clove, it seems to be similar to the result recorded by Ali *et al.* (2007). In combination of black pepper with silver nanoparticles showed a synergistic effect only against *E. coli* ATCC 8739 defined by FICI ≤ 0.5, and additive effects against *S. typhimurium* and B. subtilis ATCC 6633 defined by FICI>0.5 to ≤ 1, and against *Ps. aeruginosa* , *S. typhimurium* and *E. coli* ATCC 8739 defined by FBCI >0.5 to ≤1. There is an antagonistic effect against *C. parapsilosis* AUMC 8909 defined by FICI > 2. The other effects (indifferent or antagonistic) were uncooperative.

The study demonstrated the enhanced antimicrobial effect by combination of spices with AgNPs against the tested foodborn microorganism. Enhanced synergistic inhibitory and bactericidal activities investigated in the combination of clove with silver nanoparticles against *Ps.aeruginosa* and *S.typhimurium*. Enhanced synergistic effects against *S. typhymurium* were seen in the combination of rosemary with silver nanoparticles and in the combination of thyme with silver nanoparticles. There were enhanced synergistic inhibitory and bactericidal activities against *E.coli* ATCC 8739 by the combination of thyme with silver nanoparticles and in the combination of rosemary with nanoparticles. Conclude that the combination of spices with silver nanoparticles allows the applying of lower concentrations of both agents in a helpful approach, which is below the toxic limit for mammalian cells.

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