

The Study of Synergistic Effects of Silver Nanoparticle and Brassica Napus L. Alcoholic Extract on Pathogenic Bacteria

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ABSTRACT: By increase of people information about synthetic antibiotic dangerous effects, the demands for natural replacement of these drugs have increased. This study is done by the purpose of synergistic specification of Nano silvers and turnip extract on pathologic bacteria. In this study the turnip plant was used by scientific name of Brassica Napus L. and silver Nano particles. The alcoholic extract of plant is taken first and then concentrations of 50, 100, 200, 400 mg/ml were evaluated. In the last phase the above concentrations were combined together and used for selected bacteria. All tests procedures published by the Well Diffusion Agar, Disk Diffusion Agar and the MIC/MBC on *S. aureus*, *B. cereus*, *E. coli* and *P. aeruginosa* was performed. The results showed that silver Nano particles comparing to turnip plant alcoholic extract has more antibacterial effect. The turnip extract had more effect on positive gram bacteria while silver Nano particles had much effect on Gram negative bacteria. The effect of turnip extract was more than each of them as this composition has the most effect on *Pseudomonas aeruginosa* and the least impact on *Staphylococcus aureus*. Using this composition has considerable effect on pathologic bacteria growth. In order to use clinical application of this composition, doing clinical researches is essential.

Keywords: Brassica Napus L., silver Nanoparticles, *Pseudomonas aeruginosa*, Pathogenic bacteria

Introduction

It is more than 50 years that antibiotics are used to control and treat infectious illnesses but using incorrect and continuous of this materials leads to emergence of resistance phenomenon to antibiotic and emergence of resistant levels toward bacteria and it has made difficulty to treat human and animals curing (Hedayati,1991). Expansion of drug resistance between bacteria has led to more attention to find preventive methods for resistant occurrence and also finding proper drugs with few poisoning and subsidiary effects. And to achieve this aim today many herbal plants and also Nano particles are considered by special researchers (Dadgar, 2007).

Because herbal plants have high distribution in our country, studies on these plants has provided proper field from anti microbe feature that these evaluations have been used for replacing drugs with natural source for control and treating Bacteria infects and this issue could reduce chemical drugs consumption and the consequential results of them (yazdi, 2007)

According to the microbes' resistant subject in antibacterial drugs and the subsidiary results in natural drugs as turnip plant; there is the possibility to

use the turnip alcoholic extract to face with bacteria in body external condition.

The brassicacea family was planted all around the world in great scale and it is used widely. In this field the brassica napus type has important varieties as turnip. It is the plant from the wallflower family and it has the bush. The turnip plant is in white and green color with many slices and in flat leaves. Its root has the gland in ball shape or long format in white and purple color. It grows in cold weather perfectly and may be the reason is that it is widely used in cold season a lot for illnesses in cold weather and it is used for breathing illnesses. The usable parts include the root or gland, the leaf and seeds. Vitamins and minerals as iron, the volatile oil, rapine and Glucosinolates are the most effective materials in turnip (Sasaki and Takahashi, 2002).

Up to now about the antibacterial specification of turnip plant only a few studies have been done but in other plant specification of turnip as anti cancer, anti diabetic and ant oxidization many studies are done (Alizadeh et al, 2014, Mohajery et al, 2012).

In a study that was performed by Dastan et al in relation with anti *Pseudomonas* feature of turnip we concluded that the turnip extract prevents the growth

of *Pseudomonas* in injured tissue and showed that the metabolic extract of this plant is more effective than chloroform extract, ethanol and N hexane (Dastan et al, 2011).

Other new techniques to come up with microbes without increase of drug resistance could be application of technology and Nano technology in producing Nano particles. Nano technology researchers have realized different aspects of Nano particles that might have important role in medical application and prevention and treating illnesses. One of the Nano particles whose anti microbe effect is proved is the silver Nano particles that is provide through different methods (braydich-stolle et al, 2005).

Silver in Nano dimension scale has effect on metabolism, breathing and reproduction. In different studies, the ant microbe specification of these Nano particles and beneficial use of them in biotechnology field and prevention of microbes has been evaluated (Christian et al, 2008).

Silver Nano particles lead to prevent bacteria breathing system without drug resistant enhancement. This element has special features in anti microbe application but its preparation is difficult and expensive (Hussein et al, 2006).

Therefore researchers have found that using other material and combining them with silver is a practical way for using anti microbe specification of silver. Therefore scientists have done too many researches on silver particles Nano synthetic and they have mixed it with other materials. Using these combination is done simultaneously that could solve many problems and it could have better and more economical results so this research plan is done.

Materials and Methods

This experimental research is done in laboratory during winter of 2013 at Ahar Azad university microbiology laboratory; the turnip plant was gathered from natural regions and gardens around the Ahar city at the end of winter season. The roof and leaves of plant were dried in room temperature far away from the sun shine and then the dried plants were changed to powder for extracting by electrical mill for extracting by laboratory method (Alizadeh et al 2012). After obtaining pure extract without soluble the rotary system is used in 40 centigrade in vacuum, the provided extracts were prepared in concentrations of 50, 100, 200, 40, and milligrams to use in sink spreading and determination of MIC/MBC.

The bacteria type used in this test included:

Staphylococcus aureus (ATCC:25923), *Bacillus cereus* (ATCC:1052) ,

Escherichia coli (ATCC:25922), *Pseudomonas aeruginosa* (ATCC:27853)

These were prepared in from Tehran university biotechnological research center. The microbe samples were relived based on the standard methods. In order to prepare the microbe suspension from new and young implant of multi colony bacteria were transferred to Muller Hinton implant environment to have the same tiff similar to the pipe tiff at 0.5 MCFarland 1.5×10^8 for bacteria in each milliliter.

Test 1

To evaluate the antibacterial of turnip plant alcoholic effects, first the spreading method from sink was used. Therefore the we prepare the sterile cotton swab near the fire and under the hood and then inter it the prepared microbe suspension with pipe of 0.5 MCFarland and by pushing the swab to the pipe body, the additional amount of suspension was observed and the bacteria implant was done on the plate containing growing environment of Muller Hilton thoroughly. Then on the plate the sink is made in 5 mm in diameter and the distance of 2 cm. each of sinks were filled with different concentration of extract that at the beginning were pointed out. For the experiment positive witness, the antibiotic Streptomycin was used as the negative witness of DMSO.

After the job termination, all implant environments were put in incubator for 24 hours in 37 centigrade after this time, the bacteria implants were measured about the formation or lack of formation in millimeter by caliper (Alizadeh et al, 2014).

To determine the minimum concentration of bacteria growth MIC and the minimum bacteria concentration MBC the pipe dilution is used. To determine MIC the prepared alcoholic extract in 25, 50, 100, 200, series and 1.56, 3.125, 6.25, 12.5 ml/ml were prepared in implant environment. Hence for each of dilution 1ml of microbe suspension was added. As the positive witness a pipe with contains materials (bacteria containing environment without extract) and as the negative witness a pipe with contains without bacteria was prepared. After the job termination all pipes were transferred to incubator with 37c temperature in 24-48 hours.

After incubation time, the pipes were evaluated from the growth of inseminated bacteria. The lowest dilution of extract in which no tiff was observed was regarded as MIC. To determined the minimum concentration of extracts extension (MBC) all pipes in which the blight was seen in them were implanted in Muller Hinton and the insemination environments were incubated for 24 hours in 37 centigrade the related plate was considered to a pipe that includes the minimum extract and the bacteria

growth is not observed in as the MBC concentration of that extract (CLSI, 2011).

Test 2

To test silver Nano particles anti bacteria, the silver Nano particles were prepared from Nano Sany engineers company in 20 nm dimension. He dilution series used in 10, 20, 40, 80 micro gram on milliliter. In this test also the spreading method from sink and MIC determination test was used in Muller Hinton. The implant environment preparation, bacteria levels and the operation method was used as the methods in turnip plant alcoholic extract test.

Test 3

The most important purpose of this research is practical and scientific and to evaluate the synergistic feature of silver Nano particles and alcohol extract of turnip plant effect on pathogen bacteria. Therefore a

dilution series tests mentioned before are added together and used as an applicable concentration. The test affair method is similar to previous tests.

Results

The test 1 results (evaluation of turnip plant alcoholic extract effect)

By effecting different extract of turnip alcoholic concentration on pathologic bacteria, it was clarified that this extract has preventive effect on four bacteria tested before and as the alcoholic extract concentration increased, the preventive effect is also considerable in the form of growth prevention. This study showed that the alcoholic extract preventive effects on positive gram bacteria are more than negative gram bacteria. The results from the different concentration effects in turnip alcoholic different extract concentration is obtained in emission at Well Diffision Agar at table 1

Table 1. The zone diameter of absence of bacteria growth in milimeter in the extract of plant

Control pos	Control Neg	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	Cocentration Bacteria
17.50 mm	-	14 mm	13.32 mm	11.10mm	10.70 mm	S. aureus
18mm	-	14.64 mm	13.70 mm	12.32 mm	11mm	B. cereus
14.60 mm	-	10.90 mm	7.10mm	---	---	E. coli
15mm	-	11.70 mm	9.50 mm	8.20mm	---	P. aeruginosa

MIC/MBC determination test showed that between the tested bacteria the Bacillus cereus has the most sensitivity and E. coli has the lowest sensitivity toward turnip plant alcoholic extract. The result are presented for the minimum bacteria growth preventive

concentration and the minimum concentration of bacteria killing concentration MBC for turnip alcoholic extract against selected bacteria in pipe dilution method according to table 2.

Table 2. the MIC/MBC in mg/ml in the different concentrations of alcoholic extract

MBC mg/ml	MIC mg/ml	Cocentration Bacteria
50	12.5	S. aureus
25	12.5	B. cereus
100	50	E. coli
50	25	P. aeruginosa

Test 2 results: evaluation of silver particles effect

The results of antibacterial tests for silver Nano particles in spreading method from Well

diffision agar and MIC determination are presented orderly in tables 3 and 4.

Table 3. The zone diameter of absence of bacteria growth in milimeter in the SilverNanoparticles

Control pos	Control Neg	80 µg/ml	40 µg/ml	20 µg/ml	10 µg/ml	Cocentration Bacteria
16mm	-	15.90 mm	14 mm	12 mm	10 mm	S. aureus
16mm	-	15.95 mm	14.60mm	13 mm	10.32mm	B. cereus
16.5mm	-	16.32 mm	15 mm	11.50mm	11mm	E. coli
16.64 mm	-	16.64 mm	15 mm	12.32 mm	12mm	P. aeruginosa

The results of Well diffusion agar shows the powerful effects of silver Nano particles toward selected bacteria as it has had effect on all bacteria and

specially in negative gram bacteria it has shown considerable preventive effects.

Table 4. The MIC/MBC in mg/ml in the different concentrations of silver Nano particles

MBC $\mu\text{g/ml}$	MIC $\mu\text{g/ml}$	Cocentration	Bacteria
100	50		S. aureus
50	50		B. cereus
25	12.5		E. coli
12.5	6.25		P. aeruginosa

The result of this test shows that the silver Nano particles has had the most effect on

Pseudomonas aeruginosa bacteria and the lowest effect on the Staphylococcus aureus.

Test 3 results, evaluation of turnip plant alcoholic extract synergistic effects with silver Nano particles

By mixing prepared dilutions from silver Nano particles and turnip alcoholic extract in previous test a

composition concentration is obtained. The result of antibacterial test of this composition which his performed in sink spreading method and MIC test are presented in tables 5 and 6.

Table 5. The zone diameter of absence of bacteria growth in millimeter in the different concentration of turnip plant extract and silver Nano particle

Control Pos	Control Neg	+400 mg/ml 80 $\mu\text{g/ml}$	+200 mg/ml 40 $\mu\text{g/ml}$	+100 mg/ml 20 $\mu\text{g/ml}$	+ 50 mg/ml 10 $\mu\text{g/ml}$	Cocentration	Bacteria
16.40 mm	-	16.62 mm	15mm	13.42 mm	12mm		S. aureus
16.50mm	-	16.80mm	14 mm	13.50 mm	11.80mm		B. cereus
16mm	-	17mm	15mm	12.90 mm	11mm		E. coli
16.50mm	-	17.50mm	15.80mm	14 mm	13mm		P. aeruginosa

Table 6: The MIC/MBC in mg/ml in the different concentrations of Nano silvers and turnip extract

$\mu\text{g/ml}$ MBC mg/ml	$\mu\text{g/ml}$ MIC mg/ml	Cocentration	Bacteria
25mg.ml , 25 $\mu\text{g.ml}$	6.25 mg.ml , 6.25 $\mu\text{g.ml}$		S. aureus
12.5 mg.ml , 12.5 $\mu\text{g.ml}$	6.25 mg.ml , 6.25 $\mu\text{g.ml}$		B. cereus
12.5 mg.ml 12.5 $\mu\text{g.ml}$	6.25 mg.ml , 6.25 $\mu\text{g.ml}$		E. coli
6.25 mg.ml , 6.25 $\mu\text{g.ml}$	3.125 mg.ml , 3.125 $\mu\text{g.ml}$		P. aeruginosa

The results show that by combining the sliver Nano particles and turnip extract all bacteria will be affected by this composition as the minimum concentration of prevention and killing of bacteria had meaningful reduction compared to previous tests. This combination has had the most effect on Pseudomonas aeruginosa bacteria and the lowest effect on the Staphylococcus aureus, Bacillus cereus bacteria.

Discussion

Turnip is the plant that is paid too much attention because it has the wide application. This plant was grown 4000 years ago and after than it was used all around the world. Using this plant especially in

winter is more than other seasons because of its feature for treatment. For many years human has found the silver antibacterial feature and in ancient Greece silver was added to the drinking liquids or water was drunken in silver jars because Greece people believed that silver increase longevity that is the antimicrobial feature of silver which is proved today, Nano particles have high level and for silver this level increasing leads that one gram of Nano silvers considered adequate for antibacterial preparation of an article surface in 100 square meter(m²).

In this research it was identified that turnip extract has considerable effects on bacteria during test, between the preventive effects for positive gram

bacteria as in low concentrations of 50 mg/ml concentrations, the negative gram bacteria have high resistance while in positive gram bacteria there is considerable sensitivity toward alcoholic extract and also in high concentration of 400 mg/ml both groups of positive gram bacteria did not have capability for growth and they were sensitive. Also it was realized that among five used bacteria, *Bacillus cereus* was the most sensitive and *E. coli* was the most resistant bacteria toward plant extract.

In evaluations the silver Nano particles effect on gram negative bacteria was more than gram positive bacteria and the reason is that gram negative bacteria has the cell wall in thickness of 7 to 8 nm that is made of a solid layer, on the other hand the positive gram bacteria is thick in 20 to 80 nm which are more harsh for silver particles penetrations. The silver particles are effective for bacteria but their preventive power is lower or equal to Streptomycin antibiotic.

In a study made by Dastan et al about the relation of turnip Anti-*Pseudomonas* feature, the results showed that the methanol extract of turnip prevents *Pseudomonas* bacteria growth in injured tissues and also this plant methanol extract is more effective than chloroform, ethanol and N-hexane extract and the effects which are shown are similar to the present research outcomes, Sadeqhi et al made researches to compare antibacterial effect of silver Nano particles and found that the MIC are in silver and Chlorhexidine against the *Streptococcus sanguis* is 16 and 256 mg/ml and for *Actinomyces viscosus* it is about 4 and 64 mg/l. The MBC rate of soluble silver particles against *Streptococcus sanguis* is 64 and 512 milligrams in liter and for *Actinomyces viscosus* it is 16 and 102 mg/ml, while silver particles have perfect antimicrobial activity against mentioned bacteria, this effect is obtained comparing to Chlorhexidine with lower concentration from silver Nano particles (sadeghi et al, 2009) in his research the silver particles effect and turnip extract were compared and their influences was evaluated about the selected bacteria prevention through synergistic effect of this combination.

Niyakan et al present an article for evaluation of preventing colloid silver with an antibacterial soluble and they mentioned that the effect of use material in MIC and MBC is about 10 ppm for *Staphylococcus aureus* bacteria and it is in order 10 and 500 ppm for *Pseudomonas aeruginosa*. So in their research MIC and MBC rates were the same but about *Pseudomonas aeruginosa* the MBC rate was 5 and 20 times more than MIC. Also the effect of silver antibacterial feature was more than *Staphylococcus aureus* (Niyakan et al, 2009). In the recent research also results were similar to Nikan et al findings in

which the silver Nano particles effect on *Pseudomonas aeruginosa* was more than *Staphylococcus aureus*.

Khosravi eqhbal et al evaluated the silver Nano particles and copper and compared with sodium hypochlorite on the *Bacillus subtilis* and *Bacillus cereus* and they concluded that *Bacillus subtilis* has the most sensitivity to both Nano particles in comparison to *Bacillus cereus* to the point MIC in silver Nano particles is equal to 7 ppm and in copper Nano particles it is 50 ppm and in hypochlorite it is 700 ppm, *Bacillus subtilis* spores in lower dilutions of antimicrobial were destroyed in zero time. So it was observed that spore and growing *Bacillus subtilis* had more sensitivity to silver Nano particles and these results represents that silver Nano particles are more effective compared to other antimicrobial materials in this discussion (Khosravi eqhbal et al, 2008). The results of recent research also showed that silver Nano materials is better than turnip extract but composition of these two material leads to better and influential effects.

Conclusions

The results show that silver Nano particles and turnip extract are influential on pathological bacteria but using them simultaneously leads to synergetic feature between them and as the result the antibacterial effects is increased more.

Suggestions

It is advised for other researchers to combined the turnip alcoholic extract with other Nano particles to evaluate their synergetic effects, also we can use other techniques to reduce the particles Nano poisoning acceptably. Before preparing the synthetic drug preparation there should be some tests about the internal cellular condition.

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