Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 10, pp.21548-21552, October, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOSYNTHESIS OF SILVER NANOPARTICLES USING *SALVIA OFFICINALIS* EXTRACT AND ASSESSMENT OF THEIR ANTIBACTERIAL ACTIVITY

[1]ShahdI Daoud, [1]Mona A. M. Alqahtani, [1]Dalal H. M. Alkhalifah, [2]Mudawi M. Elobeid and [1,*]Afrah E. Mohammed

[1]Department of Biology, Faculty of Science, Princess Nourahbint Abdulrahman University, 11474 Riyadh, Saudi Arabia
[2]Department of Silviculture, Faculty of forestry, University of Khartoum, Shambat, Sudan

ARTICLE INFO

Article History:
Received 07th July, 2015
Received in revised form
22nd August, 2015
Accepted 27th September, 2015
Published online 31st October, 2015

Key words:
AgNPs, Bactericidal,
Salvia officinalis,
Ethanolic extract.

ABSTRACT

Recently, the biosynthesis of nanoparticles has been considered as a new approach of research. In the current investigation, the green chemistry of silver nanoparticles synthesis from aqueous solution of silver nitrate as a rapid eco-friendly technique was described. Extracellular biosynthesis of silver nanoparticles (AgNPs) was carried out using plant extracts of *Salvia officinalis* L. The AgNPsformation was detected by the colour change of plant extracts and confirmed with the help of UV-Vis spectroscopy where the peak values were in the range of 425– 445 nm. In this study, bio-synthesized AgNPsas well as the ethanolic extract of *S.officinalis* were tested for their antibacterial activity using a well diffusion method against some bacterial species*; Pseudomonas aeruginosa* (Gram negative) and Gram positive bacteria (*Bacillus subtilis, Streptococcus faecalis and Staphyllococcusaureus*). Results from this investigation showed that AgNPs mediated by *S. officinalis* had an inhibition zone diameter ranging between (3.4 – 9.5 mm). Inhibition zone ranged (11.1 – 16 mm) was observed effect for *S. officinalis* leaves extracted with ethanol. Furthermore, MIC (Minimum Inhibitory Concentration and MBC (Minimum Bactericidal Concentration) were also determined for the ethanolic leaves extract. The MIC was 25 mg ml and the MBC was 50 mg/ml. The observed differences among the bactericidal activities against the tested organisms might be attributed to the microbe characteristics. Our findings indicated that AgNPs synthesis mediated by *S. officinalis* extracts had an efficient bactericidal activity. Future investigations are required to perform experiments to explore the unknown mechanism for the bactericidal activity of biogenic AgNPs.

***Citation*:** ShahdI Daoud, Mona A. M. Alqahtani, Dalal H. M. Alkhalifah, Mudawi M. Elobeid and Afrah E. Mohammed, 2015. "Biosynthesis of silver nanoparticles using *salvia officinalis* extract and assessment of their antibacterial activity", *International Journal of Current Research*, 7, (10), 21548-21552.

INTRODUCTION

Silver and its compounds have strong inhibitory and microbicidal activities for bacteria, fungi, and viruses (Sahayaraj and Rajesh 2011). It is well known to be a toxic material that causes serious environmental hazards, but using silver in a form of nanoparticles reduces its negative effect since it can be discarded via hair, urine and faeces instead of deposition in human body in a form of silver ion (Di Vincenzo *et al*., 1985). Silver nanoparticles have recently become a focus of interest since they play a significant role in biological systems, living organisms and medicine (Gurunathan *et al.*, 2009). Efficiency of silver nanoparticles is typically attributed to their greater surface area per weight which makes them more reactive than other molecules (Manisha *et al.,* 2013). An eco-friendly safe methodology for the positive conversion of silver ions to silver nanoparticles has to be well selected. Using the bio-material as a mediator for this conversion is known as a green synthesis. The (*green*) chemistry approach for synthesizing biocompatible silver nanoparticles (AgNPs) has gained considerable attention in recent years (Mie *et al.*, 2014). Using plant extracts, such as bacteria, fungi, actinomycetes, yeast, algae for the biosynthesis of nanoparticles were reported (Mohammed 2014; Thakkar *et al.,* 2010, Gurunathan 2009). A recent review revealed that using plant extract as a bio-mediator for silver nanoparticles is faster than using microbes. Furthermore, plants are easily available, safe, and nontoxic in most cases (Prabhu and Poulose 2012). Different plant extracts for the biogenic synthesis of AgNPs such as *Aloe vera*, *Coriandrum sativum, Murrayakoenigii, Hibiscus rosasinensis* and *Eucalyptus*

Corresponding author: Afrah E. Mohammed
Department of Biology, Faculty of Science, Princess NourahbintAbdulrahman University, 11474 Riyadh, Saudi Arabia

camaldulensis have been intensively studied recently (Chandran *et al.,* 2006; Sathyavathi *et al.,* 2010; Philip *et al*., 2011; Philip 2010; Mohammed, 2014). The main mechanism for the process of biosynthesis of silver nanoparticles is assisted by plant phytochemicals. The water-soluble phytochemicals that are responsible for the immediate reduction of the ions are flavones, organic acids, and quinines (Prabhu and Poulose 2012). In this investigation the leaves of *Salvia officinalis* aqueous extract was used for the biogenic reduction of silver in a form of silver nitrate to silver nanoparticles. *S. officinalis* name is attributed to its medicinal importance since Salvia comes from "*salvare*" meaning "to cure" in latin and "*officinalis*" means medicinal (Chipault *et al.,* 1952).

Early studies have shown antioxidant properties, anti-bacterial, antifungal and anti-inflammatory activity of *S. officinalis*leaves and oil extracts (Salem *et al.,* 2012; Abu-Darwish 2013). The aqueous extract of *S. afficinalis* leaves showed antibacterial activity against *Bacillus, Staphylococcus aureus, E. coli, Salmonella, Pseudomonas aeruginosa, Sarcinalutea, Candida albicans, Saccharomyces cerecisiae* and *Aspergillus niger* (Stanojevic *et al.,* 2010; Viuda-Martos *et al.,* 2007; Velickovic *et al.,* 2002). The ability of photochemicals such as tannins, flavonoids, alkaloids and several other aromatic compounds to serve as defense mechanisms against microorganisms was well documented (Doughari, 2006). Regarding the phytochemical in *S. officinalis* that might enhance the conversion of silver ion to silver nanoparticles besides the antimicrobial ability, ten phenolic compounds were isolated from a butanol fraction extracts. Among which, 4-hydroxy-acetophenone-4-O- β -D-apiofuranosy l- (1-6)-O- β-D-glucopyranoside were identified. The most active compounds were found to be rosmarinic acid and luteolin-7-O- β –glucopyranoside (Wang *et al.* 1998). Furthermore, *S. officinalis* oil contain a-pinene, camphene, limonene, l, s-cineole, a- and P-thujone, camphor, linalool, linalyl acetate, bornyl acetate, and a-humulene (Velickovic *et al.,* 2004). Furthermore, the rosmarinic acid and luteolin-7-9 glucoside were detected in two *S. officinalis* extracts using water and methanolic extract which caused a cyto protective effects in HepG2 cells (Lima *et al.,* 2007).

Despite the abundance of antibacterial applications of *S.officinalis* leaf extracts besides employing silver nanoparticles, yet the mode of action and the exact mechanism of the ethanol extract and biosynthesized AgNPs on the target microbes are not clearly known. The main Objective of this study was to use *S. officinalis* leaf extracts to mediate reduction of silver ions present in the form of aqueous solution of silver nitrate to produce silver nanoparticles. Furthermore, to prepare *S. officinalis* leaves extract using organic solvent (ethanol). The specific objective was to evaluate the *in-vitro* bactericidal impact of the biogenic AgNPs and the ethanolic leaf extracts on some pathogenic bacteria by determination of the inhibition zone.

MATERIALS AND METHODS

This research work was conducted in the Department of Biology, Faculty of Science in Princess Nourah Bint Abdulrahman University, Riyath Saudi Arabia in 2014.

Materials

S. officinalis L. was collected from Jordan. Silver nitrate ($AgNO_3$) was purchased from Merck (Darmstadt, Germany). Mueller-Hinton agar, Mueller-Hinton broth, and nutrient broth were purchased from Wateenalhyaa Company (Riyadh, Saudi Arabia) for the antibacterial assays.

Synthesis of silver nanoparticles

The aqueous extract of *S. officinalis* was prepared by mixing 10 g of the dry sample with 100 ml of highly purified water. The mixture was heated for 10 minutes at 80°C to denature the enzymes in the extracts. The solution was filtered through a Whatman filter paper No. 1 (pore size 125 mm). The supernatant (filtrate) was further filtered through a Whatman filter paper No. 1 (pore size 25 μm) to remove plant residues. For synthesis of the AgNPs, 12 ml of each aqueous *S. officinalis* extract as a reducing agent was mixed with 88 ml of a 1 mM $AgNO_3$ solution in an Erlenmeyer flask and allowed to react at room temperature for 24 hours. Ultra-highly purity water was used as a reaction medium to avoid the presence of chloride ions and also to prevent precipitation of silver chloride. The AgNPs extract was stored at 4°C until further analysis.

Characterization of AgNPs

The reduction of silver ions to AgNPs in the solution was monitored by measuring the ultraviolet-visible spectrum of the solution using a UV 2450 double-beam spectrophotometer (Shimadzu, Tokyo, Japan) operated at a resolution of 2 nm in the range from 400–500 nm.

Plant ethanol extracts preparation

The ethanol extracts of *S. officinalis* was prepared by mixing 10 g of the dry sample with 100 ml of ethanol and shaken well and stayed for 24 hours and then filtered through a Whatman filter paper No. 1 (pore size 125 mm). The supernatant (filtrate) was further filtered through a Whatman filter paper No. 1 (pore size 25 μm). Heat treatment for the prepared sample was done at 80°C for concentrating the extract and removing the effect of the ethanolthen kept at 4°C for further analysis.

Evaluation of antibacterial activity

The antibacterial activity of the synthesized AgNPs and ethanol extract was determined using the well diffusion methods. Four types of pathogenic bacteria, including Gram-negative bacteria (*P.aeruginosa*) and Gram-positive bacteria (S.*aureus, B.subtilis* and *S.faecalis*) were tested. Pure cultures of the micro-organisms were sub-cultured on Mueller-Hinton agar. Each strain was swabbed uniformly onto individual agar plates using sterile swabs. Subsequently, four adequately spaced wells (holes) of 4 mm diameter each were made per plate at the culture agar surface using sterile metal cup borer. In each hole, 0.2 ml of each extract and control were put under aseptic conditions, kept at room temperature for one hour to allow the extracts to diffuse into agar medium and incubated accordingly. Sterile distilled water was used as the reference negative

control. All the plates were incubated at 37°C for 18–24 hours. The tests were repeated three times. The zone of inhibition, which appeared as a clear area around the well was measured.

Statistical analysis

Data was statistically analyzed with the statistical programme JMP 5.1 Start Statistics, third edition (SAS Institute, Inc., Cary, North Carolina, USA). Variations among the different treatments were tested using analysis of variance, ANOVA. Results presented are means (4 replicates ± SD).

RESULTS AND DISCUSSION

The biogenic synthesis of silver nanoparticles using *S. officinalis* leaf extracts was carried out in this study. Silver nitrate conversion to AgNPs was found to be successful as suggested by the change in color of the solution to brown. It has been well documented that AgNPs exhibit brown color in aqueous solution due to excitation of surface plasmon vibrations.

Furthermore, the green synthesis of AgNPs was also confirmed by the optical measurements by UV-VIS- spectroscopy. Absorbance peaks of silvernanoparticles was in the range between 425-435 nm (Figure 1). Similar trend of result regarding color change in the biogenic material and also absorbance peak of 430 were reported by Baharara *et al.* (2014) when he studied the anti-angiogenesis properties of AgNPs synthesized using *S. officinalis* extract on chick chorioalantoic membrane. Ability of *S. officinalis* extract to synthesize AgNPs might be attributed to the water soluble plant secondary metabolities such as phenolic compounds and organic acids as suggested by Prabhu and Poulose 2012. On the other hand, as shown in Table 1, *S.faecalis* treated with $AgNO_3$showed10 ± 1.1 mm inhibition zone, *B. subtilis* (10.5 ± 0.7 mm), *P. aeruginosa* (11.3 ± 1.3 mm) and *S.aureus* (9.8 ± 1.9 mm). Furthermore, AgNPs synthesized by *S. officinalis* showed an antibacterial activity against *S. faecalis* (3.4 ±0.4 mm), *B. subtilis* (6.3 ± 0.6 mm), *P. aeruginosa* (9.3 ± 0.7 mm) and *S. aureus* (9.5 ± 0.5 mm). *P. aeruginosa* had the highest

Table 1. Inhibition zone (mm) of bacteria treated with silver nitrate 1mM and silver nanoparticles mediated by *Salvia officinalis* extract

Treatment	*Microorganisms*			
	S. faecalis	*B. subtilis*	*P. aeruginosa*	*S. aureus*
AgNPs *mediated by Salvia officinalis* extract	3.4 ± 0.4 c	6.3 ± 0.6 b	9.5 ± 0.7 a	9.2 ± 0.5 a
$AgNO_3$	10 ± 1.1 b	10.5 ± 0.7b	11.3 ± 1.3 a	9.8 ± 1.9 c

Data are mean ± SD for four replicates. Letters express variation among different microorganisms

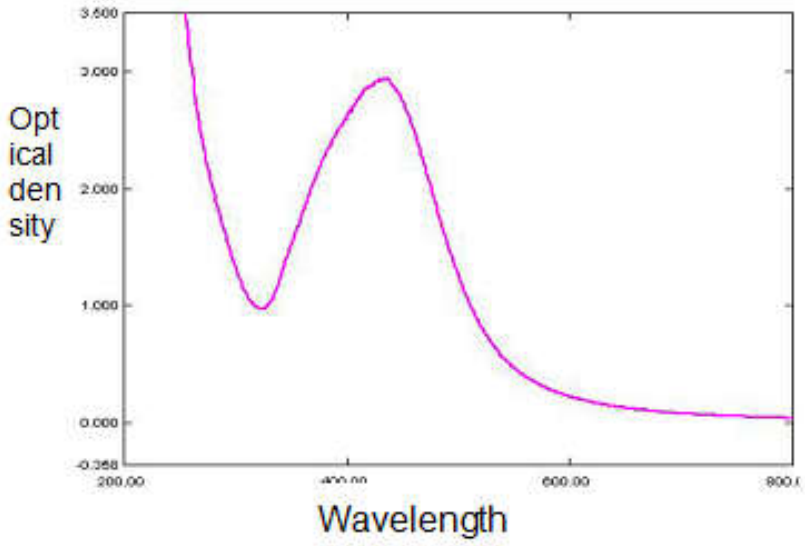

Figure 1. UV-VIS spectra of the formed silver nanoparticles using *Salvia officinalis* leaf extract

Table 2. Inhibition zone (mm) of bacterial species treated with *Salvia officinalis* ethanolic extract

Plant extract	Microorganism			
	S. faecalis	*B. subtilis*	*P. aeruginosa*	*S. aureus*
Salvia officinalis	15.5 ± 0.8 b	16 ± 0.9 a	11.2 ± 1 c	11.1± 1 c

Data are mean ± SD for four replicates. Letters express variation among different microorganisms

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in mg⁄ml of the ethanol extract of *Salvia officinalis*

Microorganism	*Salvia officinalis*extract	
	MIC	MBC
S. aureus	25 mg / ml	50 mg / ml
B. subtilis	25 mg / ml	50 mg / ml

inhibition zone in both cases (Table 1). In a recent study Salem *et al*. (2012) investigated the cytotoxic effect of nanoparticles synthesized from *S. officinalis* and *Ricinus communis* aqueous fruit extracts against vero cell line. Their findings indicated that the aqueous extracts of *S. officinalis* and *R. communis* have the potential to reduce silver nitrate ions to silver nanoparticles. Our study revealed that, Gram-negative bacteria *P. aeruginosa* had the highest zone of inhibition compared with other tested Gram-positive bacteria, Same line of observation was also recorded by Mie *et al*. (2014) who found that silver nanopartices have relatively higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria, which might be attributed to the presence of porins and the thinner peptidoglycan layer (Geoprincy *et al.* 2013). A recent observation suggested that the bactericidal activity of AgNPs might be attributed to the loss of replication and degradation of bacteria DNA when studying the AgNPs on *Proteus sp*. and *Klebsiella* sp. (Ouda 2014). It would be a great challenging task for the future research to elucidate the mechanisms of plant-mediated synthesis of silver nanoparticles.

On the other hand, results from our study showed that leaves of *S. officinalis* extracted with ethanol had an antibacterial activity against the bacterial species tested. *B. subtilis* showed the highest inhibition zone (16 ± 0.9 mm) and (11.1± 1 mm) for S. *aureus* which had the lowest zone of inhibition among the bacterials peciestested (Table 2). Aqueous extract and essential oil of *S. officinalis* showed an antibacterial activity against *B. subtilis*, *S. aureus* (Stanojevic *et al.*, 2010; Miladinovic and Miladinovic 2000). Antifungal activity against the yeast cells was clear when treated with the ethanolic extract of *S. officinalis* leaves (Farcasanu and Oprea, 2006). Phytochemical components such as flavonoids and other aromatic secondary plant metabolites act well against many microorganisms (Doughari 2006). The secondary metabolites of *S. officinalis* leaves was reported, diterpenoid, 12–O-methyl carnosol, 3-apianane terpenoids, 1 anthraquinone, 8 flavonoids (Miura *et al.* 2002).

Furthermore, Wang *et al.* 2010 isolated ten types of phenolic compounds from *S. officinalis* leaves. Furthermore, Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the leaves of *S. officinalis* ethanol extracts against *Bacillus* and *S. aureus*, 25 mg/ml and 50 mg/ml was the MIC and MBC for all the both microbes, respectively (Table3). The range between 25 – 50 mg /ml for MIC and 25 – 100 mg/ml for MBC were detected for the methanolic leaves extract of *S. officinalis* (Ali and Aboud 2010). Generally the highest MIC and MBC values is an indication that either the plant extracts are less effective on some bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the efficacy of the plant extracts (Ali and Aboud 2010).

Conclusion

Based on our current findings it can be concluded that ethanol extract and the bio-synthesized silver nanoparticles mediated by leaf extracts of *Salvia officinalis* showed bactericidal potency. Further work to validate our findings is required.

Acknowledgements

The authors wish to thank the Department of Biology, Faculty of Science, Princess Nourah bent Abdul-Rahman University, Riyadh – Saudi Arabia for providing the required facilities for the laboratory experiments.

REFERENCES

Abu-Darwish MS, Cabral C, Ferreira IV, Gonçalves MJ, Cavaleiro C, Cruz MT, Al-bdour TH, Salgueiro L. 2013. Essential oil of common sage (*Salvia officinalis* L.) from Jordan: assessment of safety in mammalian cells and its antifungal and anti-inflammatory potential. Bio Medical Research International. Article ID 538940, 9 pages. http://dx.doi.org/10.1155/2013/538940

Ali, M. R. and Aboud, A.S. 2010. Antimicrobial Activities of Aqueous and Methanolic Extracts from *Salvia officinalis* and Salix acmophylla Used in the treatment of wound infection isolates. *Journal Ibn Al-Haitham Pure and Applied Sciences,* 23 (3): 14 pages.

Baharara, J., Namvar, F., Mousavi, M., Ramezani, T. and Mohamad, R. 2014. Anti-Angiogenesis Effect of Biogenic Silver Nanoparticles Synthesized Using *Saliva officinalis* on Chick Chorioalantoic Membrane (CAM). Molecules 19 (9), 13498-13508.

Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M. 2006. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnology Progress*, 22:577–583

Chipault, J.R., Hawkins, J.M., Lundberg, W.O. 1952. The antioxidant properties of natural spices. *Food Research*, 17, 64 – 54.

Di Vincenzo, G.D., Glordano, C.I., Schriever, L.S. 1985. Biologic monitoring of workers exposed to silver. International Archive Occupation Environmental Health 56: 207 -215.

Doughari, J.H. 2006. Antimicrobial Activity of Tamarindusindica Linn. *Tropical Journal of Pharmaceutical Research,* 5 (2): 597-603.

Farcasanu, C. and Oprea, E. 2006. Ethanol extracts of Salvia officinalis exhibit antifungal properties against saccharomyces cerevisiae cells. Analele UniversităŃii din Bucuresti – Chimie, Anul XV (serienouă),1: 51-55.

Geoprincy, G, Srri, B.N.V., Poonguzhali, U, Gandhi, N.N., Renganathan, S. 2103. A review on greensynthesis of silver nanoparticles. *Asian Journal of Pharmaceutical and Clinical Research,* 6: 8-12.

Gurunathan S, Kalishwaralal K, Vaidyanathan R, Deepak V, Pandian SRK, Muniyandi J.2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. *Colloids and Surfaces B: Biointerfaces* 74:328–335.

Lima, C.F., Valentao, P.C.R., Andrade, P.B., Seabra, R.M., Fernandes-Ferreira, M., Cristina Pereira-Wilson C.2007. Water and methanolic extracts of *Salvia officinalis* protects HepG2 cells from t-BHP induced oxidative damage. Chemico-Biological Interactions 167 (2), 107-115.

Manisha, D.R., Karunakar, R.K., Jahnavi, A., Anila, M., Sreedhar, B., Pratap, R.M.P. 2013. Synthesis of silver nanoparticles using extracts of Securinega Leucopyrus and

evaluation of its antibacterial activity. *International Journal of Current Science,* 6: 1-7.

Mie, R., Samsudin, M.W., Din, L.B., Ahmad, A., Ibrahim, N., Adnan, S.N.A. 2014. Synthesis of silver nanoparticles with antibacterial activity using the lichen Parmotremapraesorediosum. *International Journal of Nanomedicine,* 9: 121 – 127.

Miladinović, D. and Miladinović, L. 2000. Antimicrobial activity of essential oil of saga from serbia. Series: *Physics, Chemistry and Technology*, 2 :97 – 100.

Miura, K, Kikuzzaki, H., Nakatani, N. 2002. Antioxidant activity of chemical components from saga (*Salvia officinalis* L.) and Thyme (Thymus vulgaris L.) Measured by the Oil Stability Index Method. *Journal of Agriculture and Food Chemistry,* 50 (7); 1845-1851.

Mohammed, A.E. 2014. Green synthesis and antimicrobial activity of *Eucalyptus camaldulensis* mediated silver nanoparticles. *Asian Pacific Journal of tropical Biomedicine,* 5(5): 382-386 (2015)

Ouda, S.M. 2014. Some Nanoparticles Effects on *Proteus* sp. and *Klebsiella* Sp. Isolated from Water. *American Journal of Infectious Diseases and Microbiology*, 2, (1): 4-10.

Philip, D. 2010. Green synthesis of gold and silver nanoparticles using *Hibiscus rosasinensis*. Physica E: Low-dimensional Systems and Nanostructures, 42 (5): 1417-1424.

Philip D, UnnibC, Aromala SA, and VidhuaVK.2011. MurrayaKoenigii leaf-assisted rapid green synthesis of silver and gold nanoparticles .Spectrochimica Acta Part A 78: 899–904.

Prabhu, S., Poulose, E.K. 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Prabhu and Poulose International Nano Letters 2 (32): 1 – 10.

Sahayaraj, K., Rajesh, S. 2011. Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas *(Ed.)*.

Salem, A.N.B., Zyed, R., Ali, L.M., Nidhal, S., Souad, S., Mahjoub, A. 2012. Cytotoxic effect of nanoparticles synthesized from *Salvia officinalis L*. and Ricinuscommunis aqueous extracts against vero cell line and evaluation of their antioxidant activities. *African Journal of Biotechnology,* 11 (52): 11530 – 11534.

Sathyavathi, R., Balamurali Krishna, M., VenugopalRao, S, Saritha, R. and NarayanaRao, D. 2010. Biosynthesis of Silver Nanoparticles Using *CoriandrumSativum* Leaf Extract and Their Application in Nonlinear Optics. Advanced Science Letters 3: 1–6.

Stanojevic, D., Čomic, L., Stefanovic, O., Sukdolak, S. 2010. In vitro synergistic antibacterial activity of *Salvia officinalisL*.and some Preservatives. *Archives of Biological Science Belgrade*, 62 (1): 175-183.

Thakkar, K.N., Mhatre, S.S., Parikh, R.Y. 2010. Biological synthesis of metallic nanoparticles. Nanomedicine; 6 (2): 257–262.

Velickovic, D.T., Ristic, M.S., Randjelovic, N.V. and Smelcerovic, A.A. 2002. Chemical Composition and Antimicrobial Characteristic of the Essential Oils Obtained from the Flower, Leaf and Stem of *Salvia officinalis* L. Originating from Southeast Serbia. *Journal of Essential Oil Research*, 14: 453-458.

Viuda- Martos, M., Ruiz- Navajas Y, Fernández- López J. and Pérez- ÁlvarezJA.2007. Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *International Journal of Food Science & Technology*, 43 (3): 526-531.

Wang, M., Jiangang, Li, Rangarajan, M., Shao, Y., Lavoie, E. J, Huang, T., Ho, C. 1998. Antioxidative Phenolic Compounds from Sage (*Salvia officinalis*). *Journal of Agriculture and Food Chemistry,* 46: 4869-4873.

****