Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2021, 10(1):118-126
https://doi.org/10.51847/oBv07gPJ5C

Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Thymus Vulgaris (Thyme) as a Natural Organic Matter to Biosynthesis Silver Nanoparticles and their Antibacterial Efficiency

Ghena Mohammad Abdrabou Mohammad Al-Jahani[1]*

[1]*Department of Nutrition and Food Science, College of Home Economics, Tabuk University, Tabuk, Saudi Arabia.*

***Email:** gmohammed@ut.edu.sa*

ABSTRACT

Thymus Vulgaris (Thyme) is a natural organic matter to biosynthesis silver nanoparticles (AgNPs) and their antibacterial efficiency. This research deals with the green synthesis of AgNPs by Thyme extract. Nanoparticles were characterized using UV-Vis spectra to analyze size and shape regulated nanoparticles in aqueous suspension, AAS to observe the varying concentricity of Ag+ ions in the solution through time, and FTIR to classify the possible Les combination's current activities in Thyme extract that are responsible for the silver reduction. The activity of antibacterial nanoparticles against pathogenic gram-positive and negative bacteria was studied. Additionally, an edible film of methyl cellulose reinforced with AgNPs to evaluate its antibacterial efficiency to utilized as alimentary packaging substances U.V. AgNPs reduced thyme extract and became globular intense peaks centered near 410 nm, thyme extract reduced the silver ions to silver nanoparticles decrease in the concentration of Ag+ ions from 5.5 ppm to 0.05 ppm for 12 minutes the reaction to occur. FTIR, the presence of carbonyl group of amino acids of proteins and -HO stretching in phenol extract of thyme leaves acts as a reducing agent and stabilizing silver nanoparticles and protecting them from changes, the antibacterial activity of biosynthetic AgNPs from thyme shows a promising area of inhibition on pathogenic gram-positive and negative bacteria. Also, as observed clear bactericidal activity of the cellulose / AgNPs mixture membranes against Escherichia coli 0157: H7 after 12 h of incubation at 37 o-C and increased with increasing concentration of AgNPs.

Key words: *Silver nanoparticles, Common thymus, Reducing agent, Antibacterial activity*

INTRODUCTION

Nanotechnology known as the Nano-scale of particles1-100 nm depend on materials [1, 2]. Nanoparticles are probably having a larger surface area and thereby, more surface atoms comparing to their micro-scale counterparts and their size [3]. Nanotechnology has risen to be popular as a cutting-edge technology including improvement of food quality, as well as in drug production, agriculture, however food is relatively new- [4]. Nanotechnology includes the fabrication, characterization, and/or manipulation of structures, materials, or devices which are approximately 1 to 100 nm in length. They are used to generate new products with various advantages for the food industry [5], such as extending shelf life, improving food safety, and monitoring food spoilage. In the past, due to their ability to protect food – stuffs and avoid rancidity caused by oxidation, antioxidant agents were taken from plants were taken into consideration. These compounds, which include flavones, vitamin C, zinc, B-carotene, phenols, vitamin E, and selenium, have shown higher antioxidant capacity than antioxidant compounds contained in animal protein [6, 7].

Researchers have recently focused on the usage of phytochemicals from plants for synthesizing green nanoparticle, which they are finding that metal nanoparticles synthesized from plants have all kind of unexpected

benefits, as they are free from toxic chemicals, easy handling, lower cost, social acceptance, develop environmentally friendly methods [8]. The concept of "green synthesis" of metal nanoparticles was introduced by development the field of nanotechnology by replacing the use of more hazardous chemicals as reducing agents such as silver and zinc oxide (ZnO), with biological decreasing agents from a plant such as Flavonoids, tannins and vitamin C, to solve price and pollution problems, among others [9]. particular methodologies have been carried out to synthesize nanoparticles of noble metals in specific size and shape because, in comparison with their micro- scale equivalents, nanomaterials have a higher surface to-volume ratio. This enables nanomaterials to bind more copies of biological molecules, conferring greater efficiency [10]. Since nanoscale materials have a higher surface-to-volume ratio than their microscale counterparts, they have a higher surface-to-volume ratio. Silver nanoparticles can be produced in many ways, including physical, biological, and chemical methods. Despite the organic synthesis required less time for the synthesis process of large nanoparticles quantities, this method has a downside in that it often necessitates the use of capping agents to keep the nanoparticles' sizes stable [11]. As a result, there is a growing need for green nanotechnology in the development of particle size. Huge research data informed that microorganisms are an efficient and powerful bio-reducing agent for metal nanoparticles, in contrast, it can increase the issues related to elevate handling of the microbial culture using in microbes which mediated biosynthesis of metal particles on a Nano-scale [12]. Spices deliver a better platform for nanoparticles synthesis such as Acalypha indica [13], Glycyrrhiza glabra [14], Cynodon Dactyion [11], Myristica Fragrans [15], Cuminum cyminum [8]. All of them were used similarity to be responsible for reducing silver ions to silver nanoparticles, which plant phytochemical with antioxidant or decreasing features are usually responsible for reducing metal compounds in their relevant nanoparticles [16].

Carbonyl group of amine acid in cell proteins have strong capability to bind with metal showing the layer formation that contains metal nanoparticles and acts as capping agent providing stability to the medium. Besides, proteins that have -SH group (thiol), reacts with silver forming a stable bond and forming a layer covering metal nanoparticles as well as lignin's groups present in phenolic compounds of plant extract inactivated silver ions by chelating and suppressing the super oxide driven reaction [17]. Furthermore, the increased surface area of the synthesized silver nanoparticles given by the metal's nanoparticle shape provides excellent microbial resistance. Nowadays, lots of consumer goods in the market contain silver nanoparticles, including containers of food stuff, cosmetics, pillows, clothes, and medical devices. This concept, has recently benefited from using nanotechnology materials including nanoparticles and Nano coatings, which have special significance in the extended shelf life foods and fresh areas [18, 19]. Recent reports emphasized that metal nanoparticles eradicate bacteria by any of these mechanisms; (i) interference with vital cellular procedures by binding to sulfhydryl or disulfide functional group on the surfaces of membrane proteins and interfering with enzymes. (ii) disruption of DNA replication.

(iii) oxidative stress through the catalysis of reactive oxygen species [4], therefore, antimicrobial properties of metal nanoparticles have been exploited in food contact materials such as plastic and stainless steel as well as deodorants and bandages [20]. Nevertheless, it is reported that some nanotechnology-related, antimicrobial agents can influence the physical features [21]. Thymus Vulgaris is known as a perennial plant characterized by small grey-green leaves with purple flowers and one of the preferable cooking herbs. It has some medicinal properties. It serves as anti-asthma, anti-cough, stomachic stimulant and intestinal catarrh. The chemical composition of its leaves contains proteins, sugars, polyphenols, terpiines and vitamins and bioactive phytochemicals such as thymol, Carvacrol, Pinene Alfathiogon, Cineol and Terpenoids were found in Thymus Vulgaris. The current research focuses on using its leaf extract as a model and temple of synthesized silver nanoparticles and exploiting their medicinal benefit in antibacterial activity (ii) To prepare methyl-cellulose/ AgNPs blend films and to determine the properties for their potentiality usage in food packaging application, because till dates up to our knowledge, there is no report the synthesis of silver nanoparticles from Thymus Vulgaris (Thyme).

MATERIALS AND METHODS

Materials

Silver nitrate and all the reagents used throughout the experiments were bought from Sigma-Aldrish (St Louis, MO, USA). Ultrapure water (from a Millipore System, Millipore, Billerice, MA. USA). Thymus Vugaris (Thyme) was supplied by Khidr El-Attar Company (Cairo-Egypt).

Bacterial strains

The four bacterial strains employed in the research included S. aureus (American Type Culture Collection [ATCC] 11988; Manassas, Va., USA), E. Coli 0157:H7 (ATCC 43895). B. subtilis and P. aeruginosa obtained from Food Sci. Dep. Agric. Collage, Ain Shams Uni. Egypt.

Methods

Preparation of aqueous silver nitrate

3.3 mg AgNO3, salt is emulsified in 30 mL distilled water, to obtain 1 mm AgNO3. The solution was allowed to be stirred in a magnetic stirrer for 15 minutes and stored in dark glass. This solution was homogenized to incorporate silver nanotechnology.

Preparation of the synthesized silver nanoparticles

The extract utilized for the synthesized silver nanoparticles was prepared by taking 25 g of well-washed thyme leaves, adding them to 100 ml of distilled water and stirring at 90 ° C for 30 minutes. The solution was then cooled and filtered with Whitman No.1 paper (pore size 25 μm) and centrifuged at 8000 rpm for 10 min. The supernatant solution obtained after centrifugation was used as thyme leaf extract. 10 ml of the extract prepared above was taken in 4 separate flasks 50 ml of aqueous AgNO3 solution (1, 2, 3 and 4 mm) was added to flasks for distillation with continuous stirring and boiling at 80 ° C. for an hour. After 1 hour of reaction, the color of the reaction mixture changed to dark brown indicating the reduction of Ag + ions to silver nanoparticles. Finally, the mixture was centrifuged at 5,000 rpm for 20 minutes. The resulting substrate was collected and the pellets were dried in a hot air oven at 65 ° C for 20 minutes.

Description of nano silver particles

Ultraviolet visible spectroscopy

Visually observed color changes in the silver nitrate solution was added to the biological extracts. The bio-reduction of primary silver ions was monitored by sampling aliquots (1 mL) at various time intervals. Absorption measurements were carried out on a UV-Visible Spectrophotometer (ELICO U. V. 165) at room temperature operating at 1 nm resolution. A UV-visible analysis of one-week-old samples was also performed to confirm the stability of the silver nanoparticles. The previous stock solution of AgNO3 at different concentrations (1 mm to 4 mm) was added to 10 ml of thyme extract and UV results were taken for these samples.

Atomic absorption spectroscopy

In a study (AAS), an ASS system was used to analyze the concentration of silver ions in solution over a specified period (GBC 932 AA). And converting Ag + to Ago during the reaction at different times, then drawing samples and centrifuging them at a rate of 14000-15000 revolutions per minute, and this solution became homogeneous to contain silver nitrate and it was found that (Ag + ions) did not interact with (Ago) because these ions became small, and did not contain Ag (Ago) nanoparticles and nanoparticles. The supernatant was analyzed by AAS to reveal the amount of Ag + ions. It depicts the rate of decrease in the conversion concentration of Ag + Ag + ions to Ago.

Fourier transform infrared spectroscopy (FTIR)

This was done by studying the chemical composition of silver nanoparticles using FTIR spectroscopy, where the measurement was performed to identify the interaction between Ag nanoparticles and the blocking agent, (as well as study the potential interaction between protein and silver nanoparticles) in thyme extract, and centrifugation of the bio-compound nanoparticles at 20000 rpm for 20 minutes to purify and remove residues of any other agglomerated compound. It does not contain nanoparticles. Then the Ag nanoparticles obtained after the centrifugation process were diffused in deionized water and washed three times with 20 ml of the same water. Samples were dried and milled with KBr granules and analyzed on a Nicolet IR200 model (Thermo Electron Corp.).

Analysis of the antimicrobial activity of solutions

Containing Bio-silver Nanoparticles: Antimicrobial activity was analyzed in solutions containing silver nanoparticles by disk diffusion method. The prepared nutrient agar was poured onto sterile Petri dishes and 17 hr culture of B. subtitles cationic bacteria, S. aurous, E. coli, and Gram-negative P. aeuginosa were swabbed onto agar plates. Meanwhile, the sterile tablets were impregnated with different

concentrations of silver nanoparticles (50 μg / ml, 100 μg / ml and 150 μg / ml aqueous solution) then pre-prepared and an antibiotic (antibiotic ciprofloxacin) was added upside down on a mop. painting. An empty sterile tablet was kept as a negative control. Dishes were incubated at 37 ° C for 24 hours. Then, the antibacterial activity was assessed by measuring the Diameter in the Inhibition Zone (DIZ) of the tested bacteria and DIZ was expressed in centimeters. All tests were conducted in triplicate.

Preparation of edible films from methylcellulose

Edible films were prepared from methylcellulose containing silver nanoparticles, and the antibacterial activity was studied by dissolving 5 gm methylcellulose (w / v) in 100 ml of distilled water at 45 ° C, and then preparing nutrient edible film solutions with the addition of glycerol (0.4%). Where elasticity is given to the edible film, then xanthan gum (0.05%) is added to stabilize a solution with the addition of lecithin (1% weight // volume) as an emulsifier and dissolve all components under a magnetic vibrating stirrer, the solution is cooled to room temperature and then an aqueous solution with a different concentration of silver nanoparticles is added Pellets) prepared in advance (50 μg / mL, 100 μg / mL and 150 μg / mL auroral solution) then mixing the solution components and then decanting by drawing 10 ml of the solution into sterile Petri plates (VWR) with 10 cm inner diameters and letting them dry throughout Overnight (18 hours) in a sterile air-drying oven at 25 ° C. A control sample was prepared from the same solution without adding the silver nanoparticle solution.

Antimicrobial activity analysis in the film

The antimicrobial activity of the membranes and the effect of inhibition on the growth of Escherichia coli 0157: H7 were examined. The tested microorganisms were inoculated in 20 ml of Nutrient Agar (NA) medium and incubated at 37 ° -C for 16 hours. Centrifugation was then worked at 2000 rpm for 10 minutes, and the cell pellets were then suspended in 100 ml of NA. Sterile and diluted 10 times with sterile distilled water. 20 ml of the dilute solution (106-107 cfu / ml) was taken in a 10 ml conical flask containing 100 mg of film sample and incubated in rocking incubation at 37 ° C for 16 h and then cell viability was inoculated into plates of NA. Then count the microorganisms by counting the bacterial colonies on the plates at 0, 4, 8, 12, 16 and 20 hours. After that, antimicrobial tests were conducted on three individually prepared films [22].

RESULTS AND DISCUSSION

UV-Vis spectrum analysis

The research was conducted to prepare solutions with different concentrations of AgNO3 ((1,2,3 and 4 mm) by adding 10 ml of thyme leaf extract to the solution and then exposing it to ultraviolet rays in the form of **Figure 1**. In the range of 400-430 nm, while the absorption length is between 400-600 nm, the high absorption length between 400-430 nm UV visible rays showed the presence of nanoparticles of silver. Hence, the reported results include the presence of nanoparticles in the solution. In addition, the UV absorbance width was about 230 nm and 310 nm, which makes the proteins and phenols in the organic extracts in the curve represent the absorption, which leads to the interaction of proteins and phenols in the extracts to the highest peak in the curve. It also indicated that the peak of absorption was found, where it was found narrow and a single peak, as it was revealed that the shape of the silver nanoparticles reduced by thyme leaf extract was found to be spherical and dispersed [23]. Also found that the Surface Plasmon Resonance (SPR) peak was also obtained from chemically synthesized silver nanoparticles concentrated near 410 nm which confirmed that thyme leaf extract reduces silver ions and converts them into silver nanoparticle. The results of the study indicated that the thyme extract changed color in the AgNO3 solution, but gradually stains from transparent to dark brown as a result of reducing Ag + ions to Ago within 12 minutes from the beginning of the reaction, as it remained stable (7 days). Short time, improved the stability of the silver nanoparticles. This caused the emergence of this modulation to change the chromaticity due to the excitation of the vibrations of the mixture on the surface with the silver nanoparticles [24].

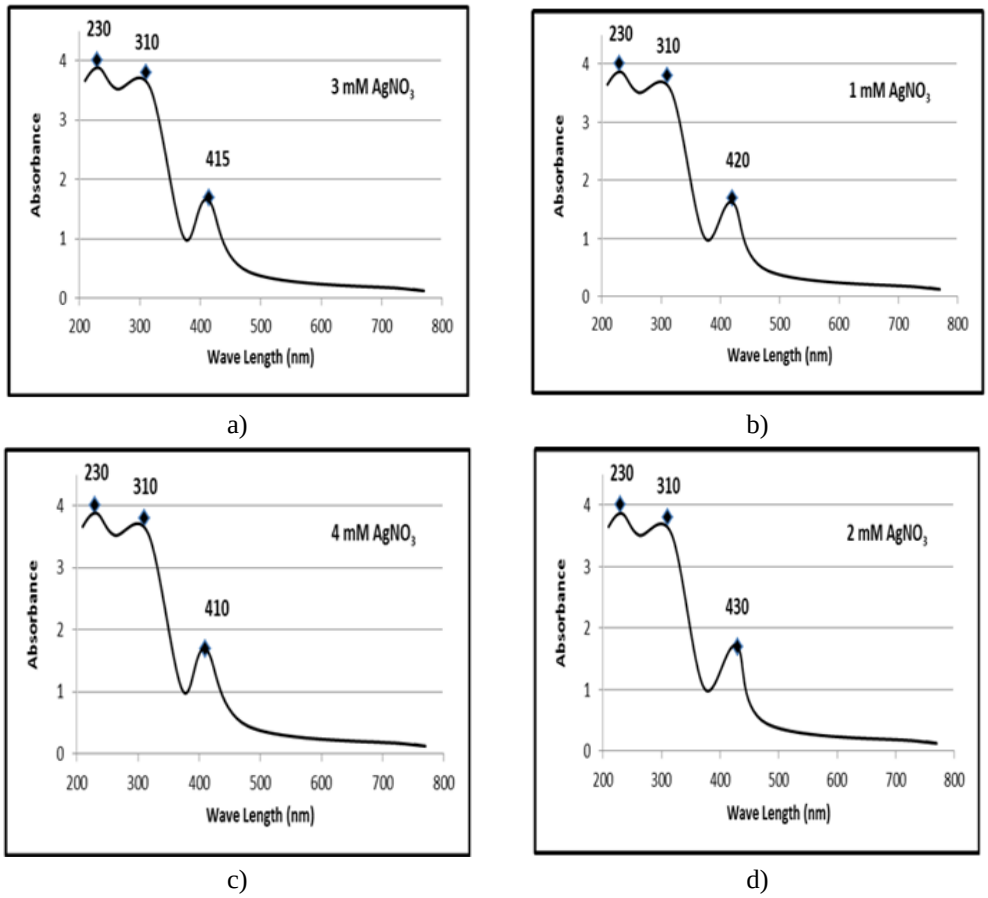

Figure 1. UV spectrum of Ag nanoparticles obtained by 1 – 4 mM AgNO3 and Thyme extract

Atomic absorption spectroscopy analysis (AAS)

This analysis indicated AAS in reaction solution that was performed at regular time intervals as in **(Figure 2)** where the Ag + ions were transformed into Ago. Initially, by preparing the standard solution of 5.5 ppm of AgNO3 and analyzing it using an atomic absorption spectroscopy at 0 min and by monitoring the concentration of Ag + ions in the reaction solution, after adding the thyme leaf extract, at regular intervals. The results indicated a decrease in the concentration of Ag + ions (5.5). , 4.5, 3.3, 2.2, 1.3 and 0.05 ppm at 0, 2, 3, 4, 8 and 12 min, respectively) indicating conversion of Ag + to Ago and its rate from Ag + and its decrease in solution. A similar pattern was also observed by [25] where the silver nano particles were synthesized using Nigella Sativa

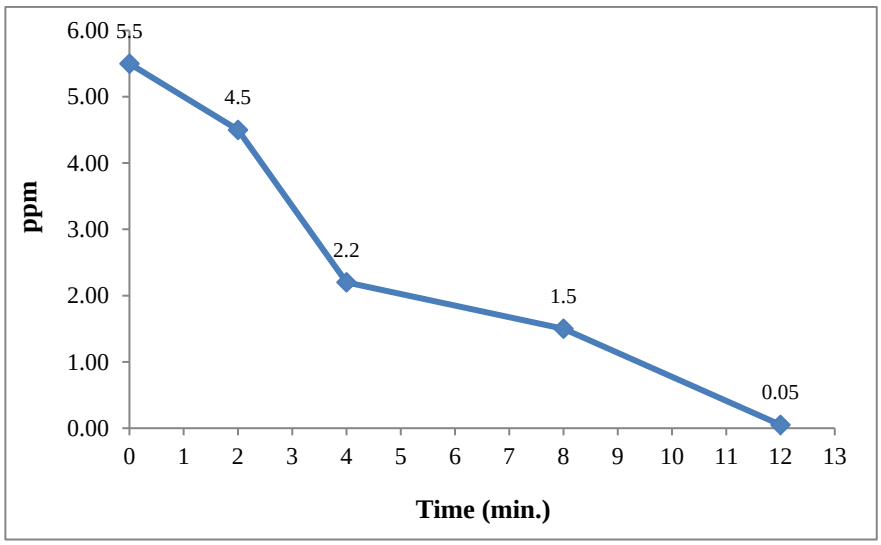

Figure 2. The Graph Shows an Atomic Absorption Spectrometer of the Ag + Concentration in the Reaction Mixture.

FTIR analysis

By studying the FTIR measurement analysis of biosynthetic silver nanoparticles was done to find out the potential interaction between protein and silver nanoparticles / or potential biosynthetic molecules of an extract from thyme leaves responsible for the formation and fixation of dissolved nanoparticles. The results of the FTIR measurements showed the occurrence of peaks in the transmittance located at lengths of about 3475 cm-1, 2388 cm-1 and 1635 cm-1 **(Figure 3)**. From the spectrum, the mapping of the main peak was shown at a wavelength of 3475 cm-1, indicating OH-stretching in alcohols and phenolic compounds [17]. The absorption peak at 1635 cm-1 led to the occurrence of an amide-1 bond in the resulting proteins compared to the carbonyl expansion of the proteins in thyme leaf extract, and the absorption peak at 1635 cm-1 is close to those of the original proteins [15]. This evidence indicates that the proteins interact with biosynthetic nanoparticles, and that their secondary structure did not have an effect during interaction with Ag + ions or after binding to Ag nanoparticles [26]. A small peak at 2388 cm-1 was determined for the NH stretch mode and it was optimized when compared with the FTIR spectrum of thyme leaf extract.

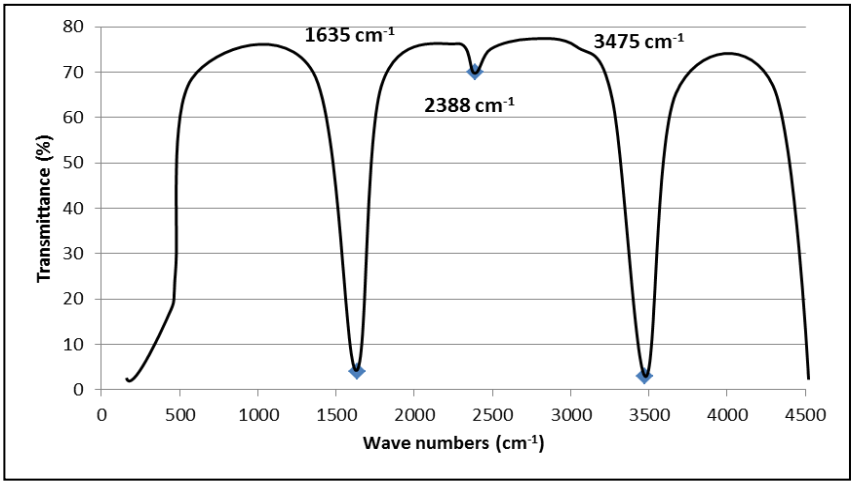

Figure 3. Demonstrated FTIR Spectroscopy of Silver Nanoparticles from Thyme Leaf Extract.

From this study, it was found that the infrared spectrum contains the carbonyl group present in the amino acids, as it can form strong cross-links with the metal, and it also covers metallic nanoparticles and provides stability [8]. The extract of phenolic compounds from thyme leaves also works to stop silver ions by claws in addition to neutralizing the reaction resulting from oxidation, and is believed to be a source of reactive oxygen [27]. The presence of proteins with phenols extracted from thyme leaves act as a reducing and stabilizing agent for silver nanoparticles and protect them from changes, as the nanoparticles produced through the biological process are more stable compared to chemical reduction.

Antibacterial activity

The antibacterial activity of biosynthetic silver nanoparticles inactivating the Gram-negative E. coli & P. aeuginosa, S. aureus and B. inhibition was performed by disc diffusion. Then, compared the inhibition of bacterial growth by nano-Ag with the standard antibiotic ciprofloxacin. The diameter of the inhibition zone was (mm) for the concentrations of silver nanoparticles containing the organisms

Table 1. Inhibition Regions for Bioactive Silver Nanoparticles Concentrations against Gram-negative and Gram-positive Bacteria.

Bacteria	Zones of inhibition for silver nanoparticles concentration (ppm)		
	50	100	150
E. coli	0	0	14
P. aenginosa	0	10	15
B. subtilis	7	12	17
S. aureus	11	15	20
Ciprofloxacin (Antibiotic)	13	18	22

The data in **Table 1** shows the diameters of the inhibition areas for three concentrations of Ag nanoparticles, where it was found that enhancing the concentration led to inhibition of Gram-positive bacteria and was the most influential comparison with gram-negative bacteria, where the areas of the inhibition range for bacteria ranged from 14 to 20 mm at a concentration of 150 ppm while the regions of inhibition of P. aeruginosa were 10 mm at a concentration of 50 ppm, while the areas of inhibition of the bacteria for E. coli were 14 µM at 150 ppm. It was also found that there were no inhibition zones at 100 and 50 ppm compared to the standard antibiotic with sufficient efficacy. The use of these silver nanoparticles in low doses with standard antimicrobials leads to the reduction and biological coverage, and these silver nanoparticles are natural, environmentally friendly and non-toxic compared to chemically synthesized silver nanoparticles.

Antimicrobial activity of cellulose / silver nanoparticles blend films

Conducting an analysis of anti-microbial phenolic extracts with membranes mixed with cellulose / Silver nanoparticles by using the method of total coli counting of pathogenic bacteria resulting from food E. coli 0.157: H7. The results are shown in **Table 2** and **Figure 4**. Significant activity against Escherichia coli after 12 hours and with increasing concentration of silver nanoparticles, as it was repeatedly observed that the greater amount of silver nanoparticles mixed with food membranes showed stronger activity against E. coli 0157: H7. Therefore, silver nanoparticles is a more environmentally friendly method than traditional chemical methods and we can add its extract to food packaging materials as an anti-microbial agent to maintain safety and extend the shelf life of packaged foods

Table 2. Antimicrobial Activity of Cellulose / Ag NPs Blend Films against E. coli 0157: H7 with Different Ratio.

Bacteria	Cell viability (Log cFu/ml)					
	Time (h)					
	0	4	8	12	16	20
Zero ppm Ag NPs (control)	4.44	7.24	8.37	9.26	10.08	11.00
50 ppm Ag NPs	4.44	6.61	7.34	8.22	7.13	6.40
100 ppm Ag NPs	4.44	5.38	6.29	7.48	6.34	5.26
150 ppm Ag NPs	4.44	4.95	5.69	5.12	4.31	3.25

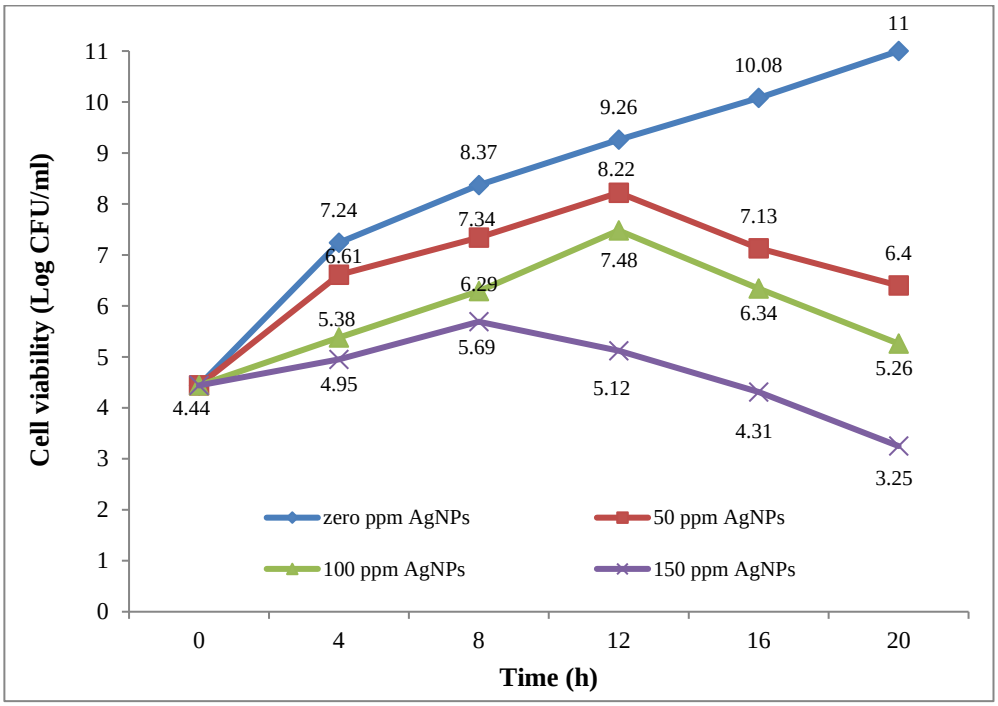

Figure 4. Antimicrobial Activity of Cellulose/ Silver nanoparticles Blend Films against *E. coli* 0.157: H7 with Different Ratio.

CONCLUSION

The study aims to produce silver nanoparticles by reducing these ions to the colloidal state. The thyme extract acts as a reducing agent for the biosynthesis process. Silver nanoparticles within 12 minutes and the ultraviolet radiation of the silver nanoparticles ranged between 400 to 430 nm. From the FTIR spectra, it was also found that the biomolecules responsible for covering and fixing the silver nanoparticles are proteins and phenolic compounds present in thyme extract, which are phenolic compounds associated with silver nanoparticles, that indicate their potential role in the bio-reduction mechanism. In addition, silver nanoparticles biocomposed using thyme extract have effective activities in resisting microbes against gram-positive and gram-negative bacteria, and these silver nanoparticles are not economically inexpensive, natural and environmentally friendly and are used in the industrial and medical field and thus are used with edible cellulose films / AgNPs or food coating. Antimicrobial activity can be used as food packaging material to maintain safety and extend the shelf life of prepackaged foods.

ACKNOWLEDGMENTS : I thank the Deanship of Scientific Research (DSR) University of Tabuk, Saudi Arabia, so the authors express their thanks and technology for DSR.

CONFLICT OF INTEREST : None

FINANCIAL SUPPORT : None

ETHICS STATEMENT : None

REFERENCES

1. Babaei H, Sepahy AA, Amini K, Saadatmand S. The Effect of Titanium Dioxide Nanoparticles Synthesized by Bacillus tequilensis on clb Gene Expression of Colorectal Cancer-causing Escherichia coli. Arch Pharm Pract. 2020;11(1):22-31.
2. Kanjikar AP. On Anti-Diabetic Potential of Phyto-nanoparticles Comparison with Hormonal Therapy and Medicinal Plants. Int J Pharm Phytopharmacol Res. 2019;9(1):103-11.
3. Uyen VN, Van NT, Van Minh N, Nguyen T, Ha HK. Fabrication of Cu_2O-ZnO Nanocomposite by the Sol-gel Technique and its Antibacterial Activity. J Biochem Technol. 2020;11(1):18-24.
4. Caro N, Medina E, Díaz-Dosque M, López L, Abugoch L, Tapia C. Novel active packaging based on films of chitosan and chitosan/quinoa protein printed with chitosan-tripolyphosphate-thymol nanoparticles via thermal ink-jet printing. Food Hydrocoll. 2016;52:520-32.
5. Mohamed FA, Salama HH, El-Sayed SM, El-Sayed HS, Zahran HA. Utilization of natural antimicrobial and antioxidant of Moringa oleifera leaves extract in manufacture of cream cheese. J Biol Sci. 2018;18(2):92-106.
6. Jeyashree R, Revathi S. Synthesis of AgNPs of Cuminum cyminum seed extract, characterization and antimicrobial activity. Int J Eng Sci Comput. 2017;7(7):14193-6.
7. Hosseinnia M, Khaledabad MA, Almasi H. Optimization of Ziziphora clinopodiodes essential oil microencapsulation by whey protein isolate and pectin: A comparative study. Int J Biol Macromol. 2017;101:958-66.
8. Jiang S, Liu C, Han Z, Xiong L, Sun Q. Evaluation of rheological behavior of starch nanocrystals by acid hydrolysis and starch nanoparticles by self-assembly: A comparative study. Food Hydrocoll. 2016;52:914-22.
9. Alparslan Y, Baygar T, Metin C, Yapici HH, Baygar T. The role of gelatin-based film coating combined with orange peel essential oil on the quality of refrigerated shrimp. Acta Aquatica Turcica. 2019;15(2):197-212.
10. Bellaouchi R, Ghomari I, Hasnaoui A, Hakkou A, Bechchari A, Chihib NE, et al. Physico-chemical and microbial properties of undervalued dates and processed dates by-products in Morocco. Int Food Res J. 2017;24(3):963-9.
11. Noori S, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiber officinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control. 2018;84:312-20.

12. Bukar A, Uba A, Oyeyi T. Antimicrobial profile of Moringa oleifera Lam. extracts against some food–borne microorganisms. Bayero J Pure Appl Sci. 2018;4:125-31.
13. Artiga-Artigas M, Acevedo-Fani A, Martín-Belloso O. Improving the shelf life of low-fat cut cheese using nanoemulsion-based edible coatings containing oregano essential oil and mandarin fiber. Food Control. 2017;76:1-2.
14. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-9.
15. Sharma G, Sharma AR, Kurian M, Bhavesh R, Nam JS, Lee SS. Green synthesis of silver nanoparticle using myristica fragrans (nutmeg) seed extract and its biological activity. Dig J Nanomater Biostruct. 2014;9(1):325-32.
16. Acevedo-Fani A, Soliva-Fortuny R, Martín-Belloso O. Nanoemulsions as edible coatings. Curr Opin Food Sci. 2017;15:43-9.
17. Rao P, Chandraprasad MS, Lakshmi YN, Rao J, Aishwarya P, Shetty S. Biosynthesis of silver nanoparticles using lemon extract and its antibacterial activity. Int J Multidiscip Curr Res. 2014;2:165-9.
18. Abdel Khafar EA. Enhancement the antibacterial agent of edible coating and films by incorporating it with green metallic nanoparticles using Luria mobiles leaves. MOJ Food Process Technol. 2020;8(2):80-97.
19. Acevedo-Fani A, Soliva-Fortuny R, Martín-Belloso O. Nanoemulsions as edible coatings. Curr Opin Food Sci. 2017;15:43-9.
20. Gundewadi G, Rudra SG, Sarkar DJ, Singh D. Nanoemulsion based alginate organic coating for shelf-life extension of okra. Food Packag Shelf Life. 2018;18:1-2.
21. Haghju S, Beigzadeh S, Almasi H, Hamishehkar H. Chitosan films incorporated with nettle (Urtica dioica L.) extract-loaded nanoliposomes: I. Physicochemical characterisation and antimicrobial properties. J Microencapsul. 2016;33(5):438-48.
22. Aboul-Anean HE. Using quinoa protein and starch nano particles to produce edible films. J Nutr Health Food Eng. 2018;8(4):297-308.
23. Patil SV, Borase HP, Patil CD, Salunke BK. Biosynthesis of silver nanoparticles using latex from few euphorbian plants and their antimicrobial potential. Appl Biochem Biotechnol. 2012;167(4):776-90.
24. Wu C, Wang L, Hu Y, Chen S, Liu D, Ye X. Edible coating from citrus essential oil-loaded nanoemulsions: physicochemical characterization and preservation performance. RSC Adv. 2016;6(25):20892-900.
25. Fayaz AM, Balaji K, Girilal M, Yadav R, Venketesan R. FTIR measurements of biosynthesized silver nanoparticles. Nanotechnol Biol Med. 2010;6:103-9.
26. Parida P, Behera A, Swain SK, Mishra SC. Characterization of nanoparticles through SEM, FTIR, XRD and DSC University, department of pharmaceutical sciences. Utkal Uni Bhubaneswar Fitoterapia. 2011;3:253-69.
27. Grazlela VL, Mirella RS, Luis FP, Marostegan CG, Camila GJ. Physicao-chemical stability of beta-carotene loaded lipid nanoparticles of cupuacu butter. J Food Eng. 2017;192:93-102.