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PRODUCTIONS OF SILVER NANOPARTICLES FROM C*ITRUS SINENSIS* PEEL AND STUDY THE ANTIBACTERIAL EFFECTS AGAINST SOME FOOD BORNE PATHOGENS

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Abstract

The objective of this study was isolation and identification of some food pathogens from some local food in Erbil city and determination the sensitivity to classical antibiotics as well as trying to synthesis of sliver nanoparticles from peel *Citrus sinensis* extracts. Two hundred food samples were collected from ready to eat food include; red meat and poultry, local dairy products and cake and pastry from December, 2016, to April 2017, in Erbil city. Different types of media were used; S.S. agar, Macconkey agar, Manitol salt agar, Nutrient agar for isolation of *Salmonella typhimurium*, *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.* from food samples. The isolates were investigated phenotypically, cultural and biochemical tests, Vitek systems, and confirmed genotypically, by using 16srRNA gene amplified by the polymerase chain reaction (PCR). Microscopically morphology and biochemical characterizations were examined for the isolated bacteria the results appeared that 31 isolates characterized as *Salmonella* spp, 59 isolates of *Escherichia coli,* 28 isolates of *Staphylococcus aureus* and 38 isolates as *Bacillus spp.* The prevalence of these bacteria in tested food samples recorded as follow; highest number of bacteria was obtained from red meat and poultry (19) isolates followed by Local dairy product(18) isolates, Ready to eat food (16) isolates and (11) isolates from cake and pastry. The antibiotics sensitivity tests using disc diffusion method reveled that all the isolates were variable in resistant to Ampicillin, Amoxicillin, Tetracycline, Erythromycin, Vancomycin, Piperacillin, except *Salmonella typhimurium* which 100% are sensitive to those antibiotics. Peel extract from *Citrus sinensis* was used for the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. Ag NPs characterized by UV–vis spectrophotometer, X-ray diffractometer (XRD), and scanning electron microscope (SEM). The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by UV vis spectrophotometer analysis. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using the Scherrer's equation. Formation of silver nanoparticle with an average size of 65 nm corresponding to *C. sinensis*. SEM determination of the brown color stable samples showed the formation of silver nanoparticles and well dispersed nanoparticles could be seen in the samples treated with silver nitrate. The inhibitory effect with different concentration; 25, 50, 75 and 100 µl. were increased according to increasing the concentration of the silver nonoparticales; the highest inhibition zone against bacteria *E. coli* and *Sal. typhimurium* were 30mm, while the highest inhibition zones were 28mm and 25mm for *Staph. aureus* and *Bacillus cereus* respectively.

Keyword **:** Synthesis of Silver NPs. Food borne pathogens, *Citrus sinensis*

Introduction

Food borne illness defined by the World Health Organization as diseases, usually food born intoxication, caused by agents that enter the body through the ingestion of food. Bacterial contamination of food represents one of the major public health problems, *Salmonella* spp., *Staphylococcus aureus, E. coli* pathotypes and *L. monocytogense* are the predominant bacteria species that cause public health problems worldwide (Lei *et al.,* 2008). Food borne diseases are main problems, particularly in developing countries and cause the majority of illnesses and death around the world. Food is the most important vehicle that transmits the microorganisms to human. Worldwide, food-borne diseases are a major health burden leading to high morbidity and mortality. The global problem of infectious diarrhea involves 3-5 billion cases and nearly 1.8 million deaths annually, mainly in young children, caused by contaminated food and water (Srivastava *et al.*, 2009). Pathogen detection system has long been used in food production as a means of process and quality control, tracking of contamination sources, and monitoring regulatory compliance. Food-borne diseases are now becoming a great concern involving a wide range of illnesses caused by bacterial, viral, parasitic or chemical contamination of food. In addition, resistance of these microorganisms to multi-drugs made this situation more of a concern to public health. One of the promising efforts to address this challenging and dynamic pattern of infectious diseases is the use of nanotechnology.

Nano technological applications in medicine have yielded a completely new field of technology that is set to bring momentous advances in the fight against a range of diseases. Nanoparticles exhibit attractive properties like high stability and the ability to modify their surface characteristics easily (Tom *et al.*, 2004). Exerting their antibacterial properties, nanoparticles attach to the surface of the cell, this interaction causes structural changes and damage, markedly disturbing vital cell functions, such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes, and finally leading to cell death (Rai andYadav 2009; Sharma *et al.,* 2009; Li *et al.,* 2010), the aim of this study were determine the incidence of pathogenic bacteria in local food products in Kurdistan region and using cultural methods and biochemical test for identification the isolated bacteria as well as using PCR. controlling the growth of pathogenic bacteria which have contaminated food, and resist to classical antibiotics by using synthesis silver nanoparticle by using peel *Citrus sinensis* extracts to control the growth of isolated pathogenic bacteria and comparing their effects with the classical antibiotics against the bacteria.

Material and Methods

Two hundred (200) local food samples were collected from Kurdistan region markets, this food samples include 50 from each of ready to eat food, red meat and poultry, local dairy products and cake and pastry. Twenty five grams of

each food samples were suspended to 225ml of buffer peptone water and incubated at 37°C for 12-18hr. serial dilution were prepared from the initial dilution, from suitable dilution (0.1) was transferred onto surface of specific media as follow (Levinson and Jawetz, 2000)

Isolation of *Salmonella* spp. : Enrichment phase and selective liquid environment was carried out, culture Rappaport Vassiliadis was used and then transport 1 ml to plate of S. S agar, incubated at 37 °C for 18-24 h (Omar *et al.,* 2014).

Isolation of *Bacillus sp.* was done by heating the samples to get *Bacillus sp.* spore and then 1ml transferred to the plate surface of nutrient agar, and incubate for 18-24 hours in 37 °C (Omar *et al.,* 2014).

Isolation of *Staphylococcus aureus:* 1 ml. was transferred onto the surface of mannitol salt agar (Levinson and Jawetz, 2000).

Isolation of *E. coli* and *E. coli* O157 H:7. The samples were diluted in tryptone water (1% tryptone, 0.5% NaCl), inoculated onto MacConkey agar, and for isolation of *E. coli* O157 was performed according to the Dentorou method, colonies was streaked on Sorbitol MacConkey agar (SMAC) which contains cefixime (50 µg/liter) and potassium tellurite 2.5 mg/liter, and incubated at 42ºC for 24 hours (Dontorou *et al.*, 2003).

Identification of isolated bacteria and determination MIC of bacteria isolates by VITEK 2 compact system (bioMerieux Vitek, Hazelwood, µ0, USA), antimicrobials are placed on plastic reagent cards that can hold microliter quantities of test media. As well as the identification confirmed by PCR. The Primers sequencing which used appeared in the following table:

Table 1 : Primers Sequencing:

Target gene	Primer	Nucleotide sequence	Product size	Reference
16srRNA for *Salmonella typhimurium*	16S FWD1 16S REV1	5-CGG.,ACG,GGT,GAG,TAA,TGT,CT3- 5-GTT,AGC,CGG,TGC,TTC,TTC,.TG3-	406	(Mahon *et al.,* 1994)
16srRNA for *Bacillus cereus*	16S FWD1 16S REV1	5-CAAGTCAAGATAAGAGGCTTC3- 5-AAAGCTCTTGCCAAATAACC3-	370	(Fangio *et al.*, 2010)
16srRNA for S*taphylococcus aureus*	16S FWD1 16S REV1	5-GTAGGTGGCAAGCGTTATCC3- 5-CGCACATCAGCGTCAG3-	228	(Awadalla *et al.,* 2015)
16srRNA for *E. coli*	16S FWD1 16S REV1	5'-GGAAGAAGCTTGCTTCTTTGCTGAC-3' 5'AGCCCGGGGATTTCACATCTGACTTA3'	594	(Magda *et al.,* 2014)

Genomic DNA extraction:

Sample preparation and bacterial culturing were carried out, pure isolated colony for each type of isolated bacteria were inoculated in 90 ml Tryptone Soya Broth-Yeast Extract (TSBYE) incubated overnight in 37c. DNA was prepared for PCR according to the method (Adwan *et al.,* 2015). Briefly, 1.5 ml from overnight TSBYE broth was centrifuged for each DNA prepared extraction, the pellet washed twice with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). The pellet was then re-suspended in 0.5 ml of sterile distilled H_2O, and boiled for 10-15 min. The cells then immediately were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 × g for 5 min. DNA concentration was determined using a spectrophotometer and DNA samples were stored at -20ºC until use. The amplified products were examined by 2% agarose gel electrophoresis. A DNA ladder of 100 bp was also included in all gels (100 bp DNA ladder RTU, GeneDireX).

Synthesis of Silver nanoparticales

Collection of plant material: Five different natural peels of *Citrus sinensis* were selected for the silver nanoparticles synthesis. The peels were washed 2–3 times with de-ionized water.

Biosynthesis of silver nanoparticles: Silver nitrate (Himedia) used in this study; 2.5 g of the peels of *C. sinensis* were boiled in 150 ml of de-ionized water. 2.5 ml of ammonium solution was added to 5 ml of 1 mM $AgNO_3$ (solution, followed by addition of peel extract 1–10 ml and the final volume was adjusted to 50 ml by adding the suitable amount of de-ionized water, the solution turned from yellowish to bright yellow and to dark brown indication of silver nanoparticles. The flasks were incubated at 37 C under shaking (200 rpm) for 24–48 h (Kasthuri *et al.,* 2009).

Description of silver nanoparticles; To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300–540 nm using a UV–vis spectrophotometer (Thermo Scientific NanoDrop 1000 labtech U-2800 spectrophotometer). The deionized water was used as the blank. The samples were air-dried and allowed to characterize by Atomic Force Microscopy (Model-Nanosurf easyscan 2 AFM, Switzerland) for its detail size, morphology and agglomeration of silver. AFM Image was taken with silicon cantilevers with force constant 0.02–0.77 N/m, tip height 10–15 nm, contact mode. To check phase formation and purity, XRD patterns were recorded using powder X-ray diffract meter (Model-D8 Advance, made in BRUKER Germany).

The samples from the exciting time point of production of silver nanoparticles were equestrian on specimen stubs with double-sided adhesive tape and coated with gold in a sputter coater to avoid charging and examined under SEM (HITACH, Model S- 3400N).

The antibacterial activity of Nanoparticles

Antimicrobial activity by well diffusion method: The silver nanoparticles (Ag NPs) synthesized from *C. sinensis* were tested for their antimicrobial activity by well diffusion method against pathogenic organisms like *Staphylococcus aureus*, *E. coli, Salmonella typhmurium* and *Bacillus cereus.* The pure cultures of the bacteria were sub cultured on Nutrient broth at 35C on rotary shaker at 200 rpm. Each

isolate was streaked uniformly on the separate plates using sterile cotton swab. Wells with size 5 mm have been made on Muller–Hinton agar plates using cork borer. Using micropipette, 50 ll, 75 ll and 100 ll of the nanoparticles solution were poured into the wells. After incubation at 37C for 20 h, the zones of inhibition were measured (Ahmed *et al.,* 2015).

Result and Discussion

In this study, 200 food samples were collected from different markets in Erbil city to determine the presence of pathogenic bacteria, types of these food appeared in table 2, out of total food samples,150(75%) were contaminated with bacteria, and according to the results the highest contaminated food was ready to eat food 42(84%), while the red meat, and cake with cream and pastry were 39 (78%), but 30 (60%) of local dairy products was contaminated with pathogenic bacteria. The food samples were taken and examined for *S. aureus, E. coli, Salmonella sp.* and *Bacillus sp*, which cultivated on specific selective media for each genera, as appeared in figure 1, 75% of the total samples were contaminated with *Bacillus sp*. 42% , 24.50% of the samples were contaminated with *E. coli* and *Staph. aureus* respectively while *Salmonella s*p. found in 21% of the total food samples.

Table 2 : Number and Percentage of Contaminated Food Samples

Type of food	Total No. of samples	No. and % of positive sample	No. and % of negative samples
Ready to eat food	50	42 (84)	8(16)
Red meat	50	39(78)	11(22)
Local dairy product	50	30(60)	20(40)
Cake with cream	50	39(78)	11(22)
Total	200	150(75)	50(25)

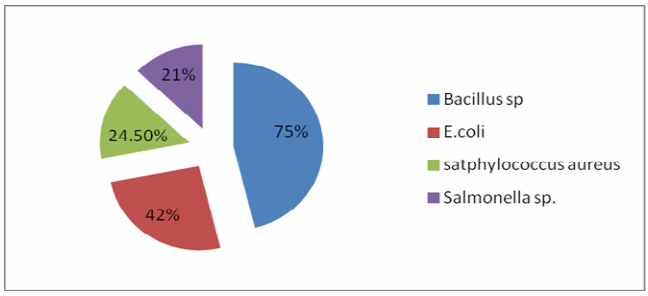

Fig. 1 : Percentage of Isolated Bacteria from Different Food Sources

Identification of Pathogenic Bacteria.

The study revealed to isolate *Salmonella* in selective media S.S. agar and XLD; identified by biochemical tests and veitek compact 2 system, the result appeared that; 15(48.%) of the total isolates were confirmed as *Salmonella enterica* (*typhimurium*) and 16(51.06%) were the other salmonella species as showed in table 3, this bacteria identified as *Sal. typhimurium* according to possessing the specific gene 16rRNA products size 406 bp.

This result was agree to Swanenburg *et al.* (2001) they performed to identification *Salmonella typhimurium* in different source of food and detection by PCR technique, 39 (31.45%) was positives from the 268 field samples. Among various foodborne pathogens, *Salmonella* serotypes are the most common bacteria responsible for foodborne gastroenteritis. There are more than 2500 serovars of *Salmonella* and all are considered as pathogenic. *Salmonella* is found anywhere in nature, including the digestive tracts of different animals, poultry products, milk products and seafood. Raw chicken meat is known to be the major source for *Salmonella* food poisoning (Chen and Schluesener 2008). The prevalence of *Salmonella* in different food products ranged from 2% to 100% (Iyer *et al.,* 2013; Anihouvi *et al.*, 2013; Adeyanju and Ishola, 2014).

Table 3 : Identification of pathogenic bacteria by PCR

Type of pathogenic bacteria	No. of isolates by vitiek	No. (%) of positive isolates by PCR
Escherichia coli	59	56
Bacillus cereus	38	33
Staphylococcus aureus	28	22
Salmonella entrica (*typhimurium*)	31	15(48)

Staphylococcus aureus identified by biochemical tests, from the total isolates; 28 isolates divided as follow: 22 isolates were confirmed and gave positive result to specific sequence 16rRNA gene as showed in table 3. *Staph. aureus* possessed specific gene 16rRNA products size (228bp) cleared as bands in the gel electrophoresis. The isolates of *Bacillus* spp. identified by biochemical tests, only 38 isolates were confirmed as *Bacillus cereus* by vitiek compact system2 and by PCR. The results were shown that 59 isolates confirmed as *E. coli* (Kiranmayi *et al.,* 2010).

Antibiotics susceptibility test

The antibacterial sensitivity test was performed according to Kirby-Bauer Method (antibiotic disc diffusion method) included 7 types of antibiotics; Ampicillin, Amoxicillin, Tetracycline, Erythromycin, Vancomycin, Piperacillin, Cefazolin. Twenty six antimicrobial agents, including the mentioned antibiotics, were applied against all the isolates by vietik kits technique, minimum inhibition concentration (MIC) was determined. The test was applied to find the multidrug resistance pattern that was usually associated with resistance of these bacteria the results in table 4 shown that *Staph. aureus* was 100% resistant to Ampicillin and 80%, 60% and 25% were resist to Erythromycin, Vancomycin and Tetracycline respectively. *Salmonella* typhimurium, on the other hand was sensitive to Amoxicillin, but 20% of this bacteria resists to, Erythromycin, and 12% to Ampicillin while 15% resist to Vancomycin. Also all *E. coli* was resistance to Erythromycin and 90, 85 and 74% of the isolated bacteria resist to Piperacillin, Vancomycin, and Amoxicillin respectively but 20% was resist to Ampicilin. as well as all *Bacillus cereus* isolates was resistance to, Erythromycin, and 75% to Ampicillin, and 70% to Amoxicillin, but 60% of the isolates resists to Cefazolin antibiotics.

Studies demonstrated that the increase in the rate of Penicillin group resistance in all Staphylococci isolates especially in the Methicillin resistance strains such as studies by (Mario *et al.,* 2016; Oliva *et al.*, 2013; Karanika *et al.,* 2015), they showed that Penicillin resistance rates in the *Staph. aureus* were 97.5%, 87.8% and 100% correspondingly. One of the reasons that may explain the increasing of Penicillin group resistance among *Staph. aureus* isolates in most cases is the production of β-lactamase

enzyme that destroyed the β-lactam ring making this antibiotic inactivated.

There were reports of resistance of *E. coli* to antibiotics associated with treatment failure (Talan *et al,* 2004). Included in the list of affected antimicrobials are penicillin, cephalosporin, sulpha drugs, and fluoroquinolones (Goettsch *et al,* 2000). Fluoroquinolone resistant *E. coli* strains often show resistance to other drugs such as ampicilin, tetracycline, chloramphenicol, trimethoprin, sulphamethoxazole and Gentamycin, and there has been a significant increase in fluoroquinolones resistant *E. coli* in many countries over the last few decades (Viroy *et al.,* 2005).

Table 4 : MIC of antibiotics and percentage resistance isolated bacteria

Type of bacteria	Antibiotics	MIC (µg/ml.)	% of bacteria resistance to antibiotics
Staph. aureus	Ampicillin	0.25	100
	Tetracycline	8	25
	Erythromycin	0.5	80
	Vancomycin	1	40
E. coli	Amoxicillin	2	74
	Ampicillin	16	20
	Erythromycin	0.5	80
	Vancomycin	1	70
	Piperacillin	0.12	55
Bacillus cereus	Amoxicillin	8	88
	Ampicillin	16	60
	Erythromycin	0.5	95
	Cefazolin	16	80
Sal. typhimurium	Amoxicillin	8	30
	Ampicillin	8	35
	Erythromycin	1	40
	Vancomycin	4	20

The characters of synthesis of silver nanoparticles

The study on biosynthesis of silver nanoparticles by using natural plants extract such as *C. sinensis* was done and described in this effort. The aqueous silver ions were reduced to silver nanoparticles when added to peel extract of *C. sinensis*. It was detected that the color of the solution turned from yellow to bright yellow and then to dark brown after 1, 24 and 48 h of the reaction, which indicated the formation of silver nanoparticles. The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The UV–vis spectra showed maximum absorbance at 420 nm, which increased with time of incubation of silver nitrate with the plants extract (Figure 2). The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were seen at 420 nm equivalents to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag+ ions took place extracellularly. It is reported earlier that absorbance at around 430 nm for silver is a characteristic of these nobel metal particles (Nestor *et al,* 2008). In order to verify the results of the UV–vis spectral analysis, the samples of the silver ions exposed to the extracts of natural plants were examined by XRD. Figure 3 shows the XRD pattern for silver nanoparticles synthesized using natural plants extract. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using Scherrer's equation (Balaji *et al.,* 2009). For peel extract synthesized silver nanoparticles the calculated average particle size of the silver was found to be 65 nm. The silver nanoparticles were characterized by Atomic Force Microscopy (AFM) for its detail size and morphology of silver. The topographical images of irregular silver nanoparticles synthesized were shown in Figure 4. The particle size of the silver nanoparticles was found to be 65 nm, Fig 4 shows the scanning electron micrograph of *C. sinensis* treated with 1 mM silver nitrate solution for 24 h. SEM determination of the brown color stable samples showed the formation of silver nanoparticles and well dispersed nanoparticles could be seen in the samples treated with silver nitrate. Many researchers have reported the biosynthesis of nanoparticles with plants extract for biosynthesis reaction. Synthesis of spherical silver nanoparticles using purified compound, extracted from henna leaf at ambient conditions (Kasthuri *et al.,* 2009). Using green tea, *C. sinensis* extract as reducing and stabilizing agents gold nanoparticles and silver nanostructures could be produced in aqueous solution at ambient conditions (Torresdey *et al.,* 2003). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis (Retchkiman-Schabes *et al.,* 2006; Shankar *et al.*, 2003b; Shankar *et al.*, 2005).

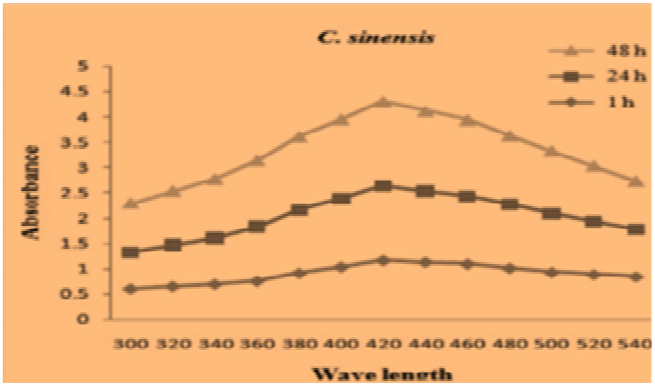

Fig. 2 : UV–vis spectra of silver nanoparticles synthesized using *Citrus sinensis* peel extracts.

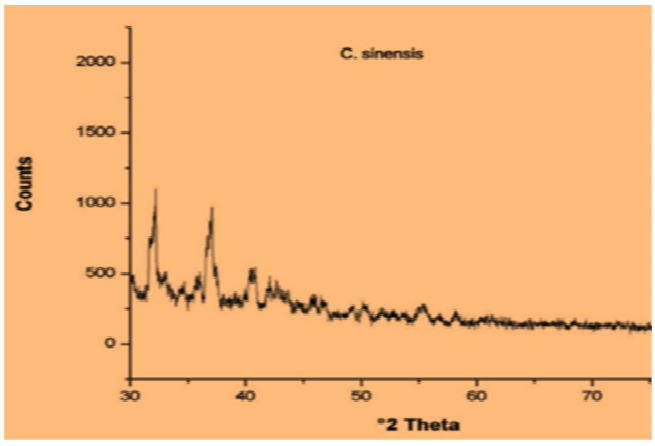

Fig. 3 : X-ray diffraction pattern of the silver nanoparticles were synthesized from *Citrus sinensis* peel extracts.

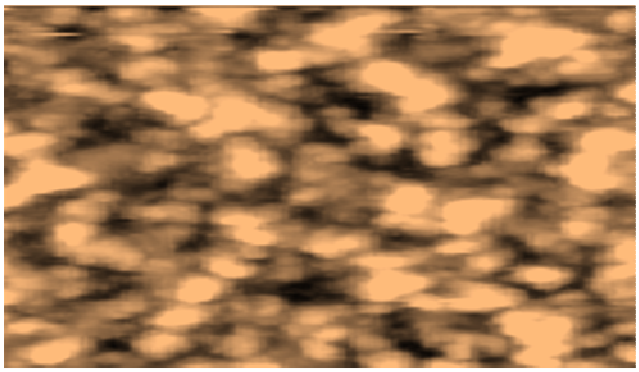

Fig. 4 : AFM images of the silver nanoparticles synthesized by *Citrus sinensis* peel extracts.

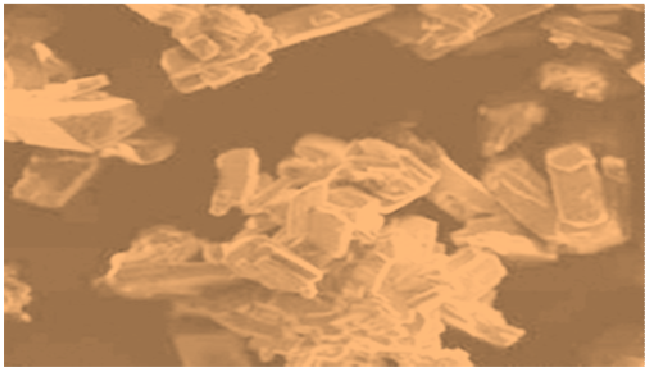

Fig. 5 : SEM images of the silver nanoparticles synthesized by *Citrus sinensis* peel extracts.

Antibacterial activity of synthesized Sliver Nanoparticles

The antimicrobial activity of silver nanoparticles synthesized by peel extract was examined against various isolated food borne pathogens such as *S. aureus*, *E. coli* and *Salmonella typhimurium* and *Bacillus cereus* using well diffusion method. The measurement of inhibition zones diameter (mm) around the well with silver nanoparticles solution was appeared in Table 5. The AgNps synthesized using peel of *C. sinensis* extracts reduction with 65nm in size have inhibitory effect against isolated pathogenic bacteria by using well diffusion method, as follow*:* the inhibitory effect of Ag Nps with different concentration; 25,50, 75 and 100 µl. were increased according to increasing the concentration of the silver nonoparticales as shown in table 5 ; the inhibition zone against bacteria *Staph aureus* was 12,19,25 and 28 mm respectively whereas the inhibition zone of *Bacillus cereus* measured as; 14, 20, 23 and 25mm for the similar concentration (25,50,75 and 100µl), and the larger inhibition zone measured 30mm and 21mm for the concentration 100 µl and 50µl against each *E. coli* and *Sal. typhimurium* isolates respectively. As well as the inhibition zone recorded 21 mm when 50 µl concentrations of the Ag Nps synthesized was used against each *E. coli* and *Sal. typhimurium* as well as; 28mm and 25mm recorded against the same bacteria when the concentration75µl were examined.

Table 5 : The antibacterial activity of synthesized nano silver against tested bacteria

AgNps con.(µl)	*Staph. aureus*	*E. coli*	*B. cereus*	*Sal. typhimurium*
25	12	15	14	17
50	19	21	20	21
75	25	28	23	25
100	28	30	25	30

The silver nanoparticles showed effective antibacterial property due to their extremely large surface area, which delivers improved contact with bacteria. The nanoparticles get attached to the cell membrane and entered inside the bacteria. The silver nanoparticles interacted with sulfide proteins in cell membrane structure as well as interact with these proteins in the cell and with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria corporations thus, protecting the DNA from the silver ions. The nanoparticles first attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Sondi *et al.,* 2004; Morones *et al.,* 2005).

The silver nanoparticles have been produced by *C. sinensis* peel extracts, which is an inexpensive, effectual and ecological friendly process. UV–vis spectrophotometer, XRD, AFM and SEM techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The zones of inhibition were formed in the antimicrobial showing test specified, that the Ag NPs made in this process has the efficient antimicrobial activity against pathogenic bacteria. The biologically synthesized silver nanoparticles could be of huge use in medical field for their effective antimicrobial function.

References

Adeyanju, G.T. and Ishola, O. (2014) *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springer Plus 3: 139.

Adwan, G.M.; Alqarem, B.R. and Adwan, K.M. (2015). Prevalence of foodborne pathogens in meat samples in Palestine. International Food Research Journal, 22(5): 1806-1812

Ahmed, S.; Ahmad, M.; Swami, B.L. and Ikram, S. (2015). Plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of Advance Research. http://dx.doi.org/10.1016/j.jare.02.007.

Anihouvi, D.; KAyodé, G.H.; Anihouvi, A.P.P.; Azokpota, V.B.; Kotchoni, S.O. and Hounhouigan, D.J. (2013). Microbial contamination associated with the processing of tchachanga, a roasted meat product. African Journal of Biotechnology 12(18): 2449-2455.

Awadalla, H.; Khalil, I.; Bassim, H.; Ahmed, M. and Wahba, L. (2010). Molecular typing of methicilin-resistant *Staphylococcus aureus* isolates at Ain Shams University Hospital, Egypt. Afr J Microbiol Res. 4: 1639-1646.

Balaji, D.S.; Basavaraja, S.; Deshpande, R.; Bedre, M.D.; Prabhakar, B.K. and Venkataraman, A. (2009). Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf. B Biointerf. 68: 88–92.

Chen, X. and Schluesener, H.J. (2008). Nano-silver: A nanoproduct in medical application. Toxicol. Lett. 176: 112.

Dontorou, C.; Papadopoulou, C. and Filioussis, G. (2003). Isolation of Escherichia coli O157:H7 from foods in Greece. Int J Food Microbiol; 82: 273-9.

Fangio, M.F.; Roura, S.I. and Fritz, R. (2010). Isolation and identification of *Bacillus* spp. and related genera from different starchy foods. J. Food Sci., 75: 218-21.

Goettsch, W.; van Pelt, W.; Nagelkerke, N.; Hendrix, M.G.; Buiting, A.G.; Petit, P.L.; Sabbe, L.J.; van Griethuysen, A.J. and de Neeling, A.J. (2000). Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the netherlands. J Antimicrob Chemother. 46(2): 223-8.

Iyer, A.; Kumosani, T.; Yaghmoor, S.; Barbour, E.; Azhar, E. and Harakeh, S. (2013). *Escherichia coli* and *Salmonella* spp. in meat in Jeddah, Saudi Arabia. Journal of Infection in Developing Countries 7(11): 812-818.

Karanika, S.; Zervou, F.N.; Zacharioudakis, I.M.; Paudel, S. and Mylonakis, E. (2015). Risk factors for meticillin-resistant *Staphylococcus aureus* colonization in dialysis patients: a meta-analysis. J Hosp Infect. 91(3): 257-63.

Kasthuri, J.; Veerapandian, S. and Rajendiran, N. (2009) Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids Surf. B: Biointerf. 68: 55–60.

Kiranmayi, Ch.; Bindu, N.; Krishnaiah, and Naga Mallika, E. (2010) *Escherichia coli* O157:H7 - An Emerging Pathogen in foods of Animal Origin. Veterinary World. 3(8): 382-389

Lei, I.F.; Roffey, P.; Blanchard, C. and Gu, K. (2008). Development of a multiplex PCR method for the detection of six common food borne pathogens, J. Food and Drug Analysis 16: 37-43.

Levinson, W. and Jawetz, E. (2000). Medical Microbiology and Immunology. 6th Edition, McGraw-Hill, New York, p. 123

Li, W.R.; Xie, X.B.; Shi, Q.S.; Zeng, H.Y.; Ou-Yang, Y.S. and Chen, Y.B. (2010). Antimicrobial activity and mechanism of silver nanoparticles on *Escherichia coli.* Appl Microbial. Biotechnol, 85: 1115-1122.

Magda, H.; Rady, B.A.; Merdan Eman Essa, M.S.; Salama, S. and Sayes, E.I. (2014) The First Record of *Escherichia coli* O157: H7 Isolated from Wastes and Associated Houseflies in Egypt. World Applied Sciences Journal 31(8): 1437-1447.

Mahon, J.; Murphy, C.K.; Jones, P.W. and Barrow, P.A. (1994). Comparison of multiplex PCR and standard bacteriological methods of detecting *Salmonella* on chicken skin. Lett. Appl. Microbiol. 10: 255–262.

Mario, M.C.; Simon, H.; Julia, M.; Matthias, M.; Jan, F.; Sven, H. and Volker, B. (2016). Antibiotic Resistance of Commensal *Staphylococcus aureus* and Coagulase-Negative Staphylococci in an International Cohort of Surgeons: A Prospective Point-Prevalence Study, PLoS One, 11(2): PMC4739597.

Morones, J.R.; Elechiguerra, J.L.; Camacho, A.; Holt, K.; Kouri, J.B.; Ramfrez, J.T. and Yacaman, M.J. (2005). The bactericidal effect of silver nanoparticles. Nanotechnology 16: 2346–2353.

Nestor, A.R.; Mendieta, V.S.; Lopez, M.A.; Espinosa, R.M. and Alatorre, J.A. (2008). Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camiellia sinensis* extract. Mater. Lett. 62: 3103–3105.

Oliva, A.; Lichtner, M.; Mascellino, M.T.; Iannetta, M.; Ialungo, A.M.; Tadadjeu, M.S.; Pavone, P.; Mengoni, F.; Mastroianni, C.M. and Vullo, V. (2013). Study of methicillin-resistant Staphylococcus aureus (MRSA) carriage in a population of HIV-negative migrants and HIV-infected patients attending an outpatient clinic in Rome. Ann Ig. 25(2): 99-107.

Omar, B.A.; Atif, H.A.; Ibrahim, H.A. and Hegazy, A.I. (2014). Detection of Salmonella in Food Samples by Culture and Polymerase Chain Reaction Methods. J Bacteriol Parasitol, 5: 3.

Rai, M. and Yadav, A. (2009). Silver nanoparticles as a new generation of antimicrobial, Biotechnology Advances, 27: 76-83.

Retchkiman-Schabes, P.S.; Canizal, G.; Becerra-Herrera, R.; Zorrilla, C.; Liu, H.B. and Ascencio, J.A. (2006). Biosynthesis and characterization of Ti/Ni bimetallic nanoparticles. Opt. Mater. 29: 95–99.

Shankar, S.S.; Ahmad, A. and Sastry, M. (2003b). Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog. 19: 1627– 1631.

Shankar, S.S.; Rai, A.; Ahmad, A. and Sastry, M. (2005). Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings. Chem. Mater. 17: 566–572.

Sharma, V.K.; Yngard, R.A. and Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci., 145: 83-96

Sondi, I. and Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloids Interface Sci. 275: 177–182.

Srivastava, R.K.; Ichhpujani, R.L.; Shashi, K. and Harit, A.K. (2009). Food-Borne Diseases: CD Alert, 13(4).

Swanenburg, M.H.; Urlings, J.M.; Snijders, D.A. and Keuzenkamp Van Knapen, F. (2001). *Salmonella* in slaughter pigs: Prevalence serotypes and critical control points slaughter in two slaughterhouses. Int. J. Food Microbiol., 70: 243–254.

Talan, D.A.; Naber, K.G.; Palou, J. and Elkharrat, D. (2004). Extended-release ciprofloxacin (Cipro XR) for treatment of urinary tract infections. Int J Antimicrob Agents., 23(1): S54-66.

Tom, R.T.; Suryanarayanan, V.; Ganapati, R.P.; Baskaran, S. and Pradeep, T. (2004). Ciprofloxacin-Protected Gold nanoparticles. Langmuir, 20: 1909-1914.

Torresdey, J.L.; Gomez, E.; Videa, J.R.; Parsons, J.G.; Troiani, H. and Yacaman, M.J. (2003). Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir 19: 1357–1361.

Viroy, M.; Lunkin, D.; Maslow, J.N.; Stieritz, D.D.; Carson, L.S. and Bilker, W.B. (2005). Longitudinal trends in antimicrobial susceptibilities across long term care facilities emergence of fluoroquinolone resistance. Infect Control Hosp Epidemiol. 26: 56–62.