Synthesis of Silver Nanoparticles Using Low-Grade Date Syrup for Production of Active Edible Films

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ABSTRACT

Nowadays more researchers are giving more attention toward eco-friendly biosynthesis of green silver nanoparticles. In this study, the possible role of bioactive compounds date syrup extract in reducing silver nitrate into silver nanoparticles is highlighted. These biosynthesized nanoparticles were characterized with the help of UV-visible spectrophotometer, atomic absorption spectroscopy and FTIR spectroscopy. It was observed that date extract can reduce silver ions into silver nanoparticles within 26 min. of reaction time. The intense peaks centered near 420 nm by UV-analysis, affirmed the reduction of Ag$^+$ to Ag$^0$. ASS analysis showed decrease in concentration of Ag$^+$ ions from 5.6 ppm to 0.04 ppm with 26 min. FTIR measurements confirms the presence of CH$_3$ and CH$_2$ stretching, C = O carbonyl group, C - S stretch and OH stretching in the analysis which exist in bioactive compounds of date syrup extract, such as phenols, all act as reducing and stabilizing agents for silver nanoparticles and protect it from further changes. Antibacterial activity of these synthesized nanoparticles was studied against gram positive and negative pathogenic bacteria. Additionally, an edible films of pectin reinforced with AgNPs were prepared to evaluate its antibacterial efficiency to be used as food packaging materials. The findings suggest that antibacterial activity of biosynthesized AgNPs from date fruit shows a promising zone of inhibition pathogenic bacteria. Also, results exhibited clear bacteriostatic activity of pactin/AgNPs blend films against all microbes tested after 5 weeks of storage a meat patty samples at 4°C and its decreasing diminution reached to about eight-fold the decreasing of control sample.

Keywords: Silver nanoparticles, Date extract, Biosynthesis, Antibacterial Activity.

1. Introduction

*Phoenix dactylifera* is the scientific name of date tree, a member of *Palmaceae* family. Date fruits are rich in antioxidants in peels and / or in fruits including: phenols, tannins, flavones, alkaloids, glycosides, saponins, resins and vitamins such as A, C, H, B$_1$, B$_2$, B$_3$ and B$_6$. Many bioactive compounds were found in date syrup such as Camphene acid, Veriolic acid, Dactileviric acid, Chlorogenic acid, Trans Cinamic acid, Comaric acid, Caphiol, Campherol, Myristin, Cirastin, Chatshi, 1, 3-beta – diglocan, Epiganic and Liotolwin. (Reem et al., 2017)

One hundred grams of dried date syrup contains: 277 calories, 75 gm carbohydrates, 7.0 gm fibers, 2.0 gm proteins, 1.74 – 2.67 mg simple polyphenols, 85 – 100 mg soluble tannis, 12.6 – 39.2 mg insoluble tannis, 80 – 100 IU vitamin A, 0.07 mg B$_1$, 0.03 mg B$_2$, 2.2 – 3.0 mg B$_3$ (Niacin), 0.8 – 3.0 mg vitamin C, and 2 – 3 mg Caviol. (Keiper, 2020)

Also date fruits contains some necessary minerals which act as antioxidants such as Selenium. Selenium have been recognized to have antioxidant potential and has been described to exceed antioxidant compounds to animal protein. (One gm of dried date syrup contains 1.47 – 2.97 microgram selenium). These minerals can save the daily human consumption need by 20% of potassium, 14% of magnesium, 18% of cupper, 15% of manganise and 5% of iron (Sahoo et al., 2019).

In the past, antioxidant agents from plant was established an amazing consideration due to their talent to protect food stuffs and avoid rancidity caused by oxidation (Fatma et al., 2018).
Recently, researchers are now focusing on use of plants for green synthesis of nanoparticles 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 environmental friendly methods (Jeyashree and Revathi, 2017).

It is believed that metal nanoparticles kill bacteria by one of the following mechanisms: (i) interference with vital cellular processes 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 (Ducan, 2011).

Specific methodologies have been used to synthesize noble metal nanoparticles of particular size and shape, this allows nanomaterials to be able to attach more copies of biological molecules, which confers greater efficiency due to materials in the nanoscale have a higher surface – to – volume ratio when compared with their microscale counterparts (Neethirajan and Jayas, 2011).

Silver nanoparticles can be synthesized using various approaches including chemical, physical and biological routs. Although chemical methods of synthesis requires short period of time for synthesis of large quantity of nanoparticles, still this method has a disadvantage that is also requires capping agents for size stabilization of the nanoparticles (Supraja et al., 2013). Thus there is an increasing demand for green nanothechnology for synthesis of stable nanoparticles.

Synthesis of nanoparticle by biological method is through microbes like Aspergillus flavus (Vigneshwaran et al., 2007), Pseudomonas spp. (Silambarasan and Jayanthi, 2013) and plant sources such as chenopodium album (Dwivedi and Gopal, 2010), Acalypha indica (Krishnaraj et al., 2010), Cynodon dactyion (Supraja et al., 2013), Glycyrrhiza glabra (Dinesh et al., 2012), Nigella sativa etc. Although, microorganisms have already been reported as efficient bio-reducing agent for metal nanoparticles, but it increases the issues related to elaborate microbial culture handling used in microbe mediated biosynthesis of metal nanoparticles (Bukar et al., 2018).

Therefore, plants and spices provide a better platform for nanoparticles synthesis, which plant phytochemical with antioxidant of reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles (Balaprasad et al., 2005).

Today, manufacturers add silver nanoparticles to hundreds of consumer products, including food storage containers, clothing, computer key boards, cosmetics, pillows, cellphones and medical appliances. The properties of these consumables can be further improvised by using silver nanoparticles synthesized via the ecofriendly green routes.

Active packaging is an innovative concept in which the package, the product, and the environment interact to prolong the shelf-life, enhance safety, or improve sensory properties, while maintaining the stability and quality of the product (Khalil et al., 2013; Anngkana et al., 2016).

This concept, which is of special importance in the area of fresh and extended shelf life foods, has benefited recently by the use of nanotechnology materials including nanocoatings and nanoparticles (Grazlela et al., 2017).

Among different plants, the seeds of some spices had shown to exhibit various medicinal properties such as antioxidant, antibacterial and anticancer activity (Bourgou et al., 2012). But till date, up to our knowledge, there is no report on synthesis of silver nanoparticles from the syrum of rich antioxidant fruits. Hence, the present study was deliberately aimed with a simple and an effective approach of synthesizing silver nanoparticles using the syrup of date fruit as a reducing agent and to treat against spoilage and diseases causing bacteria.

The present study focused on (i) The use of low grade date syrup as a template for silver nanoparticles synthesis and to exploit their medicinal importance in terms of antimicrobial activity. (ii) To prepare pectin / AgNPs blend films and to characterize their properties for their potential use as food packaging application.

2. Materials and Methods

2.1. Materials

All of reagent and ultrapure water used throughout the experiments were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Date fruits were purchased from a local market (Giza, Egypt).
2.1.1. Bacterial Strains

Three bacterial strains used in the study included *E. Coli* 0157:H7 (ATCC 43895), *S. aureus* (ATCC 11988) and *B. subtilis* (ATCC 64540) were obtained by food Sci. Dep. Agric. Collage, Mansoura Univ., Egypt.

2.2. Methods

2.2.1. Preparation of date syrup

The syrup used for the synthesis of silver nanoparticles was prepared by taking 750 gm of thoroughly washed destoned fresh low grade date, and extract the syrup by soaking at a suitable time in hot water at 65°C. The suspension was homogenized by blending for 5 min. at high speed, and the homogenized suspension was centrifuged (to separate the date extract from the fibers and insoluble matters), the supernatant fraction was filtrated through whatman No.1 and evaporated by vacuum evaporator (45°C) and made up to a concentration of 70° Brix and stored at 4°C for further use in plastic bag.

2.2.2. Preparation of AgNO₃ solutions

A weight of 3.34 mg of AgNO₃ salt was suspended in 30 ml of distilled water, to obtain 1 mM of AgNO₃, and then three another aqueous AgNO₃ solutions with different standard concentration (2 mM, 3 mM, and 4 mM) were prepared.

2.2.3. Optimization and synthesis of silver nanoparticles

Each 10 ml of the prepared date extract was taken in separate conical flask. 50 ml of each above AgNO₃ solutions was added to the respective flasks drop by drop with continuous stirring for bio-reduction process of the Ag⁺ ions at room temperature under dark conditions. The conical flasks were sealed using cotton plugs and observed for color change. The color of the reaction mixture changes to dark brown color indicating the reaction of Ag⁺ ions to AgNPs. Finally, the solution mixture was centrifuged at 5000 rpm for 20 min. and the resulting substrate was collected and the pellet formed was dissolved in 0.1 ml of toluene water and oven dried at 65°C for 20 min. (to avoid clumping of particles (Prathna et al., 2011).

2.2.4. Characterization of silver nanoparticles-UV-Vis spectroscopy:

The formation and the stability of metal nanoparticle in aqueous solution are monitored by UV-Vis Spectroscopy. The spectrum was taken for the reaction mixture immediately when it is prepared. The bio-reduction of precursor silver ions was detected and confirmed by sampling of aliquots (1 ml) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer (ELICO U.V. 165) at room temperature operated at a resolution of 1 nm between 250 nm and 800 nm.

2.2.5. Characterization of silver nanoparticles – Atomic absorption spectroscopy

AAS was used to analyze the varying concentration of Ag⁺ ions in the solution over a period of time. The conversion of Ag⁺ to Ag⁰ can be inferred with this measurement. During the course of the reaction at regular intervals, the aliquots of samples were withdrawn and centrifuged at 15000 rpm. The supernatant solution was then analyzed by AAS (GBC 932 AA) to detect the amount of Ag⁺ ions. The rate of decrease in the concentration of the Ag⁺ ions depicts the conversion of Ag⁺ to Ag⁰.

2.2.6. Characterization of silver nanoparticles – FTIR analysis

Fourier transform infrared spectroscopy (FTIR) measurement was carried out to recognize the interaction between the Ag nanoparticles and the capping agent, from the date extract. For this, the biosynthesized Ag nanoparticle solution was centrifuged at 14000 rpm for 15 min in order to remove any free biomass residue or compound that is not the capping ligand in the nanoparticles. The Ag nanoparticle pellet obtained after centrifugation was re-dispersed in water and washed three times with 20 ml of the same water. The samples were dried and grinded and analyzed on a Nicolet IR 200 (Thermo Electron Corp.) model, at a resolution of 2 cm⁻¹. FTIR is taken for the date extract and also for the silver nanoparticles prepared from date extract, to identify the functional groups present in the date extract that are responsible for reduction of silver nanoparticles.
2.2.7. Antimicrobial activity of silver nanoparticles by disc diffusion method
The prepared nutrient-agar was poured onto sterile petri plates and 17 h growing cultures of gram positive *B. subtilis* & *S. aureus* and gram negative *E. coli* were swabbed onto the agar plates. Meanwhile, the sterile discs were impregnated with different concentrations of silver nanoparticles (50, 100 and 150 µg/ml aqueous solution) previously prepared and positive control drug (antibiotic Ciprofloxacin) were placed inverted on the swabbed plat. Empty sterile disc was kept as negative control. The plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (DIZ) of the tested bacteria (mm). All tests were performed in triplicates (Cruickshank, 1968).

2.2.8. Antimicrobial activity of prepared edible film reinforced with silver nanoparticles

2.2.8.1. Edible film preparation
5 gm of high methoxy pectin were mixed with distilled water (100 ml), polyvinyl alcohol (1.25% w/v) and 2.5% glycerol (w/v) at room temperature (25°C) for 5 min., then the suspension was transferred to a water bath at 90°C for 30 min., and agitated by magnetic stirrer (500 rpm), following by cooling at 40°C. Aqueous solution of different concentration from silver nanoparticles (pellets) previously prepared (50, 100 and 150 µg/ml aqueous solution) were added. After the components of the film had been mixed, the films were cast by pipetting 10 ml of the solution into sterile petri plates (VWR) with inner diameters of 10 cm and allowed to dry overnight (18 h) in a laminar air flow cabinet under a flow of sterile air at 25°C. A control film sample was prepared by the same procedure without adding of silver nanoparticles solution.

2.2.8.2. Antimicrobial activity of film samples
Antimicrobial activity of the films were examined for their inhibitory effect on the growth of *E. coli* 0157:H7. Test microorganisms was inoculated in 20 ml of nutrient agar (NA) media and subsequently incubated at 37°C for 16h. The cultured broth was centrifuged at 2000 rpm for 10 min., and the cell pellets were suspended in 100 ml of sterile NA and diluted 10 times with sterile distilled water. 20 ml of diluted broth (10^6 – 10^7 CFU/ml) was taken into conical flask containing 100 mg of film sample and subsequently incubated at 37°C for 16h under mild shaking. The cell viability was inoculated in plates of NA. The microorganisms was calculated by counting bacteria colonies on the plates at 0, 4, 8, 12, 16 and 20 h. Antimicrobial tests were performed in triplicate with individually prepared films (CLSI, 2012).

2.2.8.3. Patty preparation and microbiological analysis
A 20 gm of homogeneous ground beef meat patty samples were prepared. The patties were separated into five groups; uncoated, coated with 50 µg/ml silver nanoparticles film, coated with 100 µg/ml silver nanoparticles film, coated with 150 µg/ml silver nanoparticles film and coated with pure pectin based materials (control film). The beef meat patties were coated by dipping them into the prepared coating solutions for 5 second at room temperature, then drying for 30 second. This dipping procedure was repeated three times, then the patties were dried for 2 h in a laminar flow hood at 25°C and kept in refrigerator for five weeks.

After the end of storage, each packaged sample from each treatment were aseptically opened and a 10-g-portion from the center of the patties was homogenized in sterile maximum recovery diluent (Merck) in a Seward stomacher for 1 min. to make the initial dilution. Appropriate serial dilutions (10^1 – 10^6) were spread plated on Plate Count Agar for total viable counts (TVC), Hi Crome agar medium for *E. coli* 0157:H7, Baird Parker agar + egg yolk tellurite emulsion for *S. aureus*, Tryptose Sulfite Cycloserine (T.S.C) agar base for cl. Perfringens and selective agar base medium (PEMBA) for *B. cereus* (Downes and Ito, 2000).

3. Results and Discussion

3.1. UV-visible spectroscopy analysis
Solutions with different concentrations of AgNO₃ (1mM, to 4 mM) were prepared and 10 ml of date extract added to each solution. As shown in Fig (1), the absorption peaks obtained for these samples are in the range of 420 – 435 nm, which is prescribed for Ag nanoparticles. Hence, the results obtained
ensure the existence of Ag nanoparticles in the solutions. Since the surface plasmon resonance SPR peak centered near 410 nm. A similar pattern was observed by Zarchi, et al., (2011), using an Andrachnea chordifolia ethanol extract as reducing agent.

Fig. 1: UV spectrum of Ag nanoparticles obtained by 1-4 mM AgNO₃ and date syrup extract

UV-Vis absorbance of date extract also showed small absorbance about 315 nm indicating the presence of phenols in the extract. The absorption peaks disappeared during the reaction which indicates the role and involvement of phenols in the reaction (Fig. 2).

The absorption peak is found to be narrow and single peaked shows that the shape of the silver nanoparticles reduced by date extract is found to be spherical and the particles are monodispersed.

Date extract changed the color of AgNO₃ solution gradually from transparent to dark brown due to the reduction of Ag⁺ to Ag⁰ within 26 min. of commencement of the reaction which further remained constant. Thus, silver ions reduction was done by date extract within a short period of time and the stability of biosynthesis silver nanoparticles was improved when the reduction was completely done.

Fig. 2: UV-visible spectra of aqueous solution of 1 mM AgNO₃ with the date extract after 12 min. of commencement of the reaction.
3.2. Atomic absorption spectroscopy analysis (AAS)

AAS analysis for the reacting solution was done at regular intervals to analyze the varying concentration of Ag\(^+\) ions in the solution over a period of time (24 min). The conversion of Ag\(^+\) to Ag\(^0\) can be inferred with this measurement. A standard solution of 6.0 ppm of AgNO\(_3\) was prepared and analyzed with AAS at 0 min. Ag\(^+\) ions concentration in the reaction solution after adding date extract was monitored at regular time intervals (zero, 4, 8, 12, 16, 20 and 24 min.). The results showed a decrease in concentration of Ag\(^+\) ions; 5.6, 4.5, 2.6, 2.4, 1.5, 0.8 and 0.04 ppm respectively, indicating the conversion of Ag\(^+\) to Ag\(^0\) and its rate of Ag\(^+\) decreasing in the solution (Fig. 3).

![Fig. 3: Atomic absorption spectroscopy graph of Ag\(^+\) concentration in reaction mixture.](image)

3.3. FTIR analysis

Results of FTIR study showed transmittance peaks located at 3315 cm\(^{-1}\), 2360 cm\(^{-1}\), 1636 cm\(^{-1}\) and 608 cm\(^{-1}\) (Fig. 4). The major peak was assigned at 3315 cm\(^{-1}\) which indicates OH stretching in alcohols and phenols. The alcohols present in the date extract are caphiol and campherol. The linkage at 2360 cm\(^{-1}\) confirms the presence of the CH\(_3\) and CH\(_2\) stretching and the peak at 1636 cm\(^{-1}\) confirms the carbonyl stretch C = O (Priti et al., 2013). The peaks formed at the 608 cm\(^{-1}\) confirms the C – S stretch in the analysis (Jeyashree and Revathi, 2017).

![Fig. 4: FTIR spectrum of silver nanoparticle from date extract](image)
Phenolic compounds belonging to the lignans group have been earlier reported to be capable of chelating with metallic elements to form complexes (Sharma et al., 2014). Thus, it can be concluded that hydroxyl and carboxyl groups present in phenolic compounds of the date extract may inactivate silver ions by chelating and additionally suppressing the superoxide driven reaction, which is believed to be the most important source of reactive oxygen species (Jayashree & Revathi, 2017). These results confirm the presence of polyphenols and flavones which may act as reducing and stabilizing agents for silver nanoparticles.

3.4. Antimicrobial activity analysis:

Antimicrobial activity of biosynthesized silver nanoparticles was analyzed against gram negative \textit{E. coli} and against gram positive \textit{S. aureus} and \textit{B. subtilis}. The inhibition tests were carried out by disc diffusion method. Growth inhibition of bacteria by Ag nanoparticles was compared with standard antibiotic Ciprofloxacin. Inhibition zone diameter (mm) of the different synthesized silver nanoparticles concentrations over the microorganisms is shown in Table (1).

Data shows the inhibition zones diameters for three concentrations from Ag nanoparticles. Increase concentrations gave increase in bacteria inhibition. The gram positive bacteria were most affected compared with gram negative. Inhibition zones of \textit{E. coli} 9 mm at concentration 150 ppm, while it was 9 mm at concentration 50 ppm for \textit{b. subtilis}. At 150 ppm, the inhibition zones for all bacteria were 9 to 12 at concentration 150 ppm. Thus the silver nanoparticles of date extract significantly inhibits the pathogens and were competent enough with the standard antibiotic indicating stronger antimicrobial activity of biosynthesized silver nanoparticles which may be due to their increased area.

| Bacteria      | Zones of inhibition for AgNPs concentration (mm) |
|---------------|-----------------------------------------------|
|               | 50 ppm | 100 ppm | 150 ppm |
| \textit{E. coli} | 0      | 0       | 9       |
| \textit{S. Aureus} | 12     | 16      | 21      |
| \textit{B. Subtilis} | 9      | 14      | 18      |
| Ciprofloxacin (Antibiotic) | 14     | 19      | 23      |

Therefore, these silver nanoparticles can be used in low doses of antimicrobial treatment in comparison to standard antimicrobial agents. Also, because of the biological reducing and capping agents, these silver nanoparticles are also environment friendly and non-toxic in comparison to chemically synthesized silver nanoparticles.

3.5. Effect of edible films with different concentration of S. nanoparticles on the growth of different microbial flora

Results in Table (2) explained the effect of three pectin films incorporated with S. nanoparticles (50, 100 and 150 µg/ml aqueous solution) after 5 weeks of storage the meat patty samples in refrigerator (4°C).

It has been found that the numbers of aerobic colonies was decreased with all biolife film tested. The highest decrease in colonies was appeared with 150 µg/ml S. nanoparticles film and the lowest decrease were found in 50 µg/ml S. nanoparticles film. This diminution represent eight-fold the decreasing of the control sample (Table 2). Similarly, all the tested biolife films were able to decrease the number of \textit{S. aureus}. All three films specimens exhibited no any colony count has been found for \textit{E. coli} 0157:H7, \textit{Cl. perfringens} and \textit{B. cereus} after 5 weeks of storage the samples at 4°C.

It has been concluded that 150 µg/ml film followed by 100 µg/ml film were found to have the best results against all tested microorganisms.

The results were agreement with many studies demonstrated that green silver nanoparticles have a strong antimicrobial activity when incorporated into the packaging edible films.
Table 2: Effect of edible films incorporated with different concentration of AgNPs on the growth of different microbial flora when used to pack meat patties after 5 weeks storage at 4°C.

| Samples | Aerobic colony count | E. coli 0157:H7 CFU/g×10 | S. aureus CFU/g×10 | Cl. Perfringens CFU/g×10 | Bacillus Cereus CFU/g×10 |
|---------|----------------------|---------------------------|-------------------|--------------------------|--------------------------|
| F.C.S   | 114 × 10⁴             | 12                        | 18                | 6                        | n/d                      |
| C.S     | 252 × 10⁴             | 25                        | 91                | 15                       | n/d                      |
| Control | 125 × 10³             | 17                        | 14                | 6                        | n/d                      |
| A       | 57 × 10³              | -                         | 10                | n/d                      | n/d                      |
| B       | 34 × 10³              | -                         | 6                 | n/d                      | n/d                      |
| C       | 15 × 10³              | -                         | 2                 | n/d                      | n/d                      |

* F.C.S: Fresh sample at zone time without film.
* C.S: Uncoated sample after storage at 4°C for 5 weeks.
* Control: Zero ppm AgNPs film
* A: 50 ppm AgNPs film
* B: 100 ppm AgNPs film
* C: 150 ppm AgNPs film
* n/d: Not detected in 25 gm.
* –: Negative in 10 gm.

Conclusion

In conclusion, the bioreduction of silver ions using date extract as reducing agent has been illustrated. From the present study, it is clear that the silver nanoparticles synthesized by date extract can serve as a suitable reducing agent for biosynthesis of silver nanoparticles within 26 min. of reaction time. UV spectrum, AAS and FTIR results confirmed the formation of silver nanoparticles. The UV spectra peaks for silver nanoparticles range 420 to 435 nm. and were found to be mono-dispersed. From FTIR spectrum, they were found to be stable due to the presence of bioactive compounds such as polyphenols in the extract which act as capping agent and prevent the particle from aggregation. AAS results showed an obvious decrease of Ag⁺ ions and an increase in Ag⁰ in the solution. This study also showed that biosynthesized silver nanoparticles using date extract have potent antimicrobial activities against E. coli (gram negative) and S. aureus & B. subtilis cells (gram positive). Thus, this approach can be applied for rapid cost effective and ecofriendly green synthesis of silver nanoparticles for industrial field without the involvement of toxic chemicals.

Results revealed that there are a significant inhibits to the pathogens and were competent enough with the standard antibiotic.

Finally, the blend of pectin/AgNPs films are expected to be used for the edible film or coating of foods with antimicrobial activity have a potential to be used as food packaging materials for maintaining the safety and extending the shelf life of packaged food.

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