Efficacy of Silver Nanoparticles Synthesized from Pineapple Fruit Extracts Against Enterococcus Faecalis and Streptococcus Sanguis (An in Vitro Study)

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Abstract
Aims of the study: the study aimed to estimate the antibacterial efficacy of biosynthesizing Ag nanoparticles (Ag Nps) from pineapple extracts against some oral bacteria. Material and methods: The aqueous and ethanolic extracts were prepared from pomace and core tissues of pineapple fruit separately and the Ag Nps were synthesized by the addition of 5 mL of 1mMolar of an aqueous solution of silver nitrate waiting for color change from transparent to black, the UV Spectrophotometer, EDX, SEM, and FTIR were used to characterize Ag Nps, turbidity method was carried out for the antibacterial activity and the absorbance of 1/1,1/2 and 1/4 dilutions of the four extracts against oral bacteria [Enterococcus faecalis and Streptococcus sanguis] was measured at 530 nm by vis-spectrophotometer. Results: Ag Nps were synthesized and characterized, and all the prepared extracts and the nanoparticles had an antibacterial effect against Enterococcus faecalis and Streptococcus sanguis, the best antibacterial effects of Ag Nps biosynthesized from aqueous extract for both pineapple tissue pomace and core extract and ethanolic pomace extract (0.00) followed by 0.114 ethanolic core extract against Streptococcus sanguis, .022 ethanolic pomace extract against Streptococcus sanguis and 0.04 for ethanolic core extract against Enterococcus faecalis.Conclusion: The results showed the sensitivity of Enterococcus faecalis and Streptococcus sanguis to biologically synthesized nanoparticles made from the aqueous and ethanolic extracts, respectively, as well as the sensitivity of both types of bacteria to the aqueous extract of pineapple more than the ethanolic extract.

Keywords: Enterococcus faecalis , Streptococcus sanguis , Nanoparticles, Pineapple, Pomace , Core, Sliver nitrate.

1. Introduction

Nanotechnology is the use of the substance on an atomic, molecular and supramolecular scale for industrial purposes. A more general description of nanotechnology was given later by the National Nanotechnology Authority, which defined nanotechnology as the processing of matter with at least one dimension in size from 1 to 100 nanometers (1).

Metal nanoparticles have received much attention because of their optical and magnetic properties. Shape and size, mono dispersion and particle morphology are vital for tuning these properties (2). Silver nanoparticles are particles between 1 nm and 100 nm in size although described as “silver”, some of them consist of a large proportion of silver oxide due to the large proportion of silver atoms in the surface (3).

Currently, most applications of silver nanoparticles are in antibacterial (4) and antifungal (5), biotechnology (6), textile engineering (7), water treatment (8), and consumer products based on silver nanoparticles (9). As for the medical fields, silver nanoparticles have been used in the treatment and preventive effect of many chronic and incurable diseases, the most important of which are in the treatment of cancer, especially breast cancer, gland cancer, Alzheimer’s and tooth decay treatments and killing bacteria and other diseases (10). In dentistry, it is an active component of Howe's solution used to prevent and arrest caries. Silver nitrate solution with sodium fluoride varnish to stop caries has been used as a medical model for a caries management strategy. Although the treatment results in permanent black spots, this treatment protocol is simple, painless, non-invasive and low-cost. It is well accepted by many clinicians and patients, and thus appears to be a promising strategy for caries control, especially for young children, the elderly, and patients with high caries risk or those with special needs (11). Pineapple is a tropical fruit with a high taste for its unique aroma and sweet taste. Famous for being a flavorful fruit (12). Pineapple contains large amounts of biologically active compounds, dietary fiber, minerals and nutrients. Additionally, pineapple has been shown to have various health benefits including anti-inflammatory and antioxidant activity, monitoring of nervous system function, and healing of bowel movement (13). . It also contains a protein known as bromelain purified from the crude aqueous extract of the pineapple (bromelain family)

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Ananassa sativa (14) , as well as minerals such as calcium, chlorine, phosphorous and sodium (15). Bromelain acts as a defense protein that protects the pineapple throughout the growth, and ripening process (16), belongs to the class of proteases also known as proteins or peptides, a group of enzymes that catalyze proteolytic reactions in which the breakdown of proteins into multiple smaller peptides or single amino acids (17,18). More specifically, it is classified as a cysteine protein also known as (a thiol protein) because of the cysteine thiols in its active site (19).The mechanism underlying the antimicrobial activity of bromelain is thought that bromelain may impede bacterial growth by hydrolysis of certain peptide bonds in the bacterial cell wall (16). The cell wall is damaged, allowing the cell Leakage, swelling and opening (14). Bromelain also inhibits the growth of some bacteria by preventing bacteria from attaching to specific glycoprotein receptors on the surface (20) and exhibits antimicrobial activity against Gram-positive and Gram-negative bacteria, and is well tolerated and considered a safe nutrient with no serious adverse effects (21,22). It has already received FDA approval for clinical use as an oral anti-inflammatory and anticoagulant (23). It is well tolerated orally even at high doses (up to 3 g/day) for long treatment periods, up to several years (23).

Aims of study

The study aimed to:

Prepare aqueous and ethanolic extracts of pineapple fruits.

2- Synthesize nanoparticles from pineapple fruit extracts (aqueous and ethanolic) using silver nitrate Ag2SO4.

3-Characterization of the synthesized nanoparticles by Ultraviolet-visible spectroscopy (UV-Vis), Energy Dispersive X-Ray Analysis (EDX), Scanning Electron Microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR) .

4- Evaluation of the antibacterial effect of aqueous and ethanolic extract of pineapple fruits and nanoparticles synthesized from extracts with silver nitrate Ag2NO3.

2. Materials and Methods

Fresh pineapple fruit was purchased from closed local fruit shops in Mosul and stored at a moderate temperature away from heat and sunlight. The fruit was washed well to be used in the preparation of the plant extract.

The aqueous extract of the pineapple fruit was prepared by peeling the pineapple fruit and separating the pomace from the core to obtain two separate parts to prepare two extracts separately. 60g of both pomace and core were taken and placed in an electric blender and 60 mL of distilled water was added and mixed in the blender to obtain a homogeneous extract, then filtered The extract using a strainer, then we filter the extract using filter paper to get rid of impurities and fine solids and obtain a clear filtrate, then we sterilize the filtrate by using a 0.2 µm millipore filter, then it is kept in sterile tubes (24). The ethanolic extract of the pomace part and core of the pineapple fruit is prepared by taking 60 g of both, adding 60ml of ethanol with a concentration of 98% (25) , mixed with an electric mixer, then the ethanol is evaporated using a rotary evaporator, then the sample is dried by cooling to preserve the active components of the pineapple, using the Lyophilizer, to obtain a dry sample. Silver nanoparticles were synthesized from the aqueous and ethanolic extract of pineapple fruit (pomace and core)with silver nitrate 1mMolar We take a volume of 5 mL of the extract in exchange for 5ml of the chemical dissolved in a tube. When it interacts, minutes are formed, and within hours a different color change occurs Where the color of the silver nanoparticles solution changes from transparent to gray and then black as shown in Fig. no.1; dilutions of nanoparticles 1/1, 1/2 and 1/4 are made to diagnose its effect on bacteria.

Antimicrobial activity was carried out against pathogenic oral bacterial strains Enterococcus faecalis, Streptococcus, which were isolated from oral swabs in the microbiology laboratory at the University of Mosul, College of Dentistry.

Where the effect of the plant extract and the nano synthesized on the bacterial activity of these two strains was tested through a turbidity test using a spectrophotometer.

We take 1g of the dry extract and dissolve it with 1mL DMSO. We sterilize it using a millipore filter to obtain a sterile ethanolic extract. We take 1/2 mL of the sterilized extract and add it to 5ml of DMSO to obtain a dilution of 1/10. Then we take 1/2mL from the first dilution and add it to 5ml of DMSO to obtain a 1/100 dilution .The ethanolic extract is used in different concentrations to measure the effectiveness of the plant extract on the oral bacteria Enterococcus faecalis and Streptococcus Sanguis. The turbidity test was used to measure the antibacterial activity of nanoparticles and the plant extract of pineapple fruit against the pathogenic oral bacteria, namely Streptococcus Sanguis and Enterococcus faecalis. The turbidity test carried out by preparing a Brain Heart Infusion broth culture medium of 4 mL per tube, then the culture medium distributed in tubes is sterilized in the device autoclave, and dilutions of the plant extract are made, where 3 dilutions were made, starting from 1/1, 1/2, and 1/4 for each of the core and pomace. A positive control is prepared. 4 mL of culture medium and 0.1 mL of culture medium containing activated bacteria are prepared for 24 hours. One hour, negative control is prepared consisting of 4mL culture medium and 0.1mL bacteria and antibiotics Penicillin G, and vancomycin. Samples are prepared, which consist of 4ml culture medium, 0.1mL bacteria, and 0.1mL of plant extract used as an antibiotic. Samples consisting of 4mL medium are prepared from 0.1mL of bacteria and 0.1mL of nanoparticles as an antibiotic after preparation are incubated for 24 hours in the incubation at 37 °C to be measured the next day with a spectrophotometer. The results

were analyzed statistically using ANOVA and Duncan test and SPSS1 Version 25.

3. Results and discussion

Preparation and characterization of silver nanoparticles

The first character appears as an indicator for the presence of nanoparticles is the color change from transparent to black as in Figure no.1:

Figure 1: Color change of the silver nanoparticles solution

We note from the examinations under the scanning electron microscope that the silver nanoparticles prepared from the aqueous extract of the core of the pineapple fruit are in the form of sticky and oval-shaped particles with a magnification of 50µm. Thus, silver nanoparticles can be produced from pineapple, as in Figure (2). It is shown in Figure (2) for examining the sample under the EDX technique, which shows the analysis of the elements of the sample under examination, as the results showed the presence of the highest percentage of silver particles, which amounted to 78.74% and chlorine 21.26%.The silver nanoparticles prepared for pomace from pineapple fruit under a scanning electron microscope are in the form of sticky balls with a magnification of 20µm. The EDX tests showed the highest percentage of silver particles in the sample, which was 76.34%, and also the presence of other elements such as aluminum, 16.49%, and chlorine, 7.17%, as in Figure (3)

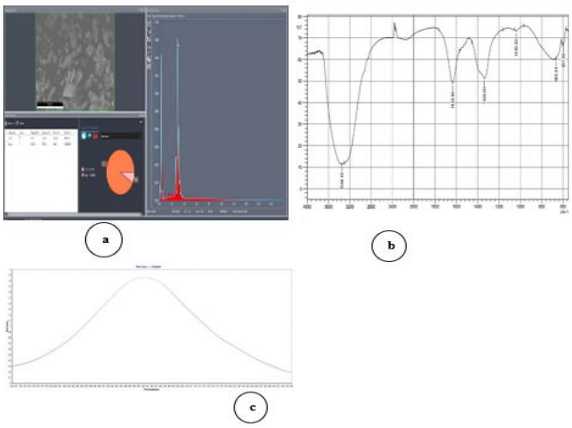

Figure (2): a) Nano-silver under a scanning electron microscope and EDX technique b) Nano-silver by FTIR c) Nano-silver absorption spectrum by UV- visible, aqueous extract from core

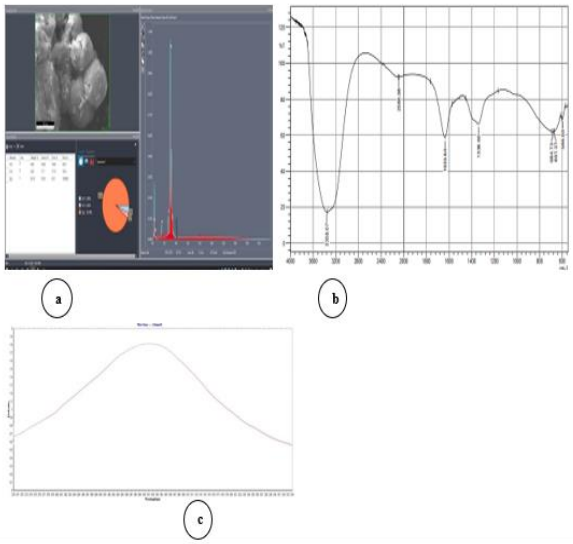

Figure (3): a) Nano-silver under a scanning electron microscope and EDX technique b) Nano-silver by FTIR c) Nano-silver absorption spectrum by UV- visible, aqueous extract from pomace.

As for the silver particles formed in the alcoholic medium, we note from the examinations under the scanning electron microscope that the silver nanoparticles prepared from the core of pineapple fruit are in the form of combined spherical porous structures with a magnification of 50µm. And with the presence of the highest percentage of silver particles 82.22% in the sample under examination, followed by aluminum at 11.20%, then chloride at 6.58%, as in Figure (4).As for the nano-silver prepared from pomace under a scanning electron microscope in an alcoholic medium, it will be in the form of clear balls with a magnification of 100 µm. The EDX technical tests showed that the highest percentage of silver particles in the prepared sample amounted to 76.01%, with the presence of the two elements aluminum by 11.18% and chlorine by 12.81%, as in Figure (5).

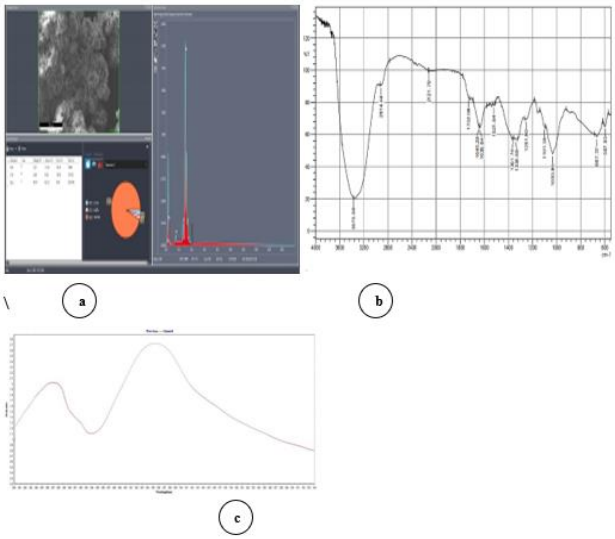

Figure (4): a) Nano-silver under a scanning electron microscope and EDX technique b) Nano-silver by FTIR c) Nano-silver absorption spectrum by UV- visible, ethanolic extract from core.

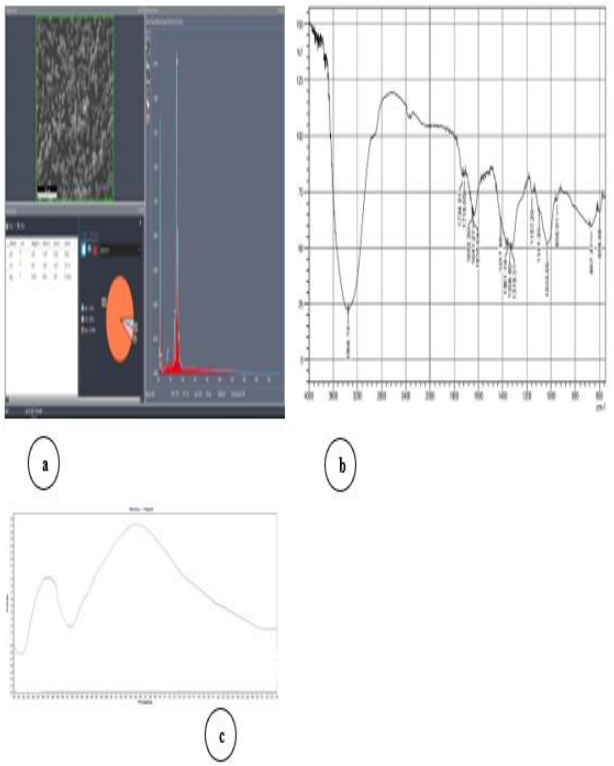

Figure (5): a) Nano-silver under a scanning electron microscope and EDX technique b) Nano-silver by FTIR c) Nano-silver absorption spectrum by UV- visible, ethanolic extract from pomace.

The appearance of black colouration confirmed by UV-visible at 295-308 nm proved the existence of silver nanoparticles. The FTIR result suggested that sugars such as sucrose, glucose etc. could be responsible for the bioreduction of the AgNO3. From the kinetics studies, the formation of the nanoparticles almost started from the second minute The scanning electron microscope, which is referred to as SEM, is one of the most important microscopic imaging devices, which has many major and important applications in the field of materials science, medical sciences and various other natural sciences, through which the surface of the sample is accurately photographed using a stream of electrons to give an image Very clear topography for the studied sample.Silver nanoparticles and compounds incorporated with silver have been shown to be very toxic to microbes, with significant biocidal properties on up to 16 species of bacteria, including bacteria, fungi, and viruses (26).

Antibacterial effect against Enterococcus faecalis and Streptococcus Sanguis

According to the results of the current study, in which a spectrophotometer was used to measure the turbidity of the medium (27), the aqueous extract of pineapple tissue, core, pomace It showed stronger antibacterial activity against the two isolated strains than the alcoholic extract (Table 1).The results also showed the bactericidal effect of biosynthetic nanoparticles from the aqueous and alcoholic extract of pineapple plant tissues as in (Table 2). Table 3 and Table 4 show a comparison between the effect of nanoparticles and aqueous and alcoholic extracts, respectively

Table1 : Antibacterial effect of aqueous and ethanolic extracts of the core and pomace from pineapple (Absorbance at 530 nm)

Bacteria	aqueous extract of pomace	ethanolic extract of pomace	aqueous extract of core	ethanolic extract of core	Control +ve (bacteria alone)	Vancomycin	Penicillin G
Enterococcus faecalis	0.088	0.137	0*	0.159	0.605	0	0
Streptococcus Sanguis	0.156*	0.504	0.021*	0.531	0.694	0.255	0.413
*significant The difference was considered significant at p ≤ 0.05							

Table 2: Antibacterial effect of Ag Nps from different extracts of the pineapple (Absorbance at 530 nm)

Bacteria	Ag Nps from aqueous extract of pomace	Ag Nps from ethanolic extract of pomace	Ag Nps from aqueous extract of core	Ag Nps from ethanolic extract of core	Control +ve (bacteria alone)	Vancomycin	Penicillin G
Enterococcus faecalis	0*	0*	0*	0.04	0.605	0	0
Streptococcus Sanguis	0*	0.022*	0*	0.114*	0.694	0.255	0.413
*significant The difference was considered significant at p ≤ 0.05							

Table 3 : Comparison between antibacterial effect of aqueous extracts of the pineapple and Ag Nps synthesized from them (Absorbance at 530 nm)

bacteria	aqueous extract of pomace	aqueous extract of core	Ag Nps from aqueous extract of pomace	Ag Nps from aqueous extract of core
Enterococcus faecalis	0.088	0	0*	0*
Streptococcus Sanguis	0.156	0.021	0*	0*
*significant The difference was considered significant at p ≤ 0.05				

Table 4 : Comparison between antibacterial effect of ethanolic extracts of the pineapple and Ag Nps synthesized from them (Absorbance at 530 nm).				
bacteria	ethanolic extract of pomace	ethanolic extract of core	Ag Nps from ethanolic extract of pomace	Ag Nps from ethanolic extract of core
Enterococcus faecalis	0.137	0.159	0*	0.04*
Streptococcus Sanguis	0.504	0.531	0.022*	0.114*
***significant** The difference was considered significant at p ≤ 0.05				

Meanwhile, the advent of nanotechnology in the twenty-first century has given rise to a new view on the dental use of silver nitrate-type Ag NPs, which exhibit antibacterial properties due to the slow discharge of silver ions (28).The past few years have also witnessed a remarkable development in the clinical applications of bio-nanomaterials in healthcare and dentistry. Nanotechnology is a valuable tool for the healthcare industry, and its applications have led to a significant improvement in modern medicine and dental practices (29). These new nanomaterials can mimic the surface and interface properties of dental tissues (30).The use of nanoparticles in root-end sealants and fillings provides increased strength and luster, similarly, incorporation of antimicrobial nanoparticles into restorative materials ensures protection against caries forming bacteria and maintains a healthy oral environment, in addition to a nanoparticle-based system An attractive method for topical drug delivery in gingivitis and oral squamous cell carcinoma (31). Plant extracts are widely used as antimicrobial agents. The effect of pineapple extract is attributed to the presence of bromelain, which is considered one of the most important factors that have been widely used as an anti-inflammatory drug in the field of medicine and dentistry. The discharge of silver ions, which increases uptake and causes infection of bacterial DNA and cytoplasm, is responsible for the antibacterial activity. By interfering with bacterial metabolism, the development of complexes between silver nanoparticles and proteins may lead to bacterial death. The bactericidal effect may come from the interaction of the particles with bacterial DNA, which inhibits cell proliferation (32). The results of the current study show its antibacterial effectiveness However, more clinical trials need to be done in order to validate this hypothesis.

4. Conclusion

This study has shown that pineapple (core, pomace) aqueous and ethanolic extracts have transformed silver nitrate to nanoparticles with great stability, also showed a good antibacterial effect on *Enterococcus faecalis* , *Streptococcus sanguis* with an inhibition rate of 100% compared to effect of vancomycin and penicillin G.

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