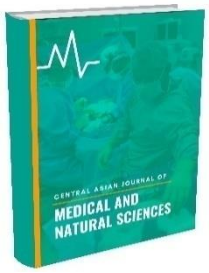
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 02 | Mar-Apr 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Antibacterial Efficacy of Ammonium Nanoparticles Synthesized from *Ananas Comosus* Fruit Extracts Against *Streptococcus Sanguis* and *Enterococcus Faecalis* (An in Vitro Study)

1. **Ebtihal Safi Mohamad**
2. **Ghada Younis Abdulrahman**

Received 2nd Jan 2023,
Accepted 3rd Feb 2023,
Online 20th Mar 2023

[1] BSc Microbiology

[2] BSc, MSc &PhD Microbiology

Key words: Nanoparticles, Ananas comosus, Pineapple, Pomace, Core, Ammonium sulfate, Enterococcus faecalis, Streptococcus sanguis.

Abstract: the study aimed to biosynthesize NH nanoparticles (NH Nps) from *Ananas comosus* (pineapple fruit) extracts and estimate their antibacterial efficacy against *Streptococcus sanguis* and *Enterococcus faecalis*.

Material and methods: The aqueous and ethanolic extracts were prepared from the core and pomace tissues of pineapple fruit separately and the NH Nps were synthesized by the addition of 5ml of 1mMolar of an aqueous solution of ammonium sulfate then we notice color change from white to light pink, The EDX, UV Spectrophotometer, FTIR and SEM were used to characterize NH 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: NH 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 NH Nps biosynthesized from aqueous core extract (0.172) , (0.234) for ethanolic core extract,(0.251) for ethanolic pomace extract and (0.252) for aqueous pomace extract against *Enterococcus faecalis* , (0.201) aqueous extract of core,(0.301) aqueous extract of pomace,(0.601) ethanolic extract of core and (0.610) ethanolic extract of pomace against *Streptococcus sanguis*.

Conclusion: The results showed the bactericidal effect of the aqueous extract of pineapple more than the ethanolic extract on *Enterococcus faecalis* and *Streptococcus sanguis*, as well as the sensitivity of *Enterococcus faecalis* and *Streptococcus sanguis* to biologically synthesized nanoparticles (NH NPs) made from the aqueous and ethanolic extracts.

Introduction

Nanotechnology is defined as the technology that studies and works on the precise adaptation of the composition of materials at the molecular level down to the nanoscale and the resulting change in the size of materials and the behavior of molecules and atoms that show different properties from materials of larger size than them the micrometric scale or larger. These differences are related to chemical reactivity, electrical conductivity, magnetic and optical effects, and thus obtaining new applications and uses for these materials [1,2] .Ammonium sulfate is a chemical compound represented by the following chemical formula (H8N2O4S), and its preferred formula is (NH4) 2SO4. It is in the form of colorless crystals, and its crystals are not hygroscopic, and they do not clump on each other [3].

The ammonium sulfate compound dissolves well in water and its aqueous solutions react in a weakly acidic manner, and the ammonium sulfate compound does not dissolve in organic solutions. However, it decomposes to ammonium bisulfate, liberating ammonia ،Ammonium sulfate is used in the field of fertilizers, as well as in processing and fire treatment [4,5].The size of nanomaterials is similar to that of most biological molecules and structures, so nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. To date, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physiotherapy applications, and drug delivery means. Nanomedicine seeks to offer a valuable set of clinically useful research tools and devices in the near future [6]. Nanotechnology is expected to have new commercial applications in the pharmaceutical industry that may include advanced drug delivery systems [7]. Vaccines [8]. and new treatments against viruses, especially respiratory viruses [9]. and incurable diseases such as cancer [10]. Pineapple (Ananas comosus) 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 [11].

Aims of study

The study aimed to:

1. Prepare ethanolic and aqueous extracts of pineapple fruits.
2. Synthesize nanoparticles from pineapple fruit extracts (ethanolic and aqueous) using ammonium sulfate $(NH_4)_2SO_4$.
3. Characterization of the synthesized nanoparticles by Ultraviolet-visible spectroscopy (UV-Vis) , Scanning Electron Microscope (SEM) , Energy Dispersive X-Ray Analysis (EDX) and Fourier-transform infrared spectroscopy (FTIR) .
4. Evaluation of the antibacterial effect of ethanolic and aqueous extract of pineapple fruits and nanoparticles synthesized from extracts with ammonium sulfate $(NH_4)_2SO_4$.

Materials and Methods

Pineapple fruit was purchased from the local market in Mosul and stored at a cool place away from heat, then washed well before the preparation of the pineapple extracts. The ethanolic and aqueous extracts of the pineapple fruit part (core, pomace) were prepared by peeling the pineapple and separating the core from the pomace to obtain two separate parts to prepare two separate extracts. The ethanolic extract of the pomace part and core of the pineapple fruit is prepared by taking 50g of both, adding 50ml of ethanol with a concentration of 98% [12] , mixing 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. Ammonium nanoparticles were synthesized from the aqueous and ethanolic extract of pineapple fruit (pomace and

core)with silver nitrate 1mMolar We take a volume of 5ml of the extract in exchange for 5ml of the chemical dissolved in a tube. 50g of both pomace and core were taken and placed in an electric blender and 50ml 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 [13]. When it interacts, minutes are formed, and within hours a different color change occurs Where the color of the ammonium nanoparticles solution changes from white to light pink 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 *Streptococcus sanguis*, *Enterococcus faecalis* 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 1 ml DMSO. We sterilize it using a millipore filter to obtain a sterile ethanolic extract. We take 1/2ml 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 4ml 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.

Results and discussion:

Preparation and characterization of ammonium nanoparticles:

The first character appears as an indicator for the presence of nanoparticles is the color change from white to light pink for the ammonium sulfate solutions as in Figure no.1:

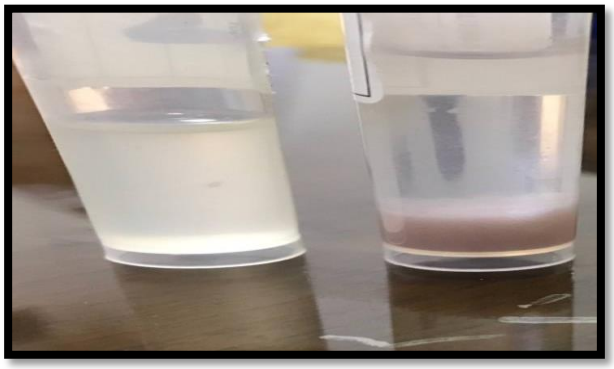


Figure 1.color change of the ammonium nanoparticles solution

The particles were observed as smooth and highly crystalline in nature .Nanoscale NHNPs was characterized by means of SEM Synthesized using the core and pomace of the pineapple fruit in the aqueous and ethanolic extract .Figure no. 2,3,4,5 shows a typical image of the nanoscale NHNPs synthesized via coordination n precipitation n method . It can be observed that it appears to have a platelet-like shape and with a dimension of 30 nm, and weak agglomeration can be seen NH NPs. The result shows the nanoparticles are in the same sizes as it is shown in the SEM images .In figure no.2 (a) shows the examination of nano ammonium prepared from the aqueous extract of the core under the EDX technique, which shows the analysis of the elements of the sample, The results show the presence of the highest percentage of sodium particles, in the amount of 42.99%, sulfur 29.72%, thorium 10.85%, silica 7.53%, aluminum 5.73%, and iron 3.17%.As for the ammonium nanoparticles prepared from the aqueous extract of pomace, the EDX technical tests show the highest percentage of sulfur particles in the sample, which was 49.13%, and also the presence of other elements such as sodium 38.25%, potassium 5.04%, rubidium 3.83%, and aluminum 3.74%, as in figure no.3 (a).The examination of nano-ammonium prepared from the ethanolic extract of the core under the EDX technique, which shows the elemental analysis of the sample appeared the presence of the highest percentage of sodium particles 46.01% and other elements are sulfur by 11.20%, indium 10.64%, then silica 5.43%, as in the figure no.4(a).As for the ammonium nanoparticles prepared from the ethanolic extract of pomace, the EDX technical examinations show the highest percentage of minutes to minutes. The highest percentage is for sulfur particles in the prepared sample, as it was 49.13%, and also the presence of other elements such as sodium 38.25%, potassium 5.04%, rubidium 3.83%, and aluminum 3.74%, as in the figure no.5 (a).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 100µm. The EDX technical tests showed that the highest percentage of silver particles in the prepared sample amounted to 76.01%.The FTIR appears peaks around 1600-1700 cm-1 are due to the C=C stretching vibrations in the aromatic ring, and around 1000-1300 cm-1 due to the C-O stretching vibrations in the sugar moiety. also, it is important to mentioned that peaks around 1300-1500 cm-1 are due to the N-H bending and stretching vibrations in the amine group. All of these functional group confirm the existence of phytochemical in the extract. For figure no. 2,3,4,5 (b), the peaks related to the ammonia could be characterized. A strong peak around 3300-3500 cm-1 corresponds to the N-H stretching vibration in NH3. A weak peak around 1600-1700 cm-1 may be observed due to the N-H bending vibration in NH3 and a weak peak around 1000-1200 cm-1 is due to the C-N stretching vibration in some NH3 derivatives, such as

ammonium salts .The appearance of to light pink for the ammonium sulfate solutions coloration confirmed by UV-visible at 203-205 nm proved the existence of ammonium nanoparticles.

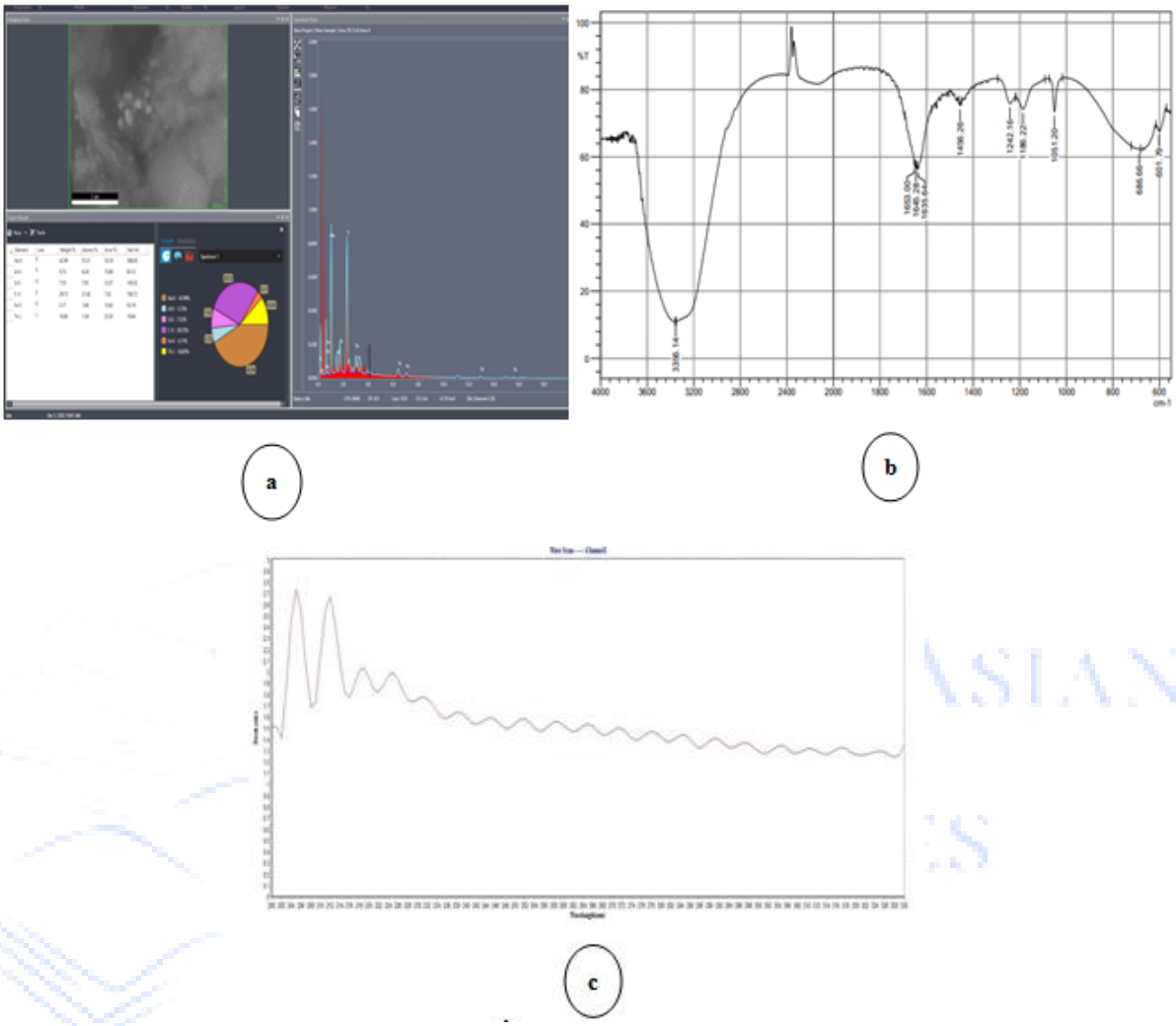
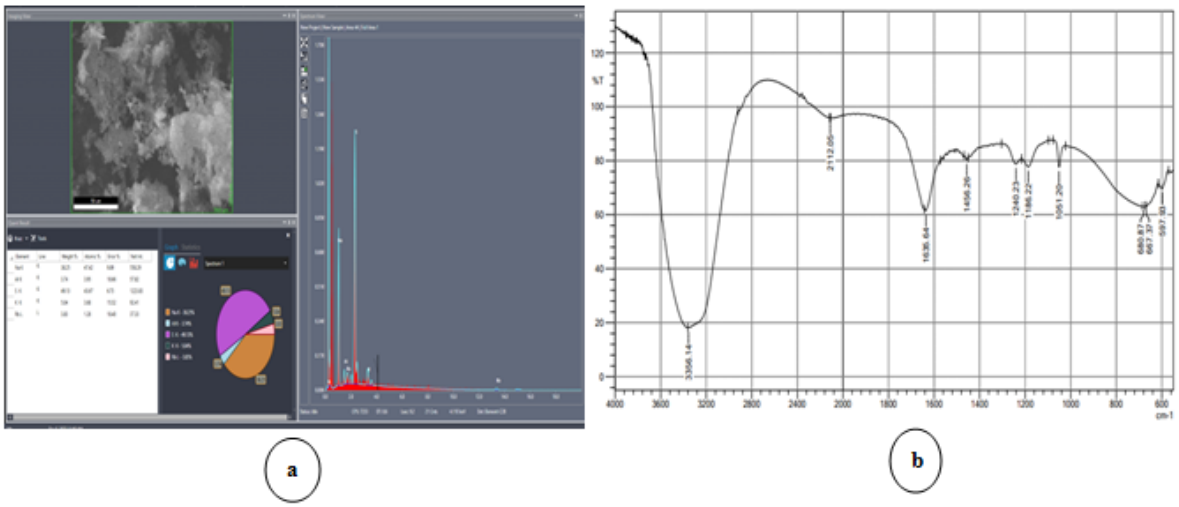
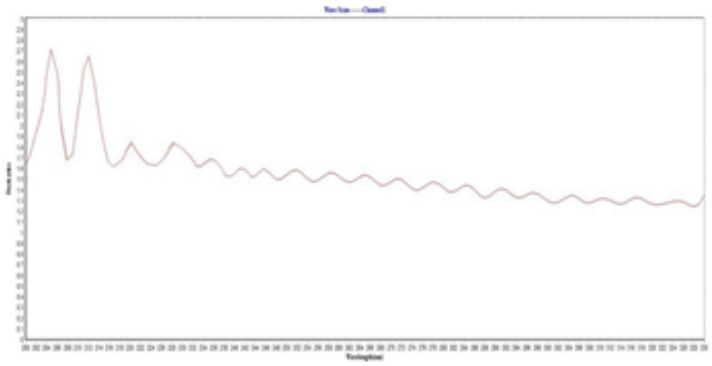


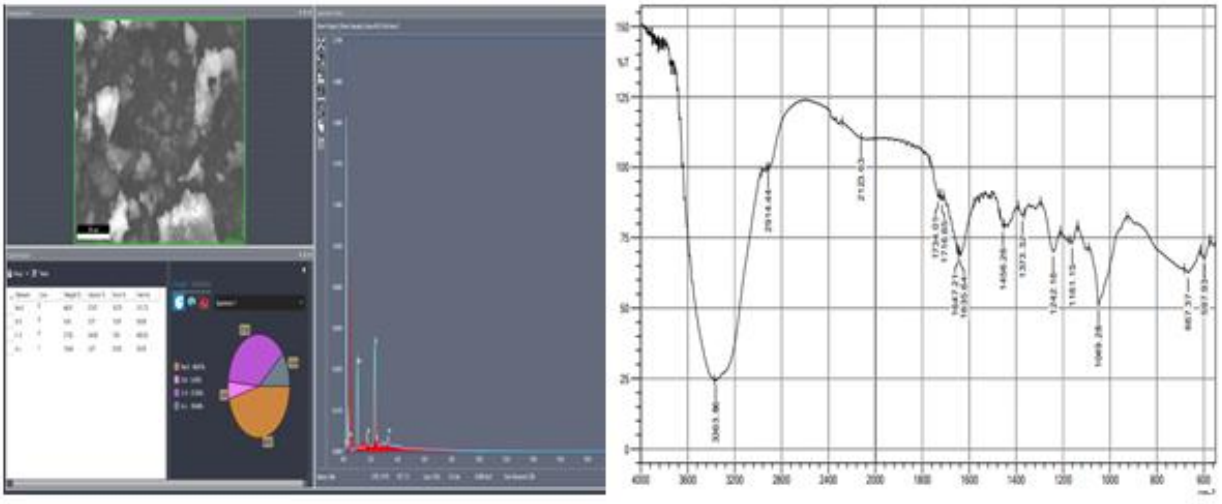
Figure (2) a) Nano- ammonium under a scanning electron microscope and EDX technique **b)** Nano- ammonium by FTIR **c)** Nano- ammonium absorption spectrum by UV- visible, aqueous extract from core



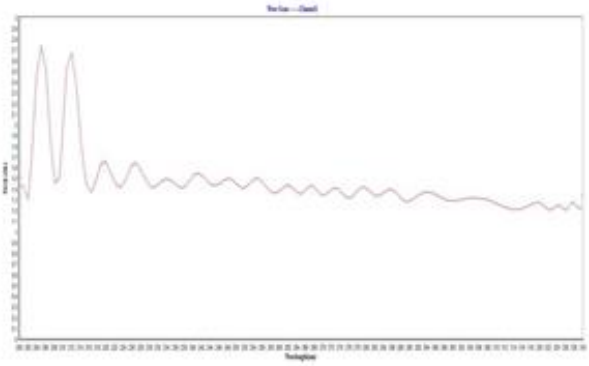


c

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



a b



c

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

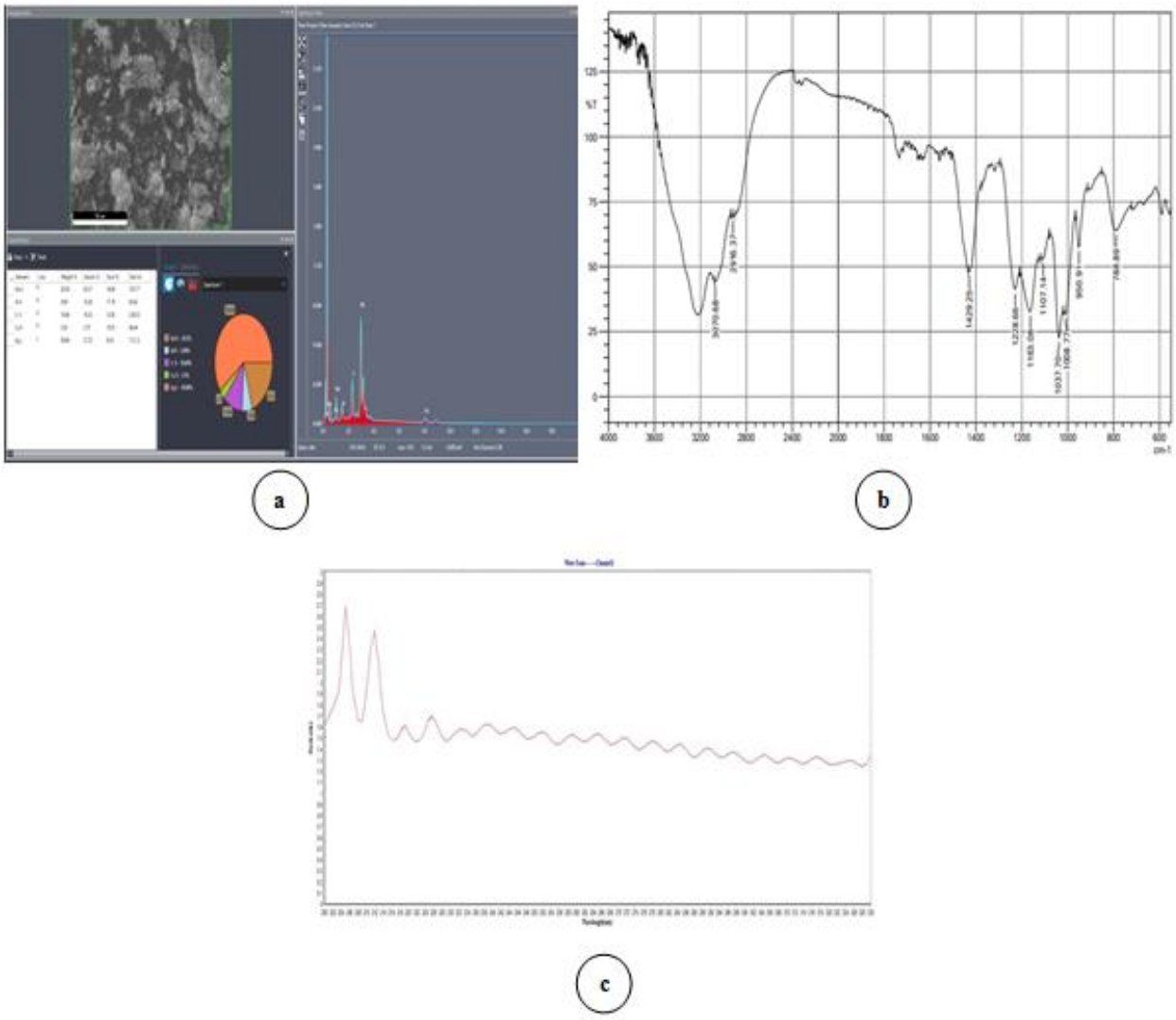


Figure (5) a) Nano- ammonium under a scanning electron microscope and EDX technique **b)** Nano- ammonium by FTIR **c)** Nano- ammonium absorption spectrum by UV- visible, ethanolic extract from pomace

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 [(14)], 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 biosynthetic nanoparticles (NH NPs) from the aqueous and ethanolic extract of pineapple plant tissues showed various and differentiated results of bactericidal effect as in (Table 2). Table 3 and Table 4 show a comparison between the effect of nanoparticles and aqueous and alcoholic extracts, respectively

Table 1: Antibacterial effect of aqueous and ethanolic extracts of the core and pomace from pineapple (Absorbance at 530nm)

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 $p \leq 0.05$

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

Bacteria	NH Nps from aqueous extract of pomace	NH Nps from ethanolic extract of pomace	NH Nps from aqueous extract of core	NH Nps from ethanolic extract of core	Control +ve (bacteria alone)	Vancomycin	Penicillin G
Enterococcus faecalis	0.252	0.251	0.172	0.234	0.605	0	0
Streptococcus Sanguis	0.301	0.610	0.201*	0.601	0.694	0.255	0.413

***significant** The difference was considered significant at $p \leq 0.05$

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

bacteria	aqueous extract of pomace	aqueous extract of core	NH Nps from aqueous extract of pomace	NH Nps from aqueous extract of core
Enterococcus faecalis	0.088	0	0.252	0.172
Streptococcus Sanguis	0.156	0.021	0.301	0.201

***significant** The difference was considered significant at $p \leq 0.05$

Table 4: Comparison between antibacterial effect of ethanolic extracts of the pineapple and NH Nps synthesized from them (Absorbance at 530nm).

bacteria	ethanolic extract of pomace	ethanolic extract of core	NH Nps from ethanolic extract of pomace	NH Nps from ethanolic extract of core
Enterococcus faecalis	0.137	0.159	0.251	0.234
Streptococcus Sanguis	0.504	0.531	0.610	0.601

***significant** The difference was considered significant at $p \leq 0.05$

The development in the past few years clinical applications of bio-nanomaterials in healthcare and dentistry confirmed that the nanotechnology is a valuable tool for the healthcare industry, and its applications have led to a significant improvement in modern medicine and dental practices [15]. These new nanomaterials can mimic the surface and interface properties of dental tissues [16].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 [17]. 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 results of the current study show its antibacterial effectiveness Bromelain also inhibits the growth of some bacteria by preventing bacteria from attaching to specific glycoprotein receptors on the surface [18] 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 [19,20]. It has already received FDA approval for clinical use as an oral anti-inflammatory and anticoagulant [21]. It is well tolerated orally even at high doses (up to 3 g/day) for long treatment periods, up to several years [21]. However, more clinical trials need to be done in order to validate this hypothesis.

Conclusion This study has shown that pineapple (core, pomace) aqueous and ethanolic extracts good antibacterial effect on *Streptococcus sanguis, Enterococcus faecalis* and they have transformed ammonium sulfate to nanoparticles with great stability when they reacted ,also ammonium nanoparticles showed a various antibacterial effect against *Streptococcus sanguis & Enterococcus faecalis.*

References:

1. Joseph, T., & Morrison, M. (2006). Nanotechnology in Agriculture and food Nanoforum report.org, European nanotechnology gateway.
2. Nilesh, J., Ruchi, J., Navneet ,T., Brham, P. Gupt; Deepak, K. Jain ; Jeetendra, B. &Surendra, J . (2010) . Nanotechnology: A safe and effective drug delivery system. Asian Journal of Pharmaceutical and Clinical Research . Vol. 3, Issue 3.
3. Sanford, J. o ; Lancaster ; J. D. Biological and chemical evaluation of the readily available sulfur status of Mississippi soils . soil sci. Am. Proc. 1962, 26, 63-65.
4. Karl-Heinz Zapp "Ammonium Compounds" in Ullmann's Encyclopedia of Industrial Chemistry 2012, Wiley-VCH, Weinheim .
5. Tisdate , S. and W. Nelson (1975) ; soil fertility and fertilizers n. New York . Macmilltn publishing Co. 1975 .
6. Jia, J. Interactions between nanoparticles and dendritic cells: from the perspective of cancer immunotherapy. Front. Oncol. 2018;8, 404–404
7. Fatemeh.O,Fahimeh.C,Maryam.H,Amir,A.RezvanRobati *et al* Recent advances in nanotechnology-based drug delivery systems for the kidney. Journal of Controlled Release . 321, 10 .2020: 442-462.
8. Sulczewski, F. B., Liszbinski, R.B., Romao, P. R. T. & Rodrigues Junior, L. C. Nanoparticle vaccines against viral infections. Arch. Virol. 2018. 163, 2313–2325.
9. Al-Halifa, S., Gauthier, L., Arpin, D., Bourgault, S. & Archambault, D. Nanoparticle-based vaccines against respiratory viruses. Front. Immunol.2019. 10, 22.

10. Landgraf, M. Targeted camptothecin delivery via silicon nanoparticles reduces breast cancer metastasis. Biomaterials.2020.240, 119791.

11. Vipul Chaudhary, Vivak Kumar, Sunil, Vaishali, Kavindra Singh, Ratnesh Kumar and Vikrant Kumar. Pineapple (Ananas cosmosus) product processing: A review. Journal of Pharmacognosy and Phytochemistry 2019; 8(3): 4642-4652.

12. Al-Hasso, Z. Y. K., Al-Katib, M. A., & Alrahman, G. Y. A. (2022). Biosynthesis of gold Nanoparticles by Lyngbya sp. Clone Zen / Mira 16S ribosomal RNA gene and its antibacterial activity. International Journal of Health Sciences, 6(S6), 10810–10819. https://doi.org/10.53730/ijhs.v6nS6.12920

13. Sarinya Poadang, Niti Yongvanich and Siriporn Phongtongpasuk .Synthesis, Characterization, and Antibacterial Properties of Silver Nanoparticles Prepared from Aqueous Peel Extract of Pineapple, Ananas comosus. Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakornpathom, Thailand. Doi: 10.12982/cmujns.2017.0010.

14. Kinnear, C.; Moore, T.L.; Rodriguez-Lorenzo, L.; Rothen-Rutishauser, B.; Petri-Fink, A. Form Follows Function: Nanoparticle Shape and Its Implications for Nanomedicine. Chem. Rev. 2017; 117, 11476–11521.

15. Kah, M., Tufenkji, N. & White, J. C. Nano-enabled strategies to enhance crop nutrition and protection. Nat. Nanotechnol. 2019.

16. Palazzolo, S.; Hadla, M.; Spena, C.R.; Bayda, S.; Kumar, V.; Lo Re, F.; Adeel, M.; Caligiuri, I.; Romano, F.; Corona, G.; *et al*. Proof-of-Concept Multistage Biomimetic Liposomal DNA Origami Nanosystem for the Remote Loading of Doxorubicin. ACS Med. Chem. Lett. 2019, 10, 517–521.

17. Sharon Mitchell, Ruixuan Qin, Nanfeng Zheng & Javier Pérez-Ramírez , Nanoscale engineering of catalytic materials for sustainable technologies Nature Nanotechnology ,2020.

18. Cloete, T. E. (2003). Resistance mechanisms of bacteria to antimicrobial compounds. International Biodeterioration and Biodegradation, 51(4): 277-282.

19. Moura, I.; Torres, C.; Silva, N.; Somalo, S.; Igrejas, G.; and Poeta, P. (2013). Genomic description of antibiotic resistance in Escherichia coli and enterococci isolates from healthy Lusitano horses. Journal of Equine Veterinary Science, 33(12): 1057-1063.

20. Levinson, W.; and Jawetz, E. (2002). Medical Microbiology and Immunology: Examination and Board Review.7th edition. McGraw-Hill Co.,USA.pp:77:96-98 .

21. Depardieu, F.; Perichon, B.; and Courvalin, P. (2004). Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. Journal of Clinical Microbiology, 42(12): 5857-5860.