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THE INHIBITORY EFFECT OF *BOSWELLIA SERRATE* NANOPARTICLES ON THE FORMATION OF BIOFILM PRODUCED BY SOME ORAL PATHOGENIC BACTERIA

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Abstract
gum olibanum (*Boswellia serrate*) is one of traditional remedy used for a long time to cure many diseases. Current study was carried out to propose a green approach for the synthesis of ZnO nano particles using (*Boswellia serrate*). ZnO nanoparticles have a very broad range of applications especially as antimicrobial agent. There are various methods are available for the synthesis of ZnO nanoparticles, but among them the synthesis of ZnO nanoparticles by using plant material is a very good alternative and eco-friendly method. Leaves extract was used as a biological reducing agent for the synthesis of ZnO nano particles from the zinc nitrate. The prepared nano particles were characterized by using various analytical and spectroscopic tools such as X-ray diffraction (XRD) and scanning electron microscopy (SEM) analysis. Along with this study we also investigate the antimicrobial activity of bio synthesized nanoparticles by using *Boswellia serrate* and evaluation of their Inhibition of biofilm formation produced form clinical isolates of Periodontitis bacterial causative agents *Streptococcus pneumonia, Streptococcus gordonii, Streptococcus mitis, Gemella adicens*. the highest value of bacteria (Streptococcus) was 0.435 nanometers, which decreased to 0.137 nanometers. The antibacterial test was carried out following the method done by Perez and others. Using 100 µl, the results showed a great inhibition zone (46 mm and 41 mm) against pathogenic isolates. This *Boswellia serrate* nanoparticles contains active chemical components that contribute to biological activity thereby assisting to combat bacterial infections and the potential for maintaining and promoting total health.

Keywords: *Boswellia serrate*, nanoparticles, biofilm, oral pathogenic bacteria

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抽象的

乳香（Boswellia serrate）是一种长期用于治疗多种疾病的传统药物。目前的研究是为了提出一种使用（乳香锯齿）合成 ZnO 纳米颗粒的绿色方法。 ZnO 纳米粒子具有非常广泛的应用范围，尤其是作为抗菌剂。 ZnO纳米粒子的合成有多种方法，但其中利用植物材料合成ZnO纳米粒子是一种很好的替代和环保方法。叶子提取物被用作生物还原剂，用于从硝酸锌合成 ZnO 纳米颗粒。通过使用各种分析和光谱工具，例如 X 射线衍射 (XRD) 和扫描电子显微镜 (SEM) 分析来表征制备的纳米颗粒。随着这项研究，我们还通过使用乳香锯齿状乳香研究了生物合成纳米颗粒的抗菌活性，并评估了它们对牙周炎细菌病原体肺炎链球菌、戈登链球菌、缓和链球菌、Gemella adicens 的临床分离株产生的生物膜形成的抑制作用。细菌（链球菌）的最高值是 0.435 纳米，下降到 0.137 纳米。抗菌试验按照佩雷斯等人的方法进行。使用 100 µl，结果显示对病原分离物有很大的抑制区（46 毫米和 41 毫米）。这种乳香锯齿状纳米颗粒含有有助于生物活性的活性化学成分，从而有助于对抗细菌感染以及维持和促进整体健康的潜力。

关键词：乳香锯齿，纳米颗粒，生物膜，口腔致病菌

Introduction

The rise in the emergence and spread of antimicrobial resistance among the different microorganisms (bacteria, fungi, virus, and parasites) is one of the most important health problems worldwide today so synthesis of metal nanoparticles using biological systems is an expanding research area in nanotechnology. Nanotechnology involves the use of materials having nanoscale dimensions in the range of 1–100 nm. Operating with Nanomaterials has allowed researchers to have a much better understanding of biology. The green synthesis of nanoparticles has greatly reduced the use of physical and chemical methods. Various chemical methods have been proposed for the synthesis of zinc oxide nanoparticles (ZnO NPs), such as reaction of zinc with alcohol, vapor transport, hydrothermal synthesis, precipitation method etc [1–4]. Biosynthesis of ZnO NPS from plants such as Aloe vera, Sargassum muticum, Eichhornia crassipes, Borassus flabellifer fruit, and also in some bacterial and fungal species such as *Bacillus* subtilis and *Escherichia coli* Ureolytic bacteria, Lactobacillus plantarum have been reported [5] . Nanoparticles synthesis within the size range of 10–100 nm have become an extensive research and concern due to their potential application in wide areas of science and technology. Metal oxide nanoparticles have been extensively used for medicinal purposes in the past decades. Metal oxide nanoparticles has environmental applications as it can act as catalyst which is helpful in reduction or elimination of the toxic hazardous chemicals from the environment [6].There are so many reports are available in the last few years which clearly indicate the

considerable antimicrobial activity of inorganic TiO2, SiO2, MgO, CaO, and CeO2. Specially, TiO2, ZnO, MgO, and CaO are of particular concern because they are not only stable under harsh process conditions, but also are considered as safe materials to humans .In the present work we study the Zinc oxide nanoparticles. The reason for selecting ZnO nanoparticles (ZnO nps) for the present study is that ZnO is a metal oxide, which is very much stable and having longer life as compared to the organic based disinfectants and antimicrobial agents[7].

Streptococcus which include (*pneumonia, gordonii, mitis*) and *Gemella morbillorum* are strongly associated It is the main cause of human tooth caries.(8). The dental plaque on the surfaces of the teeth is a result of the ability of these bacteria to form biofilms (are communities of microorganisms that grow immersed in a matrix of three-dimensional structure in a solid surface on a film, adhering to living tissues) and is considered one of the most important virulence properties (9). In response to appropriate environmental cues, biofilm formation begins with interactions between plankton bacteria and the surface (10) And also the responses to chemical and physical signals, bacteria regulate diverse physiological processes in a manner dependent on a single cell density called quorum sensing. (11).

2. Materials and methods

2.1Materials

Zinc Chloride (ZnCL2), ethanol and all other chemicals used in

Zinc Chloride ($ZnCl_2$), ethanol and all other chemicals used in the experiment were of analytical grade and purchased from Merck. All glassware were washed with sterile distilled water and dried in an oven before use.

2.2 Synthesis of *Boswellia serrate* nanoparticles

Prepared by hydrolysis (dissolving) 5 g/100 ml in Distilled water a 200 ml flask placed on a Hot plate stirrer at a temperature not exceeding 50oC for one hour.

Then it is filtered by filtering funnel and left for the second day. *Boswellia serrate* is easy to dissolve water. Soluble binder does not leave residues and when added to nanomaterials, high adhesion can be obtained.

2.3 Synthesis of ZnO nanoparticles 2.32220

Prepared by hydrolysis dissolving (6.05 g/100 ml) of distilled water in a 200 ml according to the molarity equation Previous (1).

$$\boldsymbol{M = \frac{Wt}{MWt \times V/1000}}$$

Where: M: Molar concentration, Wt: Weight of the materials used Mwt: Molecular weight of materials (g / mole) and V: Volume of distilled Water (ml) . by adding 100 micro liters of HCL (to increase solubility) flask placed on a Hot plate stirrer at a temperature not exceeding 80 oC for one hour. 50 ml of the Arica gum was mixed with 0.2 M Zinc Chloride dehydrates and the solution was dissolved using magnetic stirrer at 80 C˚ for 2 h. Then, the formed light yellow coloured precipitate was allowed to settle for 18 h. The precipitate was separated from the mixture by centrifugation at 10,000 rpm for 25 min and rinsed with distilled water again and again to remove the impurities and dried in a hot air oven at 90 C ˚for overnight. The crystallinity and other organic impurities are removed in calcinations process. The powder was subjected to calcinied at 400 C˚ for 2 h [4].

2.4 Characterizations

Scanning electron microscopy(SEM)

Morphological study of Zno nanoparticles surface was performed using scanning electron microscopy, SEM (Scanning Electron Microscope Inspect 550, Netherland).

Powder X-Rays diffraction(PXRD)

XRD patterns for CNTs was investigated using powdered X-Rays diffraction, XRDXRD6000, Shimadzu, Japan.

Fourier transform infrared spectroscopy (FTIR)

Functional groups in Zno nanoparticles samples were investigated using FTIR spectroscopy. FTIR spectra were recorded with Perkin Elminer Spectrophotometer. All Samples were prepared for san by grounding with KBr crystal and mixed Zno nanoparticles samples to form uniform pellets using Perkin Elmer hydrolytic pump.FTIR Fourier – Transform FTIR-8400S Shimadzu, Japan.

2.5 Bacterial isolates:

27samples were collected from the Faculty of Dentistry, University of Kufa, from periodontitis patients and it was diagnosed using the VITEC device.

2.6 Preparation of the Bacterial Suspension:

All bacterial isolates were cultured on Brain heart infusion broth and the turbidity of each of the bacterial suspensions was prepared to match the standard of 0.5 McFarland (1.5x108 CFU / ml). Turbidity was measured with a spectrophotometer at turbid suspension at 625 nm as per Bauer-Kirby Method (1966).

2.7 Antimicrobial activity:

The antibacterial test was carried out following the method done by Perez and others (19). On Muller Hinton Agar spread 0.1ml of the culture with a sterile swab, dry at room temperature for (10-15) minutes. Inhibitory activity was detected by the agar-well diffusion method , after sterilizing with the cork borer, four wells were made on the surface of the culture media with a diameter of 10 mm then add (100 µl) to each well in *Boswellia serrate* nanoparticles, The plate was incubated for 18-24 hours at 37 ° C. The diameter of the inhibition zones was measured. The tested nanoparticles from this medicinal plant with the concentrations 100 µl were screened. Streptococcus orails showed the most isolate affected by the nanoparticles.

2.8 Inhibition of biofilm formation:

200 µl of BHI medium were transferred to (MTP 96-wells), 100 µl of nanoparticles and 20 µl of suspension, To each well that

contains culture media prepared with the use of control well containing only (BHI) broth, The plates were incubated at 37 ° C for 18-24 hours, then the contents of the wells were removed and washed 3 times with buffer solution phosphates, ethanol-adherent cells were stabilized at 95% concentration and left for 10 minutes. The wells is dyed with Crystal violet at a concentration of 1%, 100 µl of dye was added to each hole for 15 minutes. Then the drill was washed with sterile distilled water three times to remove the non-sticking dye.

Thus, the ability of bacteria to adhere to quantitatively can be estimated by observing the amount of dye attached to the wells, and to qualitatively estimate the ability of bacteria to produce the mucous material, the adhesive dye was removed by adding 200 µl at a concentration of 100% of methanol to each hole. Absorbance was measured at the wavelength of 630 nm with an ELIZA instrument. (Mathur et al., 2006).

2.9 Statistical analysis:

The data gathered and exported to a Microsoft Excel spreadsheet where descriptive statistics were performed. The data was analyzed processed using SAS version 9.1. Two-way ANOVA was also carried to determine if there was any interaction between the effect of extracts concentration and the pathogenic bacteria. $P \leq 0.05$ is considered significant in both tests (Tukey test).

Results and Discussion

The FT IR spectrum of compound is generally used for the determination of functional group present in the compound Fig. 1. shows the FTIR spectrum of synthesized ZnO NPs.

the peaks spectrum of the prepared compound beam Frequency absorption(754.17),(817.82),(1440.83),(1662.64) cm-1 bundles belonging to the gum Arabic spectrum [14]. The broad stretch at 3481.51 cm^{-1} shows the presence of O–H stretch and hydrogen bonded groups in alcohol or phenolic or water molecules in the extract [15]. The absorption peaks at 1635.64 cm -1 indicates the stretching vibration of C=O hydroxyl (or) carboxyl groups on the surface of the sample. The peak around 1379.15cm^{-1} the asymmetric stretching vibration of Chloride (Cl-) ions[16]. The peak in the region between 600 and 800 cm-1 is allotted to Zn–O. The stabilization and capping agent of synthesized ZnO NPs may be due to the coordination of ZnO NPs with –OH and C=O groups. It may also conclude that the presence of phenolic and flavonoid group molecules is responsible for the reduction process[17].

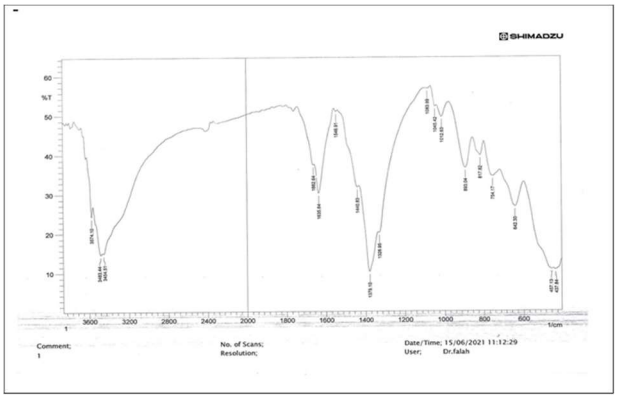


Fig 1. FT-IR spectra of synthesized ZnO nanoparticles using Boswellia serrate

X-rays diffraction (XRD)

X-ray diffraction pattern of synthesized nanoparticles is used

for the calculation of Crystal lattice indices and particle size

Diffraction peaks were observed at 2Θ values of 31.88˚, 34.54˚ , 36.43˚ ,47.58˚,56.68˚,62.98˚and 68.03˚ corresponding to lattice planes (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3) and (1 1 2) respectively as shown in Fig. 2. The peaks have been attributed to hexagonal phase of ZnO [18].

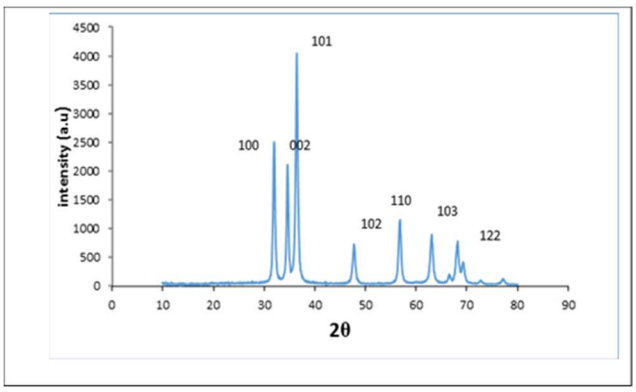
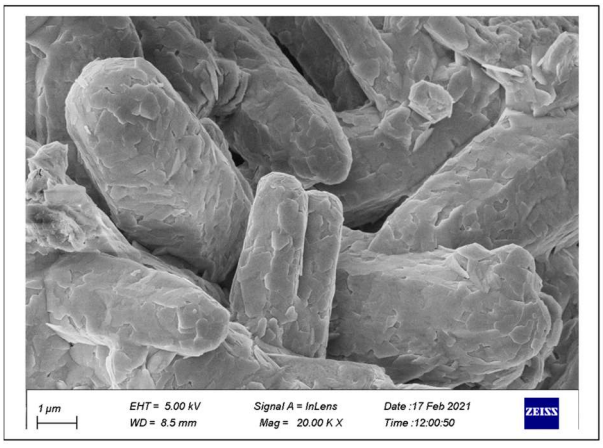


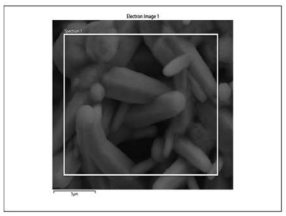
Figure 2: XRD Patterns for Zinc oxide nanoparticles Zno

Scanning electron microscope (SEM)

Scanning electron microscope is type of electron microscope which is used to capture the images

of sample by using high energy electron beam. High energy electron beam interact with the constituent of the compound and give signals reveals important information regarding the composition, surface topography and other properties such as electrical conductivity [19]. Therefore the SEM is a useful analytical technique to analyze the surface morphology and size of the synthesized ZnO
nanoparticles. SEM image illustrate individual ZnO nanoparticles as well as number of aggregates. Fig. 3 illustrates the particles are predominantly spherical in shape and aggregates into larger particles with no well-defined morphology. The SEM image shows the size of the ZnO nanoparticles ranging from 25 to 35 nm. Fig. 4 shows the EDAX analysis, confirmed the presence of metallic zinc oxide in biosynthesized ZnO NPs. The composition obtained from EDAX analysis was Zinc NPs. The composition obtained from EDAX analysis was Zinc (74.26%) , Oxygen (25.00%), chloride (0.51%) and calcium (0.23%) [20] .





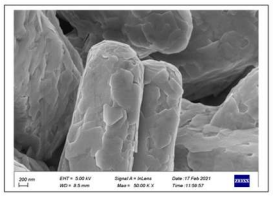


Figure3: SEM images for the Zinc oxide nanoparticles Zno

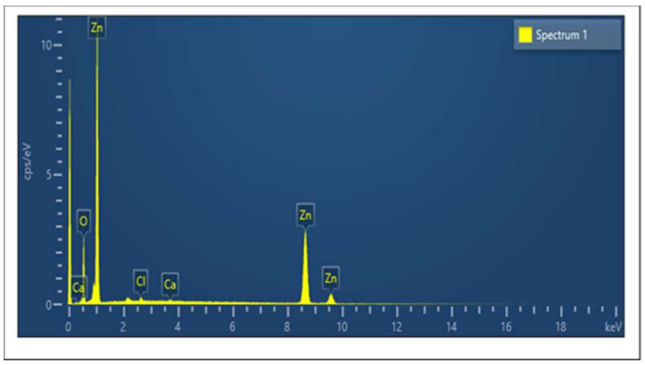


Figure 4: EDX for the Zinc oxide nanoparticles Zno

Effect of *Boswellia serrate* Zno nanoparticles on biofilm formation

The results in this study showed that the biofilm significantly decreased according to the bacterial isolates after treatment with the male frankincense compared to the control treatments, as (A) *Streptococcus gordnii* had the highest value of 0.435 nm decreased 0.137 nm As in Figure 5.

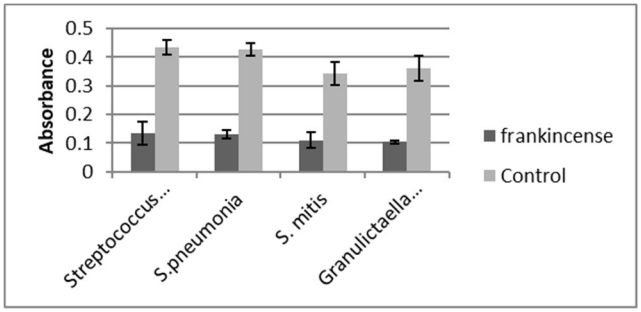


Figure (5) :The (X) axis represents the pathogenic bacterial isolates and the (Y) axis represents the readings at the wavelength of 630 nm using an ELIZA device.

The ability of bacteria to adhere to can be quantified by observing the amount of dye adhering to the Wells. Figure (6).

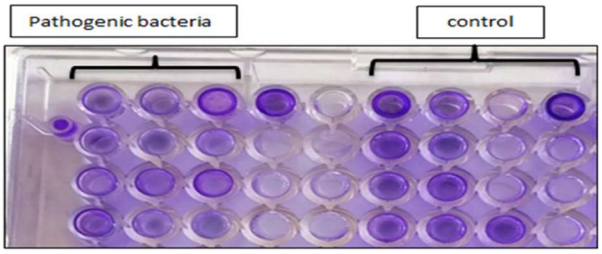


Figure (6) shows the treatment of the bacterial isolates with the *Boswellia serrate* nanoparticles, where the clear gradient of the dye is observed because it caused the breakdown of the biofilm.

Medicinal plants for a long time have been applied as substitutable agents to medicine in many societies (Álvarez-Martínez et al, 2020). The results show that the effect of BZnNPs was negative on the formation of the biofilm, and the formation of the biofilm decreases according to the different bacterial isolates compared to the control treatments. As shown in (Figure 1) that *Streptococcus gordnii* had the highest value of 0.435 nm decreased 0.137 nm , *S.pneumonia, S. mitis* and *Granulictaella adicens* (0.122, 0.137and 0.119) respectively. In this study (BZnNPs) showed a different inhibitory activity for the formation of the biofilm of oral bacteria(Figure 2), due to the fact that the chemical composition of the biofilm differs between types of bacteria in general and even between species belonging to the same family, as each bacteria has its own structure and peptides may interfere with these membranes and cause in the presence of an imbalance in its composition and some bacterial species consume these peptides, contributing to the formation of factors the virulence of bacteria (Arvanati et al., 1994). the amounts of ZnO NPs is essential for growth inhibition and the inhibition of metabolic activity was approximately equal, and this result indicates that the predominant antimicrobial targets of the NPs are the metabolic pathways of bacteria. Thus the mechanism of NPs inhibition of bacterial biofilm formation is related to the regulation of bacterial metabolism (Salem et al., 2015).

Antimicrobial properties:

BZnNPs have very strong inhibitory action against, Gram-positive bacteria Fig. 7,8).

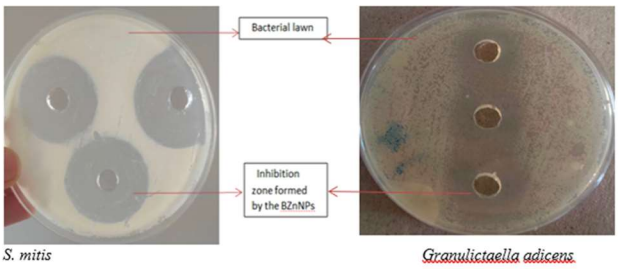


Fig. 7,8: Biological activity of BZnNPs against pathogenic bacteria

The BZnNPs showed to be considerably active against *Streptococcus* spp and *Granulictaella adicens*. that being affected by the extract with a diameter of inhibition (47mm and 38mm), Increase the available surface area for interactions this can be attributed to the enhanced antimicrobial activity of the nanoparticles, which enhances the bactericidal effect. The molecules are large in size and therefore, they transport cytotoxic microorganisms. (Adams et al. 2006).

The results showed that the nanocomposite was significantly effective against clinically isolated bacteria with an inhibition diameter of 45mm compared to Salman et al (2021). Many studies suggest that when bacteria were treated with zinc nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane (Auffan et al. 2009). leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death.

the interaction of nano-materials with the biological macromolecule is the possible mechanisms that thought the metal oxides carry a positive charge while microorganisms carry a negative charge. This creates an ‘‘electromagnetic’’ attraction between the microbe and treated surface. Zinc nanoparticles synthesized from the B. ovalifoliolata stem bark extract proved to be more potent the antimicrobial activity is probably derived, through the electrostatic attraction between negative-charged cell membrane of microorganism and positive-charged nanoparticles (Prasad et al. 2011).

Many of the components of BZnNPs are active antibacterial agents and have shown activity against oral bacterial infections as reported in some literature.

Conclusion:

Synthesis of zinc oxide nanoparticles from *Boswellia serrate* was demonstrated. Zinc oxide nanoparticles were synthesized from *Boswellia serrate* showed good antimicrobial activity against oral bacteria. Moreover, BZnNPs showed good Inhibits the formation of biofilm produced by pathogenic oral bacteria. The findings in this study may lead to the development of BZnNPs-based (*Boswellia serrate*) new antimicrobial systems for medical applications especially with regard to dentistry.

References:

1- H. Mirzaei, M. Darroudi, Zinc oxide nanoparticles: biological synthesis and biomedical applications, Ceram. Int. 43 (2017) 907–914, doi: 10.1016/j.ceramint. 2016.10.051.

2- Pulit-Prociak, J. Chwastowski, A. Kucharski, M. Banach, Functionalization of textiles with silver and zinc oxide nanoparticles, Appl. Surf. Sci. 385 (2016) 543–553, doi: 10.1016/j.apsusc.2016.05.167.

3- T.C. Taranath, B.N. Patil, Limonia acidissima L. leaf mediated synthesis of zinc oxide nanoparticles: a potent tool against Mycobacterium tuberculosis, Int. J. Mycobacteriol. 5 (2016) 197–204, doi: 10.1016/j.ijmyco.2016.03.004.

4- P. Jamdagni, P. Khatri, J.S. Rana, Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity, J. King Saud Univ.-Sci. (2016), doi: 10.1016/j.jksus.2016.10.002.

5- S. Vijayakumar, G. Vinoj, B. Malaikozhundan, S. Shanthi, B. Vaseeharan, Plectranthus tranthus amboinicus leaf extract mediated synthesis of zinc oxide nanoparticles and its control of methicillin resistant Staphylococcus aureus biofilm and blood sucking mosquito larvae, Spectrochimica Acta–Part A 137 (2015) 886–891, doi: 10.1016/j.saa.2014.08.064.

6- S. Gunalan, R. Sivaraj, V. Rajendran, Green synthesized ZnO nanoparticles against bacterial and fungal pathogens, Prog. Nat. Sci. 22 (2012) 693–700, doi: 10.1016/j.pnsc.2012.11.015.

7-S. Stankic, S. Suman, F. Haque, J. Vidic, Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties, J. Nanobiotechnol. 14 (2016) 73, doi: 10.1186/s12951- 016- 0225- 6.

8- 3. Hamada, S., and H. D. Slade. 1980. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol. Rev. 44:331–384.

9- Yamashita, Y., W. H. Bowen, R. A. Burne, and H. K. Kuramitsu. 1993. Role of the Streptococcus mutans gtf genes in caries induction in the specificpathogen-free rat model. Infect. Immun. 61:3811–3817).

10- 5. Costerton, J. W., P. S. Stewart, and E. P. Greenberg. 1999. Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322.

11- Surette, M. G., M. B. Miller, and B. L. Bassler. 1999. Quorum sensing in Escherichia coli, Salmonella typhimurium, and Vibrio harveyi: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA 96:1639–1644.

14- Ibekwe, C. A., Oyatogun, G. M., Esan, T. A., Oluwasegun, K. M. Synthesis and characterization of chitosan/gum Arabic nanoparticles for bone regeneration. American Journal of Materials Science and Engineering, 5(1), 28-36(2017). (

15-Singh, P., Kim, Y.J., Zhang, D., Yang, D.C., 2016. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. 34 (7), 588–599.

16- Yuvakkumar, R., Suresh, J., Saravanakumar, B., Nathanaeld, A.J., Honga, S.I Rajendran, 2015. Rambutan peels promoted biomimetic synthesis of bioinspired zinc oxide nanochains for biomedical applications. Spectrochim Acta Part A Mol. Biomol. Spectrosc. 137, 250–258.

17- Awwad, A.M., Salem, N.M., Abdeen, A.O., 2013. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. Int. J. Ind Chem. 4, 29.

18- Khoshhesab, Z.M., Sarfaraz, M., Asadabad, M.A., 2011. Preparation of ZnO nanostructures by chemical precipitation method. Synth. React. Inorg., Met- Org., Nano-Met. Chem. 41 (7), 814–819.

19- Kumar, S.S., Venkateswarlu, P., Rao, V.R., Rao, G.N., 2013. Synthesis, characterization and optical properties of zinc oxide nanoparticles. Int. Nano Lett. 3, 3.

20- G.D. Venkatasubbu, R. Baskar, T. Anusuya, C.A. Seshan, R. Chelliah, Toxicity mechanism of titanium dioxide and zinc oxide nanoparticles against food pathogens, Colloids Surf. B 148 (2016) 600–606, doi: 10.1016/j.colsurfb.2016.09. 042 .

21- Mathur, T., S. Singhal., S. Khan., D.J. Upadhyay., T. Fatma and A. Rattan.2006. Detection of Biofilm Formation among The Clinical Isolates of Staphylococci: An Evaluation of Three Different Screening Methods.Indian *J. Med. Microbiol*. 24(1):25-29.

22- Álvarez-Martínez, F., Barrajón-Catalán, E. and Micol, V., 2020. Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. *Biomedicines*, 8(10), p.405.

23- Arvanati, A., N. K. Karamanos., G. Dimitracopoulos and E. D.Anastassiou.1994. Isolation and characterization of a novel 20 kDasulfated polysaccharide from the extracellular slime layer of *Staphylococcus epidermidis*. Arch. *Biochem. Biophys*. 308:432–438.

24- Salem W, Leitner DR, Zingl FG, et al. Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli*. *Int J Med Microbiol*. 2015;305(1):85–95.

25- Adams LK, Lyon DY, Alvarez PJJ (2006) Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. Water Res 40:3527–3532.

26- Prasad TNVKV, Elumalai EK, Khateeja S (2011) Evaluation of the antimicrobial efficacy of phytogenic silver nanoparticles. Asian Pac J Trop Biomed 82–85.