RESEARCH ARTICLE OPEN ACCESS

Bactericidal Activity of Oleo-gum Resins Doped with Metal Oxides

MSc Diogo José Horst[1], Dr. Sergio Mazurek Tebcherani[1], Dr. Evaldo Toniolo Kubaski[2], Dr. Rogério de Almeida Vieira[3]
[1](*Department of Production Engineering), Federal University of Technology (UTFPR), Brazil.*
[2](*Department of Materials Engineering), Ponta Grossa State University (UEPG), Brazil.*
[3](*Department of Exact and Earth Sciences), Federal University of São Paulo (UNIFESP), Brazil.*

ABSTRACT
This work aimed to evaluate the bactericidal activity of vegetable oil-gum resins (*Styrax benzoin*, *Commiphora mirrha*, and *Boswellia papyrifera*) doped with metal oxides (TiO_2, P25, MoO_3 e Cu_2O) in nanometric dimension. The in vitro susceptibility of pathogenic Gram-negative bacteria *E. coli* was accessed. The antibiogram essay was performed using the semi-quantitative method Kirby-Bauer (KB) agar diffusion test. The materials obtained were characterized by ultraviolet visible (UV-Vis) spectrophotometry, additionally; its morphology was characterized by scanning electron microscopy (SEM). The results show that in most cases the materials present bactericidal activity, thereby inhibiting both planktonic and surface associated growth of this microorganism.
Keywords: Bactericidal activity, Biopolymers, Nanotechnology, Plant resins, Ceramic metal oxides.

I. INTRODUCTION

The history of medicine and pharmacy is well known to use extracts and oil-gum resins from plants to cure diseases. These are known to have analgesic, antioxidant, antifungal, antiseptic, antibacterial, astringent, sedative and stimulant action among other therapeutic properties [1-2-3-4-5].

A demonstration of the presence of natural products such as terpenes, alkaloids, flavonoids, coumarins and other medicinal plant secondary metabolites provides scientific validation for the popular use of these plants [6-7-8].

Resins mainly contain terpenoid compounds with a few or no carbohydrates and are generally insoluble in water, unlike gums that are soluble in water and predominantly composed of numerous carbohydrates [9].

These characteristics means that resins play a good role for binding/bonding purposes [10]. From the analytical point of view, a majority of studies presented in the literature are focused on the characterization of aromatic compounds of monolignol type that constitute a major part of the resins [11].

Recently, the use of resins for the greener synthesis of new antibacterial compounds has been widely studied by several researchers [12-13-14-15-16].

In fact, the discovery and synthesis of new antimicrobial agents has been gaining more interest in academic research and also in industry due to its potential applications, providing quality and safe benefits for many materials.

However, the low molecular weights of the antimicrobial agents suffer from many disadvantages, such as the toxicity to the environment and the short-term antimicrobial activity [17].

Metal/ceramic nanoparticles have demonstrated determined bioactivity due to oligodynamic action. The addition of metal particles such as gold, silver and titanium has been suggested by several researchers as efficient in combating various pathogenic microorganisms [18].

Different composites have been studied in order to obtain a material with specific properties desired for biomedical applications, normally polymer matrices doped with ceramic/glass particles have been investigated [19-20-21].

However, while numerous materials have been investigated, the optimum (ideal) material has yet to be developed due to the complex nature of the intended application [22].

Nature can combine fragile minerals and organic molecules into hybrid composites that are highly organized to achieve exceptional fracture resistance [23-24].

Hybrid nanostructures with organic-inorganic phase and materials on their base are presented as a promising class of advanced multifunctional materials [25-26].

Within this context, the aim of this research was to study the bactericidal activity of vegetable resins in pure state and also doped with nanoparticles of metallic oxides in the formulation of hybrid materials (inorganic/organic) in an attempt to inhibit the growth of Gram-negative bacillary bacteria *Escherichia coli.*

II. MATERIALS AND METHODS

2.1 Materials

The oleo-gum resins from benzoin (*Styrax benzoin*) originally harvested from Singapore, myrrh (*Commiphora myrrha*) from Somalia, and olibanum (*Boswellia papyrifera*) from Ethiopia were purchased from Mountain Rose Herbs (Eugene, Oregon, USA). The *Escherichia coli* ATCC-8739 was original from the American Type Culture Collection (ATCC, Manassas, VA, USA). Muller-Hilton agar media was purchased from Kasvi, Brazil.

2.2 Method

In vitro tests were performed following the Kirby-Bauer semi-quantitative methodology [27-28]. Mueller-Hinton agar (MHA) plates were used to determine the antimicrobial activity of the resins in pure state and also doped with the metal oxides: titanium (TiO_2), anatase (P25), molybdenum trioxide (MoO_3), and copper I (Cu_2O) oxide were separately added to the resins at a fixed concentration of 5%, this value was defined by the sensitivity of the microorganisms to the antimicrobial Through the minimum inhibitory concentration (MIC) [29].

In the tests, the agar medium was prepared in Petri dishes; in sequence the material to be tested was inserted in form of disks measuring 1cm x 1 cm. Utilizing a transfer swab 10^6 cells mL^{-1} suspension containing microorganisms were inoculated into the agar medium. Then plates were incubated into bacteriological greenhouse at 37 °C during 48 h, with growth controls performed at every 6 hours. During essays, the evaluation of bacterial biofilm formation by the microorganism in question was also evaluated. The antibacterial efficacy was measured by taking into account the halo created between the materials and the colony forming units (CFU). To obtain a more accurate count of viable cells, the software ImageJ with the automated plug-in for colony counting was used [30-31-32].

Microscopy analysis was performed using a Jeol electronic microscope (JSM-6610LV). The tests were carried out in accordance with ISO-16700: 2004 and ASTM E2809-13.

Spectroscopy analyses in the UV-visible region were performed using a Shimadzu equipment (IR-Prestige-21) calibrated in the spectral range of 300nm to 900nm.

For statistical analysis of the antibiogram assay the content used was the experimental design of contextualized blocks [33-34]. In this research the blocks are as 16 samples (raw data) and the treatments are the 15 types of bactericidal material + test control plate. For the treatment of the obtained data, the statistical software SASM Agri-8.1 was used [35].

III. RESULTS AND DISCUSSION

The Figure 1, Figure 2, Figure 3 and Figure 4 present the results regarding the antibiogram tests for the Gram-negative pathogenic microorganism *Escherichia coli*, using the resins of *S. benzoim* (resin B), *C. myrrha* (resin M) and *B. papyrifera* (resin P) in pure state and also doped with oxides titanium (TiO_2 - P25), molybdenum (MoO_3), and copper I (Cu2O).

During the initial period of 6 hours, the analyzes were similar for the compositions under study, except for the benzoin resin doped with titanium oxide ($B+TiO_2$), which presented greatest bactericidal activity during this time interval, however after 12 hours of test this material presented a decrease in activity.

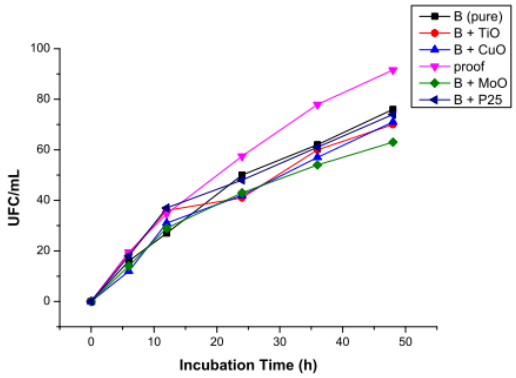

Fig. 1. Antibiogram of *E. coli* using the benzoin resin in pure state and also doped with metal oxides.

During the 12 hour time period, the plate without bactericidal material (control plate) differed significantly (p = 5%) from most cases, except for *S. benzoin* doped with molybdenum trioxide ($B+MoO_3$), *S. benzoin* containing copper oxide ($B+Cu_2O$), *B. papyrifera* doped with titanium oxide ($P+TiO_2$), *B. papyrifera* doped with molybdenum oxide ($P+MoO_3$), *B. papyrifera* containing copper oxide ($P+Cu_2O$), *C. myrrha* in pure state (pure M), and *C. myrrha* doped with titanium oxide (M+P25).

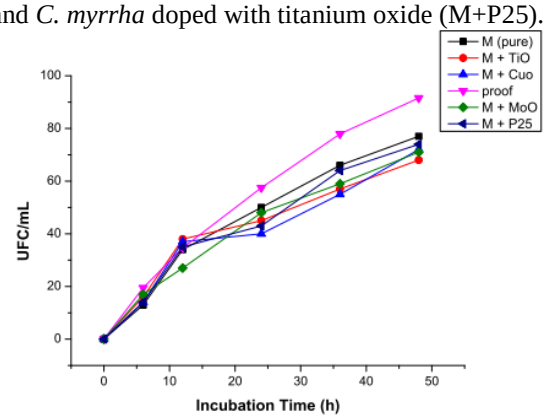

Fig. 2. Antibiogram of *E. coli* using the myrrh resin in pure state and also doped with metal oxides.

In the 24-hour essay period, it was observed that the plate containing the *E. coli* microorganism without addition of bactericidal material (test plate) differed significantly (p = 5%) from most cases, except for the plate containing the *S. benzoin* in pure state (B pure), *S. benzoin* containing anatase oxide (B+P25), *B. papyfera* in pure state (P pure), *B. papyrifera* resin doped with molybdenum trioxide (P+MoO_3), *C. myrrha* in pure state (M pure), and *C. myrrha* containing molybdenum trioxide (M+MoO_3). Therefore, these materials showed the highest bactericidal activity in regarding to the initial colonization and biofilm formation by *E. coli.*

During the initial 36 hours of test, in most cases the test plate differed significantly from the other plates containing the materials in question, except in *B. papyrifera* containing anatase (P+P25), *C. myrrha* in pure state (M pure), and *C. myrrha* containing anatase oxide (M+P25), thus indicating that these materials showed more efficiency in regarding to prevent the initial colonization and biofilm formation of the microorganism during this period of time.

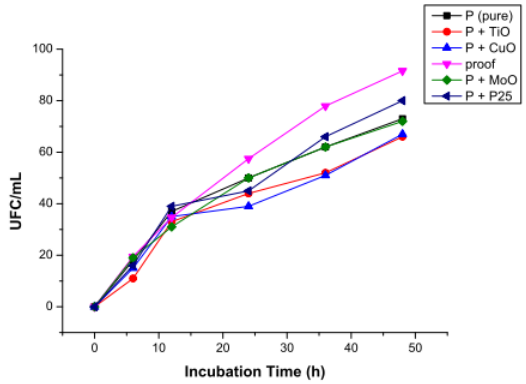

Fig. 3. Antibiogram of *E. coli* using the olibanum resin in pure state and also doped with metal oxides.

During the last 48 hours of the test, there was a significant difference between the test plate and the most cases, with a single exception in the resin *B. papyrifera* doped with titanium oxide (P + P25).

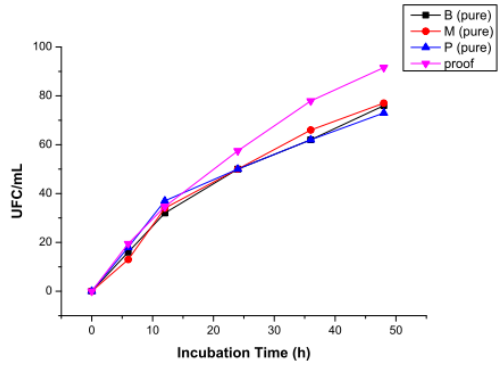

Fig. 4. Antibiogram of *E. coli* using the resins in pure state

Summarizing, it can be seen that in most cases the materials presented bactericidal activity, mainly during the first 24 hours of the test, thus inhibiting the initial growth and proliferation of the microorganism in question.

In relation to the formation of bacterial biofilms, there is a first yet reversible adhesion maintained by non-specific physic-chemical interactions constituent of the foundation for the biofilm growth. A second phase of adhesion consists of the transition from the reversible to the irreversible stage, at which point the bacteria begin to secrete substances that are responsible for maintaining the adhesion and the layer that surrounds the biofilm. Still in this phase, there is the beginning of formation of micro-colonies and the development of the architecture of the mature biofilm [36-37].

Figure 5 shows the *E. coli* biofilm during the first 24 hours of assay.

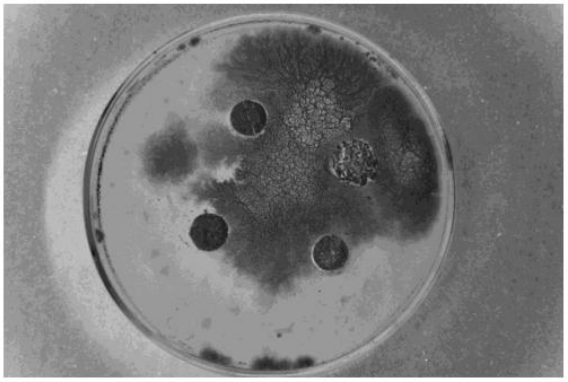

Fig. 5. Formation of *E. coli* biofilm on Petri dish containing pure *B. papyrifera* resin

Mature bacterial biofilms are surrounded by pores and water channels that function as a system for the exchange of nutrients, oxygen, and metabolites that need to be secreted out of the biofilm [38]. The irreversible biofilm fixation of *E. coli* normally occurs during the first hours of colonization of this microorganism [39-40].

Figure 6 shows the spectrograms in the UV-vis region for the samples of *B. papyrifera* (resin P), *C. myrrha* (resin M) and S. *benzoin* (resin B) in pure state. The peak identified between ~350 nm and ~420 nm confirms the presence of olibanum gum (*Boswellia papyrifera*) [16]. Important to emphasize that *B. papyrifera* resin presents several diterpenes and tetranortriterpenoids [41].

The phytochemical analysis of the bark and stem strata of *B. papyrifera* found several stilbene and triterpene glycoside groups along with known compounds: boswellic acid, beta-elemonic acid, beta-boswellic acid, and beta-sitosterol [42].

Noteworthy, stilbene glycosides exhibit significant inhibition of phosphodiesterase I and xanthine oxidase. The triterpenes (3-9) exhibited endopeptidase inhibitory activities [43]. So the endopeptidases break down amino acid peptides bonds [44], thus the production of high molecular weight extracellular polymer substances (SPE) secreted by *E. coli* is therefore reduced, inhibiting their growth.

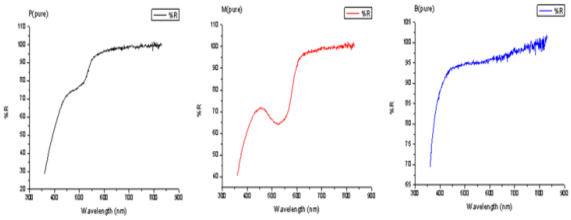

Fig. 6. Spectrogram of plant resin samples in pure state: *B. papyrifera, C. myrrha* and *S. Benzoin*

The SPE's establish the functional and structural integrity of biofilms, being considered fundamental components that determine the physical properties of a biofilm [45]. Most of the SPE's are composed of extracellular polysaccharides (PECs) (exopolysaccharides) and proteins, including other macromolecules such as DNA, lipids and humic substances [46].

PECs (bacterial extracellular polymers) are the building material of bacterial settlements, to remain attached to the outer surface of the cell. These compounds are important in the formation of biofilm and surface-bound cells. PECs make up 50% to 90% of the total organic matter of a biofilm [47-48].

Still in relation to Figure 6, the peak identified around 400nm with a prominent secondary peak around 480nm proves the presence of myrrh extract [49]. As shown in the present work, other studies confirm the antimicrobial activity present in resin oil and myrrh extracts, mainly due to the presence of monoterpenes and sesquiterpenes [50-51]. The triterpenoids are the main constituents isolated from the resin of the genus Commiphora while the flavonoids and lignans commonly occur in the stems of this species [52]. In addition, the chemical composition of the essential oils of several species of Commiphora mainly presents monoterpenes, oxygenated sesquiterpenes and hydrocarbons that invariably differ from species to species [53-54].

Still according to Figure 6, the prominent peak found around 350nm with molecular vibration extending to a peak near at 450nm confirms the presence of the resin oil of *Sryrax benzoin* [55].

The oleoresin of benzoin is composed of benzoic acid, cinnamic acid, oleanoic acid and terpenes. Several compounds present in the resin oil of the genus Styrax present antibacterial and antifungal activity, mainly due to the presence of triterpenes and sesquiterpenes [56].

Therefore, the presence of monoterpenes, diterpenes and triterpenes justifies the results presented in this work considering the bactericidal action identified in the plates containing the resins of *C. myrrha, S. benzoin* and *B. papyrifera* in the pure state.

Figure 7 shows the spectrograms in the UV-visible region for samples *B. papyrifera* (resin P), *C. myrrha* (resin M) and *S. Benzoin* (resin B) doped with titanium and anatase (TiO2 and P25) oxides:

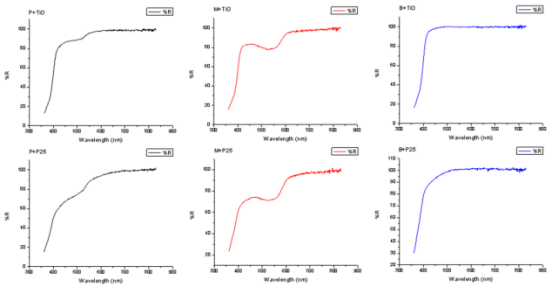

Fig. 7. Spectrogram of the resin samples *B. papyrifera, C. myrrha* and S. *benzoin* doped with titanium oxides (TiO2 - P25).

The spectra with wavelength intensities >350 nm are typical for titanium dioxide (TiO_2), and anatase + rutile (P25), confirming the results presented in Figure 7 [57].

Figure 10 shows spectrograms in the UV-vis region for samples of *B. papyrifera* (resin P), *C. myrrha* (resin M), and *S. benzoin* (resin B) containing molybdenum trioxide (MoO_3) and copper (Cu_2O) oxide.

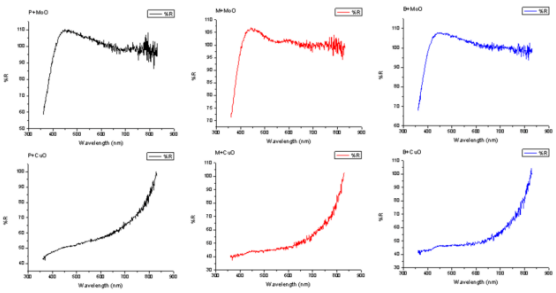

Fig. 8. Spectrogram of the resin samples *B. papyrifera, C. myrrha* and S. *benzoin* doped with molybdenium trioxide (MoO3) and copper (Cu_2O).

According to Figure 8, the UV-Vis spectrum with wavelength intensity > 300 nm is typical for diametric and/or oligomeric species, thereby confirming the identification of MoO_3 [57]. In the same figure, distinct peaks are observed

around ~ 600 nm and the peak at the intensity of 800 nm confirms the presence of copper oxide I [58].

Figure 9 and Figure 10 show micrographs of the surface structure of *C. myrrha* resin containing copper oxide and *B. papyrifera* containing titanium oxides, respectively.

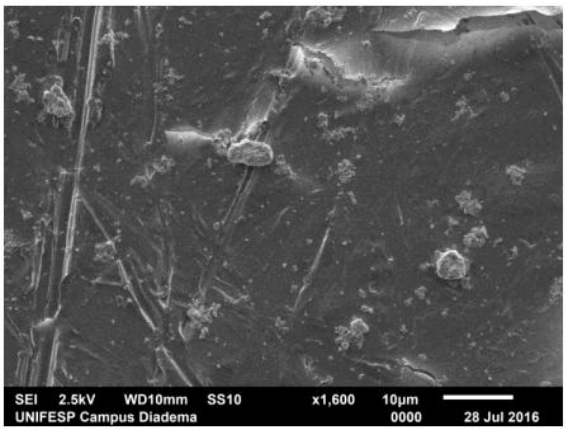

Fig. 9. Surface morphology of the resin sample C. myrrha doped copper oxide I (Cu_2O)

According to Figure 9 it can be seen that the incorporation of the oxides into the polymer matrix of the *C. myrrha* resin was not efficient, the copper oxide I remained heterogeneously dispersed on its surface.

Figure 10 shows the deposition of the titanium oxide particles (P25) on the surface of the *B. papyrifera* resin, in which case the dispersion was more uniform, but in the same way the oxide remained superficially pooled on the surface of the material. It can also be seen that this resin has high porosity.

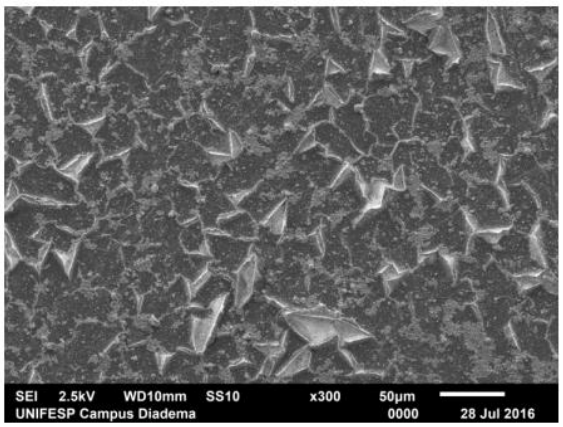

Fig. 10. Surface morphology of resin sample *B. papyrifera* doped with anatase oxide (P25).

IV. CONCLUSION

In the majority of the cases analyzed, the *B. papyrifera*, *C. myrrha* and *S. benzoin* resins in the pure state as well as titanium and anatase oxides (TiO2-P25), molybdenum trioxide (MoO_3) and copper oxide (Cu_2O) showed bacteriostatic activity against the pathogen *E. coli*, inhibiting its growth and biofilm formation. The incorporation of the oxides in the polymeric matrix of the resins doesn't showed homogeneity, being these unordered dispersed on its surface.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Industrial Engineering from the University of Santa Catarina State (UDESC) and the Department of Materials from Federal University of São Paulo (UNIFESP) for the availability of laboratories and helping in testing, and also the Brazilian Agency for the Coordination of Improvement of Higher Level Personal (CAPES) for the doctoral scholarship granted.

REFERENCES

[1] H. Kong, J. Yang, Y. Zhang, Y. Fang, K. Nishinari, G.O. Phillips. Synthesis and antioxidant properties of gum arabic-stabilized selenium nanoparticles. *International Journal of Biological Macromolecules*, *65*:155–162, 2014.

[2] A.N. Shikov, O.N. Pozharitskaya, V.G. Makarov, H. Wagner, R. Veerporte, M. Heinrich. Medicinal Plants of the Russian Pharmacopoeia; their history and applications. *Journal of Ethnopharmacology*, *154*:481–536, 2014.

[3] S. Sultana, H.M. Asif, N. Akhtar, K. Ahmad. Medicinal plants with potential antipyretic activity: A review. *Asian Pac. J. Trop. Dis., 5(1):*202-208, 2015.

[4] E. Özdemir & K. Alpinar. An ethnobotanical survey of medicinal plants in western part of central Taurus Mountains: Aladaglar (Nigde–Turkey). *Journal of Ethnopharmacology, 166*:53-65, 2015.

[5] M. Bahmani, K. Saki, S. Shahsavari, M. Rafieian-Kopaei, R. Sepahvand. Identification of medicinal plants effective in infectious diseases in Urmia, northwest of Iran. *Asian Pac. J. Trop. Biomed., 5(10)*:858–864, 2015.

[6] A.A. Mohamed, S.I. Ali, F.K. El-Baz, A.K. Hegazi, M.A. Kord. Chemical composition of essential oil and in vitro antioxidant and antimicrobial activities of crude extracts of *Commiphora myrrha* resin. *Industrial Crops and Products, 57*:10–16, 2014.

[7] S. Su, T. Wang, J-A, Duan, W. Zhou, Y-Q. Hua, Y-P. Tang, L. Yu, D-W. Quian. Anti-inflammatory and analgesic activity of different extracts of *Commiphora myrrha*. *Journal of Ethnopharmacology, 134:*251–258, 2011.

[8] S.D. Caetano da Silva, M.G. Mendes de Souza, M.J. Oliveira Cardoso, et al. Antibacterial activity of *Pinus elliottii* against anaerobic bacteria present in primary endodontic infections. *Anaerobe, 30*:146-152, 2014.

[9] P. Velmurugan, J. Shim, K. Kim, O. Byung-Taek. Prunus × yedoensis tree gum mediated synthesis of platinum nanoparticles with antifungal activity against phytopathogens. *Materials Letters, 174*:61-65, 2016.

[10] C.D. Matheson, A.J. McCollum. Characterizing native plant resins from Australian Aboriginal artifacts using ATR-FTIR and GC/MS. *Journal of Archaeological Science, 52:*116-128, 2014.

[11] J.H. Langenheim. *Plant Resins: Chemistry, Evolution, Ecology, and Ethno botany*. (Timber Press, Portland, 2003).

[12] C. Dong, X. Zhang, H. Cai, C. Cao. Facile and one-step synthesis of monodisperse silver nanoparticles using gum acacia in aqueous solution. *Journal of Molecular Liquids, 196*:135–141, 2014.

[13] D. Pooja, S. Panyaram, H. Kulhari, S.S. Rachamalla, R. Sistla. Xanthan gum stabilized gold nanoparticles: Characterization, biocompatibility, stability and cytotoxicity. *Carbohydrate Polymers, 110:*1–9, 2014.

[14] S.K. Bajpai, & M.A. Kumari. Green approach to prepare silver nanoparticles loaded gum acacia/poly (acrylate) hydrogels. *International Journal of Biological Macromolecules, 80:*177–188, 2015.

[15] Y-F. Li, W.P. Gan, J. Zhou, Z-Q. Lu, C. Yang, T-T. Ge. Hydrothermal synthesis of silver nanoparticles in Arabic gum aqueous solutions. *Trans. Nonferrous Met. Soc. China, 25:*2081−2086, 2015.

[16] A.J. Kora, & L. Rastogi. Green synthesis of palladium nanoparticles using gum ghatti (*Anogeissus latifolia*) and its application as an antioxidant and catalyst. Arabian *Journal of Chemistry, Appl. Nanosci., 5:*315-320, 2015.

[17] N. Nichat, T. Ahamad, S.M. Alshehri, S. Parveen. Synthesis, characterization, and biocide properties of semicarbazide–formaldehyde resin and its polymer metal complexes. *European Journal of Medicinal Chemistry 45:*1287–1294, 2010.

[18] H. Kong, J. Yang, Y. Zhang, Y. Fang, K. Nishinari, G.O. Phillips. Synthesis and antioxidant properties of gum arabic-stabilized selenium nanoparticles. *International Journal of Biological Macromolecules, 65*:155–162, 2014.

[19] M. Wang. Developing bioactive composite materials for tissue replacement. *Biomaterials, 24(13):*2133–2151, 2003.

[20] M. Venkatesham, D. Ayodhia, A. Madhusudhan, A.S. Kumari, G. Veerabhadram, K.G. Mangatayaru. A Novel Green Synthesis of Silver Nanoparticles Using Gum Karaya: Characterization, Antimicrobial and Catalytic Activity Studies. *Journal of Cluster Science, 25(2):*409-422, 2014.

[21] R.L. Simpson, S.N. Nazhat, J.J. Blaker, A. Bismarck, R. Hill, A.R. Boccaccini, U.N. Hansen, A.A. Amis. A comparative study of the effects of different bioactive fillers in PLGA matrix composites and their suitability as bone substitute materials: A thermo-mechanical and in vitro investigation. *Journal of the mechanical behavior of biomedical materials, 50*:277–289, 2015.

[22] K. Rezwan, Q.Z. Chen, J.J. Blaker, A.R. Boccaccini. Biodegradable and bioactive porous Polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials, 27(18)*:3413–3431, 2006.

[23] G. Mayer. Rigid biological systems as models for synthetic composites. *Science, 310:*1144-1147, 2005.

[24] E. Munch, M.E. Launey, D.H. Alsem, E. Saiz, A.P. Tomsia, R.O. Richie. Tough, bioinspired hybrid materials. *Science, 322:*1516-1520, 2008.

[25] J. Yuan, A.H.E. Muller. One-dimensional organic–inorganic hybrid nanomaterials. *Polymer, 51:*4015-4036, 2010.

[26] T. Zima, N. Baklanova, I. Bataev. Synthesis and characterization of hybrid nanostructures produced in the presence of the titanium dioxide and bioactive organic substances by hydrothermal method. *Journal of Solid State Chemistry, 198:*131–137, 2013.

[27] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol., 45:*493–496, 1966.

[28] B. Bonev, J. Hooper, J. Parisot. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *J. Antimicrob. Chemother., 61(6):*1295-301, 2008.

[29] J.M. Andrews. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother., 48(1):*5-16, 2001.

[30] J. Marotz, C. Lubbert, W. Eisenbeiss. Effective object recognition for automated counting of colonies in Petri dishes (automated colony counting). *Comput*

*Methods Programs Biomed., 66:*183–198, 2001.

[31] M. Putman, R. Burton, M.H. Nahm. Simplified method to automatically count bacterial colony forming unit. *J. Immunol. Methods, 302*:99–102, 2005.

[32] S. Siewerts, F.A.M. De Bok, E. Mols, W.M. De Vos, J.E.T. Van HylckamaVlieg. A simple and fast method for determining colony forming units. *Letters in Applied Microbiology, 47(4):*275-278, 2008.

[33] M.I. Rodrigues, & A.F. Lemma. *Planejamento de experimentos e otimização de processos*. (Editora: Cárita, 2ª ed.) Campinas-SP, 2009.

[34] C.A. Mucelin. *Estatística Elementar e experimental aplicada às tecnologias*. (Editora: Valério, 2ª ed.) Medianeira-PR, 2006.

[35] M.G. Canteri, R.A. Althaus, J.S. Virgens Filho, E.A. Giglioti, C.V. Godoy. SASM - Agri: Sistema para análise e separação de médias em experimentos agrícolas pelos métodos Scoft - Knott, Tukey e Duncan. *Revista Brasileira de Agrocomputação, 1(2):*18-24. 2001.

[36] R.M. Donlan. Biofilms: microbial life on surfaces. *Emerging Infect. Dis., 8(9):*881–90, 2002.

[37] R.M. Donlan, & J.W. Costerton. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev., 15(2):*167–93, 2002.

[38] P. Stoodley et al. Biofilms as complex differentiated communities. *Annual Reviews in Microbiology, 56:1:*187-209, 2002.

[39] M.Q. Carter, J.W. Louie, D. Feng, W. Zhou, M.T. Brandl. Curli fimbriae are conditionally required in *Escherichia coli* O157:H7 for initial attachment and biofilm formation. *Food Microbiology, 57*:81-89, 2016.

[40] A.H.P. Anvarian, M.P. Smith, T.W. Overton. The effects of orange juice clarification on the physiologyof *Escherichia coli*; growth-based and flow cytometric analysis. *International Journal of Food Microbiology, 219:*38–43, 2016.

[41] D. Bekana, T. Kebede, M. Assefa, K. Habtemarian. Comparative Phytochemical Analyses of Resins of Boswellia Species (*B. papyrifera* (Del.) Hochst., B. *neglecta* S. Moore, and *B. rivae* Engl.) from Northwestern, Southern, and Southeastern Ethiopia. *ISRN Analytical Chemistry, Vol. 2014*, 9 pages, 2014.

[42] E.M. Abdalah. & A.E. Khalid. A preliminary evaluation of the antibacterial effects of *Commiphora molmol* and *Boswellia papyrifera* oleo-gum resins vapor. *International Journal of Chemical and Biochemical Sciences, 1*:1-5, 2012.

[43] H. Safayhi. & E.R. Sailer. Anti-inflammatory actions of pentacyclic triterpenes, *Planta Medica, 63*:487-493, 1997.

[44] H-C. Flemming, J. Wingender, T. Griebe, C. Mayer. P*hysico-Chemical Properties of Biofilms*, in EVANS, L.V. *Biofilms: Recent Advances in their Study and Control*. (CRC Press) ISBN: 978-9058230935, 2000.

[45] C. Staudt, H. Horn, D.C. Hempel, T.R. Neu. Volumetric measurements of bacterial cells and extracellular polymeric substance glycoconjugates in biofilms. *Biotechnol. Bioeng., 88(5):*585–92, 2004.

[46] M. Ullrich. *Bacterial Polysaccharides: Current Innovations and Future Trends*. (Caister Academic Press). ISBN: 978-1-904455-45-5. 2009.

[47] Surech & Mody. *Microbial Exopolysaccharides: Variety and Potential Applications*. *Microbial Production of Biopolymers and Polymer Precursors*. (Caister Academic Press). ISBN: 978-1-904455-36-3, 2009.

[48] A.D. Welman. *Exploitation of Exopolysaccharides from lactic acid bacteria*. *Bacterial Polysaccharides: Current Innovations and Future Trends*. (Caister Academic Press). ISBN: 978-1-904455-45-5. 2009.

[49] I.M. El-Sherbini, E. Salih, F.M. Reicha. Green synthesis of densely dispersed and stable silver nanoparticles using myrrh extract and evaluation of their antibacterial activity. *Journal of Nanostructure in Chemistry, 3:8:1-*7, 2013.

[50] D.A. Tipton, B. Lylea, H. Babichb, M.K.H. Dabbousa. In vitro cytotoxic and anti-inflammatory effects of myrrh oil on human gingival fibroblasts and epithelial cells. *Toxicol. In Vitro, 17*:301–310, 2003.

[51] A.M. Massoud, F.H. El Abiary, M.M. Abou-Gamra, G.F. Mohamed, S.M. Shaker. Evaluation of schistosomicidal activity of myrrh extract: parasitological and histological study. *J. Egypt. Soc. Parasitol., 34*:1051–1076, 2004.

[52] T. Shen, G-H. Li, X-N. Wang, H-X. Lou. The genus Commiphora: a review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol., 142*:319–330, 2012.

[53] K.H.C. Baser, B. Demirci, A. Dekebo, A. E. Dagne. Essential oils of some Boswellia ssp. myrrh and opopanax. *Flavour Fragance J., 18*:153–156, 2003.

[54] B. Marongiu, A. Piras, S. Porcedda, A. Scorciapino. Chemical composition of the essential oil and supercritical CO 2 extract of Commiphora myrrha (Nees) Engl. and of Acorus calamus L. *J. Agric. Food Chem., 53:*7939–7943, 2005.
[55] M. Houvaneissian, P. Archier, C. Mathe, G. Culioli, C. Vieillescazes. Analytical investigation of styrax and benzoin balsams by HPLC- PAD-fluorimetry and GC-MS. *Phytochem. Anal., 19:*301–310, 2008.
[56] F. Modugno, E. Ribechini, M.P. Colombini. Aromatic resin characterization by gas chromatography–mass spectrometry Raw and archaeological materials. *Journal of Chromatography A, 1134*:298–304, 2006.
[57] J.P. Thieleman, T. Ressler, A. Walter, G. Tzolova-Muller, C. Hess. Structure of molybdenum oxide supported on silica SBA-15 studied by Raman, UV–Vis and X-ray absorption spectroscopy. *Applied Catalysis A: General, 399*:28–34, 2011.
[58] G. Varuguese, V. Rini, S.P. Suraj, K.T. Usha. Characterization and optical studies of copper oxide nanostructures doped with lanthanum ions. *Advances in Materials Science, 14(42):*49-60, 2014.