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Studies on the possibilities of useing of the Epilobium parviflorum Schreb species in alternative endodontic therapy

Abstract of PhD Thesis

PhD Coordinator

Prof. PhD. Victoria Badea

PhD Student

Erdogan Elvis Șachir

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Motto:

" The doctor cares, the nature heals."

(Hippocrates)

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INTRODUCTION

Currently, there is an upward trend regarding the use of herbs in the substitution and/or supplementation of the action of the synthetic drugs, because these besides the beneficial therapeutic effects also have side effects and adverse effects. Specialists in the field recommend the use of natural remedies as an adjunct to classical therapy, all the more so as the antibacterial action of the plant extracts could limit the expansion of bacterial resistance to antibiotics, a problem that has dominated the second millennium and persists in an upward trend in the one of the third millennium [1].

Pierre Fauchard introduced for the first time the concept of endodontics and is called the father of modern dentistry; wrote the first book on dentistry called "Le Chirurgien Dentiste" in 1728, in which he scientifically describes pulp pathology as well as the possibility of pulp tissue removal [2].

Periapical endodontic disorders have always been a priority topic for dentists, who have been constantly concerned about the alleviation of these diseases by means that have improved technically over time. Numerous experiments have been carried out over time, ranging from empirical treatments to modern techniques used today. These were based on the new

discoveries related to the total deciphering of the structure of the component tissues of the pulpo-dentinary and periradicular complex as well as an increasing understanding of the impact that endodontic disorders have on disturbing the homeostasis of the oral-dental cavity as well as of homeostasis of the whole organism [3].

Currently, it is unanimously accepted worldwide that AP is a consequence of bacterial infection at the level of the endodontic space; for these reasons, identifying the most effective techniques for removing the bacterial biofilm from the endodontic space could be one of the most effective therapeutic solutions for the prevention of endodontic infections [5].

The discovery of new antibiotics remains a major challenge for physicians, pharmacists, chemists and geneticists; In this context, planets can be a valuable source of biologically active natural compounds that can be used as alternative therapeutic agents or as basic nuclei for new synthetic products with increased antibacterial activity and as few side effects as possible [6,7].

In the category of plants with antibacterial, anti-inflammatory and antioxidant properties, Epilobium parviflroum Schreb is also found. In the accessed literature there are

empirical data showing that this plant has been used in traditional medicine to treat different diseases, without specifying the active principles that generate the processes of improvement or cure of diseases [8,9,10] .

GENERAL PART

The first chapter of the thesis describes bacterial physiology, bacterial biofilm and bacterial species involved in endodontic pathology.

An element of the news is the inter-bacterial communication that determines a new perspective in the application of the therapeutic schemes, improving the bactericidal effect of the antibiotics; communication inhibition can be achieved by interfering with signaling pathways or by using signal molecules.

Another important element described in the general part is the endodontic diseases in the context of the oral-dental pathology and in the context of systemic diseases.

Towards the end of the general part were presented the morphological and chemical properties of the plant Epilobium parviflorum Schreb.

PERSONAL PART

The second part of the thesis is structured in 4 studies as follows:

- identification and quantification of the substances with antibacterial effect in the plant extracts obtained from Epilobium parviflorum Schreb and demonstrating the antibacterial properties;

- evaluation of the in vitro cytotoxicity of the plant extract obtained from Epilobium parviflorum Schreb;

- evaluation of the in vivo toxicity of the plant extract obtained from Epilobium parviflorum Schreb;

- complex study by radioimagistic exams, regarding the effectiveness of the antibacterial activity of the plant extract obtained from Epilobium parviflorum Schreb.

Study I. Evaluation of the antibacterial effect of the plant extracts obtained from Epilobium parviflorum Schreb

Hypothesis of this study: the extracts obtained from Epilobium parviflorum Schreb have an effect on bacterial species involved in endodontic pathology, as compared to the usual antiseptics used in current endodontic practice.

Study purpose: the use of plant extracts, obtained from Epilobium parviflorum Schreb, as a basis for alternative perspective use in the treatment of endodontic pathology.

1.1. Material and method

1.1.1. Harvesting the plant and making extracts from Epilobium parviflorum Schreb

Epilobium parviflorum Schreb is a plant that is part of the Romanian area. The plant is found in the humid areas along the lower hilly and mountain valleys, in Transylvania, Muntenia, Moldova, as well as in the Romanian Plain, Dobrogea [99].

- **Obtaining the aqueous extract**

Description of the technique of double maceration: the extracted product is first mixed with 1 / 2-2 / 3 of the total amount of solvent, after which the liquid is separated and the residue will be pressed. This will contact the rest of the solvent, thus obtaining a new amount of extractive solution. The two extractive liquids will come together and filter after a 24 hour rest [101].

- **Obtaining the hydroalcoholic extract**

The extraction time with alcohol and water is 10 days, shaking 3-4 times a day. After a more active extraction period due to the penetration of the solvent through the walls and the diffusion of the more concentrated solution externally, a balance is established between the two concentrations, inside and outside the plant product and the extractive dissolution stagnates [101].

- **Obtaining the ultrasonic hydroalcoholic extract**

Extraction assisted by ultrasound allows the solvent to penetrate through the cell walls, and the bubbles produced by the acoustic cavitation favor the breakdown of the cell wall and the release of the active compounds, thus increasing the extraction efficiency [104].

1.1.2. Determination of the content of bioactive compounds from plant extracts obtained from Epilobium parviflorum Schreb

- **Determination of total polyphenols**

The Folin-Ciocalteau test method is the simplest method available for measuring the phenolic content of organic products [106].

- **Determination of total flavonoids**

The Folin-Ciocalteau test method was used to determine total flavonoids [106].

1.1.3. Conducting bacteriological tests

- **Harvesting of pathological products**

The pathological product, infected dentine, was harvested using a sterile Kerr cell-type needle, which was subsequently placed in a culture medium container and transported to the Microbiology laboratory of the Faculty of Dental Medicine at the "Ovidius" University of Constanța.

- **Bacteriological identification technique**

- **The technique of testing the antibacterial effect of the aqueous and hydroalcoholic solutions obtained from Epilobium parviflorum Schreb**

- **Sampling technique for semi-quantitative evaluation of the antibacterial effect of the tested solutions**

1.2. Results obtained and discussions

The largest amount of polyphenols and flavonoids was hydroalcoholic extract, followed by ultrasound. The aqueous extract of Epilobium parviflorum Schreb contains the smallest amount of total polyphenols and flavonoids.

The plant extracts obtained from Epilobium parviflorum Schreb have certain antibacterial properties.

STUDY II. In vitro evaluation of the cytotoxicity of the plant extract obtained from Epilobium parviflorum Schreb

Hypothesis of this study: is that the toxicity of the plant extract obtained from Epilobium parviflorum Schreb is lower than the usual antiseptics used in endodontic lavage.

Study purpose: is the in vitro evaluation of the cytotoxicity of the plant extract obtained from Epilobium parviflorum Schreb, which can be used as an alternative to conventional antiseptic irrigant solutions in endodontic therapy.

2.1. Material and method

○ Processing of vegetable extract from Epilobium parviflorum Schreb. Cell cultures

In the cytotoxicity assays, a cell culture line purchased from the ATCC cell bank, namely L929 with fibroblast morphology, was used (ATCC® CCL-1™).

○ MTT test method. Microscopic evaluation

The viability of the cells treated with the extract for 24 hours at different concentrations was measured using 3- (4,5-Dimethylthiazol-2-yl) -2,5-Diphenyl tetrazolium (MTT). The analysis is based on the reduction of a yellow MTT tetrazolium salt to blue-formazan, made by intracellular enzymes, especially those in the mitochondria [117].

2.2. Results obtained and discussions

The absence of cytotoxicity of the plant extract offers the possibility of its use in endodontic therapy, as an alternative to the classic irrigant solutions.

STUDY III. In vivo study of the toxicity of the plant extract obtained from Epilobium parviflorum Schreb

Hypothesis of this study: is that the toxicity of the plant extract obtained from Epilobium parviflorum Schreb is lower than the usual antiseptics used in endodontic lavage.

Study purpose: is to demonstrate the absence of in vivo toxicity in laboratory animals.

3.1. Material and method

- **Biological material used**

The animals used in our experimental model were rats the albino line from Wistar, which were raised and maintained in the Biobaza of the "Ovidius" University of Constanţa, respecting the hygiene, food and accommodation rules imposed by the Community legislation.

3.1.1. Post-extraction dental alveolar toxicity assessment

• Extraction of the maxillary incisors

• Animal sacrifice and preparation of histological examination

3.1.2. Evaluation of liver toxicity

➢ **Preparation of resorbable antiseptic pastes**

Toxicity testing

It was performed according to the method of testing the toxicity of the drugs by placing the test substances under the dermis [127].

➢ **Animal sacrifice and lamella production for histological examination**

The harvested fragment was fixed in 10% formaldehyde for a period of 24 hours and then proceeded to process the samples for inclusion in paraffin, sectioning and staining with HE [128].

The histological preparations were evaluated using the optical microscope and the results are presented and interpreted, experimental group in relation to the control group.

3.2. Results obtained and discussions

The post-extraction healing process occurred faster in the batch where the dehydrated vegetable extract was applied.

The vegetable extract of Epilobium parviflorum Schreb is not liver toxic.

STUDY IV. Radioimaging in the evaluation of the therapeutic effect of the plant extract obtained from Epilobium parviflorum Schreb

Hypothesis of this study: is that the toxicity of the plant extract obtained from Epilobium parviflorum Schreb is lower than the usual antiseptics used in endodontic lavage.

Study purpose: is to verify, by radioimagistic examinations, the antibacterial activity of the plant extract obtained from Epilobium parviflorum Schreb in comparison with three resorbable antiseptic pastes.

4.1. Material and method

- **Delimitation of the study lot**
- **Clinical examination**

The clinical examination aimed to obtain as complete information as possible in order to establish the diagnosis based on anamence stages, exo and endoral clinical examinations.

- **Examination of the retroalveolar radiographs of the endodontic preoperation**

During the paraclinical examination, digital retro-alveolar radiographs were used in a radio imaging center accredited by the National Commission for the Control of Nuclear Activities using a radiation cannon and an RVG sensor.

- **Recording of the Periapical Index Score (PAI)**

The periapical status was evaluated based on the periapical index introduced by Ørstavik D. et al. it is made up of a system of templates with which the comparison of the apical periodontites on the radiographs is performed and based on the similarity between the reference and the comparative radiographs, the classification in a certain class is validated.

- **Chemo-mechanical and endodontic therapy**

4.2. Results obtained and discussions

The clinical examination revealed the absence of pain in the axial and transverse percussion, the absence of dental mobility where preoperative existed, with no visible clinical signs of failure of endodontic treatment.

The plant extract of Epilobium parviflorum Schreb used in endodontic therapy had a positive effect by reducing the periapical index (PAI).

FINAL CONCLUSIONS

1. All three plant extracts obtained from Epilobium parviflorum Schreb contain polyphenols and flavonoids.

2. The highest amount of polyphenols and flavonoids was hydroalcoholic vegetable extract.

3. There are no significant differences regarding the total polyphenols and flavonoids content between the hydroalcoholic vegetable extract and the ultrasonic hydroalcoholic vegetable extract.

4. Herbal extracts obtained from Epilobium parviflorum Schreb certainly have antibacterial properties.

5. There are no differences regarding the effectiveness of the antibacterial effect between the hydroalcoholic vegetable extract and the ultrasonic hydroalcoholic vegetable extract.

6. Hydroalcoholic vegetable extract had the best antibacterial activity.

7. The most sensitive bacterial species were those belonging to the group of Gram positive cocci.

8. The absence of cytotoxicity of the plant extract has been demonstrated both in vitro and in vivo (Wistar rats), which offers the possibility of its use in endodontic therapy as an alternative irrigant solution.

9. Dehydrated vegetable extract from Epilobium parviflorum Schreb decreases the intensity of the post-extraction inflammatory process.

10. The post-extraction healing process occurred faster in the lot where the dehydrated vegetable extract was applied.

11. The plant extract of Epilobium parviflorum Schreb used in endodontic therapy had a positive effect by reducing the periapical index and the extent of the periapical lesion.

12. The therapeutic efficiency of the dehydrated plant extract of Epilobium parviflorum Schreb is close to that of the substances used in classical endodontic therapy.

13. The results support the idea of using the plant extract of Epilbium parviflorum Schreb in endodontic therapy as an alternative to conventional substances or in combination with them to enhance periapical healing.

TABLE OF REFERENCES

1. Fatemeh Jamshidi-Kia, Zahra Lorigooini, Hossein Amini-Khoei, Medicinal plants: Past history and future perspective, J Herbmed Pharmacol. 2018; 7(1): 1-7, doi: 10.15171/jhp.2018.01, https://doi.org/10.15171/jhp.2018.01
2. Aliuddin, Syed & Prakash, Prashanth & Mohiuddin, Sana & Ravula, Sandeep & Nallamilli, Leela & Dutt, Anil. (2017). Historical Milestones in Endodontics: Review of Literature. International Journal of Preventive and Clinical Dental Research. 4. 56-58. 10.5005/jp-journals-10052-0081.
3. Gutmann, James. (2008). History of endodontics. Ingle's Endodontics.
4. Patel, S., Arias, A., Whitworth, J., & Mannocci, F. (2020). Outcome of endodontic treatment – the elephant in the room. International Endodontic Journal, 53(3), 291–297. doi:10.1111/iej.13238, https://doi.org/10.1111/iej.13238
5. Constantin Mihai, Cristian Constantin Budacu, Gheorghe Raftu, SURGICAL TREATMENT PRINCIPLES IN PERIAPICAL ENDODONTIC PATHOLOGY, Romanian

Journal of Oral Rehabilitation, Vol. 11, No. 1, January - March 2019.

6. Oprică L., Metaboliți secundari din plante, origine, structură, funcții, Editura Universității Alexandru Ioan Cuza Iași, 2016; ISBN:978-606-714-253-2.
7. Kunjachan S, Rychlik B, Storm G, Kiessling F, LammersT. Multidrug resistance: Physiological principles and nanomedical solutions. Adv Drug Deliv Rev 2013;65:1852-1865.
8. Cando, D., Morcuende, D., Utrera, M., Estevez, M., 2014. Eur. Food Res. Technol.238, 741.
9. Vilma Kaškonienė, Audrius Maruška, Ieva Akuņeca, Mantas Stankevičius, Ona Ragažinskienė, Violeta Bartkuvienė, Olga Kornyšova, Vitalis Briedis & Rasa Ugenskienė (2016), Screening of antioxidant activity and volatile compounds composition of Chamerion angustifolium(L.) Holub ecotypes grown in Lithuania, Natural Product Research, 30:12, 1373-1381, DOI: 10.1080/14786419.2015.1058792, https://doi.org/10.1080/14786419.2015.1058792
10. V. Steenkamp, M.C. Gouws, M. Gulumian, E.E. Elgorashi and J. van Staden, Studies on antibacterial, anti-

inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prostatic hyperplasia and prostatitis, JOURNAL OF ETHNOPHARMACOLOGY, FEBRUARY 2006, Impact Factor: 3. doi: 10.1016/j.jep.2005.07.007. Source: PubMed., https://doi.org/10.1016/j.jep.2005.07.007

11. Dumitru Buiuc, Marian Neguţ, Tratat de microbiologie clinică, Ediţia a III-a, 2017, Editura Medicală.
12. Harpreet Singh, Microbiology of Endodontic Infections, Journal of Dental and Oral Health, 2016, ISSN: 2369-4475
13. Dioguardi, M.; Di Gioia, G.; Illuzzi, G.; Arena, C.; Caponio, V.C.A.; Caloro, G.A.; Zhurakivska, K.; Adipietro, I.; Troiano, G.; Lo Muzio, L. Inspection of the Microbiota in Endodontic Lesions. Dent. J. 2019, 7, 47, DOI: 10.3390/dj7020047, https://doi.org/10.3390/dj7020047
14. Tronstad, Leif & Barnett, Frederic & Riso, Kenneth & Slots, Jorgen. (1987). Extraradicular endodontic infection. Endodontics & dental traumatology. 3. 86-90. doi: 10.1111/j.1600-9657.1987.tb00549.x, https://doi.org/10.1111/j.1600-9657.1987.tb00549.x

15. Bammann LL, Estrela C., Microbiological aspects in endodontics: Endodontic Science,(edition 2) 2009;Vol 1:258–81.
16. Gomes, B. P.F.A. and Pinheiro, E. T. (2017) Extraradicular Endodontic Infections, in Endodontic Microbiology (ed A. F. Fouad), John Wiley & Sons, Inc., Hoboken, NJ, USA., doi: 10.1002/9781119080343.ch6, https://doi.org/10.1002/9781119080343.ch6
17. Jhajharia, Kapil & Parolia, Abhishek & Shetty, K & Mehta, Lata. (2015). Biofilm in endodontics: A review. Journal of International Society of Preventive & Community Dentistry. 5. 1-12. 10.4103/2231-0762.151956, https://doi.org/10.4103/2231-0762.151956
18. Miquel S, Lagrafeuille R, Souweine B, Forestier C (2016) Anti-biofilm activity as a health issue. Front Microbiol 7:592. https://doi.org/10.3389/fmicb.2016.00592
19. Jamal M, Ahmad W, Andleeb S, Jali F, Imran M, Nazaw MA, Hussain T, AliM, RafiqM, KamilMA (2018) Bacterial biofilm and associated infections. J Chin Med Assoc 81(1):7–11. https://doi.org/10.1016/j.jcma.2017.07.012

20. Song F, Koo H, Ren D (2015) Effects of material properties on bacterial adhesion and biofilm formation. J Dent Res 94(8):1027–1034. https://doi.org/10.1177/0022034515587690

21. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L (2017) Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol 15(12):740–755. https://doi.org/10.1038/nrmicro.2017.99

22. Rewak-Soroczyńska J, Paluch E, Siebert A, Szałkiewicz K, Obłąk E(2019) Biological activity of glycine and alanine derivatives of quaternary ammonium salts (QASs) against microorganisms. Lett Appl Microbiol 69(3):212–220. https://doi.org/10.1111/lam.13195

23. Lear, G; Lewis, GD (editor) (2012). Microbial Biofilms: Current Research and Applications. Caister Academic Press. ISBN 978-1-904455-96-7.

24. Marsh PD1, Zaura E J.Clin.Periodontol. 2017 Mar;44 Suppl 18:S12-S22. doi: 10.1111/jcpe.12679. Dental biofilm: ecological interactions in health and disease, https://doi.org/10.1111/jcpe.12679

25. Yeon-Jee Yoo, Hiran Perinpanayagam, Soram Oh, A-Reum Kim, Seung-Hyun Han, Kee-Yeon Kum,

Endodontic biofilms: contemporary and future treatment options, Restor Dent Endod. 2019 Feb;44(1):e7, pISSN 2234-7658·eISSN 2234-7666, https://doi.org/10.5395/rde.2019.44.e7

26. Prasanna Neelakantan, Monica Romero, Jorge Vera, Umer Daood, Asad U. Khan, Aixin Yan and Gary Shun Pan Cheung, Biofilms in Endodontics—Current Status and Future Directions, Int. J. Mol. Sci. 2017, 18, 1748; doi:10.3390/ijms18081748, https://doi.org/10.3390/ijms18081748
27. Xiuliang Huang, Olivia P Duddy, Justin E Silpe, Jon E Paczkowski, Jianping Cong, Brad R. Henke and Bonnie L Bassler, Mechanism underlying autoinducer recognition in the Vibrio cholerae DPO-VqmA quorum-sensing pathway, J. Biol. Chem. published online January 21, 2020, doi: 10.1074/jbc.RA119.012104, https://doi.org/10.1074/jbc.RA119.012104
28. Li T, Wang D, Liu N, Ma Y, Ding T, Mei Y, Li J (2018) Inhibition of quorum sensing-controlled virulence factors and biofilm formation in Pseudomonas fluorescens by cinnamaldehyde. Int J Food Microbiol 269:98–106. https://doi.org/10.1016/j.ijfoodmicro.2018.01.023

29. Derek Fleming and Kendra P. Rumbaugh, Approaches to Dispersing Medical Biofilms, Microorganisms 2017, 5, 15; doi: 10.3390/microorganisms5020015, https://doi.org/10.3390/microorganisms5020015

30. Buret, A.G., Motta, J., Allain, T. et al. Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: a role for iron?. J Biomed Sci 26, 1 (2019). https://doi.org/10.1186/s12929-018-0495-4

31. Karatan E, Watnick P (June 2009). "Signals, regulatory networks, and materials that build and break bacterial biofilms". Microbiology and Molecular Biology Reviews 73 (2): 310–47. doi:10.1128/MMBR.00041-08. PMC 2698413. PMID 19487730. http://mmbr.asm.org/cgi/pmidlookup?view=long&pmid=19487730

32. Davies DG, Marques CN (March 2009). "A fatty acid messenger is responsible for inducing dispersion in microbial biofilms". Journal of Bacteriology 191 (5): 1393–403. doi:10.1128/JB.01214-08. PMC 2648214. PMID 19074399. http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=2648214

33. Shadaba Asad and Steven M Opal, Bench-to-bedside review: Quorum sensing and the role of cell-to-cell communication during invasive bacterial infection. Critical Care 2008, 12:236 doi:10.1186/cc7101, published 35, 10.1177/0022034519880157, https://doi.org/10.1186/cc7101

34. Charlton, S., White, M. A., Jana, S., Eland, L. E., Jayathilake, P. G., Burgess, J. G., Chen, J., Wipat, A., & Curtis, T. P. (2019). Regulating, Measuring, and Modeling the Viscoelasticity of Bacterial Biofilms. Journal of bacteriology, 201(18), e00101-19. https://doi.org/10.1128/JB.00101-19

35. Diaz PI, Valm AM., Microbial Interactions in Oral Communities Mediate Emergent Biofilm Properties., J.Dent.Reschers 2020 Jan;99(1):18-25. doi: 10.1177/0022034519880157. Epub 2019 Oct 7, https://doi.org/10.1177/0022034519880157

36. Miller DP, Fitzsimonds ZR, Lamont RJ., Metabolic Signaling and Spatial Interactions in the Oral Polymicrobial Community, J.Dent.Reschers 2019 Nov;98(12):1308-1314. doi: 10.1177/0022034519866440.

Epub 2019 Jul 29, https://doi.org/10.1177/0022034519866440

37. Abisado RG, Benomar S, Klaus JR, Dandekar AA, Chandler JR (2018) Bacterial quorum sensing and microbial community interactions. mBio 9(3): e02331-17. https://doi.org/10.1128/mBio.02331-17
38. Reuter K, Steinbach A,HelmsV(2016) Interferingwith bacterial quorum sensing. Perspect Medicin Chem 8:1–15. https://doi.org/10.4137/PMc.s13209
39. E. Paluch, J. Rewak-Soroczyńska, I. Jędrusik, E. Mazurkiewicz, K. Jermakow, Prevention of biofilm formation by quorum quenching, Applied Microbiology and Biotechnology (2020) 104:1871–1881, https://doi.org/10.1007/s00253-020-10349-w
40. Papenfort K, Bassler B (2016) Quorum-sensing signal-response systems in gram-negative bacteria. Nat Rev Microbiol 14(9):576–588. https://doi.org/10.1038/nrmicro.2016.89.
41. https://thebiologynotes.com/quorum-sensing-in-bacteria/
42. Rehman ZU, Leiknes T (2018) Quorum-quenching bacteria isolated from Red Sea sediments reduce biofilm

formation by Pseudomonas aeruginosa. Front Microbiol 9:1354. https://doi.org/10.3389/fmicb.2018.01354

43. Sumaya Abusrewil, Om Alkhir Alshanta, Khawlah Albashaireh, Saeed Alqahtani, Christopher J. Nile, James Alun Scott & William McLean (2020): Detection, treatment and prevention of endodontic biofilm infections: what's new in 2020?, Critical Reviews in Microbiology, https://doi.org/10.1080/1040841X.2020.1739622
44. July Fong, Chaodong Zhang, Renliang Yang, Zhao Zhi Boo, Soon Keat Tan, Thomas E. Nielsen, Michael Givskov, Xue-Wei Liu, Wu Bin, Haibin Su & Liang Yang, Combination Therapy Strategy of Quorum Quenching Enzyme and Quorum Sensing Inhibitor in Suppressing Multiple Quorum Sensing Pathways of P. aeruginosa, SCIeNTIfIC REPOrTS,(2018),8:1155, https://doi.org/10.1038/s41598-018-19504-w
45. Xihong Zhao, Zixuan Yu, Tian Ding, Review Quorum-Sensing Regulation of Antimicrobial Resistance in Bacteria, Microorganisms 2020, 8, 425; https://doi.org/10.3390/microorganisms8030425
46. Bodede O, Shaik S, Chenia H, Singh P, Moodley R (2018) Quorum sensing inhibitory potential and in silico

molecular docking of flavonoids and novel terpenoids from Senegalia nigrescens. J Ethnopharmacol 216:134–146. https://doi.org/10.1016/j.jep.2018.01.031

47. Asfour HZ. Anti-Quorum Sensing Natural Compounds. J Microsc Ultrastruct. 2018 Jan-Mar;6(1):1-10. doi: 10.4103/JMAU.JMAU_10_18. PMID: 30023261; PMCID: PMC6014249, https://doi.org/10.4103/JMAU.JMAU_10_18
48. Liu Wenzheng, Røder Henriette L., Madsen Jonas S., Bjarnsholt Thomas, Sørensen Søren J., Burmølle Mette,Interspecific Bacterial Interactions are Reflected in Multispecies Biofilm Spatial Organization, Frontiers in Microbiology, 2016, doi: 10.3389/fmicb.2016.01366, https://www.frontiersin.org/article/10.3389/fmicb.2016.01366
49. Brackman G, Coenye T (2015a) Inhibition of quorum sensing in Staphylococcus spp. Curr Pharm Des 21(16):2101–2108, https://doi.org/10.2174/1381612821666150310101014
50. Dahlen G, Moller A., Jr . Microbiology of endodontic infection. In: Slots J, Taubman MA, editors.

Contemporary Oral Microbiology and immunology. St. Louis: Mosby year Book Inc; 1991. pp. 444–55.

51. Pavena Chivatxaranukul, Stuart G. Dashper, Harold Henry Messer, Dentinal tubule invasion and adherence by Enterococcus faecalis., Materials Science, MedicinePublished in International endodontic journal, 2008, DOI:10.1111/j.1365-2591.2008.01445.x, https://doi.org/10.1111/j.1365-2591.2008.01445.x
52. Anderi Iliescu, Tratat de Endodonţie, Editura Medicală 2015, volumul 1, volumul 2.
53. Ujjwal Das, Saswati Mukherjee Das, An Overview on Endo-Perio Interrelationship - A Multidisciplinary Approach, IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 12 Ver. 9 (December. 2018), PP 15-21, DOI: 10.9790/0853-1712091521, www.iosrjournals.org
54. Ayush Goyal, John V. George, Ritu Singh, The Endodontic-Periodontal Interrelationships - A comprehensive review, LAP LAMBERT Academic Publishing, 2016, ISBN-13: 978-3-659-87082-8, ISBN-10: 365987082X, EAN: 9783659870828.

55. Khalid S. Al-Fouzan, ”A New Classification of Endodontic-Periodontal Lesions”, International Journal of Dentistry, vol. 2014, Article ID 919173, 5 pages, 2014.doi:10.1155/2014/919173, https://doi.org/10.1155/2014/919173
56. Hand AR, Frank ME, Goldberg M. Dentin, pulp, and tooth pain. In ”Fundamentals of oral histology and physiology”. First edition. Chapter 5. John Wiley & sons, Blackwell inc. 2014;85-112.
57. Goldberg M. Pulp anatomy and characterization of pulp cells, in: ”Biology, pathology, and regenerative therapies”. Springer Vertag, Berlin, Heidelberg. 2014; 13-33.
58. Goldberg M. Root Canal Tratment (RCT): From Traditional Endodontic Therapies to Innovating Pulp Regeneration. Journal of Dentistry & Oral Disorders.2016:2(5):1024.
59. Colombo JS, Moore AN, Hartgerink JD, D`Souza RN, Scaffolds to control inflammation and facilitate dental pulp regeneration. J Endod. 2014; 40:6-12.
60. Bun San Chong, Harty's Endodontics in Clinical Practice, Elsevier 2017, Seventh Edition

61. TMA, Saoud & Ricucci, Domenico & Lin, Louis & Gaengler, Peter. (2016). Regeneration and Repair in Endodontics—A Special Issue of the Regenerative Endodontics—A New Era in Clinical Endodontics. Dentistry Journal. 4. 1-15. doi: 10.3390/dj4010003, https://doi.org/10.3390/dj4010003

62. European Society of Endodontology developed by:Segura-Egea JJ, Gould K, Hakan Şen B, Jonasson P, Cotti E, Mazzoni A, Sunay H, Tjäderhane L, Dummer PMH. European Society of Endodontology position statement: the use of antibiotics in endodontics. International Endodontic Journal, 2017, doi:10.1111/iej.12781, https://doi.org/10.1111/iej.12781

63. Nadia Chugal, Louis M Lin , Endodontic Prognosis Clinical Guide for Optimal Treatment Outcome, 2017,ISBN: 978-3-319-42410-1 (Print) 978-3-319-42412-5 (Online).

64. Saoud, T. M., Martin, G., Chen, Y.-H. M., Chen, K.-L., Chen, C.-A., Songtrakul, K., Lin, L. M. (2016). Treatment of Mature Permanent Teeth with Necrotic Pulps and Apical Periodontitis Using Regenerative Endodontic Procedures: A Case Series. Journal of Endodontics, 42(1),

57–65. doi:10.1016/j.joen.2015.09.015, https://doi.org/10.1016/j.joen.2015.09.015

65. Vishal Sahayata (2016), Endodontic Infections: Risk for Cardiovascular Diseases??, Journal of Dental and Oral Health, Volume 2, issue 6, e108, www.scientonline.org

66. European Society of Cardiology (2015) ESC Guidelines for the management of infective endocarditis. European Heart Journal 36, 3075–123, https://doi.org/10.1093/eurheartj/ehv319

67. J.M. Liljestrand, P. Mäntylä, S. Paju, K. Buhlin, K.A.E. Kopra, G.R. Persson, Association of Endodontic Lesions with Coronary Artery Disease, SAGE journals, Nov 01, 2016, doi: 10.1177/0022034516660509, https://doi.org/10.1177/0022034516660509

68. Paridhi Garg, Chandrakar Chaman, Apical Periodontitis – Is It Accountable for Cardiovascular Diseases?, Journal of Clinical and Diagnostic Reaserch, 2016 Aug, doi: 10.7860/JCDR/2016/19863.8253, https://doi.org/10.7860/JCDR/2016/19863.8253

69. J. J. Segura-Egea, J. Martin-Gonzalez & L. Castellanos-Cosano, Endodontic medicine: connections between apical periodontitis and systemic diseases, International

Endodontic Journal, 48, 933-951, 2015, doi: 10.1111/iej.12507, https://doi.org/10.1111/iej.12507

70. Cintra LT, Samuel RO, Azuma MM et al. (2014 a) Relationships between oral infections and blood glucose concentrations or HbA1c levels in normal and diabetic rats. International Endodontic Journal 47, 228-37, DOI: 10.1111/iej.12136, https://doi.org/10.1111/iej.12136
71. Alzahrani, Mohammed. (2016). Sodium Hypochlorite Accident in Endodontics: An Update Review. International Journal of Dentistry and Oral Health. 2. 10.16966/2378-7090.168, https://doi.org/10.16966/2378-7090.168
72. Arias-Moliz MT, Ordinola-Zapata R, Baca P, Ruiz-Linares M, García García E, Hungaro Duarte MA, Monteiro Bramante C, Ferrer-Luque CM. Antimicrobial activity of Chlorhexidine, Peracetic acid and Sodium hypochlorite/etidronate irrigant solutions against Enterococcus faecalis biofilms. International Endodontic Journal, 48, 1188–1193, 2015, doi: 10.1111/iej.12424, https://doi.org/10.1111/iej.12424

73. Deliverska E. Oral mucosa damage because of hypochlorite accident – a Case report and literature review. J of IMAB. 2016 Jul-Sep;22(3):1269-1273. DOI: http://dx.doi.org/10.5272/jimab.2016223.1269

74. Perotti S, Bin P, Cecchi R. Hypochlorite accident during wndodontic therapy with nerve damage - A case report. Acta Biomed. 2018;89(1):104–108. Published 2018 Mar 27. doi:10.23750/abm.v89i1.6067, https://doi.org/10.23750/abm.v89i1.6067

75. Fitzgerald M, Heinrich M and Booker A(2020) Medicinal Plant Analysis: AHistorical and Regional Discussion ofEmergent Complex Techniques.Front. Pharmacol. 10:1480. doi: 10.3389/fphar.2019.01480, https://doi.org/10.3389/fphar.2019.01480

76. Fawzi, Muntaha & Kadhem, Zena & Farhan, Sabah. (2017). Anti-Inflammatory Effect of Sage (Salvia Officinalis) Extracts ABSTRACT on Oral Health. Iraqi Dental Journal. 39. 1. 10.26477/idj.v39i1.111, https://doi.org/10.26477/idj.v39i1.111

77. Osman M. El-Maghraby, Magdy A. Abu-Gharbia, El-Sayed M. Soltan, Walaa M. Abd El- Raheem and Emad A. Shalaby, Antimicrobial activity of selected medicinal

plants against clinically isolated multi-drug resistant human oral pathogens, J. Microbiol. Biotech. Res., 2014, 4 (6):9-24, ISSN : 2231 –3168, www.scholarsresearchlibrary.com

78. Jain I, Jain P, Bisht D, Sharma A, Srivastava B, Gupta N. Use of traditional Indian plants in inhibition of caries causing bacteria- Streptococcus mutans. Braz Dent J. 2015;26:110–5, doi: 10.1590/0103-6440201300102, https://doi.org/10.1590/0103-6440201300102
79. Puvača, Nikola & Cabarkapa, Ivana & Petrovic, Aleksandra & Bursic, Vojislava & Prodanovic, Radivoj & Solesa, Dragan & Lević, Jovanka. (2019). Tea tree (Melaleuca alternifolia) and its essential oil: Antimicrobial, antioxidant and acaricidal effects in poultry production. World's Poultry Science Journal. 75. 1-12, DOI: 10.1017/S0043933919000229. https://doi.org/10.1017/S0043933919000229
80. Sgorbini, B, Cagliero, C, Argenziano, M, Cavalli, R, Bicchi, C, Rubiolo, P. In vitro release and permeation kinetics of Melaleuca alternifolia (tea tree) essential oil bioactive compounds from topical formulations. Flavour

Fragr J. 2017; 32: 354– 361. https://doi.org/10.1002/ffj.3403

81. Dandekar, Shivani & Deshpande, Neeraj & Dave, Deepak. (2017). Comparative Evaluation of Anti-Microbial Efficacy of Cranberry Extract and Chlorhexidine Mouthwash on Periodontal Pathogens: An In-vitro Study. Journal of Periodontal Practice. 2. 05-08. 10.20936/jpp/170102, http://dx.doi.org/10.20936/jpp/170102

82. Shabbir J, Qazi F, Farooqui W, Ahmed S, Zehra T, Khurshid Z. Effect of Chinese Propolis as an Intracanal Medicament on Post-Operative Endodontic Pain: A Double-Blind Randomized Controlled Trial. International Journal of Environmental Research and Public Health. 2020; 17(2):445, doi:10.3390/ijerph17020445, https://doi.org/10.3390/ijerph17020445

83. Trevisan, L., Huerta, I. R., Michelon, C., Bello, M. C., Pillar, R., & Souza Bier, C. A. (2017). The Efficacy of Passive Ultrasonic Activation of Organic Solvents on Dissolving Two Root Canal Sealers. Iranian endodontic journal, 12(1), 25–28. https://doi.org/10.22037/iej.2017.05

84. S Golmohammadi, Z Khalilak, M Vatanpour, A Mahboubi .Comparing the Effect of Green Tea, Calcium Hydroxide and Chlorhexidine on Enterococcus faecalis biofim in Root Canal system (Ex vivo). Adv. Biores., Vol 8 [2] Mach 2017: 51-57, DOI: 10.15515/abr.0976-4585.8.2.5157, https://doi.org/10.15515/abr.0976-4585.8.2.5157

85. Vishnuvardhini.S, Sivakumar A, Ravi V, Prasad A. S, Sivakumar J. S. Herbendodontics–Phytotherapy In Endodontics: A Review. Biomed Pharmacol J 2018;11(2), DOI : http://dx.doi.org/10.13005/bpj/1468

86. Tewari RK, Kapoor B, Mishra SK, Kumar A. Role of herbs in endodontics. J Oral Res Rev 2016;8:95-9, DOI: 10.4103/2249-4987.192248, https://doi.org/10.4103/2249-4987.192248

87. Chandwani M, Mittal R, Chandak S, Pimpale J. Effectiveness of Morinda citrifolia juice as an intracanal irrigant in deciduous molars: An in vivo study. Dent Res J 2017;14:246-51, DOI: 10.4103/1735-3327.211630, https://doi.org/10.4103/1735-3327.211630

88. MUNTEAN L. S., TĂMAŞ M., MUNTEAN S., MUNTEAN L., DUDA M. VÂRBAN DAN, FLORIAN

S., 2007, Tratat de plante medicinale cultivate și spontane, - Ed. Risoprint, Cluj-Napoca

89. Pignatti S. - Flora d'Italia – Edagricole – 1982. Vol. II, pag. 155.
90. https://www.soin-et-nature.com/en/medicinal-plants/5171-fireweed-plant-with-small-flowers-cut-iphym-herb-epilobium-parviflorum.html
91. https://www.pinterest.com/daramaletsas/zdravlje/
92. Ursula Stănescu, Monica Hăncianu, Oana Cioancă, Ana Clara Aprotosoaie, Anca Miron, PLANTE MEDICINALE DE LA A LA Z, Editura Polirom, 2014.
93. Franz-Christian Czygan, Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis, CRC Press, 2004, ISBN 0849319617, 9780849319617
94. Hevesi BT, Houghton PJ, Habtemariam S, Kéry A, Antioxidant and antiinflammatory effect of Epilobium parviflorum Schreb, Phytother Res. 2009 May;23(5):719-24. doi: 10.1002/ptr.2725, https://doi.org/10.1002/ptr.2725
95. Kim JH, Park KM, Lee JA. Herbal medicine for benign prostatic hyperplasia: A protocol for a systematic review

of controlled trials. Medicine (Baltimore). 2019;98(1):e14023. doi: 10.1097/MD.0000000000014023, https://doi.org/10.1097/MD.0000000000014023

96. Epilobium species, PDR for Herbal Medicines, 2nd ed., Medical, Economics Company, Montvale, New Jersey, p. 818-819.
97. Topbaş, Celalettin & Adıgüzel, Özkan. (2017). Endodontic Irrigation Solutions: A Review. International Dental Research. 7. 54. 10.5577/intdentres.2017.vol7.no3.2., https://doi.org/10.5577/intdentres.2017.vol7.no3.2
98. James L. Gutmann,Vivian Manjarrés, Historical and Contemporary Perspectives on the Microbiological Aspects of Endodontics, Dent. J. 2018, 6, 49; doi:10.3390/dj6040049, www.mdpi.com/journal/dentistry
99. Nicolae Doniţă, Mihaela Paucă-Comănescu, Aurel Popescu, Simona Mihăilescu, Iovu-Adrian Biriş, Habitatele din România, Editura Tehnică Silvică, 2005.
100. Farmacopeea Română Ediția a X-a, Editura Medicală, București, 2008.
101. United Nations Industrial Development Organization., Handa, S. S., Khanuja, S. P. S., Longo, G., Rakesh, D. D.,

United Nations Industrial Development Organization., & International Centre for Science and High Technology. (2008). Extraction technologies for medicinal and aromatic plants. Trieste (Italy): Earth, Environmental and Marine Sciences and Technologies.

102. Vilkhu, Kamaljit & Mawson, Raymond & Simons, Lloyd & Bates, Darren. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry--A review. Innovative Food Science & Emerging Technologies. 9. 161-169. 10.1016/j.ifset.2007.04.014, https://doi.org/10.1016/j.ifset.2007.04.014

103. M. Vinatoru, An overview of the ultrasonically assisted extraction of bioactive principles from herbs, Ultrasonics Sonochemistry,Volume 8, Issue 3, July 2001, Pages 303-313, https://doi.org/10.1016/S1350-4177(01)00071-2

104. Z. Hromàdkovà and A. Ebringerovà, Ultrasonic extraction of plant materials–investigation of hemicellulose release from buckwheat hulls, Ultrasonics Sonochemistry Volume 10, Issue 3, May 2003, Pages 127-133, https://doi.org/10.1016/S1350-4177(03)00094-4

105.European Pharmacopoeia , 9th ed. , EDQM, European Pharmacopoeia, Council of Europe, B.P. 907, F - 67029 , Strasbourg, France , 2016 .

106.El-Mahrouk, M.E. and Dewir, Y.H. (2016) Physico-Chemical Properties of Compost Based Waste-Recycling of Grape Fruit as Nursery Growing Medium. American Journal of Plant Sciences, 7, 48-54, http://dx.doi.org/10.4236/ajps.2016.71005

107.Gabriel A Agbor, Joe A Vinson and Patrick E. Donnelly (2014) Folin-Ciocalteau Reagent for Polyphenolic Assay.3:801., IJFS,ISSN 2326-3350, DOI: 10.19070/2326-3350-1400028, https://doi.org/10.19070/2326-3350-1400028

108.O.R. Bancuta et al., Improvement of spectrophotometric method for determination of phenolic compounds by statistical investigations, Rom. Journ. Phys., Vol. 61, No. 7–8, P. 1255–1264, 2016.

109.Gabriela Gegiu, Andrei-Dan Branza, Laura Bucur, Mircea Grigorian,Traian Tache, Victoria Badea, Contributions to the antimicrobial and antifungal study of the aqueous extract of Prunus Spinosa L., Farmacia, 2015, Vol. 63, 2, http://www.revistafarmacia.ro/issue22015.html

110.Bagul, Uddhav & Sivakumar, Sivagurunathan. (2016). ANTIBIOTIC SUSCEPTIBILITY TESTING: A REVIEW ON CURRENT PRACTICES. international journal of pharmacy. 6. 11-17, www.pharmascholars.com

111.Sabharwal S, Bhagat SK, Gami KS, Siddhartha A, Rai K, Ahluwalia Y. An in vivo study to compare anti microbial activity of triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on microorganisms in the root canal of immature teeth. J Int Soc Prevent Communit Dent 2019;9:263-8, doi: 10.4103/jispcd.JISPCD_400_18, https://doi.org/10.4103/jispcd.JISPCD_400_18

112.Monica Licker, Elena Hogea, Mihaela Crăciunescu, Microbiologie specială Îndreptar de lucrări practice, 2019, Editura „Victor Babeş", CNCSIS: 324, ISBN 978-606-786-115-0.

113.Remmel, Indrek & Vares, Lauri & Toom, Lauri & Matto, Vallo & Raal, Ain. (2012). Phenolic Compounds in Five Epilobium Species Collected from Estonia. Natural product communications. 7. 1323-4.

114.Bajer, Tomas & Šilha, David & Ventura, Karel & Bajerová, Petra. (2017). Composition and antimicrobial activity of the essential oil, distilled aromatic water and

herbal infusion from Epilobium parviflorum Schreb. Industrial Crops and Products. 100. 95-105. 10.1016/j.indcrop.2017.02.016, https://doi.org/10.1016/j.indcrop.2017.02.016

115. Kondreddi N, Venigalla BS, Singh TV, Kamishetty S, Reddy S, Cherukupalli R. Antibacterial activity of chitosan and its combination with other irrigants on Enterococcus faecalis: An in vitro study. Endodontology 2019;31:133-7., www.endodontologyonweb.org, 10.4103/endo.endo_110_18, https://doi.org/10.4103/endo.endo_110_18

116. Hans, Manoj. (2018). A COMPARATIVE EVALUATION AND EFFECTIVENESS OF DIFFERENT ANTIMICROBIAL HERBAL EXTRACTS AS ENDODONTIC IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS AND CANDIDA ALBICANS- AN IN-VITRO STUDY, University J Dent Scie; Vol. 4, Issue 2

117. Tolosa L., Donato MT, Gómez-Lechón MJ, General Cytotoxicity Assessment by Means of the MTT Assay, Methods Mol Biol. 2015;1250:333-48. doi: 10.1007/978-

1-4939-2074-7_26, https://doi.org/10.1007/978-1-4939-2074-7_26

118. Karkehabadi H, Yousefifakhr H, Zadsirjan S. Cytotoxicity of Endodontic Irrigants on Human Periodontal Ligament Cells. Iran Endod J. 2018;13(3):390-4. Doi: 10.22037/iej.v13i3.20438., https://doi.org/10.22037/iej.v13i3.20438

119. Ravinanthanan M, Hegde MN, Shetty V, Kumari S. Cytotoxicity effects of endodontic irrigants on permanent and primary cell lines. Biomed Biotechnol Res J 2018;2:59-62, DOI: 10.4103/bbrj.bbrj_92_17, https://doi.org/10.4103/bbrj.bbrj_92_17

120. https://www.iso.org/obp/ui/#iso:std:iso:10993:-12:ed-4:v1:en

121. Hussain et al., Determination of cell viability using acridine orange/propidium iodide dual-spectrofluorometry assay, Cogent Food & Agriculture (2019), 5: 1582398, https://doi.org/10.1080/23311932.2019.1582398

122. Ok E, Adanir N, Hakki S. Comparison of cytotoxicity of various concentrations origanum extract solution with 2% chlorhexidine gluconate and 5.25% sodium hypochlorite.

Eur J Dent 2015;9:6-10, DOI: 10.4103/1305-7456.149630, https://doi.org/10.4103/1305-7456.149630

123. Memet Gafar, Andrei Iliescu, Endodonție clinică și practică, Ediția a II-a revizuită și adăugită, Editura Medicală, 2010.

124. Elena Ciudin şi Dan Marinescu,Animale de laborator,Editura All, 1996.

125. Okamoto T, Russo MC. Wound healing following tooth extraction. Histochemical study in rats. Rev FacOdontolAraçatuba.1973 (2): 153-164.

126. Al-Obaidi, Mazen & Al-Bayaty, Fouad & Al Batran, Rami & Ibrahim, Omar & Daher, Aqil. (2016). Ellagic Acid Increases Osteocalcin and Alkaline Phosphatase After Tooth Extraction in Nicotinic-Treated Rats. Current Pharmaceutical Design. 22. 2403-2410. 10.2174/1381612822161604280028 42, https://doi.org/10.2174/1381612822161604280028 42

127. I.S. Beșchea Chiriac, Testarea toxicităţii medicamentelor de uz veterinar, MEDICHUB MEDIA, 2017, 10.26416/PV.29.8.2017.1249

128. Turkki R, Linder N, Kovanen PE, et al., editors. Identification of immune cell infiltration in hematoxylin-

eosin stained breast cancer samples: Texture-based classification of tissue morphologies. Proceedings of the International Society for Optics and Photonics (SPIE) Conference on Medical Imaging, San Diego, CA, USA; 2016, https://doi.org/10.1117/12.2217040

129. Fouad Hussain AL Bayaty and et al, Effect of Salvadora Persica (Miswak) on Alveolar Bone, Journal of International Dental and Medical Research ISSN 1309-100X, 2018; 11(3): 770- 777, http://www.jidmr.com.

130. Pereira YCL, Issa JPM, Watanabe E, Nascimento GC, Iyomasa MM, et al. (2018) The Therapeutic Use of Propolis Extract in Alveolar Bone Contaminated with Bacterial Endotoxin. Dentistry 8: 473. doi:10.4172/2161-1122.1000473, https://doi.org/10.4172/2161-1122.1000473

131. Ilyas, Muhammad & Fahim, Ayesha & Awan, Uzma & Athar, Yousaf & Sharjeel, Nida & Arshad, Anas & Alam, Mohammad. (2015). Effect of Honey on Healing of Extracted Tooth Socket of Albino Wista Rats. International Medical Journal (1994). 22. 422-425.

132. Pankaj Prasad., et al. "To Compare the Clinical and Radiographic Evaluation of Single Visit and Multivisit

Endodontic Treatment of Teeth with Periapical Radiolucency. An In – Vivo Study". Acta Scientific Dental Sciences 3.8 (2019): 03-11.

133. Yuying Xue, Shanshan Zhang, Yang Yang, Minyu Lu, Yiqing Wang, Ting Zhang, Meng Tang and Haruo Takeshita, Acute pulmonary toxic effects of chlorhexidine (CHX) following an intratracheal instillation in rats, Human and Experimental Toxicology, 30(11) 1795–1803, DOI: 10.1177/0960327111400104, https://het.sagepub.com.

134. Ioana ROMAN, Mircea A. RUSU, Constantin PUICĂ, Maria BORŞA, CITOTOXIC EFFECTS OF THREE SPECIES OF EPILOBIUM (ONAGRACEAE) HERBAL EXTRACTS IN RATS, Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii, Vol. 20, issue 1, 2010, pp. 19-23, www.studiauniversitatis.ro.

135. Omatali N, Roghanizad N, Moshari A, Sadaghiani M, Kalantari M. An Assessment of Coronal Restoration Quality, Root Canal Filling Quality, and Apical Health Status of Endodontically Treated Teeth at an Iranian Dental School in 2011-12. J Res Dentomaxillofac Sci. 2019; 4 (1):16-23., http://www.jrdms.dentaliau.ac.ir.

136.Liu J, Que KH, Xiao ZH, Wen W. Endodontic management of the maxillary first molars with two root canals: A case report and review of the literature. World J Clin Cases 2019; 7(1): 79-88, DOI: https://dx.doi.org/10.12998/wjcc.v7.i1.79.

137.Ahmed HM. Guidelines to enhance the interpretation of two-dimensional periapical radiographic images in endodontics. European Journal of General Dentistry 2015;4:106-12, DOI: 10.4103/2278-9626.163320, https://doi.org/10.4103/2278-9626.163320

138.Alhashimi, Raghad & Al-Huwaizi, Hussain & Bds,. (2015). Standardized Protocol for Endodontic Treatment (Iraqi Endodontic Society). Iraqi Dental Journal. 37. doi: 10.26477/idj.v37i2.46, https://doi.org/10.26477/idj.v37i2.46

139.Kirkevang LL, Ørstavik D, Wenzel A, Væth M. Prognostic value of fullscale Periapical Index. Int Endod J 2014 Oct 29. doi:10.1111/iej.12402, https://doi.org/10.1111/iej.12402

140.Bojana Ćetenović, Dejan Marković, James Gutmann, Tamara Perić, Vukoman Jokanović, Endodontic treatment of traumatized teeth with chronic periapical lesions using

antibiotic paste and mineral trioxide aggregate obturation – a preliminary study, Srp Arh Celok Lek 2019, Online First March 14, 2019, DOI: https://doi.org/10.2298/SARH180301018C

141. Hasan Ayberk Altug and Aydin Ozkan (2011). Diagnostic Imaging in Oral and Maxillofacial Pathology, Medical Imaging, Dr. Okechukwu Felix Erondu (Ed.), ISBN: 978-953-307-774-1, InTech, DOI: 10.5772/27416, https://doi.org/10.5772/27416

142. Nikola Stojanović, Jelena Krunić, Irena Mladenović, Zorica Stojanović, Sonja Apostolska, Slavoljub Živković, Influence of different forms of calcium hydroxide and chlorhexidine intracanal medicaments on outcome of endodontic treatment of teeth with chronic apical periodontitis, Srp Arh Celok Lek 2017, DOI: https://doi.org/10.2298/SARH170221139S

143. Gheorghiu Irina-Maria, Mitran Loredana, Mitran M., Temelcea Anca-Nicoleta, Scarlatesc Sanziana, Calenic Bogdan, Perlea Paula, IN VIVO STUDY OF CALCIUM HYDROXIDE ENDODONTIC TREATMENT IN CHRONIC APICAL PERIODONTITIS, ARS Medica

Tomitana - 2018; 4(24): pag. 164 -167, doi: 10.2478/arsm-2018-0034, https://doi.org/10.2478/arsm-2018-0034

144. Ertugrul Ercan, Mehmet Dalli, Ç. Türksel Dülgergil, Ferhan Yaman, Effect of Intracanal Medication with Calcium Hydroxide and 1% Chlorhexidine in Endodontic Retreatment Cases with Periapical Lesions: An In Vivo Study, Journal of the Formosan Medical Association · April 2007, DOI: 10.1016/S0929-6646(09)60243-6, https://doi.org/10.1016/S0929-6646(09)60243-6

145. Mohammed Saadi Alarbeed., et al. "Effect of Combination of Calcium Hydroxide and Chlorhexidine Gel 2% as Intracanal Medication in Comparison to Calcium Hydroxide Paste as Intracanal Medication on Postoperative Pain and Bacterial Endotoxin in Necrotic Teeth: A Randomized Controlled Trial". Acta Scientific Dental Sciences 3.2 (2019): 124-130.

146. Imani Z, Imani Z, Basir L, Shayeste M, Abbasi Montazeri E, Rakhshan V. Antibacterial Effects of Chitosan, Formocresol and CMCP as Pulpectomy Medicament on Enterococcusfaecalis, Staphylococcus aureus and Streptococcusmutans. Iran Endod J.

2018;13(3):342–350, doi:10.22037/iej.v13i3.20791,
https://doi.org/10.22037/iej.v13i3.20791