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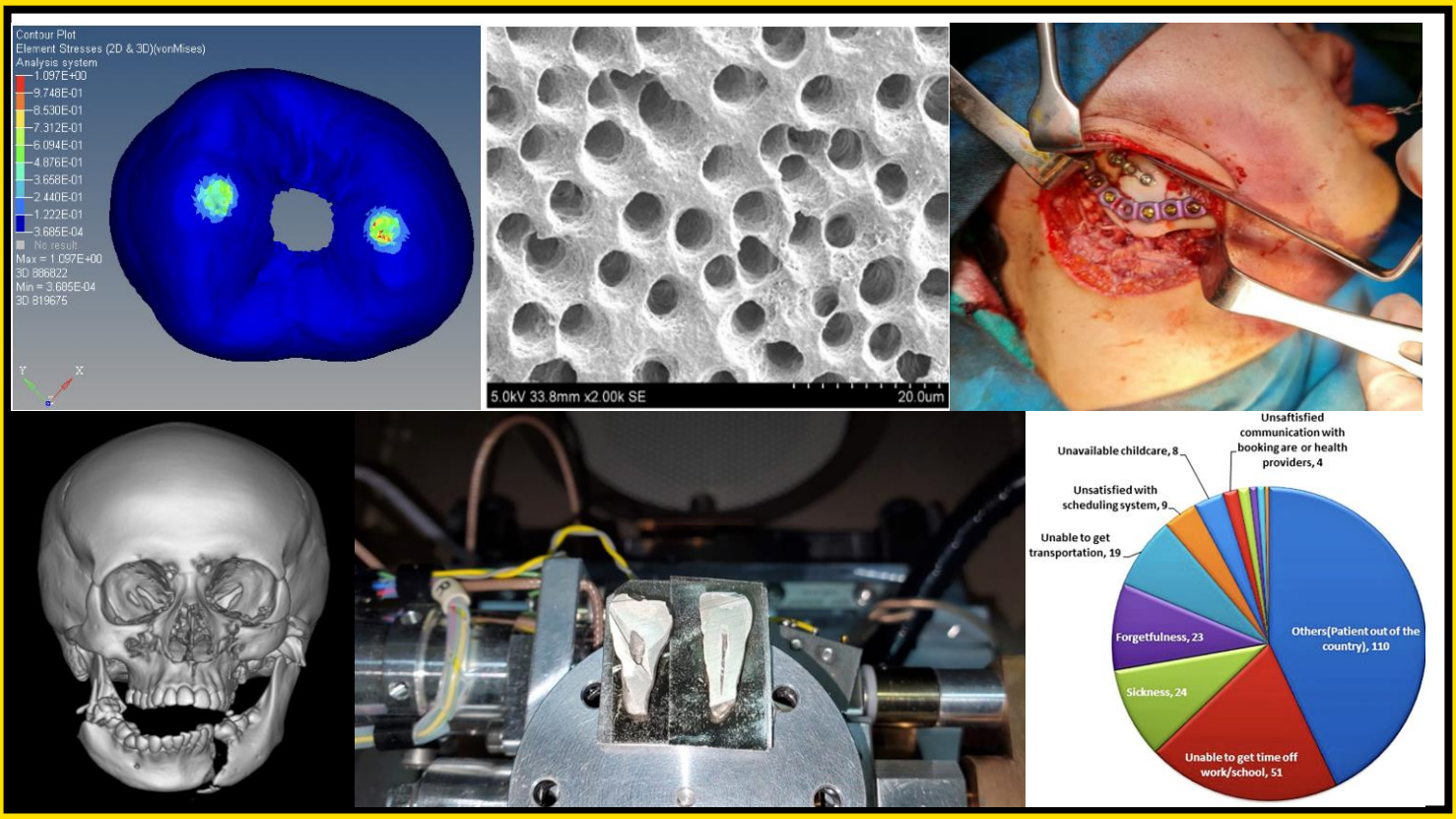

2022 - Vol. 15 – No. 1

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Current Issue of JIDMR

Table of Contents 2022 Vol.15–No.1

DENTISTRY

EXPERIMENTAL ARTICLE

1. **Changes in Cell Size and Dimension Characterized by Crystal Violet Staining and Simple Imagej Analysis**

Somying Patntirapong, Pattranith Charoensukpatana, Thitaree Thaksinawong

Full Text PDF

Pages 1-6

EXPERIMENTAL ARTICLE

2. **Synergistic Test of Chitosan-Ozonated Olive Oil and Chitosan-Coenzyme Q10 as Bone Graft Materials**

Dahlia Herawati, Ainun Mardiah Babgei, Aprilia Yuanita Anwaristi, Supriatno

Full Text PDF

Pages 7-14

EXPERIMENTAL ARTICLE

3. **Scanning Electron Microscopy Evaluation of the Smear Layer Removal Using Various Chelating Agents -An invitro Study**

Shreya Hegde, Harish K Shetty, Shourya Hegde, Kundabala Mala, Roma M

Full Text PDF

Pages 15-20

EXPERIMENTAL ARTICLE

4. **The Effect of α-Mangostin on TLR-4 and PGE2 in the Dentin-Pulp Complex Exposed to HEMA**

Febriastuti Cahyani, Widya Saraswati, Eric Priyo Prasetyo, Meircurius Dwi Condro Surboyo, Wibi Riawan, Karina Erda Saninggar,
Ninuk Hariyani, Anisa Kusumawardani, Adioro Soetojo, Soetjipto

Full Text PDF

Pages 21-26

EXPERIMENTAL ARTICLE

5. **Comparative Characteristics of Physical and Mechanical Properties of Adhesive Systems**

Khashanova L.M., Razumova S.N., Brago A.S., Bragunova R.M., Serebrov D.M., Gureva Z.A., Razumov N.M., Bait Said O.M.H.

Full Text PDF

Pages 27-30

EXPERIMENTAL ARTICLE

6. **Flexural Strength Evaluation of Chitosan-Gelatin-B-Tricalcium Phosphate-Based Composite Scaffold**

Tansza Setiana Putri, Maab Elsheikh

Full Text PDF

Pages 31-34

EXPERIMENTAL ARTICLE

7. **The Influence of Chronic Hyperhomocysteinemia on Connective Tissue Disorders in Case of Lipopolysaccharide-Induced Periodontitis**

Roman Khudan, Mariia Kyryliv, Iryna Bekus, Anna Demyd, Maria Koval, Vitaliy Luchynskyi

Full Text PDF

Pages 35-41

EXPERIMENTAL ARTICLE

8. **Median Lethal Dose (Ld50) Test of Analgesic Herbal Jatropha Curcas L. Latex on Animal Models**

Irmaleny, Narlan Sumawinata, Dewi Fatma, Marline Abdassah

Pages 42-45 Full Text PDF

EXPERIMENTAL ARTICLE

9. **The Effect of Cocoa Pod Husk and Green Tea Extract on SMAD3 and FGF2 Expressions in Exposed Dental Pulp**

Tamara Yuanita, Irfan Prasetyo, I Dewa Ayu Listiana, Irma Drismayanti, Nawira, Galih Sampoerno, Sukaton

Pages 46-52 Full Text PDF

EXPERIMENTAL ARTICLE

10. **Evaluation of the Stress Pattern in the Resin-Based Composite Restoration of an Endodontically Treated Premolar Tooth: A Finite Element Analysis Study**

Musliana Mustaffa, Arsalan Marghoub, Shirin Shahrbaf, Nicolas Martin

Pages 53-60 Full Text PDF

EXPERIMENTAL ARTICLE

11. **Bisphenol A in Hawley and Vacuum-Formed Retainers: An In-Vitro Study**

Arnis Jenny A, Purwanegara Miesje K, Anggani Haru S

Pages 61-65 Full Text PDF

EXPERIMENTAL ARTICLE

12. **The Toothpaste's Containing Coffee bean Skin Demonstrated the Specific Physicochemical Properties, Inhibited the Growth of Streptococcus mutans and Increased the Viability of Neutrophil**

I Dewa Ayu Ratna Dewanti, Ristya Widi Endah Yani

Pages 66-71 Full Text PDF

EXPERIMENTAL ARTICLE

13. **Impact of Different Surface Treatments of CAD/CAM Fabricated Glass Fiber Posts on Push-Out Bond Strength to Root Canal Dentin**

Sayed M Ghorab, Ahmed El-Kabbaney, Abdelraheem Abdelkader, Massimo Del Fabbro

Pages 72-80 Full Text PDF

EXPERIMENTAL ARTICLE

14. **Anti-cancer Activities of β-mangostin Against Oral Squamous Cell Carcinoma**

Mohamad Zakkirun Abdullah, Latifah Munirah Bakar, Noratikah Othman, Muhammad Taher, Mohammad Syaiful Bahari Abdull Rasad, Solachuddin Jauhari Arief Ichwan

Pages 81-87 Full Text PDF

EXPERIMENTAL ARTICLE

15. **A Subchronic 90-day Oral Toxicity Study of Sivanar Vembu Khuzhi Thailam in Rats**

Ganesh Shenoy, Smita Shenoy, Sathish Pai B, Nitesh Kumar, Arul Amuthan, Manjunath Shetty, Mohandas Rao, Dr Kanthilatha Pai, Bharath Rao K

Pages 88-93 Full Text PDF

EXPERIMENTAL ARTICLE

16. **Osteogenesis Effect of Human Adipose-derived Mesenchymal Stem Cell with Chitosan Scaffold in White Rat (Rattus norvegicus) Bone Defect on Serum Alkaline Phosphatase**

Dian Agustin Wahjuningrum, Setyabudi, Fauzan, Mirza Bahar Firnanda, Devi Eka Juniarti, Purwati, Fery Setiawan, Anuj Bhardwaj,
Ajinkya M. Pawar

Pages 94-102 Full Text PDF

EXPERIMENTAL ARTICLE

17. **The Pilot Study of Proximal Initial Dental Caries Measurement Correlates with Grayscale Values of Digital Bitewing Radiography**

Niraya Kornsombut, Potjaman Totiam, Sirilawan Tohnak, Kornchanok Wayakanon, Mayurach Pipatphatsakorn, Siras Sungkapreecha, Teeraphan Narakaew

Pages 103-106 Full Text PDF

EXPERIMENTAL ARTICLE

18.**Scanning Electron Microscopy**

Khaskhanova L.M., Razumova S.N., Serebrov D.M., Gureva Z.A., Vetchinkin A.V., Rebrii A.V., Bait Said O.M.H.

Full Text PDF

Pages 107-110

EXPERIMENTAL ARTICLE

19.**The Hardness of Tooth Enamel Post-Application of CPP-ACP as Remineralization Material on Demineralized Tooth**

Opik Taofik Hidayat, Lini Meriani, Irmaleny

Full Text PDF

Pages 111-118

EXPERIMENTAL ARTICLE

20.**Prevalence, Biofilm Forming Ability and Antifungal Susceptibility of Candida Species in Primary Root Canal Infections**

Elhalabi H., Newishy H., and Elsewify T.

Full Text PDF

Pages 119-123

EXPERIMENTAL ARTICLE

21.**Neurogenic Inflammation Pathway on the Up-Regulation of Voltage-Gated Sodium Channel NaV1.7 in Experimental Flare-Up Post-Dental Pulp Tissue Extirpation**

Galih Sampoerno, Anuj Bhardwaj, Putu Yuri Divina, Nathania Nurani Fripertiwi, Naufal Hafizh Adipradana

Full Text PDF

Pages 124-130

EXPERIMENTAL ARTICLE

22.**Sulfonated PEEK Characteristic after Various Surface Cleaning Techniques**

Parin Jeeranun, Nonnatee Chalongkuakul, Mansuang Arksornnukit, Patcharawan Silthampitag, Pisaisit Chaijareenont

Full Text PDF

Pages 131-139

EXPERIMENTAL ARTICLE

23.**Stimulation of Osteoblast and Osteocalcin in the Bone Regeneration by Giving Bonegraft Golden Sea Cucumber**

Mardiana Adam, Harun Achmad, Mansjur Nasir, Sri Wahyu Putri, Azizah Azizah, Dian Eka Satya

Full Text PDF

Pages 140-147

CLINICAL ARTICLE

24.**Indicators of Basal Blood Flow in Periodontal Microvessels in Children with Diabetes Mellitus**

Abdulkadyr Abdulkadyrovich Ildarov, Guriya Sanchoevna Abdullayeva, Nargiz Nizamievna Kuhmazova, Gasan Magomedalievich Budaichiev, Khadizhat Omarovna Omarova, Zuleikha Alirzaevna Alirzaeva, Naida Medzhidovna Medzhidova, Patimat Magomedalievna Budaichieva

Full Text PDF

Pages 148-150

CLINICAL ARTICLE

25.**Dental Caries Status and Dietary Characteristics During the Covid-19 Pandemic Towards Increased Risk of Stunting among Preschool Children**

Ristya Widi Endah Yani, Isa Ma'rufi, Titah Rahayu

Full Text PDF

Pages 151-157

CLINICAL ARTICLE

26.**Effect of Bio Mechanical Preparation on Endotoxin Levels in Patients with Chronic Apical Periodontitis**

Nezar Boreak

Full Text PDF

Pages 158-164

CLINICAL ARTICLE

27.**Maxillary-arch Shape Associated with the Position of the Greater Palatine Foramen**

Margot de los Ríos Argumedo, Hanny Gonzales-Olaza, Dian Agustin Wahjuningrum, Amanda, Violeta Malpartida-Carrillo,
Maria Eugenia Guerrero

Full Text PDF

Pages 165-171

CLINICAL ARTICLE

28.**A Comparative Evaluation of the Retentive Effect of Four Different Forms of Denture Adhesives on Maxillary Dentures an in vivo Study**

Jafar Abdulla Mohamed Usman, R Haribabu

Full Text PDF

Pages 172-177

CLINICAL ARTICLE

29.**Averting Crippling Fungal Super-Infection in Era of Viral Pandemic: A Case-Control Retrospective Evidence-Based Study**

Needa Ansari, Jaiprathiksha Venkatasubramanian Iyer, Dharamveersinh Yadav, Pooja Atigre, Pushpa Kumari, Leena Jobanputra

Full Text PDF

Pages 178-183

CLINICAL ARTICLE

30.**Comparative Analysis of Clinical and Functional Parameters of the Dental Gingival Complex in Patients with a Low Height of the Clinical Crown of the Tooth**

Sergey A. Veremeenko, Yuliya A. Makedonova, Aleksey D. Mikhalchenko, Denis Yu. Dyachenko, Oksana N. Kurkina, Marya V. Kabytova, Olga Yu. Afanaseva

Full Text PDF

Pages 184-189

CLINICAL ARTICLE

31.**Self-Reported Oral Health Problems and Risk Factors Associated with 3–6-year-old Children Nutritional Status in Indonesia**

Nuzulisa Zulkifli, Anton Rahardjo, Risqa Rina Darwita, Melissa Adiatman

Full Text PDF

Pages 190-198

CLINICAL ARTICLE

32.**Evaluation of Serum CXCL2 in Health, Periodontitis and Type 2 Diabetes Mellitus with Periodontitis**

Anirudh B. Acharya, Noopur Sahu, Srinath Thakur, Betul Rahman, Nadia Khalifa

Full Text PDF

Pages 199-204

CLINICAL ARTICLE

33.**Linical and Radiographic Evaluation of Crestal Ridge Splitting Versus Osseodensification Techniques for Dental Implant Placement into the Narrow Alveolar Ridge**

Ahmed Mohamed Bilal, Mohamed Mohamed bioumy, Akram Abbass Al-Awady, Mahmoud Taha El-Destawy, Mostafa Mohamed Hosny

Full Text PDF

Pages 205-211

CLINICAL ARTICLE

34.**A Study on the Attitude of Patients Towards Dental Appointments in Qatar**

Ghadah Abu-Shawish

Full Text PDF

Pages 212-217

CLINICAL ARTICLE

35.**Mothers' Role in Dental Health Behaviors of Their Children in the Low-Income Community, Blitar Rural Area**

Thalca Hamid, Satiti Kuntari

Full Text PDF

Pages 218-222

CLINICAL ARTICLE

36.**Relationship of Patients' Perception of Malocclusion Related Quality of Life and Orthodontic Treatment Need in Adults**

Papon Chongthanavanit, Suwannee Luppanapornlarp, Siripen Arunpraphan, Irin Sirisoontorn

Full Text PDF

Pages 223-229

CLINICAL ARTICLE

37.**Color Match Clinical Evaluation and Patients' Acceptability for a Single Shade Universal Resin Composite in Class III and V Anterior Restorations**

Shaymaa M. Nagi, Lamiaa M. Moharam

Full Text PDF

Pages 230-236

CLINICAL ARTICLE

38.**The Influence of the Vertical Position of the Canine on Smile Esthetic Perceptions by Orthodontists, Restorative Dentists, and Laypersons**

Lubna Alkadi, Amal Mohammed Alhamid, Haifa Abdulaziz Almthen, Nora Mohammed Almofadhi, Rayda Ali Alhabeb, Shahad Kamal Saeed, Fathima Fazrina Farook

Full Text PDF

Pages 237-242

CLINICAL ARTICLE

39.**Knowledge and Perceptions on Molar Incisor Hypomineralization among Dental Students in Indonesia**

Enrita Dian, Lisa Rinanda Amir, Diah Ayu Maharani

Full Text PDF

Pages 243-249

CLINICAL ARTICLE

40.**The use of an Ozone Generator that Produces Ozone Using Ultraviolet Radiation for Ozonize the Contact Medium in the Treatment of Gingivitis of Young People**

Mkhoyan G. R., Razumova S. N., Volkov A. G., Dikopova N. J., Brago A.S., Bait Said O.M.H., Akhmedbaeva S.S., Serebrov K.D.

Full Text PDF

Pages 250-254

CLINICAL ARTICLE

41.**A Likert Scale Versus a Visual Analogue Scale and the Participant Response: A cross Sectional Study**

Lubna Tarek Alkadi, Emad Masuadi, Tarig Awad Mohamed, Mohamud Salaad Mohamud, Fathima Fazrina Farook

Full Text PDF

Pages 255-262

CLINICAL ARTICLE

42.**Oral Health Status and Quality of Life Among Head and Neck Cancer Subjects Receiving Radiotherapy**

Aparna K S, Manjunath P Puranik, Sowmya K R

Full Text PDF

Pages 263-267

CLINICAL ARTICLE

43.**The Role of Mastication in Improving TGF- β Levels on the Inhibition of Streptococcus sanguinis and Streptococcus mutans in Gingivitis**

Rini Sitanaya, Hans Lesmana, Jenny Sunariani, Harjanto JM, Harun Achmad, Surya Irayani, Nurwiyana Abdullah

Full Text PDF

Pages 268-273

CLINICAL ARTICLE

44.**Saliva crystallography in patients receiving chemoradiotherapy in virtue of head and neck tumors results**

Hadi Darawsheh, Artem Kashtanov researcher, Yuriy Vasil'ev., Artur Heigetyan, Evgenia Gvozdikova, Olesya Kytko, Alexandra Lyakisheva, Valeria Brichagina, Darina Shirokova, Olga Melnikova, Ali Mustafaev, Muhammet Rozyyev, Igor Zhukovets, Karina Esina,
Anastasia Solovyeva

Full Text PDF

Pages 274-279

CLINICAL ARTICLE

45.**Clinical Outcomes of Implant Supported Mandibular Overdenture with Bar and Cantilevered Ball Bar Attachment: Crossover Study**
Ahmed K. Khalifa, Ahmed Ragab Elbauomy, Diab Fattoh El Haddad
Full Text PDF
Pages 280-286
CLINICAL ARTICLE

46.**Magnification in contemporary dental practice: The case of Kazakhstan**
Madina A. Kurmanalina, Aliya A. Taganyazova, Aigul M. Sumanova, Zhanara A. Shabanbayeva, Gulnar K. Isaeva, Dinara Zh. Maratova
Full Text PDF
Pages 287-290
CASE REPORT

47.**Fixation Sequence in a Patient with Multiple Mandibular and Midface Fractures: A Case Report**
Vera Julia, Andrianto Soeprapto, Wenny Yulvie, Dwi Ariawan, Lilies Dwi Sulistyani
Full Text PDF
Pages 291-296
CASE REPORT

48.**3D Printed Retainer in Case of Bruxism**
Dobromira Shopova, Miroslava Yordanova, Svetlana Yordanova, Desislava Bakova
Full Text PDF
Pages 297-300
CASE REPORT

49.**Actinic Cheilitis vs Squamous Cell Carcinoma of the Lip: Two Case Reports and a Brief Review**
Embun Manja Sari, Devi Nasution, Irna Sufiawati
Full Text PDF
Pages 301-305
CASE REPORT

50.**Computer-aided Design and Computer-aided Manufacturing Fabricated Polyoxymethylene used for Maxillary Removable Partial Denture Framework**
Pranmas Teeranuwat, Chutimon Jindachua, Pimduen Rungsiyakull, Pisaisit Chaijareenont
Full Text PDF
Pages 306-311
CASE REPORT

51.**The Effectiveness of Corticosteroid and Diode Laser Combination Therapy in the Treatment of Severe Oral Lichen Planus: A Case Report**
Irna Sufiawati, Helen Christine, Fika Faradillah Drakel
Full Text PDF
Pages 312-314
CASE REPORT

52.**Malignant Transformation of Longstanding Dentigerous Cyst in the Antrum: A Case Report**
Chithra Aramanadka, Adarsh Kudva, Srikanth Gadicherla, Monica Charlotte
Full Text PDF
Pages 315-318
REVIEW

53.**Visualisation of Dental Technicians Distribution in Indonesia: A Review Article**
Nanda Rachmad Putra Gofur, Sianiwati Goenharto, Eny Inayati, Sri Redjeki Indiani, Okti Setyowati, Endang Kusdarjanti, Sujati,
Achmad Zam Zam Aghasy, Mochamad Nur Ramadhani, Zamros Yuzadi Bin Mohd Yusof
Full Text PDF
Pages 319-322
REVIEW

54.**The Importance of Saliva Total Protein and a-amylase on Cerebral Palsy Children**
Mega Moeharyono Puteri, Sindy Cornelia Nelwan, Brian Maulani, Barnabas Bonardo, Nita Naomi, Alit Rahma Estu, Nunthawan Nowwarote
Full Text PDF
Pages 323-329
REVIEW

55.**The Comparison Analysis of Orthophosphoric and Maleic Acids in the Smear Layer Removal Techniques**
Zurab Khabadze, Zamina Suleimanova, Saida Abdulkerimova, Yusup Bakaev, Yunus Bakaev, Khadizhat Omarova, Elmira Makaeva,
Daria Nazarova, Ekaterina Shilyaeva, Shushanik Minasyan, Oleg Mordanov
Pages 330-333 Full Text PDF
REVIEW

56.**Abutment Modification in Cemented Retained Restoration for Increasing Retention: A Scoping Review of a Current Instrument**
Tri Ardi Mahendra, Ratna Sari Dewi, Yudan Whulanza, Lindawati S. Kusdhany
Pages 334-343 Full Text PDF
REVIEW

57.**Management Congenital Heart Disease Surgery during COVID-19 : A Review Article**
Nanda Rachmad Putra Gofur, Aisyah Rachmadani Putri Gofur, Soesilaningtyas, Rizki Nur Rachman Putra Gofur, Mega Kahdina,
Hernalia Martadila Putri, Zamros Yuzadi Bin Mohd Yusof
Pages 344-348 Full Text PDF
REVIEW

58.**Biodentine as an Apical Plug Material in Immature Teeth: A Rapid Review**
Irmaleny, Ayu Rahma Dania, Diani Prisinda
Pages 349-357 Full Text PDF
REVIEW

59.**A Systematic Review of the Impact of Malocclusion on the Quality of Life among Young Adults**
Papon Chongthanavanit, Suwannee Luppanapornlarp, Narumol C. Mamani, Irin Sirisoontorn
Pages 358-364 Full Text PDF
REVIEW

60.**Teledentistry for Improving Oral Medicine Diagnosis: A Systematic Review**
Anandina I Soegyanto, Kemal N Siregar, Diah A Maharani, Yuniardini S Wimardhani
Pages 365-369 Full Text PDF
REVIEW

61.**Hodgkin's Lymphoma: Current Views on the Problem**
Nataliia I. Vynnyk, Mykhailo M. Koptev, Borys M. Filenko, Ivan I. Starchenko, Serhiy M.
Bilash, Antonina H. Sydorenko, Oksana V. Kokovska, Svitlana M. Sovhyria, Serhiy A. Proskurnya, Olena M. Pronina
Pages 370-375 Full Text PDF
REVIEW

62.**Efficacy of Platelet-rich Plasma on Promoting Bone Healing in Maxillofacial Defects: A Systematic Review**
Lilies Dwi Sulistyani, Vera Julia1, Dwi Ariawan, Yudy Ardilla Utomo, Muhammad Ramaditto Reksoprodjo, Wayan Hari Swarjaya Sandi
Pages 376-382 Full Text PDF
REVIEW

63.**Differences of the Success of Apexification with Calcium Hydroxide and Mineral Trioxide Aggregate: Scoping Review**
Irmaleny, Yolanda, Mahyuni Fitriani
Pages 383-391 Full Text PDF
REVIEW

64.**The Relationship Between Sleep Bruxism and Temporomandibular Disorders: A Systematic Review**
Christian Lokita, Lia Kartika Wulansari, Ira Tanti

Pages 392-402 Full Text PDF

REVIEW

65. **Bibliometric Analysis of the 100 Most Cited Articles on Oral Biology**
Nanda Rachmad Putra Gofur, Achmad Zam Zam Aghasy, Zamros Yuzadi Bin Mohd Yusof
Pages 403-411 Full Text PDF

REVIEW

66. **Craniofacial and upper Airway Morphology in Pediatric Obstructive Sleep Apnea Patients: A Systematic Review**
Ann Chianchitlert, Nithivoot Luengthamchat, Thanakorn Saengphen, Irin Sirisoontorn
Pages 412-421 Full Text PDF

REVIEW

67. **Ozonotherapy as a Method of Alleviating Symptoms of Oral Mucositis and other Selected Oral Diseases– Literature Review**
Michalina Zylkiewicz [Sd], Sylwia Kuderewska, Grazyna Marczuk-Kolada
Pages 422-432 Full Text PDF

MEDICINE

EXPERIMENTAL ARTICLE

68. **Survival of Methicillin-Resistant Coagulase-Negative Staphylococci from Floor Dust**
Plykaeow Chaibenjawong, Kannipa Tasanapak, Sutthirat Sitthisak, Thaniya Wongwan, Suttipalin Suwannakul
Pages 433-440 Full Text PDF

EXPERIMENTAL ARTICLE

69. **The Effects of Mobile Phones on Diabetes and Appetite**
Hava Bektas
Pages 441-447 Full Text PDF

CLINICAL ARTICLE

70. **Mother's Eating Behavior During Pregnancy and Family Income with Malnutrition: Stunting Prevention in Madura, Indonesia (Mother's Eating and family Income with Stunting Prevention)**
Esti Yunitasari, Ribka Putri Sholecha, Ni Ketut Alit Armini, Retnayu Pradanie, Wahyuni Tri Lestari, Bih-O Lee
Pages 448-453 Full Text PDF

CLINICAL ARTICLE

71. **Functional Resources and Psychological Comfort of Students from Central Asia Studying at the Northern University**
Artyom Borisovich Kiryanov, Mikhail Alekseevich Kunavin, Anna Andreevna Farkova
Pages 454-460 Full Text PDF

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Gulten UNLU (TURKEY)
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Guven ERBIL (TURKEY)
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Ilker ETIKAN (TURKEY)
Isil TEKMEN (TURKEY)
Isin ULUKAPI (TURKEY)
Jalen DEVECIOGLU KAMA (TURKEY)
Kemal CIGDEM (TURKEY)
Kemal NAS (TURKEY)
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Mehmet Emin ERDAL (TURKEY)
Mehmet Sinan DOGAN (TURKEY)
Mehmet Ünal (TURKEY)
Mehmet Zulkuf AKDAG (TURKEY)
Meral ERDİNC (TURKEY)
Michele CALLEA (ITALY)
Mohamed TREBAK (USA)
Mohammad Khursheed Alam (KSA)
Mohammad SUHRON (INDONESIA)
Mohammed Mustahsen URREHMAN (UAE)
Moschos A. PAPADOPOULOS (GREECE)
Mostaphazadeh AMROLLAH (IRAN)
M.S. Rami REDDY (INDIA)
Muhammad FAHIM (INDIA)
Mukadder ATMACA (TURKEY)
Murat AKKUS (TURKEY)
Murat SOKER (TURKEY)
Mustafa KELLE (TURKEY)
Mustafa ZORTUK (TURKEY)
Muzeyyen YILDIRIM (TURKEY)
Neval Berrin ARSERIM (TURKEY)
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The Toothpaste's Containing Coffee bean Skin Demonstrated the Specific Physicochemical Properties, Inhibited the Growth of Streptococcus mutans and Increased the Viability of Neutrophil

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Abstract

Coffee bean skin contains amino acids, proteins, flavonoids, catechins equivalent to the seeds and leaves, which is thought to be used as toothpaste. As a basic ingredient of toothpaste, coffee bean skin must have good quality.

This study to analyze the Physicochemical properties (organoleptic, pH, homogeneity, spreadability, viscosity), the inhibition to S. mutans and cell viability of toothpaste's contained Robusta coffee bean skin.

Concentrations of toothpaste were 5%, 10%, 15%. Organoleptic test observed shape, color and odor, pH used a pH meter, viscosity test used vicotester VT 03. Spreadability test by measuring the diameter of toothpaste that was smear on the glass. Inhibition was carried out by measuring the diameter of the barriers against S. mutans. Cell viability used neutrophil cells. The cells were placed in 24-well microtiter plate, treated according to group. Viability was stained with Trypan Blue. Cells were counted under an inverted microscope with magnification 400 times from an average of 4 fields of view. The data obtained were ANOVA followed by LSD test and descriptive. Toothpaste preparation was cream colored, soft texture, homogeneous, smells of coffee, has a pH of 7 in accordance with the pH requirements for toothpaste preparations according to SNI 12-3524-1995, namely 4.5-10.5. Inhibition and cell viability tests showed significant differences, but not significant between 15% concentration with commercial toothpaste (ANOVA and LSD analysis, 0.05<p).

Toothpaste's contain coffee bean skin has physico-chemical properties, inhibited to S. mutans and increase cell viability equivalent to commercial toothpaste.

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Introduction

Brushing your teeth with toothpaste is important to prevent dental and oral infections. Toothpaste serves to clean teeth, reduce plaque formation, prevent caries, clean and polish tooth surfaces, eliminate or reduce bad breath, give a fresh taste to the mouth and maintain oral health. Toothpaste products will continue to change their chemical composition due to producer competition. Toothpaste contains antibacterial properties of various substances with different abilities to inhibit the growth of germs in the oral cavity, as well as a number of ingredients with specific purposes to solve certain problems. The many choices of toothpaste with various ingredients make it difficult for patients to choose the right toothpaste[1,2]. On the other hand, toothpaste products on the market can cause side effects such as canker sores, allergies, fluorosis, thyroid disorders to cancer. Some whitening products have been reported to have a pH as low as 4.0, while others have been reported to have a pH of 7.5. It has been reported that the higher the peroxide concentration, the more acidic the pH of the bleaching product.26 Some office whitening products containing 35% hydrogen peroxide may have a low pH[3]. The use of natural ingredients such as coffee bean husks is expected to minimize side effects.

Coffee bean skin contains amino acids, proteins, flavonoids, catechins equivalent to

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seeds and leaves (results of previous research). The skin of coffee beans like coffee beans contains an antioxidant, Trigonelline which is useful for protecting teeth. Although coffee drinkers generally have a slightly black tooth color due to these substances, this compound will protect teeth against S. mutans[4,5]. On the other hand, coffee does not interfere with the results after teeth whitening, so there is no need for dietary restrictions[6]. Previously we also researched about coffee plants, including: Steeping from green and black robusta coffee beans increase viability of Peripheral Blood Mononuclear Cells (PBMC) and salivary leukocytes which is induced by streptococcus mutans,Robusta Coffee Beans (Coffea canephora) Decrease IL-1α (Interleukin-1α) Expression and Increase the Number of Fibroblasts in Healing Process in Dental Pulp in Wistar Rat, the effect of steeping robusta coffee beans on monocytes:expression of IL-1β and TNF-α against streptococcus mutans[7-9]. Meanwhile, as a basic ingredient for toothpaste, coffee bean rind must have qualities regarding physical properties and biocompatibility that are equivalent to or better than toothpastes on the market. Therefore, this study aimed to analyze the quality (organoleptic, pH, homogeneity, spreadability, viscosity), microorganism inhibition and cell viability of Robusta coffee bean skin toothpaste.

Materials and methods

Research tools include: mortar and stamper, pH meter, rotary evaporator, a set of macerator tools, viscometer, digital caliper, autoclave, petri dish, incubator, ose needle, pH indicator paper, pH meter, glass object, microtiter plate, inverted microscope, micro pipette. The ingredients used were DMSO, Robusta coffee bean skin, 96% ethanol, menthol, calcium carbonate, sodium CMC, NaCL, sodium benzoate, sodium lauryl sulfate, saccharin, sorbitol, NA media, Streptococcus mutans isolate, Histopaque (Sigma), ficolhipaque (Sigma), HBSS (Hank's Balanced Salt Solution/Gibco), fungizone (Sigma), Trypan Blue, heparin, RPMI (Sigma).

Before making toothpaste, the coffee bean skin was screened for phytochemicals using the TLC method (Thin Layer Chromatography for flavonoids, polyphenols, triterpenoids, alkaloids, anthraquinones),

1. Making Coffee Bean Skin Toothpaste

Sodium CMC is sprinkled over hot water as much as 20 times the amount of Na CMC, let stand for 30 minutes, then grind until homogeneous. Menthol is dissolved in ethanol. The extract was diluted with ethanol, added sorbitol and menthol solution, added Na CMC dispersion, stirred until homogeneous. Added calcium carbonate, sodium benzoate, sodium lauryl sulfate and saccharin, stirred until toothpaste is formed. Coffee bean skin toothpaste used with concentrations of 5%, 10%, 15%.

2. Organoleptic Test

Organoleptic test observed shape, color and odor.

3. pH measurement

The paste preparation (1 gram) was dissolved with 10 ml of distilled water and measured using an OHAUSS pH meter.

4. Viscosity test

Viscosity test using vicotester VT 03.

5. Spreadability test

Spreading power by measuring the diameter of toothpaste smeared on a glass (1 gram), covered with a glass and then given a load of 200 g.

6. Inhibitory Test.

The method used is disc diffusion, by measuring the diameter of the resistance against, S. mutans (pure isolate cultured in the Biomedical Lab, Faculty of Dentistry, University of Jember). The microorganisms were standardized with standard Mc. Farland 0.5 and obtained a concentration of 1.5x108CFU/mL. A paper disk (6mm) was inserted into the sample, then placed in a petri dish containing the solidified media. Incubated for 24 hours at a temperature of 35ºC-37ºC in an incubator, then observed the area of bacterial growth inhibition using a digital caliper (Balouir et al., 2016). David and Stout (1971) in Ouchari et al., 2019, classified the zone of inhibition (ZOI): >20 mm (very strong); 10-20 mm (strong), 5-10 mm (medium); and <5 mm (no response).

7. Cell viability test

Peripheral blood collection of healthy people as much as 6 cc is then mixed with anticoagulant (heparin). Layer 3 ml of histopaque on the falcon, 3 ml of ficoll on top of histopaque, 6 ml of blood on it carefully. ficoll-hypaque centrifugation, take the monocyte layer, add 1:1

HBSS and pipette. Centrifugation, add HBSS, fungizone and penstripe Cells were placed on a 96-well microtiter plate 8 x 105 cells/well for 45 minutes at 37°C and washed 4 times with medium. Cells were placed in 24-well microtiter plates, treated with S. mutans (100µl/well) + toothpaste (5%, 10%, 15%), except C- (S. mutans only), then incubated for 5 hours. was stained with Trypan Blue. Live cells (in white) were counted under an inverted microscope with magnification 400 times from an average of 4 fields of view.

The data obtained were ANOVA followed by LSD test and descriptive.

Results

Analysis of the levels of polyphenols (393.0166 ± 85.5224 mg GAE/g and flavonoids (0.788592 ± 0.114787 mb QE/g) using 425 nm wavelength spectrophotometry with comparison of Quercetin (flavonoid) and gallic acid (polyphenol).

The results of the physicochemical test: the preparation of coffee bean skin toothpaste has a soft, homogeneous texture, smells of coffee, has a pH of 7, in accordance with the pH requirements for toothpaste preparations according to SNI 12-3524-1995, namely 4.5-10.5. All toothpastes are homogeneous. The viscosity of the coffee bean skin toothpaste is 200 dPa, according to the standard (200-500 dPa's). The spreadability that meets the standards is 15% (5) coffee bean husk toothpaste, while 5% (4) and 10% (4.5) gels are good between 5 to 7 cm.

sample name	Organoleptic
Coffee bean skin Toothpaste 5 %	Shape: Toothpaste Color: light brown Smell: Mint coffee scent
Coffee bean skin Toothpaste 10 %	Shape: Toothpaste Color: Brown Smell: Coffee, mint
Coffee bean skin Toothpaste 15 %	Shape: Toothpaste Color: dark brown Smell: Aroma of coffee, mint
Commercial toothpaste	Shape: Toothpaste White color Smell: Mint

Table 1. Organoleptic of Coffee bean skin.

Inhibition against S. mutans, there was a significant difference from the Anova analysis and LSD test (p 0.05). All groups can inhibit S. mutans except C-. The largest diameter was found at C+ followed by T1, T2 and T3, categorized as having a strong inhibitory power (>10). The higher the concentration of coffee bean rind toothpaste, the larger the diameter of the barrier (figure 1).

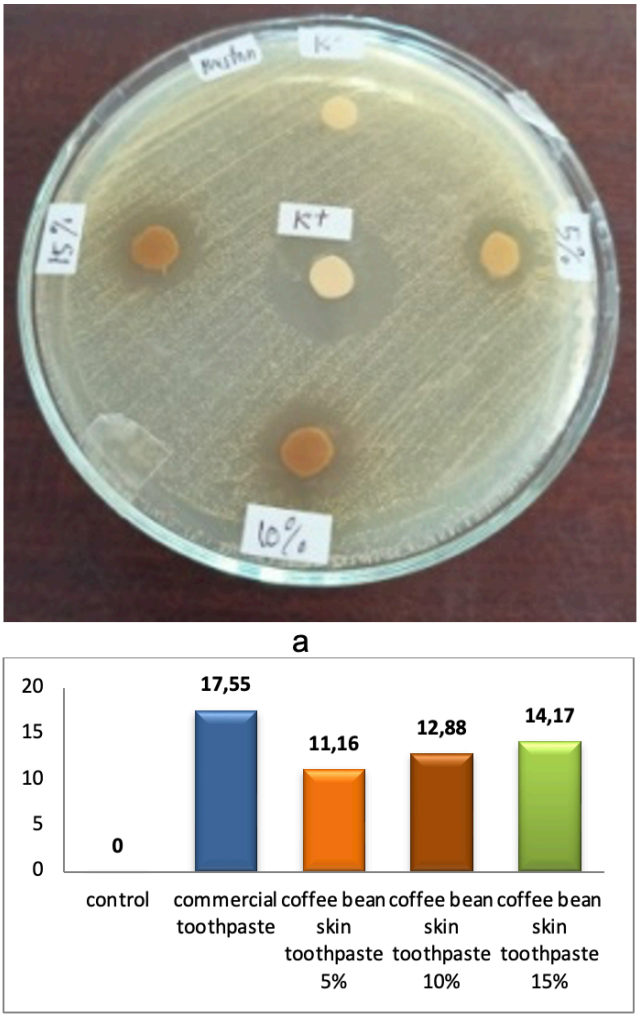

Figure 1. barrier zone of coffee bean skin toothpaste against S. mutans. The zone of inhibition (a), Bar chart (b).

The results of Anova and LSD analysis showed cell viability, C-(S. mutans) had more dead cells (dark in color), while C+(commercial toothpaste) had the most live cells (light/white in color). However, the number of live C+ cells with T1(Coffee bean skin Toothpaste 5 %), T2(Coffee bean skin Toothpaste 10 %), T3(Coffee bean skin Toothpaste 15 %) was not significantly different (Figures 2 and 3).

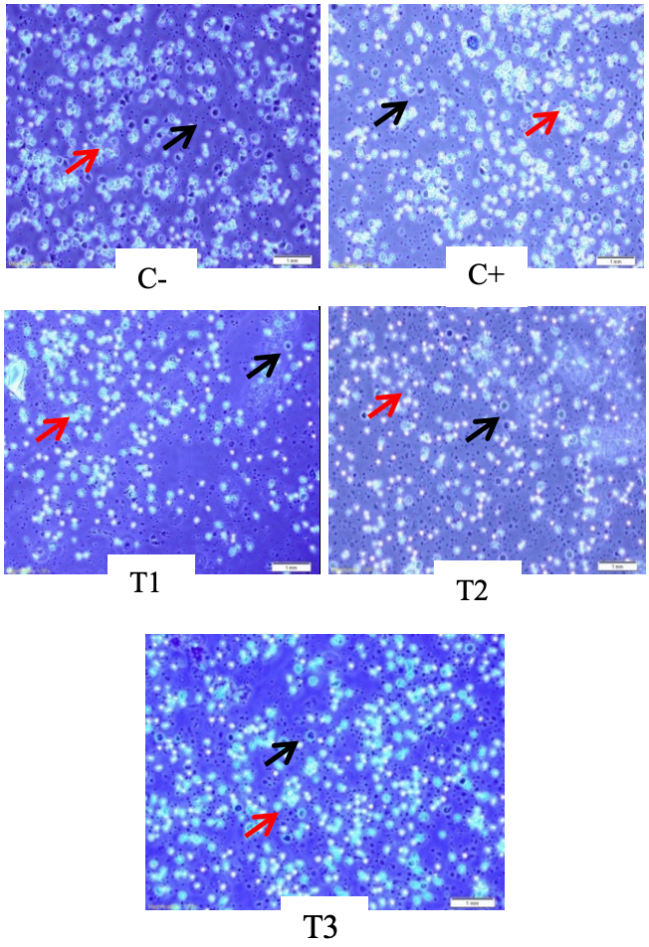

Figure 2. Cell viability was depicted with white cells (red arrows), while dead cells were dark (black arrows).

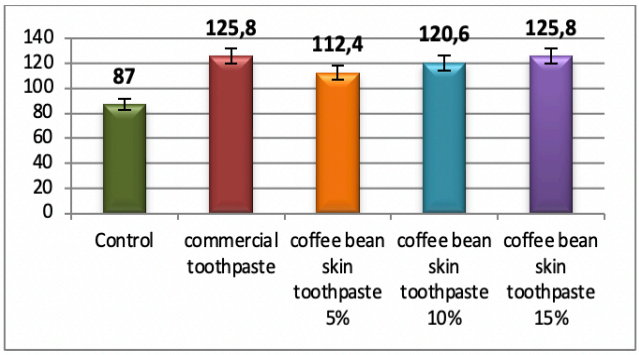

Figure 3. Bar chart of the cell viability of the coffee bean shell toothpaste.

Discussion

The preparation of coffee bean skin toothpaste is soft, homogeneous, has a distinctive coffee smell, has a pH of 7 in accordance with the pH requirements for toothpaste preparations according to SNI 12-3524-1995, namely 4.5-10.5, pH so that it does not irritate the oral mucosa. The smell of this coffee can be a special attraction, because in this world there are many coffee lovers, so we hope that many people will like it. Meanwhile, the dispersion power that most meets the standards similar to commercial toothpaste is coffee bean husk toothpaste with a concentration of 15%, meaning that the ability to spread toothpaste when applied to a toothbrush is good.

Coffee bean skin toothpaste with concentrations of 5%, 10%, 15%, and commercial toothpaste had a strong ability to inhibit S. mutans bacteria, although it was not as strong as commercial toothpaste. The higher the concentration of the coffee bean husk, the stronger the ability to inhibit the growth of S. mutans. This is presumably because of its bioactive content, the higher the concentration, the higher the bioactive content. Antioxidants (caffeic acid, chlorogenic acid, coumaric acid, sinapat acid, chlorogenic acid, flavonoids, melanoidin, faran, pitol and maltol) react directly with free radicals and form stable molecules. Chemical content of coffee such as flavonoids, xanthine, antioxidants, alkaloids, polyphenols act as anti-inflammatory, antibacterial; platelet aggregation inhibits the growth of Streptococcus mutans[7-11]. The content of alkaloids in coffee include caffeine and trigonelin. Caffeine is a purine alkaloid, has antimicrobial activity, which inhibits esterase enzymes along with DNA and RNA polymerase, inhibits cellular respiration, plays a role in DNA intercalation, causes damage and lysis[12-15]. Trigonelin which is a pyridine alkaloid can disrupt the stability of the bacterial cytoplasmic membrane which causes an imbalance in the metabolic function of bacteria so that the growth of bacteria inhibited[16]. Flavonoids are antioxidants that have antibacterial and antifungal effects because they contain phenol groups. Phenol groups can coagulate proteins, and reduce the surface tension of microbial cells[13.17]. In addition, the lipophilic nature of flavonoids interferes with microbial membranes, which will inhibit bacteria from forming a defense system[18]. Triterpenoids react with porins (trans membrane proteins) on the outer membrane of the bacterial cell wall, forming a strong polymeric bond, resulting in the destruction of the porin[7-9.19]. The ability to maintain cell viability by coffee bean rind toothpaste, especially at a concentration of 15%, is thought to be due to the presence of

flavonoids. Flavonoids activate Ca2+ in mitochondria which makes cells able to produce ATP so they can survive[20]. In addition, flavonoids have the function of preventing precipitation, damage to cell synthesis, protein denaturation, damage to cell metabolism, increasing the work of Transforming Growth Factor-beta 1 (TGF-1) which is the main factor to stimulate fibroblast proliferation. This antioxidant content is thought to work through suppression. on the production of NO (Nitric Oxide). This suppression mechanism can go through several possibilities. First, it may inhibit the action of the cytokine-induced NO synthase (iNOS) enzyme by controlling iNOS mRNA. Another possibility is to inhibit arginine transport through the CAT-2 mRNA (Cationic Amino Acid Transporter-2 mRNA) control mechanism. This can occur because it is suspected that monocyte cell damage is caused by the effects of nitric oxide (NO) free radicals. Usually NO can be produced by cells exposed to pathogens, which is caused by the formation of various cytokines and endotoxins of pathogenic bacteria that are damaging to cells. K attack pathogens, NO will be synthesized in large quantities. NO which is synthesized in large quantities will cause impaired function and cell proliferation (cytostatic)[21.22].

Conclusions

Toothpaste's contain coffee bean skin has physico-chemical properties, inhibited to S. mutans and increase cell viability equivalent to commercial toothpaste.

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Declaration of interest

There is no conflict of interest between all authors and all involved in the research or writing of articles.

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