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Efficacy Study of Eco-Friendly Bleaching Agent for Skeleton Preservation in Animal Skeleton

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Abstract

The currently used bleaching materials in skeletal preservation are hydrogen and carbamide peroxide materials with specific concentrations and techniques that impact environmental sustainability. This research describes a simple and eco-friendly technique for preserving skeletons. This research used a qualitative approach. Data were collected through observation and documentation. The collected data were analyzed descriptively. The results show that natural extracts, such as lemon combined with baking soda and commercial bleaching cloth agent, can bleach the skull and bone to preserve the skeleton. The commercial bleaching cloth agent is more capable of bleaching animal skulls and bones to preserve skeletons than natural extracts combined with baking soda do; however, the result is more brittle skeletons. Although specimens in lemon extract combined with baking soda solution more slowly clean and bleach skeleton than commercial bleaching solution, skeletal animals have bond strength and environment-friendly processes. This study recommends that an eco-friendly bleaching agent for skeletal preservation should be applied because it can cope with biological practices about osteology and other science subjects.

Keywords: Biology practice, bleaching agent, skeletal animals, skeleton preservation

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Introduction

Biology is a part of natural science that explore natural phenomena in systematically, this science is not only a collection of reliable knowledge in the form of facts, concepts, or principles but also includes scientific methods and scientific attitudes (Bonney et al., 2016; Nuangchalerm & El Islami, 2018). Students should directly experience a learning process based on science process skills and scientific attitudes. These aspects can be developed through discussion, experimentation, observation, simulation, and nature project activities in the field. In the twentieth century, these aspects become more crucial and thus, teachers should prioritize to skills-based learning (Prachagool et al., 2016; Ritter et al., 2018). Skills-based learning play an essential role in students' practical participation and individual awareness of the surrounding environment (Hayati et al., 2021). Therefore, students should be prepared to have that skills through laboratory activity-based learning for instance animal skeleton preservation.

A skeletal system is an animal structure and development that is major in biology learning. A skeletal system is built by the rigid structures of the body that play an essential role in protecting, supporting, giving, and maintaining the flexibility of body shape and helping for the movement of an animal organism (Herrmann et al., 2020; Mukund & Subramaniam, 2020). Preservation is a technique used by humans and uses material that is not easily damaged. In principle, animal preservation aims to eliminate or inhibit the process of destruction (decomposition) by microorganisms. The skeleton is preserved through several processes: preparing the materials, dissecting the organ, skinning and defleshing, decomposing, arranging of bones, bleaching, drying, varnishing of bones, articulating bones, and positioning the skeleton (Hasan & Islam, 2019). This present study

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bleaching techniques of skeleton preservation to strengthen and promote veterinary anatomy learning or to teach animal structures.

Bleaching is a common stage in skeletal preservation and various fields, such as forensic science, zoology, conservation, and anthropology (Bello & Aisha, 2021; Horák et al., 2022; Mazur et al., 2022; Simonsen et al., 2011). Generally, bleach agents use harsh, dangerous, volatile, and quite expensive chemicals, such as hydrogen peroxide, trichloroethylene, benzene, and carbamide peroxide materials with specific concentrations and techniques that impact environmental sustainability (Simonsen et al., 2011; Soares et al., 2014). These limitations, especially the character of bleaching agents, should be studied to find an alternative to manufacture a simple and environmentally friendly preservation framework. This framework can be utilized as a method of storing animal frame collections and preparing animal skeletons by students in learning processes, researchers in conservation processes, and medical sciences.

Currently, many scientists work extremely with the natural bleaching agent, such as fruits, vegetables and salts. This agent is used for teeth whitening and practice learning in osteology. Lemon fruits are extensively researched due to their bioactive composition and health benefits. Lemons contain citric acid and malic acid; the acidity of lemon juice is mainly attributed to these acids (Kang et al., 2023). Citric acid is the most abundant organic acid in lemons, while malic acid is the main acidic component of numerous fruits, such as apples, bananas, and plums, and is identified as a secondary acid substituting for citric acid in citrus fruits (Jamil et al., 2020; Savarkar et al., 2019). Malic acid is a group of carboxylic acids that can whiten teeth by oxidizing and neutralizing the surface of tooth enamel to produce a whitening effect (Junior et al., 2018). Antioxidant include 10% sodium bicarbonate solution and has been presented by manufacturers of bleaching agents as a neutralizer for the adverse effects in soft tissues. Sodium bicarbonate (SBC) that combines with other antimicrobials can increase their potential (Ramos et al., 2014). According to Keyes et al. (2023), chemical maceration using laundry detergent and sodium carbonate can macerate the remains of infant skeletal animals for forensic anthropological analysis

This study aims to analyze the use of safe, affordable, and environmentally friendly bleaching agents as a biology practice. The bleaching agent used was commercial fabric bleach, which was often used in the surrounding community. In addition, lemon extract and baking soda were selected due to their availability, low prices, environmentally friendly nature, and effectiveness as bleaching agents. The results of this study can ultimately improve the successful rate of skeletal preservation using natural bleaching agents, particularly for biology teaching and other purposes

Research Methods

Materials

The materials were skeletal animals, such as eel (*Clarias batrachus*), and domestic pigeon (*Columba domestica*). Other materials included lemon, papaya leaf, baking soda and a commercial bleaching cloth agent for bleaching solution (Proclin), water, a sample box, set dissection, gloves, mask, glue, wire, and clean paper. This research employed a qualitative approach with various bleaching agents.

Bleaching agent solution preparation

Natural bleach solution was made from two tablespoons (15 g) of baking soda ($NaHCO_3$) and two tablespoons (15 mL) of lemon extract to the distilled water (500 mL). The bleaching agent solution was prepared according to the method of Jeffers (1943) with some modifications. Meanwhile, commercial bleaching cloth were sodium hypochlorite ($NaClO_2$) and dissolved water.

Skeletal preservation preparation

Firstly, all tools and materials were prepared. Then, the animals were sedated by damaging parts of the brains of fish and by properly and accordingly slaughtering domestic pigeons. After death, the samples were boiled for about 10 minutes. The bird sample was boiled in papaya leaves so that the skinning step was easy. Afterward, the animals' bone was cleaned and kept in a bleaching agent for about 30 minutes, and then

the samples were sun-dried. The bones were arranged and connected using glue and wire. Finally, the skeleton was fixed on a wooden stand; thus, animal structure can be stored easily for teaching purposes or animal veterinary practices.

Method of Analysis

This research collected data, including bone color post-bleaching, bone strength, and characteristics. The data were then analyzed descriptively.

Result and Discussion

The present study revealed that natural extracts, such as lemon combined with baking soda, can bleach skull and bone and create eco-friendly skeleton preservation. This method can enhance biology teaching, osteology practices, and other purposes in other sciences. The present study confirms the findings of Allouch (2014), who revealed that dissection, skinning, boiling, drying, bleaching, and skeleton installation were the steps of skeleton preservation and preparation techniques. The animal skeletons were prepared by dissecting and skinning processes and removing the muscles, ligaments, tendons on the bones, and visceral organs. This stage requires thoroughness and patience to produce clean bones. Thereafter, the bone was boiled in water to complete the bone cleanliness. Next, the skinned skeleton was boiled at a high temperature to increase the breakdown of animal skeleton. The high temperatures (60°C and 55°C) had a shorter duration of maceration (one day) the low temperatures, which had three days (Mahon et al., 2021; Triaca et al., 2022).

The next step was bleaching skeletons to avoid further decomposition by microorganisms. The decomposition occurs because bones generally contain a lot of grease. This study revealed that the total duration for the animal preparation required different soaking times in different bleaching agents. A commercial bleach cloth agent needs 15 minutes to soak skeletal animals while natural bleach needs 30 minutes. According to Bemis et al. (2004), household ammonia or detergent strongly degreases skeletons of small animals and birds so that it needs a shorter time than natural bleach. For large skeletons or exceptionally greasy ones, further degreasing may be needed. After bleaching, the bones were dried using sunrays. Every process was finished very timely and quickly; thus, the bleaching bones in water solution required less time (Gofur & Khan, 2010). The results showed the color of skeletal animals in commercial bleaching cloth solution was whiter than that in natural bleaching solution. The skeleton in commercial bleaching solution is fast and clean, but the condition of the bones begins to brittle, such as the ribs, which are easily broken for two reasons. First, commercial bleach contains 5.25% sodium hypochlorite, which serves as a color pigment remover in fibers. Second, commercial bleach has high oxidation, which results in thinning bone if used too much or soaked for too long. The skeleton becomes pungent-scented due to immersion. In addition, the content can reduce the number of microorganisms on the specimen. Sodium hypochlorite is one of the ingredients that have a high oxidation power and a large redox potential. The substance is yellowish-white with a pungent aroma. This substance can be stable below 20°C (Muslim & Inayah, 2018). Sodium hypochlorite effectively removes fat and whitens specimens, although the bones will continue to break down after the process is completed (Thompson, 2015). Hot water used in the maceration step effectively and quickly removes soft tissue, and as heat increase the rate of tissue breakdown (Mairs et al., 2004; Steadman et al., 2006).

Commercial detergents containing additives enzymes, bleaching agents, and corrosion inhibitors can improve the bone maceration process and lift the stench in the sample. However, applying these agents for too long may damage the specimen and decalcification (Malesky et al., 2009). Moreover, it has been proved that detergent agents have side effects, namely causing irritation and soft tissue peeling (Api et al., 2016; Mazur et al., 2022).

Although specimens in natural bleach solution have a slower process of cleaning and bleaching than commercial bleach solution, it results in stronger bone and offers environment-friendly processes. Baking soda or sodium bicarbonate is biologically compatible with acid-buffering capacities, a

safe ingredient in dentifrice, and an antibacterial material. Its low abrasiveness can remove stains on the bones so that the bones become more sturdy and cleaner (Ahrari et al., 2017; Buelo et al., 2016). Baking soda containing $NaHCO_3$ can be useful as a stain remover on teeth and whitener because it contains dentifrices (Li, 2017). Bicarbonate ions from sodium bicarbonate are attached and can be incorporated into the surface layers of the bones so that they affect the process of whitening skeleton (Müller-Heupt et al., 2023). Lemon (*Citrus limon*) extract is the major fruit crops cultivated and consumed worldwide and contains natural antioxidant agents; for example, Oligomeric Pro-anthocyanidin Complexes (OPCs) are a class of polyphenolic bioflavonoids. The majority (>90%) of the ingredients are bioflavonoids, including hesperidin (vitamin P), neo hesperidin, and naringin. Meanwhile, the structures of more than 60 ingredients have been revealed. The ingredients not only have physiological activities, such as antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic, and vasodilatory actions (Mukka et al., 2016; Šafranko et al., 2023) but also function as their whitening agent. Jeong (2021) and Savarkar et al. (2019) state that lemon extract is an effective and safe natural tooth bleaching and result in the greatest teeth-bleaching effect when combined with $NaHCO_3$ or salt. The natural agents combined with peroxide potentially improve tooth bleaching (Ram, 2023) and reduce its toxicity (Soares et al., 2019).

After the bleaching step, stuffing and mounting steps were conducted to sundry bone animals and arrange and connect bones using glue and wire according to the concept of Van Cleave (2010) as cited in Gofur & Khan (2010) and Jannat et al. (2023). Finally, the skeleton was fixed on a wooden stand so that it could be stored easily for animal structure teaching purposes (Figure 1). The result shows that preserving skeletons using a natural bleaching agent is a simple, fast, eco-friendly, and low-cost method because this method uses materials available at home and does not require laboratory facilities. This method is preferable to biological and physical processes and can be an alternative in teaching biology, especially animal structure studies. Moreover, this method enables students to understand the characteristics of skeletal structure in the observed animals. According to Eshach and Fried (2005), using original specimens in science learning has more significant advantages than using sketches, drawings, or books. Moreover, using original specimens can attract learners' interest in understanding specimens. In addition, the skeleton preparation and preservation techniques of skeletal models of small and large animals enhance the teaching process in veterinary anatomy courses (Javid, 2023) or medical college and provide a deep understanding of osteology and scientific research for bone-related designs (Kaliyamoorthy et al., 2023).

Using a natural bleaching agent as material in animal skeletal preservation is important information for those concerned with ecological and environmental issues. This objective agrees with the three principles of the Sustainable Development Goals (SDGs), namely improving health and welfare, creating clean water, and developing economic, social, and environmental conditions for a complete global vision. A simple skeletal preservation technique using natural resources in the bleaching step can minimize toxic materials used as well as waste and pollutants generated from production and consumption processes. The use of safer and environmentally friendly chemicals can address economic and social impacts of the chemical cycle on life (Chen et al., 2020; Marion et al., 2017). The knowledge about the efficacy of skeletal preservation for teaching and learning also needs to know archaeological animal skeletal remains and learn veterinary anatomy (Javid, 2023). Further research should study the various types of animal skeletons under various conditions. It offers great advantages for coping with skeletal pathology and applying morphogenetic contexts.

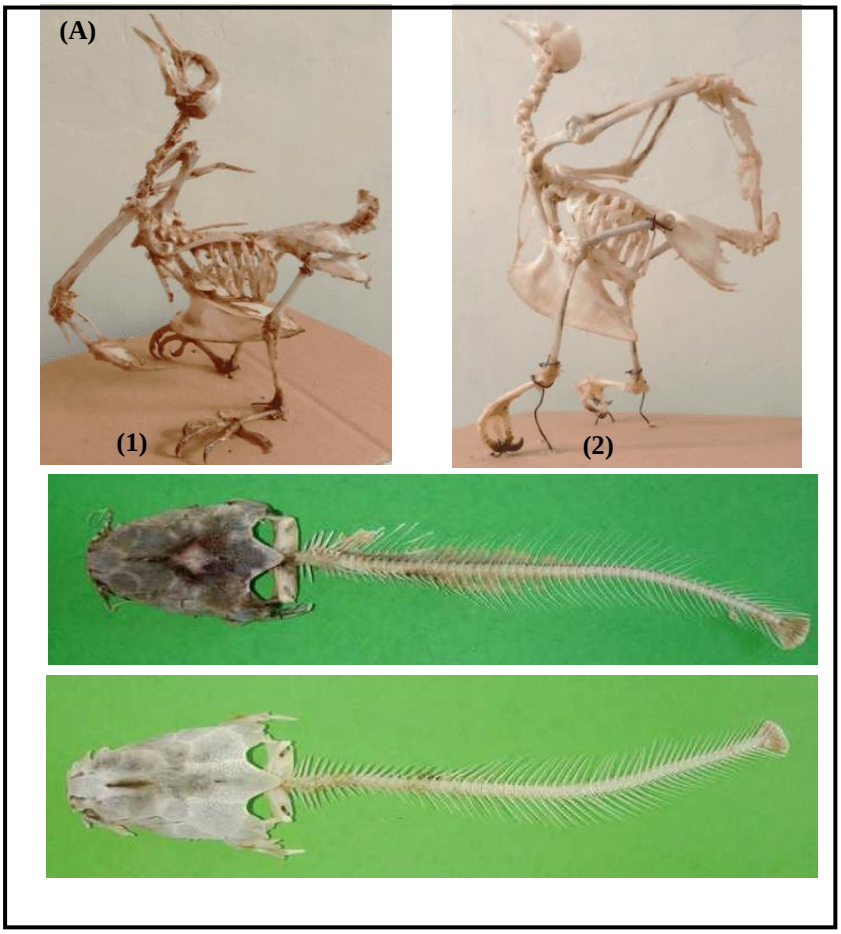

Figure 1. Skeletal preservation (A) domestic pigeon (*Columba domestica*) and (B) Eel (*Clarias batrachus*); (1) Baking soda and lemon extract solution and (2) Bleach cloth solution.

Conclusion

Commercial bleaching cloth agent more significantly preserves the skeleton of bleaching skull and bone animals than lemon extract combined with baking soda. Although specimens in lemon extract combined with baking soda solution more slowly clean and bleach bone than commercial bleach solution, the previous method results in stronger bonds and offers an environmentally friendly process. This study proved that eco-friendly bleaching agents can bleach skull and bone in skeleton preservation and significantly cope with osteology practices in biology education and other science subjects.

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References

Ahrari, F., Hasanzadeh, N., Rajabi, O., & Forouzannejad, Z. (2017). Effectiveness of sodium bicarbonate combined with hydrogen peroxide and CPP-ACPF in whitening and microhardness of enamel. *Journal of Clinical and Experimental Dentistry*, *9*(3), e344–e350. https://doi.org/10.4317/jced.53108

Allouch, G. (2014). Scientific Technique for Skeletons Preservation and Preparation of Anatomical Models to Promote Veterinary Anatomy. *Journal of Veterinary Anatomy*, *7*(2), 133–139. https://doi.org/10.21608/jva.2014.44817

Api, A. M., Belsito, D., Bhatia, S., Bruze, M., Calow, P., Dagli, M. L., Dekant, W., Fryer, A. D., Kromidas, L., La Cava, S., Lalko, J. F., Lapczynski, A., Liebler, D. C., Miyachi, Y., Politano, V. T., Ritacco, G., Salvito, D., Schultz, T. W., Shen, J., … Wilcox, D. K. (2016). RIFM fragrance ingredient safety

assessment, Eugenol, CAS Registry Number 97-53-0. *Food and Chemical Toxicology*, *97*, S25–S37. https://doi.org/10.1016/j.fct.2015.12.013

Bello, A., & Aisha, Y. (2021). Comparative Osteometric study of some selected bones of local domestic turkey and guinea fowl. *Insights in Veterinary Science*, *5*(1), 008–014. https://doi.org/10.29328/journal.ivs.1001029

Bemis, W. E., Hilton, E. J., Brown, B., Arrindell, R., Richmond, A. M., Little, C. D., Grande, L., Forey, P. L., & Nelson, G. J. (2004). Methods for preparing dry, partially articulated skeletons of osteichthyans, with notes on making ridewood dissections of the cranial skeleton. *Copeia*, *3*, 603–609. https://doi.org/10.1643/CI-03-054R1

Bonney, R., Phillips, T. B., Ballard, H. L., & Enck, J. W. (2016). Can citizen science enhance public understanding of science? *Public Understanding of Science*, *25*(1), 2–16. https://doi.org/10.1177/0963662515607406

Buelo, A., Ghassemi, A., Vorwerk, L., Hooper, W., & Nathoo, S. (2016). Clinical Study to Determine the Stain Removal Effectiveness of a New Dentifrice Formulation. *Journal of Clinical Dentistry*, *27*(3), 80–83.

Chen, T. L., Kim, H., Pan, S. Y., Tseng, P. C., Lin, Y. P., & Chiang, P. C. (2020). Implementation of green chemistry principles in circular economy system towards sustainable development goals: Challenges and perspectives. *Science of the Total Environment*, *716*, 136998. https://doi.org/10.1016/j.scitotenv.2020.136998

Eshach, H., & Fried, M. . (2005). Should science be taught in early childhood? *Journal of Science Education and Technology*, *14*(3), 315–336. https://doi.org/10.1007/s10956-005-7198-9

Gofur, M., & Khan, M. (2010). Development of a Quick, Economic and Efficient Method for Preparation of Skeleton of Small Animals and Birds. *Dasnetgroup.Com*, *2*(7), 13–17. http://dasnetgroup.com/IJBR/data/2010/July/13-17.pdf

Hasan, I., & Islam, M. R. (2019). Preparation of indigenous duck (Anas platyrhynchos) skeleton to enhance veterinary anatomy teaching. *Asian Journal of Medical and Biological Research*, *5*(3), 192–196. https://doi.org/10.3329/ajmbr.v5i3.43587

Hayati, A., Juanengsih, N., & Fadlilah, D. R. (2021). Laboratory activity-based learning to improve generic science skills on the concept of sensory systems. *Journal of Physics: Conference Series*, *1836*(1), 1–9. https://doi.org/10.1088/1742-6596/1836/1/012077

Herrmann, M., Engelke, K., Ebert, R., Müller-Deubert, S., Rudert, M., Ziouti, F., Jundt, F., Felsenberg, D., & Jakob, F. (2020). Interactions between muscle and bone—where physics meets biology. *Biomolecules*, *10*(3), 1–30. https://doi.org/10.3390/biom10030432

Horák, O., Pyszko, M., Páral, V., & Šandor, O. (2022). Degreasing and bleaching bones using light sources as a tool to increase the safety of teaching osteology at the University of Veterinary Sciences Brno. *PeerJ*, *10*, 1–9. https://doi.org/10.7717/peerj.14036

Jamil, N., Jabeen, R., Khan Musarat Riaz, M., Naeem, T., Khan, A., Sabah, N. U., Ghori, S. A., Jabeen, U., Bazai, Z. A., Mushtaq, A., Rizwan, S., & Fahmid, S. (2020). Quantitative Assessment of Juice Content, Citric Acid and Sugar Content in Oranges, Sweet Lime, Lemon and Grapes Available in Fresh Fruit Market of Quetta City. *International Journal of Basic & Applied Sciences*, *15*(1). https://www.researchgate.net/publication/348381824

Jannat, N., Islam, R., & Sultana, N. (2023). Preparing and Presenting a Pigeon Skeleton for Gross Anatomical Study Using Boiling Maceration Method: A Quick and Effective Method. *American Journal of Life Science and Innovation*, *2*(1), 26–32. https://doi.org/10.54536/ajlsi.v2i1.1269

Javid, M. A. (2023). Improvement of veterinary anatomy by using recent scientific techniques for skeleton preparation and preservation. *Pure and Applied Biology*, *12*(2), 848–853. https://doi.org/10.19045/bspab.2023.120084

Jeffers GW. (1943). Formulas for Biological Science. *The American Biology Teacher*, *6*(3), 1–71. https://doi.org/10.2307/4452043

Jeong, S.-J. (2021). Effects of Citrus limon Extract on Oxidative Stress-Induced Nitric Oxide Generation and Bovine Teeth Bleaching . *Journal of Dental Hygiene Science*, *21*(2), 96–103. https://doi.org/10.17135/jdhs.2021.21.2.96

Junior, M. T., Rodrigues, C. A., Bernardes, V. L., Berlanga de Araujo, T. S., Antonio Nicoli, G., & dos Reis Derceli, J. (2018). Dental Bleaching and New Possibilities: Literature

Review. *Health Science Journal*, *12*(6), 1–6. https://doi.org/10.21767/1791-809x.1000600

Kaliyamoorthy, K., Rajasekhar, S., & Kumar V, D. (2023). Procurement and processing of human bones in medical schools for teaching purpose: A narrative review. *Indian Journal of Clinical Anatomy and Physiology*, *9*(4), 245–251. https://doi.org/10.18231/j.ijcap.2022.052

Kang, H., Lee, S., Kim, J., Park, H., & Kim, S. (2023). Physicochemical characteristics and volatile profile of novel lemon varieties, Minimon and Jeramon. *Korean Journal of Food Preservation*, *30*(5), 770–784. https://doi.org/10.11002/kjfp.2023.30.5.770

Keyes, C. A., Giltrow, K. R., & Mahon, T. J. (2023). A comparison of maceration methods for the preparation of infant skeletal remains for forensic anthropological analysis. *International Journal of Legal Medicine*, *0123456789*, 1–8. https://doi.org/10.1007/s00414-023-03137-4

Li, Y. (2017). Stain removal and whitening by baking soda dentifrice: A review of literature. *Journal of the American Dental Association*, *148*(11), S20–S26. https://doi.org/10.1016/j.adaj.2017.09.006

Mahon, T. J., Maboke, N., & Myburgh, J. (2021). The use of different detergents in skeletal preparations. *Forensic Science International*, *327*, 110967. https://doi.org/10.1016/j.forsciint.2021.110967

Mairs, S., Swift, B., & Rutty, G. N. (2004). Detergent: An alternative approach to traditional bone cleaning methods for forensic practice. *American Journal of Forensic Medicine and Pathology*, *25*(4), 276–284. https://doi.org/10.1097/01.paf.0000147320.70639.41

Malesky, S. M., Horne, A. K., J.S.Pendergraft, & Ross, S. (2009). Evaluation of Two Degreasing Techniques used in Preserving an Equine Skeletal System. *Journal of Equine Veterinary Science*, *29*(5), 477–478. https://doi.org/10.1016/j.jevs.2009.04.169

Marion, P., Bernela, B., Piccirilli, A., Estrine, B., Patouillard, N., Guilbot, J., & Jérôme, F. (2017). Sustainable chemistry: How to produce better and more from less? *Green Chemistry*, *19*(21), 4973–4989. https://doi.org/10.1039/c7gc02006f

Mazur, M., Ndokaj, A., Bietolini, S., Nissi, V., Duś-Ilnicka, I., & Ottolenghi, L. (2022). Green dentistry: Organic toothpaste formulations. A literature review. *Dental and Medical Problems*, *59*(3), 461–474. https://doi.org/10.17219/dmp/146133

Mukka, P. K., Komineni, N. K., Pola, S., Soujanya, E., Karne, A. R., Nenavath, B., Shiva, S., & Vuppunuthula, P. (2016). An in-vitro comparative study of shear bond strength of composite resin to bleached enamel using three herbal antioxidants. *Journal of Clinical and Diagnostic Research*, *10*(10), ZC89–ZC92. https://doi.org/10.7860/JCDR/2016/19262.8676

Mukund, K., & Subramaniam, S. (2020). Skeletal muscle: A review of molecular structure and function, in health and disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *12*(1), 1–46. https://doi.org/10.1002/wsbm.1462

Müller-Heupt, L. K., Wiesmann-Imilowski, N., Kaya, S., Schumann, S., Steiger, M., Bjelopavlovic, M., Deschner, J., Al-Nawas, B., & Lehmann, K. M. (2023). Effectiveness and Safety of Over-the-Counter Tooth-Whitening Agents Compared to Hydrogen Peroxide In Vitro. *International Journal of Molecular Sciences*, *24*(3). https://doi.org/10.3390/ijms24031956

Muslim, I., & Inayah, K. (2018). Penggunaan Pemutih Pakaian Komersial (BAYCLIN) Sebagai Zat Etsa Alternatif Pada Pencapan Etsa Kain Kapas Yang Telah Dicelup Zat Warna Reaktif Dingin (Drimarene Blue K2-RL). *Prosiding Seminar Nasional I Hasil Litbangyasa Industri*, 15–20.

Nuangchalerm, P., & El Islami, R. A. Z. (2018). Comparative study between Indonesian and Thai novice science teacher students in content of science. *Journal for the Education of Gifted Young Scientists*, *6*(2), 23–29. https://doi.org/10.17478/JEGYS.2018.75

Prachagool, V., Nuangchalerm, P., Subramaniam, G., & Dostál, J. (2016). Pedagogical decision making through the lens of teacher preparation program. *Journal for the Education of Gifted Young Scientists*, *4*(1), 41–52. https://doi.org/10.17478/JEGYS.2016116351

Ram, S. (2023). Non-Peroxide Bleaching Materials: A Review. *Journal of Pharmaceutical Negative Results ¦*, *14*(02), 503–507. https://doi.org/10.47750/pnr.2023.14.02.64

Ramos, C. M., Bim Junior, O., Rodrigues, R. F., Maenosono, R. M., Alencar, M. S., Wang, L.,

& Borges, A. F. S. (2014). Bonding to bleached enamel treated with 10% sodium bicarbonate: a one-year follow-up. *Brazilian Dental Science*, *17*(4), 119–124. https://doi.org/10.14295/bds.2014.v17i4.1046

Ritter, B. A., Small, E. E., Mortimer, J. W., & Doll, J. L. (2018). Designing Management Curriculum for Workplace Readiness: Developing Students' Soft Skills. *Journal of Management Education*, *42*(1), 80–103. https://doi.org/10.1177/1052562917703679

Šafranko, S., Šubarić, D., Jerković, I., & Jokić, S. (2023). Citrus By-Products as a Valuable Source of Biologically Active Compounds with Promising Pharmaceutical, Biological and Biomedical Potential. *Pharmaceuticals*, *16*(8). https://doi.org/10.3390/ph16081081

Savarkar, S., Sankar, J., & Andrea, F. M. (2019). Efficacy Study of whitening Toothpaste containing Lemon (Citrus Limon (L) and Salt (Sodium Carbonate). *Online Journal of Dentistry & Oral Health*, *2*(3), 2–5. https://doi.org/10.33552/ojdoh.2019.02.000538

Simonsen, K. P., Rasmussen, A. R., Mathisen, P., Petersen, H., & Borup, F. (2011). A fast preparation of skeletal materials using enzyme maceration. *Journal of Forensic Sciences*, *56*(2), 480–484. https://doi.org/10.1111/j.1556-4029.2010.01668.x

Soares, D. G., Basso, F. G., Pontes, E. C. V., Garcia, L. D. F. R., Hebling, J., & De Souza Costa, C. A. (2014). Effective tooth-bleaching protocols capable of reducing H2O 2 diffusion through enamel and dentine. *Journal of Dentistry*, *42*(3), 351–358. https://doi.org/10.1016/j.jdent.2013.09.001

Soares, D. G., Marcomini, N., Duque, C. C. de O., Bordini, E. A. F., Zuta, U. O., Basso, F. G., Hebling, J., & Costa, C. A. de S. (2019). Increased whitening efficacy and reduced cytotoxicity are achieved by the chemical activation of a highly concentrated hydrogen peroxide bleaching gel. *Journal of Applied Oral Science*, *27*, 1–10. https://doi.org/10.1590/1678-7757-2018-0453

Steadman, D. W., DiAntonio, L. L., Wilson, J. J., Sheridan, K. E., & Tammariello, S. P. (2006). The effects of chemical and heat maceration techniques on the recovery of nuclear and mitochondrial DNA from bone. *Journal of Forensic Sciences*, *51*(1), 11–17. https://doi.org/10.1111/j.1556-4029.2005.00001.x

Thompson, M. C. (2015). *Preparing Skeletons for Research and Teaching from Preserved Human Specimens* (Issue December). California State University, East Bay.

VanCleave,J. (2010). How to prepare a chicken skeleton. Available: http://scienceprojectideasforkids.com.