Trop J Nat Prod Res, December 2025; 9(12): 6235 - 6238 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Structural and Morphological Characterization of Bovine Pericardium–*Nigella sativa* Composite using XRD and SEM

Ernie M. Setiawatie[1]*, Irma J. Savitri[1], Ferlina D.A.Y.P Asano[2], Aulia G. Syahria[3], Basril Abbas[4], Yessy Warastuti[4], Tantin R. Dwidjartini[4]

[1]*Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[2]*Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[3]*Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[4]*National Research and Innovation Agency, Jakarta, Indonesia*

ARTICLE INFO

Article history:
Received 10 October 2025
Revised 08 November 2025
Accepted 27 November 2025
Published online 01 January 2026

ABSTRACT

This study investigated the characterization of a bovine pericardium membrane combined with 3% *Nigella sativa* extract as a potential biomaterial for the purpose of guided bone growth. Bovine pericardium has been widely used as a membrane in regenerative procedures due to its biocompatibility and favorable mechanical properties; however, its biological activity remains limited. The addition of *Nigella sativa* extract, known for its antioxidant and antimicrobial properties, was expected to enhance the membrane's biofunctional performance. Characterization of the modified membrane was conducted using Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD). SEM analysis demonstrated a well-distributed porous morphology that supports cellular adhesion and proliferation, while XRD results revealed a semi-crystalline structure, demonstrating the composite membrane's structural stability. The incorporation of *Nigella sativa* extract enhanced the membrane's biological properties without compromising its mechanical integrity or surface structure. Overall, the findings suggest that the bovine pericardium membrane enriched with *Nigella sativa* extract exhibits enhanced bioactivity and maintains the physical characteristics necessary for use in guided bone regeneration as a barrier membrane. This material shows potential as a biocompatible and multifunctional scaffold for periodontal and other regenerative applications, warranting further in vivo investigation to evaluate its clinical effectiveness and long-term performance.

Keywords: Bovine Pericardium Membrane, *Nigella sativa*, Scanning Electron Microscopy, X-Ray Diffraction, Biomaterial, Guided Bone Regeneration

Introduction

A chronic and multifactorial inflammatory disease linked to dysbiotic biofilms. Periodontitis is characterized by gradual damage to the teeth's supporting tissues, leading to periodontal pockets, alveolar bone loss, and clinical attachment loss.[2] Periodontitis is a health problem with a high global prevalence, making identifying the best treatment options for managing this disease a critical global need.[1] The use of tissue regeneration methods to treat periodontitis has advanced significantly in the field of periodontology.[4] In line with these advances, tissue engineering is a multidisciplinary approach that supports the process of tissue regeneration in periodontitis treatment by integrating the principles of biology and engineering and utilizing various basic materials, such as biological materials, organic biomaterials, and alloplastic grafts.[10] Bovine Pericardium Membrane (BPM) is an organic biomaterial that has long been used for tissue engineering in dentistry.[11]

*Corresponding author. Email: ernie-m-s@fkg.unair.ac.id.
Tel: +62 816-520-412

Citation: Setiawatie EM, Savitri IJ, Asano FDAYP, Syahria AG, Abbas B, Warastuti Y, Dwidjartini TR. Structural and Morphological Characterization of Bovine Pericardium–*Nigella sativa* Composite using XRD and SEM. Trop J Nat Prod Res. 2025; 9(12): 6235 – 6238 https://doi.org/10.26538/tjnpr/v9i12.42

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

BPM has great potential due to the abundant availability of raw materials, customizable sizes, physical characteristics that support tissue reconstruction goals, high biocompatibility, and the ability to accelerate tissue healing.[12] Collagen, the most crucial aspect of the extracellular matrix is present in BPM, thereby supporting cell growth with a low immune response and contributing to elastic properties that can adjust to the shape of the recipient tissue.[13] The disadvantages of BPM include poor mechanical strength and rapid degradation in the tissue.[14] Numerous biological actions, such as antioxidant, antibacterial, anti-inflammatory, and osteogenic qualities, have been thoroughly investigated in this traditional medicinal plant, *Nigella sativa* (black cumin) .[8] The primary bioactive component of *Nigella sativa*, thymoquinone, has been shown to modulate inflammatory cytokines, scavenge reactive oxygen species, and stimulate osteoblastic proliferation while suppressing osteoclastic activity. These properties highlight its potential role in enhancing tissue regeneration and preventing bacterial colonization at surgical sites.[9] The integration of *Nigella sativa* extract into bovine pericardium membranes is therefore hypothesized to provide synergistic effects, combining the mechanical strength of the membrane with the bioactive therapeutic properties of the extract. In this context, BPM collagen fibers' surface shape and integrity are assessed using scanning electron microscopy examinations after the addition of *Nigella sativa*. In contrast, X-ray diffraction (XRD) tests help identify changes in crystallinity that may affect the mechanical properties of BPM. [15,16,17] Research specifically combining *Nigella sativa* at a concentration of 3% with BPM remains limited, particularly in the context of integrated characterization using SEM and XRD.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Materials

Bovine pericardium was obtained from fresh bovine tissue supplied by a certified local abattoir (Batan Research Tissue Bank, Jakarta, Indonesia). The *Nigella sativa* seeds were sourced from farmers in 8CRV+RGG, Wukirsari Village, Cangkringan District, Sleman, Yogyakarta, Indonesia. The *Nigella sativa* seeds were harvested in August 2023 and then processed into extract in December 2023. Using specimens and photos, BRIN botanists evaluated the botanical identity of the species' scientific name. A specimen bearing the voucher number (FODMNS0936) was placed in the faculty's herbarium. The 3% *Nigella sativa* extract was prepared at Materia Medika, Malang, Indonesia. Analytical-grade reagents and solvents were used throughout the experiment.

Preparation of Bovine Pericardium Membrane with Nigella sativa

The bovine pericardium was processed to obtain decellularized collagen-based membranes. After decellularization, the membranes were treated with *a* 3% *Nigella sativa* extract and stored under sterile conditions until further testing at Badan Riset dan Inovasi Nasional (BRIN), Jakarta, Indonesia. Untreated bovine pericardium membranes served as controls.

Scanning Electron Microscopy (SEM)

The Laboratory of Materials and Metallurgical Engineering, Faculty of Industrial Technology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, employed scanning electron microscopy, including SEM, JEOL, and Japan, to examine the membranes' surface morphology and porosity. Before imaging, samples were divided into tiny pieces, placed on stubs, and sputter-coated with gold. Images were acquired at various magnifications to evaluate fiber orientation, surface roughness, and pore distribution.

X-Ray Diffraction (XRD)

X-ray diffraction (XRD, Rigaku, Japan) was used to examine the crystalline structures of the membranes at the Laboratory of Materials and Metallurgical Engineering, Faculty of Industrial Technology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. The analysis was carried out at room temperature using Cu-Kα radiation (λ = 1.54 Å) operating at 40 kV and 30 mA. The 2θ range of 5–80° was used to record diffraction patterns, and the peaks obtained were compared with standard reference patterns to determine crystalline phases.

Results and Discussion

SEM Data Interpretation

The study was conducted on two samples, namely an untreated bovine pericardium membrane (BPM) and a bovine pericardium membrane with a 3% Nigella sativa extract added (BPM-NS). SEM was performed at magnifications of 2000× and 4000×. Figure 1 shows the SEM BPM results, in which collagen fibers appear with rough surfaces and non-uniform thickness.

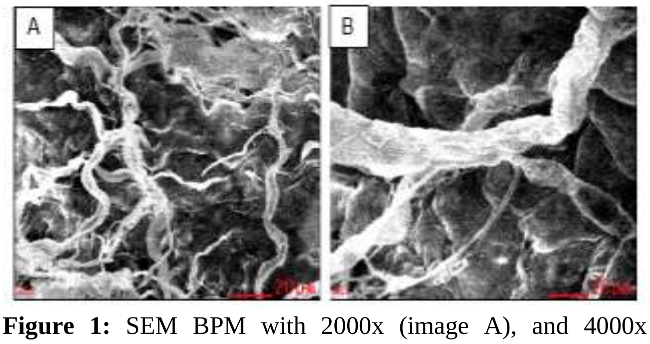

Figure 1: SEM BPM with 2000x (image A), and 4000x magnification (image B). The collagen fibers appear with rough surfaces and non-uniform thickness.

Figure 2 shows the SEM results of BPM-NS, showing denser, tighter collagen fibers with a smoother surface and uniform thickness. However, the collagen fiber diameter in both samples remained unchanged at 20 µm.

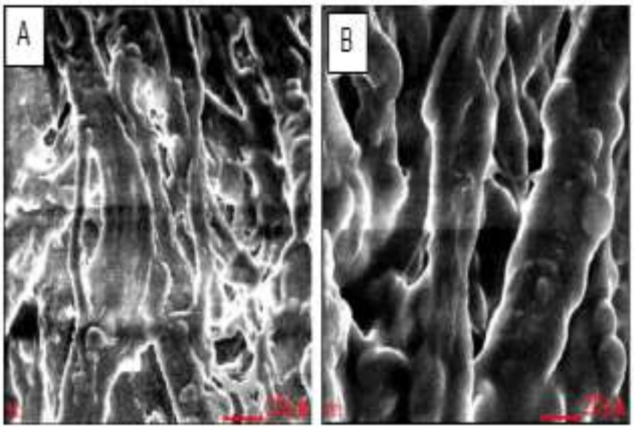

Figure 2: SEM BPM-NS with 2000x (image A), and 4000x magnification (image B). The SEM results of BPM-NS, showing denser, tighter collagen fibers with a smoother surface and uniform thickness.

XRD data interpretation

Microstructure characterization of BPM and BPM-NS was carried out using X-ray diffraction (XRD). Figure 3 shows that BPM has a semi-crystalline structure, characterized by a broad peak at a 2-Theta angle of about 20°. This is very common for collagen biomaterials, where amorphous character dominates and only a small crystalline fraction is visible. Figure 4 shows the triclinic unit cell parameters were a = 6.73728(8) A, b = 6.91560(8), according to the results of high-resolution X-ray powder diffraction and molecular localization techniques based on a simulated annealing approach following Rietveld refinement: a = 6.73728(8) A, b = 6.91560(8) A, c = 10.4988(2) A, alpha = 88.864(2) degrees, beta = 82.449(1) degrees, gamma = 77.0299(9) degrees; cell volume = 472.52(1) A3, Z = 2. The X-ray diffraction patterns showed that BPM and BPM-NS differ in their crystalline peaks. Significant results were obtained for a series of collagen fibers with uniformly homogeneous shapes in the 20 µm size range, as indicated by SEM. Finally, the infrared spectrum of the BPM-NS ensures that the highly crystalline compound with a triclinic unit cell has increased.

The current study's findings showed that the alteration of the bovine pericardium membrane with a 3% *Nigella sativa* extract (BPM-NS) significantly influenced the ultrastructural and crystalline properties of the membrane compared with the unmodified BPM. SEM analysis revealed that BPM-NS exhibited a denser, smoother, and more uniform collagen fiber arrangement, while maintaining a consistent fiber diameter of approximately 20 µm. These findings indicate that incorporation *Nigella sativa* enhanced collagen fiber packing without altering the intrinsic fibrillar dimensions. A similar stabilization and compaction effect on collagen matrices following incorporation of phytochemical bioactives has previously been reported,[9] supporting the hypothesis that thymoquinone *Nigella sativa*'s key bioactive, interacts with collagen to improve structural integrity.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

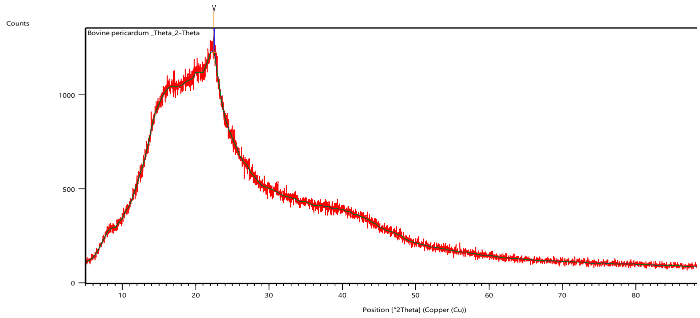

Figure 3: XRD BPM. The tested bovine pericardial membrane has a semi-crystalline structure, characterized by a broad peak at a 2-Theta angle of about 20°. This is very common for collagen biomaterials, where amorphous character dominates and only a small crystalline fraction is visible. (Source : Author's research documentation)

Thymoquinone (TQ) also inhibits the expression and activity of MMPs (MMP-1, MMP-3, MMP-9, and MMP-13) that typically break down collagen and ECM components, thereby helping to maintain matrix integrity and reduce excessive ECM degradation in inflammatory conditions or degenerative diseases such as osteoarthritis.[18,19,20]

The molecular mechanism of TQ in type I collagen synthesis involves several major cellular regulatory pathways. TQ is known to increase type I collagen mRNA expression, thereby directly increasing type I collagen production in fibroblasts or related tissues.[21] One of these mechanisms is through the activation of the PPAR-γ (peroxisome proliferator-activated receptor gamma) signaling pathway, which is an important transcription factor in the regulation of genes related to lipid metabolism, inflammation, and cell differentiation.[22] PPAR-γ activation by TQ is also associated with modulation of autophagy, which can improve cellular homeostasis and of extracellular matrix (ECM) synthesis, including type I collagen.[18]

The denser collagen network observed in BPM-NS is clinically relevant, as increased membrane compactness is associated with improved barrier functionality and reduced epithelial and fibroblast penetration, which are critical factors in guided bone regeneration (GBR) success.[5] Comparative membrane studies have shown that enhanced collagen density contributes to improved space-maintaining ability and slower enzymatic degradation rates.[6] Consistent with those findings, the present study suggests that *Nigella sativa* functionalization may serve as a bioactive strategy to reinforce biologically derived membranes without requiring cross-linking agents, which have been associated with cytotoxicity and inflammatory responses.[7]

XRD analysis further confirmed the structural modification, as evidenced by the appearance of a more defined semi-crystalline pattern in BPM-NS. This shift indicates improved molecular order and suggests chemical interaction between *Nigella sativa* constituents and collagen fibrils. Crystallinity is closely associated with membrane mechanical strength and durability; thus, the observed profile may imply enhanced physicochemical stability, which is desirable for regenerative applications.[4] Importantly, no detrimental changes in collagen fiber diameter were detected, emphasizing that the functionalization did not compromise the membrane microstructure. This aligns with modern tissue-engineering approaches favoring biofunctional enhancement over synthetic reinforcement, aiming to maintain biocompatibility while improving regenerative capacity.[1]

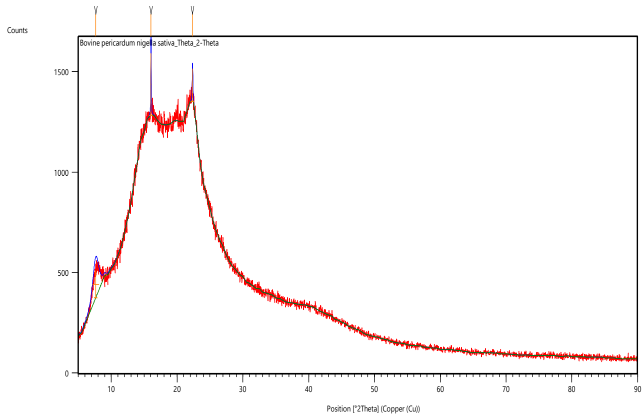

Figure 4: XRD BPM-NS. The triclinic unit cell parameters were a = 6.73728(8) A, b = 6.91560(8), according to the results of high-resolution X-ray powder diffraction and molecular localization techniques based on a simulated annealing approach following Rietveld refinement: a = 6.73728(8) A, b = 6.91560(8) A, c = 10.4988(2) A, alpha = 88.864(2) degrees, beta = 82.449(1) degrees, gamma = 77.0299(9) degrees; cell volume = 472.52(1) A3, Z = 2. (Source : Author's research documentation)

From a biological perspective, the addition of *Nigella sativa* offers potential therapeutic advantages beyond structural improvements. Thymoquinone exhibits documented antimicrobial, antioxidant, and pro-osteogenic activity,[8] which may reduce bacterial colonization and oxidative stress at defect sites and support osteoblastic differentiation. Such multifunctionality aligns with emerging biomaterial trends emphasizing scaffold bioactivity rather than serving solely as passive barriers. Integrating phytochemical-based bioactives into collagen scaffolds has been shown to enhance osteogenesis and modulate inflammation, further supporting the translational relevance of the present findings.[9]
While SEM and XRD data provide compelling evidence of improved material characteristics, additional in-depth biological evaluations, including protein material interaction assays, cell adhesion studies, and in vivo regeneration analyses are warranted to fully elucidate clinical benefits. Nevertheless, the current study supports the promising role of *Nigella sativa*-functionalized bovine pericardium as a next-generation GBR membrane with enhanced structural stability and potential biological advantages.

Conclusion

These results suggest that the bovine pericardium membrane enriched with *Nigella sativa* may serve as a promising biocompatible material for regenerative applications, particularly in periodontal therapy. Further in vivo evaluation is recommended, including Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), differential thermal analysis (DTA), differential scanning calorimetry (DSC), and a biodegradation test.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This project was funded by the Department of Periodontology Universitas Airlangga and the National Research and Innovation

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Agency (BRIN) Indonesia as stated in the letter number 084713/KRJ-UNI/III/2025.

References

1. Needleman I, Almond N, Leow N, Phillips J. Outcomes of periodontal therapy: Strengthening the relevance of research to patients. A co-created review. Periodontol 2000. 2023:1–15. doi:10.1111/prd.12483
2. Hamasni FM, El Hajj F. Treatment of a Localized Stage III Periodontitis in the Esthetic Zone with Guided Tissue Regeneration Technique on a Heavy Smoker Patient with 12 Year Follow-up: A Case Report. J Dent. 2024;25(2):183–9. doi:10.30476/dentjods.2024.100483.2224
3. Wahyuni PS, Rahardjo A, Novrinda H. Determinants of Periodontal Status in Adolescents in Indonesia: Analysis of 2018 Riskesdas Data. Cakradonya Dent J. 2024;16(1):7–16. doi:10.24815/cdj.v16i1.32134
4. Swanson WB, Yao Y, Mishina Y. Novel approaches for periodontal tissue engineering. Genes (United States). 2022;60(8–9):1–16. doi:10.1002/dvg.23499
5. Baek SH, Yang BE, Park SY, On SW, Ahn KM, Byun SH. Efficacy of Cross-Linked Collagen Membranes for Bone Regeneration: In Vitro and Clinical Studies. Bioengineering. 2025;12(8):1–15. doi: 10.3390/bioengineering12080876
6. Ren Y, Fan L, Alkildani S, Liu L, Emmert S, Najman S, Rimashevskiy D, Schnettler R, Jung O, Xiong X, Barbeck M. Barrier Membranes for Guided Bone Regeneration (GBR): A Focus on Recent Advances in Collagen Membranes. Int J Mol Sci. 2022;23(23): 14987. doi: 10.3390/ijms232314987
7. Ottenbacher N, Alkildani S, Korzinskas T, Pissarek J, Ulm C, Jung O, Sundag B, Bellmann O, Stojanovic S, Najman S, Zechner W, Barbeck M. Novel histomorphometrical approach to evaluate the integration pattern and functionality of barrier membranes. Dent J. 2021;9(11). doi:10.3390/dj9110127
8. Maduratna E, Sari DS, Rahayu RP, Masya RN, Adinar N. The Anti-Inflammatory Effect of *Nigella sativa* Toothpaste on *Porphyromonas gingivalis* Bacteria Through Decreased TNF-α, MMP-9, PGE-2 Expression in Wistar Rats. Eur J Dent. 2024;808–14. doi:10.1055/s-0043-1772700
9. Rahmani-Moghadam E, Talaei-Khozani T, Zarrin V, Vojdani Z. Thymoquinone loading into hydroxyapatite/alginate scaffolds accelerated the osteogenic differentiation of the mesenchymal stem cells. Biomed Eng Online [Internet]. 2021;20(1):1–20. doi:10.1186/s12938-021-00916-1
10. Tahmasebi E, Alam M, Yazdanian M, Tebyanian H, Yazdanian A, Seifalian A. Current biocompatible materials in oral regeneration: A comprehensive overview of composite materials. J Mater Res Technol. 2020;9(5):11731–55. doi:10.1016/j.jmrt.2020.08.042
11. Gao Y, Wang S, Shi B, Wang Y, Chen Y, Wang X . Advances in Modification Methods Based on Biodegradable Membranes in Guided Bone/Tissue Regeneration: A Review. Polymers (Basel). 2022;14(5). doi:10.3390/polym14050871
12. Amirrah IN, Lokanathan Y, Zulkiflee I, Wee MFMR, Motta A, Fauzi MB. A Comprehensive Review on Collagen Type I Development of Biomaterials for Tissue Engineering: From Biosynthesis to Bioscaffold. Biomedicines. 2022;10(9). doi:10.3390/biomedicines10092307
13. Dasuki NDNM, Taib H, Berahim Z, Hassan A. Bovine Pericardium Membrane as a Barrier Material for Periodontal Tissue Regeneration: A Retrospective Study. J Heal Sci Med Res. 2025;43(5):1–8. doi:10.31584/jhsmr.20251179
14. Yamanaka JS, Oliveira AC, Bastos AR, Fernandes EM, Reis RL, Correlo VM. Collagen membrane from bovine pericardium for treatment of long bone defect. J Biomed Mater Res - Part B Appl Biomater. 2023;111(2):261–70. doi:10.1002/jbm.b.35148
15. El-Husseiny HM, Mady EA, Kaneda M, Shimada K, Nakazawa Y, Usui T. Comparison of Bovine- and Porcine-Derived Decellularized Biomaterials: Promising Platforms for Tissue Engineering Applications. Pharmaceutics. 2023;15(7):1–17. doi:10.3390/pharmaceutics15071906
16. Nogueira GM, Rodas ACD, Weska RF, Aimoli CG, Higa OZ, Maizato M, l. Bovine pericardium coated with biopolymeric films as an alternative to prevent calcification: In vitro calcification and cytotoxicity results. Mater Sci Eng C. 2010;30(4):575–82:1-8. doi:10.1016/j.msec.2010.02.011
17. Alberts A, Moldoveanu ET, Niculescu AG, Grumezescu AM. Nigella sativa : A Comprehensive Review of Its Therapeutic Potential, Pharmacological Properties, and Clinical Applications. Int. J. Mol. Sci. 2024; *25*(24):1-28. doi:10.3390/ijms252413410
18. Soltanfar A, Meimandi Parizi A, Foad-Noorbakhsh M, Sayyari M, Iraji A. The healing effects of thymoquinone on experimentally induced traumatic tendinopathy in rabbits. J Orthop Surg Res. 2023;18(1):1–9. doi:10.1186/s13018-023-03706-8
19. Ulfah N, Sintano J, Arkan Yarus AM, Wardani PA, Maduratna E, Krismariono A. Antibacterial Activity Evaluation of Mouth Rinse containing Nigella sativa Extract Compared to Doxycycline 0,1% against Porphyromonas gingivalis and Agregatibacter actinomycetemcomitans. Res J Pharm Technol. 2023;16(9):4336–40. doi:10.52711/0974-360X.2023.00710
20. Chen WP, Tang JL, Bao JP, Wu LD. Thymoquinone inhibits matrix metalloproteinase expression in rabbit chondrocytes and cartilage in experimental osteoarthritis. Exp Biol Med. 2010;235(12):1425–31. doi:10.1258/ebm.2010.010174
21. Baştuğ AY, Tomruk CÖ, Güzel E, Özdemir İ, Duygu G, Kütan E. The effect of local application of thymoquinone, Nigella sativa's bioactive component, on bone healing in experimental bone defects infected with Porphyromonas gingivalis. J Periodontal Implant Sci. 2022;52(3):206–19. doi:10.5051/jpis.2101360068
22.