JDB Journal of Dental and Biosciences
Volume 03, Number 02, July 2026, pp.7-12
ISSN: 3089-2619 DOI 10.20884/1.jdentbios.2026.3.02.21671

ORIGINAL ARTICLE

Effect of topical *Apis trigona* honey on FGF-2 expression, fibroblast, and capillary blood vessel counts in post-extraction wound healing in a guinea pig (*Cavia porcellus*) model

Anita Dyah Septiani[1], Haris Budi Widodo[1], Helmi Hirawan[1], Ali Taqwim[1]
[1]School of Dentistry, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto-Indonesia
Correspondence e-mail to: ali.taqwim@unsoed.ac.id

Abstract

Background: Tooth extraction creates a socket wound that may develop complications and delayed healing. Fibroblast growth factor-2 (FGF-2) expression, fibroblast proliferation, and capillary formation are important indicators of tissue repair. Trigona honey contains flavonoids with antibacterial and antioxidant activities that may promote wound healing. This study evaluated the effect of topical *Apis trigona* honey on FGF-2 expression, fibroblast count, and capillary blood vessel count in post-extraction wounds. **Methods:** Thirty-two male guinea pigs (*Cavia porcellus*) were randomly allocated to control and treatment groups (n=16 each). After incisor extraction, the treatment group received 0.5 mL topical *Apis trigona* honey, whereas the control group received sterile distilled water. On day 3, mandibular tissues were collected. FGF-2 expression was assessed by immunohistochemistry, while fibroblasts and capillary blood vessels were quantified using haematoxylin-eosin staining. Data were analysed using an independent t-test with $p<0.05$. **Results:** FGF-2 expression did not differ significantly between groups ($p=0.078$). However, fibroblast and capillary blood vessel counts were significantly higher in the treatment group (both $p<0.001$). **Conclusion:** Topical *Apis trigona* honey did not significantly increase FGF-2 expression but enhanced fibroblast proliferation and angiogenesis, indicating its potential as an adjunctive agent for post-extraction wound healing.

Key words: *Apis trigona* honey, fibroblast growth factor-2, fibroblast, capillary blood vessel, wound healing

Pengaruh aplikasi topikal madu *Apis trigona* terhadap ekspresi FGF-2 serta jumlah fibroblas dan pembuluh darah kapiler pada penyembuhan luka pascapencabutan gigi pada model marmut *(Cavia porcellus)*

Anita Dyah Septiani[1], Haris Budi Widodo[1], Helmi Hirawan[1], Ali Taqwim[1]
[1]School of Dentistry, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto-Indonesia
Correspondence e-mail to: ali.taqwim@unsoed.ac.id

Abstrak

Latar belakang: Pencabutan gigi menimbulkan luka pada soket gigi yang dapat mengalami komplikasi dan keterlambatan penyembuhan. Ekspresi fibroblast growth factor-2 (FGF-2), proliferasi fibroblas, dan pembentukan pembuluh darah kapiler merupakan indikator penting dalam proses perbaikan jaringan. Madu trigona mengandung flavonoid yang memiliki aktivitas antibakteri dan antioksidan sehingga berpotensi mempercepat penyembuhan luka. Penelitian ini bertujuan mengevaluasi pengaruh aplikasi topikal madu Apis trigona terhadap ekspresi FGF-2, jumlah fibroblas, dan jumlah pembuluh darah kapiler pada luka pascapencabutan gigi. **Metode:** Sebanyak 32 marmut jantan (Cavia porcellus) dibagi secara acak menjadi kelompok kontrol dan perlakuan (masing-masing n=16). Setelah pencabutan gigi insisivus, kelompok perlakuan diberi 0,5 mL madu Apis trigona secara topikal, sedangkan kelompok kontrol diberi akuades steril. Pada hari

ke-3, jaringan mandibula diambil. Ekspresi FGF-2 dinilai dengan imunohistokimia, sedangkan jumlah fibroblas dan pembuluh darah kapiler dihitung menggunakan pewarnaan hematoksilin-eosin. Data dianalisis menggunakan uji t independen dengan p<0,05. **Hasil:** Ekspresi FGF-2 tidak menunjukkan perbedaan bermakna antarkelompok (p=0,078). Namun, jumlah fibroblas dan pembuluh darah kapiler secara signifikan lebih tinggi pada kelompok perlakuan (keduanya p<0,001). **Simpulan:** Aplikasi topikal madu Apis trigona tidak meningkatkan ekspresi FGF-2 secara signifikan, tetapi meningkatkan proliferasi fibroblas dan angiogenesis, sehingga berpotensi digunakan sebagai terapi pendukung dalam penyembuhan luka pascapencabutan gigi.

Kata kunci: Madu Apis trigona, fibroblast growth factor-2, fibroblas, pembuluh darah kapiler, penyembuhan luka

INTRODUCTION

Tooth extraction is one of the most frequently performed procedures in dentistry, and a large proportion of patients with dental pain choose extraction to relieve their complaints.[1] Extraction leaves a wound in the tooth socket that usually heals uneventfully, but complications that delay healing are not uncommon.[2] Complications after extraction generally arise when the components of wound healing do not form synergistically and completely. Wound healing is a complex and systematic cascade involving the activity of blood cells, tissues, cytokines, and growth factors.[3]

Normal wound healing proceeds through three phases: inflammation, proliferation, and maturation. Delayed healing increases the risk of infection and pain.[4] The acceleration of wound regeneration can be observed through fibroblast growth factor-2 (FGF-2) expression, the number of fibroblasts, and the number of capillary blood vessels; an increase in these three variables may serve as a marker of accelerated healing. Fibroblasts are among the key components of wound healing and are responsible for the collagen deposition required for tissue repair.[5] FGF-2 is a growth factor that marks fibroblast formation,[6] while angiogenesis (the growth of new blood vessels) occurs naturally under both healthy and pathological conditions.[7] FGF-2 functions to stimulate fibroblast proliferation and the formation of new blood vessels during wound healing.[8]

The administration of chemically based drugs may have adverse effects, particularly in patients with certain systemic conditions.[9] Antibiotic therapy plays an important role in achieving satisfactory outcomes after extraction, yet inappropriate antibiotic use can increase resistance.[10] These limitations highlight the need for a natural, topical alternative that supports wound healing without contributing to antibiotic resistance or systemic drug-related adverse effects. One of the natural materials that can be applied topically to support wound healing is honey. Trigona honey, derived from the stingless bee *Apis trigona*, is a product traditionally used in folk medicine.[11] Honey produced by *Apis trigona* exhibits antibacterial and antioxidant effects owing to its flavonoid content.[12] Based on this background, this study aimed to determine the effect of topical *Apis trigona* honey on FGF-2 expression, fibroblast count, and capillary blood vessel count in post-extraction wound healing.

METHODS

This study was a laboratory experimental study with a post-test only control group design, conducted in 2015. Ethical clearance for the use of animals was obtained prior to the study (No. 511/VII/HREC/2015, Health Research Ethics Committee, Dr. Moewardi General Hospital / School of Medicine, Sebelas Maret University, Surakarta), and all procedures were carried out in accordance with applicable institutional guidelines for the care and use of laboratory animals at the time the study was conducted.

Animals and study design

The subjects were male guinea pigs (*Cavia porcellus*). The minimum sample size was determined using the Federer formula [(t−1)(n−1) ≥ 15], resulting in 16 animals per group. The animals were allocated randomly into two groups using simple randomisation with sequentially numbered cards drawn from a sealed container: a control group (n=16) that did not receive topical *Apis trigona* honey and a treatment group (n=16) that received topical *Apis trigona* honey. Inclusion criteria were an age of approximately 4–6 months, a body weight of 400–500 g, male sex, and a healthy condition. Exclusion criteria were the presence of periodontal abnormalities and tooth luxation.

Experimental procedures

The *Apis trigona* honey used in this study was obtained from a local beekeeper in Pabelan, Mungkid, Magelang, Central Java, Indonesia. The study began with the preparation of *Apis trigona* honey, followed by obtaining permits and ethical clearance, preparation of the 32 guinea pigs, and the treatment stage. The treatment stage began with extraction of the

guinea pig incisor; guinea pigs were selected as the animal model because of their relatively large sockets, which facilitated honey application. After extraction, each socket in the treatment group received 0.5 mL of *Apis trigona* honey, whereas the sockets in the control group were cleaned with distilled water only. On day three after extraction, the animals were anaesthetised with 10% ether, decapitated, and the mandibular tissue was harvested.

Histological and immunohistochemical analysis

Tissue processing and haematoxylin-eosin (HE) staining were performed to observe the number of fibroblasts and capillary blood vessels, while immunohistochemistry was performed to observe FGF-2 expression. Immunohistochemical staining used a commercially available primary anti–FGF-2 antibody, detected with a species-matched HRP-conjugated secondary antibody and visualised with a diaminobenzidine (DAB) substrate kit, producing a brown reaction product. Fibroblasts and FGF-2 expression were observed across five fields of view, whereas capillary blood vessels were observed in the apical third of the post-extraction socket area. All observations were performed at 400× magnification. FGF-2 expression was quantified as the number of brown (DAB-positive) cells, and fibroblasts and capillary blood vessels as the number of cells or vessels per field of view; the values reported for each animal represent the mean of the five fields examined.

Data analysis

Data normality was assessed with the Shapiro-Wilk test and homogeneity of variance with the Levene test. Normally distributed and homogeneous data were analysed using the independent t-test. A *p*-value of <0.05 with a 95% confidence interval was considered statistically significant. The two independent observers were blinded to group allocation during quantification of FGF-2 expression, fibroblasts, and capillary blood vessels. Inter-observer agreement between the two observers was assessed using the Pearson correlation test.

RESULTS

This study evaluated the effect of topical *Apis trigona* honey application on FGF-2 expression, fibroblast count, and capillary blood vessel count during post-extraction wound healing. To reduce subjectivity, these three variables were assessed by two observers. The inter-observer validity test using Pearson correlation showed a strong, statistically significant agreement for all three variables (r=1.000 for FGF-2 expression, r=0.880 for fibroblast count, and r=0.936 for capillary blood vessel count; all p<0.001), indicating a significant agreement between the two observers. All variables were normally distributed (Shapiro-Wilk p>0.05 for all groups: FGF-2 expression p=0.219 and p=0.872; fibroblast count p=0.962 and p=0.665; capillary blood vessel count p=0.059 and p=0.051 for the control and treatment groups, respectively) and had homogeneous variances (Levene test: p=0.986 for FGF-2 expression, p=0.774 for fibroblast count, and p=0.224 for capillary blood vessel count), allowing parametric analysis with the independent t-test. The results of the independent t-test analysis for all three variables are presented in Figure 1.

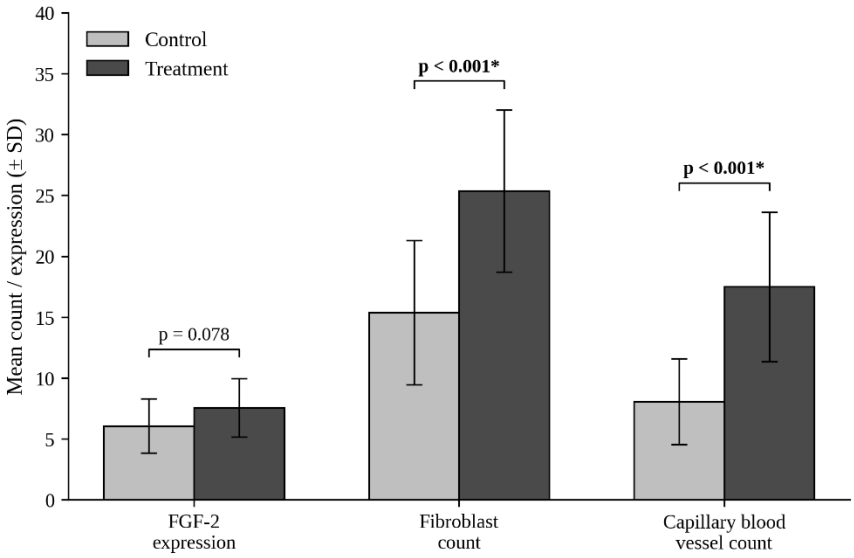

Figure 1. Comparison of FGF-2 expression, fibroblast count, and capillary blood vessel count between the control and treatment groups on day 3 post-extraction. Bars represent group means; error bars denote standard deviations. p-values were obtained from the independent t-test; asterisks (*) indicate statistical significance (p<0.05).

On day 3 post-extraction, the treatment group exhibited higher mean values than the control group for all three variables. The increase in FGF-2 expression did not reach statistical significance ($p=0.078$), whereas the fibroblast and capillary blood vessel counts were significantly higher in the treatment group (both $p<0.001$), corresponding to approximately 1.6- and 2.2-fold increases relative to the control group, respectively. These findings indicate that topical *Apis trigona* honey significantly increased fibroblast and capillary blood vessel counts during the early proliferative phase of post-extraction wound healing, whereas its effect on FGF-2 expression showed only a non-significant increasing trend.

The histological features of FGF-2 expression, fibroblasts, and capillary blood vessels in both groups are presented in Figures 2–4, allowing a qualitative comparison of the wound-healing process.

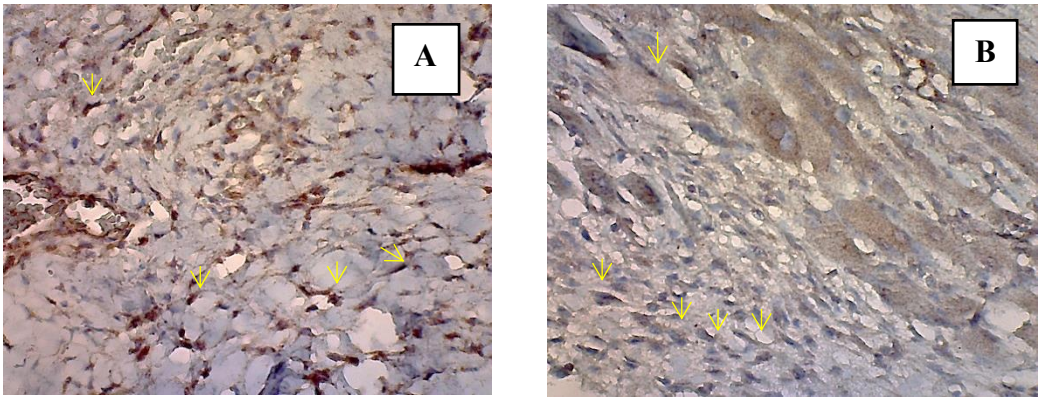

Figure 2. FGF-2 expression (arrow) with immunohistochemical staining: (A) control group; (B) treatment group; magnification 400×. FGF-2 expression, indicated by brown-stained cells, was compared between groups.

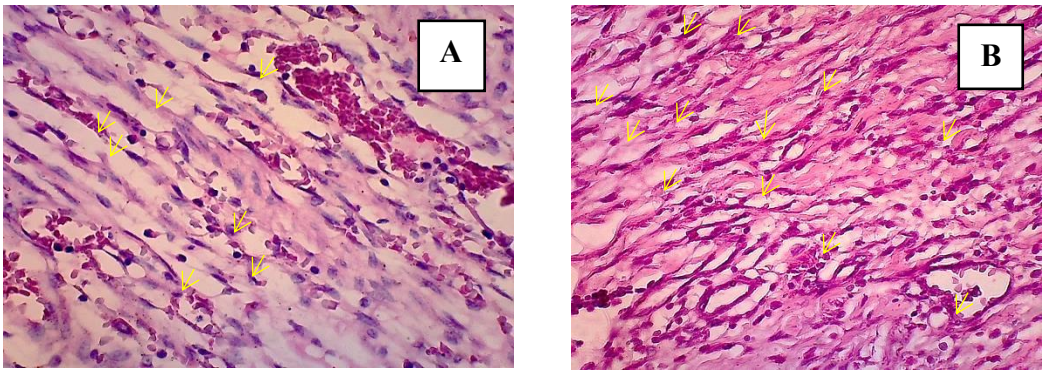

Figure 3. Microscopic appearance of fibroblasts (arrow) with HE staining: (A) control group; (B) treatment group; magnification 400×. Fibroblasts appeared as elongated, spindle-shaped cells with oval nuclei distributed among the collagen fibers.

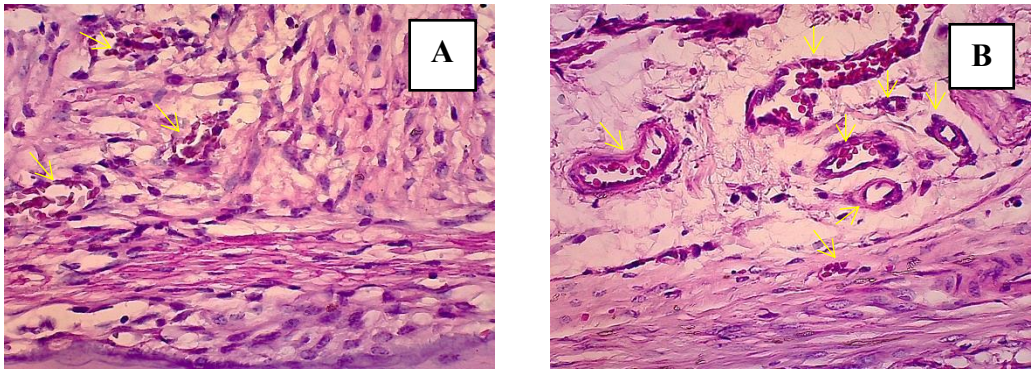

Figure 4. Microscopic appearance of capillary blood vessels (arrow) with HE staining: (A) control group; (B) treatment group; magnification 400×. Capillary blood vessels appeared as small, round to oval lumina lined by a single layer of endothelial cells, some containing erythrocytes.

DISCUSSION

The principal bioactive constituents of *Apis trigona* honey are flavonoids, which are widely reported to underlie its antioxidant and anti-inflammatory activities.[12] In the present study, however, topical application of *Apis trigona* honey did not significantly increase FGF-2 expression, suggesting that FGF-2 signaling is not a principal target of the flavonoid fraction of this honey. This interpretation is consistent with a previous report in which saponin (ginsenoside Rb1), rather than flavonoids, upregulated repair-associated growth factors including FGF-2 and PDGF;[13] stingless bee honey has not been reported to contain appreciable saponin, which may partly account for the unchanged FGF-2 expression observed here. Because cutaneous and mucosal wound healing is orchestrated by multiple growth factors acting in concert, the absence of an FGF-2 response does not preclude an overall pro-healing effect, indicating that FGF-2 is neither the sole nor the principal mediator of the responses observed in this model.

In contrast to FGF-2 expression, fibroblast counts were significantly higher in the treatment group. This difference may be attributable to the flavonoid content generally reported in stingless bee honey, although the flavonoid concentration of the honey used in this study was not directly quantified. The anti-inflammatory activity commonly attributed to flavonoids is thought to limit the influx of inflammatory cells into the wound, shortening the inflammatory phase so that TGF-β activity is less suppressed and the proliferative phase proceeds more rapidly.[14] The observation that fibroblast counts increased without a corresponding rise in FGF-2 expression is consistent with the concept that fibroblast proliferation and chemotaxis are driven not only by FGF-2 but also by other growth factors such as PDGF and TGF-β.[6] These findings suggest that *Apis trigona* honey may promote fibroblast proliferation and thereby support the early proliferative phase of wound healing.

Capillary blood vessel counts were likewise significantly higher in the treatment group. Angiogenesis during wound repair is closely coupled to fibroblast activity: proliferating fibroblasts secrete pro-angiogenic factors and remodel the provisional matrix, creating a microenvironment that supports endothelial cell proliferation and the sprouting of new vessels.[7] Flavonoids have been reported to enhance the expression of angiogenic mediators, including TGF-β and VEGF, the latter being a principal inducer of new vessel formation that acts directly on endothelial cells.[15] The parallel increases in fibroblasts and capillaries observed here are therefore biologically coherent, even though FGF-2 expression remained unchanged, because VEGF rather than FGF-2 is the dominant driver of angiogenesis, with FGF-2 playing a secondary and broader supporting role.[7]

These results indicate that the pro-healing effect of *Apis trigona* honey in this model is mediated primarily through enhanced fibroblast proliferation and angiogenesis rather than through FGF-2 upregulation. Functionally, an increased fibroblast population is expected to augment collagen deposition and accelerate wound closure, while a denser capillary network improves the delivery of oxygen and nutrients to newly forming granulation tissue. These observations are in line with earlier reports describing improved wound healing with honey and other flavonoid-rich natural products, and they support the potential use of *Apis trigona* honey as an adjunctive agent for post-extraction wounds. Several limitations should nonetheless be acknowledged. First, a single observation point on day 3 captures only the early proliferative phase and not the later remodeling phase, and no dose variation or positive comparator (e.g., a standard wound-healing agent) was evaluated. Second, the flavonoid content of the *Apis trigona* honey was inferred from prior literature rather than directly quantified; because flavonoid concentration in stingless bee honey varies with floral source, geographic origin, and harvest season, the specific composition of the batch used here is unknown. Future studies should therefore incorporate multiple time points, dose–response designs, direct phytochemical characterization (e.g., HPLC or total flavonoid content assay), and additional molecular markers such as VEGF, TGF-β, and collagen density to clarify the mechanisms underlying these effects.

CONCLUSION

Topical application of *Apis trigona* honey significantly increased fibroblast and capillary blood vessel counts in post-extraction wounds, without a corresponding increase in FGF-2 expression. These findings indicate that the wound-healing effect of *Apis trigona* honey is not mediated primarily through FGF-2, and support its potential as a supportive material for post-extraction wound healing.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support and facilities provided by the following institutions during the conduct of this research: (1) the Integrated Research and Testing Laboratory 4, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta; (2) the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta; and (3) the Anatomical Pathology Laboratory, Dr. Sardjito General Hospital, Yogyakarta. Their assistance was invaluable to the completion of this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Cao Y, Wong RCW, Jansisyanont P, Hariri F, Amir LR, Segarra MS, et al. Burden and trends of common oral disorders across the Association of Southeast Asian Nations from 1990 to 2021. *Int Dent J* 2026; 76 (1): 109395. DOI 10.1016/j.identj.2025.109395
2. Vishwanath V, Vidhale G, Sonkurla S, Mhaske A. Non-healing extraction socket: a diagnostic dilemma and contemporary review of delayed socket healing. *J Contemp Dent Pract* 2020; 21 (5): 578–583.
3. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. *Physiol Rev* 2019; 99 (1): 665–706. DOI 10.1152/physrev.00067.2017
4. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. *Open Biol* 2020; 10 (9): 200223. DOI 10.1098/rsob.200223
5. Talbott HE, Mascharak S, Griffin M, Wan DC, Longaker MT. Wound healing, fibroblast heterogeneity, and fibrosis. *Cell Stem Cell* 2022; 29 (8): 1161–1180. DOI 10.1016/j.stem.2022.07.006
6. Maddaluno L, Urwyler C, Werner S. Fibroblast growth factors: key players in regeneration and tissue repair. *Development* 2017; 144 (22): 4047–4060. DOI 10.1242/dev.152587
7. Veith AP, Henderson K, Spencer A, Sligar AD, Baker AB. Therapeutic strategies for enhancing angiogenesis in wound healing. *Adv Drug Deliv Rev* 2019; 146: 97–125. DOI 10.1016/j.addr.2018.09.010
8. Farooq M, Khan AW, Kim MS, Choi S. The role of fibroblast growth factor (FGF) signaling in tissue repair and regeneration. *Cells* 2021; 10 (11): 3242. DOI 10.3390/cells10113242
9. Little JW, Miller CS, Rhodus NL. *Little and Falace's dental management of the medically compromised patient*. 9th ed. St. Louis: Elsevier; 2018.
10. Siddique S, Chhabra KG, Reche A, Madhu PP, Kunghadkar A, Kalmegh S. Antibiotic stewardship program in dentistry: Challenges and opportunities. *Journal of family medicine and primary care* 2021; 10(11): 3951–3955. DOI 10.4103/jfmpc.jfmpc_837_21
11. Al-Hatamleh MAI, Boer JC, Wilson KL, Plebanski M, Mohamud R, Mustafa MZ. Antioxidant-based medicinal properties of stingless bee products: recent progress and future directions. *Biomolecules* 2020; 10 (6): 923. DOI 10.3390/biom10060923
12. Nordin A, Sainik NQAV, Chowdhury SR, Saim AB, Idrus RBH. Physicochemical properties of stingless bee honey from around the globe: a comprehensive review. *J Food Compost Anal* 2018; 73: 91–102. DOI 10.1016/j.jfca.2018.06.002
13. Zhang L, Hu Q, Jin H, Yang Y, Yang Y, Yang R, Shen Z, Chen P. Effects of ginsenoside Rb1 on second-degree burn wound healing and FGF-2/PDGF-BB/PDGFR-β pathway modulation. *Chin Med* 2021; 16(1): 45. DOI 10.1186/s13020-021-00455-w
14. Carvalho MTB, Araujo-Filho HG, Barreto AS, Quintans-Junior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: a systematic review highlighting the mechanisms of action. *Phytomedicine* 2021; 90: 153636. DOI 10.1016/j.phymed.2021.153636
15. Ren J, Yang R, Zhang T, Wang X, Xu B. Flavonoids as potential wound-healing molecules: emphasis on pathways perspective. *Int J Mol Sci* 2023; 24 (5): 4607. DOI 10.3390/ijms24054607