

THE EFFICACY OF USING A HONEYDEW HONEY, PROPOLIS EXTRACT AND THYME ESSENTIAL OIL DRESSING AS TOPICAL TREATMENT FOR CHRONIC WOUND IN CAT: A CASE REPORT

Octavia TAMAS-KRUMPE^{1*}, Cornelia DOȘTEȚAN ABĂLARU², Lucian PASCAL³, Cecilia Gabriella DANCIU¹, Cristina ȘTEFĂNUȚ¹, Cristina TODORAN¹ and Laurenț OGNEAN¹

¹Department of Physiology, University of Agricultural Science and Veterinary Medicine, Mănăștur Street no. 3-5, Cluj-Napoca

²Manager of S.C. Apilife Ro SRL, Taberei Street, no 17, Săliște, Sibiu county, Romania

³Veterinarian in S.C. Alvet SRL, Izlazului Street, no. 6, Cluj-Napoca, Romania

* Corresponding author, e-mail: negreaoctavia89@yahoo.com

Abstract

The healing properties of honey have been known for hundreds of years, proving its effectiveness, particularly in the wound healing process. Nowadays, a major importance was attributed to honeydew honey, due to its increased polyphenol content, antioxidant and antimicrobial activity. Propolis and thyme essential oil also represent unfailing aids in wounds' therapy, thanks to their chemical composition. The aim of the present study was to determine the clinical efficacy of a mixture consisting of honeydew honey, propolis extract and thyme essential oil in the treatment of an open chronic wound in a cat patient. The subject of our paperwork was represented by a cat, neutered male, aged one, European breed, weighing four kilograms. According to its medical history, the patient suffered from a traumatic accident by hanging in a wire fence, which resulted in an open wound in the left inguinal area. The cat was brought to ALVET veterinary clinic, because of the following clinical manifestations: apathy, loss of appetite, pain when handling the inguinal area, expressed by both aggressive behavior and vocalization. The clinical exam revealed a laceration wound in the inguinal area, which involved the whole tegument and subcutaneous tissue, while the abdominal wall remained intact. At first attempt, the wound was treated surgically, including pain medication and sedation for proper wound management, without good outcome and therefore, after two weeks we chose a traditional remedy, based on a mixture of honeydew honey, propolis soft extract and thyme essential oil. The dressing was applied once a day, for 25 days and as a result, the wound healed completely without complication and with a very good cosmetic result. We assess that apitherapy should not be simply considered as a 'folk remedy', but as a modern therapy, with a large efficacy. This research intends to inform the clinicians about the therapeutic potential of honeydew honey, propolis soft extract and thyme essential oil dressing, when applied on a chronic wound in cat.

Keywords: wound, honeydew honey, propolis, thyme, cat.

Introduction

The current situation, in which pathogens are increasingly immune to the abundance of drugs and particularly, of antibiotics and their overwhelming ability to mutate, has allowed many natural 'forgotten' remedies to once again regain their place in the medical field.

Throughout time, honey proved to meet all the criteria for its usage as a topical treatment in different types of wounds, thanks to its antibacterial, anti-inflammatory and antioxidant properties (Martinotti et al., 2015). It is worth mentioning that honey offers broad-spectrum antimicrobial properties and promotes wound healing process and consequently, the antibacterial potential of honey has been considered the exclusive criterion for its wound healing properties. Therefore, antibacterial activity of honey from different floral sources has been exclusively investigated over the past few years (Majtan et al., 2011). Moreover, numerous research have revealed the efficacy of honey in rapidly clearing wound infections and enhancing the healing process (Chirife et al., 1983; Molan, 2002; Molan, 2006; Majtan et al., 2014; Vica et al., 2014).

Nowadays, a major importance is attributed to the differentiation between floral and honeydew honey. Floral honey is made by honeybees from the nectar of blossoms, while honeydew honey is prepared from secretions of plants or excretions of plant-sucking insects (Sanz et al., 2005). Another notable difference between floral, respectively honeydew honey refers to their chemical composition. Studies pointed out that the differences appear predominantly because of the passage of juice through insect's intestine. Therefore, honeydew honey contains enzymes of saliva glands and intestine that induce differences regarding the spectrum of carbohydrates. In addition to this, honeydew honey has been found to contain higher amounts of di- and trisaccharides, as well as lower mean contents of glucose and fructose than nectar honey. Moreover, honeydew honey also presents a high polyphenol content, antioxidant and antibacterial activity (Rodriguez et al., 2015).

The treatment of wounds represents a challenge in both human and veterinary medicine field and as a consequence, the development of more effective treatment strategies is certainly required. In this regard, another promising candidate besides honeydew is propolis, a natural resinous substance collected by honey bees from the tree buds or other botanical sources such as poplar, willow, birch, elm, alder, beech, conifer, and horse-chestnut trees (Kuropatnicki et al., 2013). Furthermore, a plethora of studies have demonstrated that propolis possesses anti-inflammatory, antioxidant, antibacterial (Bosio et al., 2000) and antifungal (Bezerra et al., 2015) activities based upon their constituents including flavonoids such as galangin and caffeic acid (Cushnie and Lamb, 2005; Qiao and Chen, 1991). Additionally, recent research has emphasized that dressings consisting of propolis and honeydew honey have a synergistic effect and can accelerate tissue repair (Takzaree et al., 2016).

According to the literature, thymus oil, derived from the herb *Thymus vulgaris* has been widely reported to have significant antibacterial and antifungal properties and to promote the healing of wounds and burns (Bozin et al., 2006).

The present study was designed to determine the clinical efficacy of a dressing consisting of fir honeydew honey, propolis soft extract and thyme essential oil as topical treatment for an open chronic wound in a male cat patient. Moreover, this research intends to inform the clinicians about the valuable therapeutic potential of natural products, regarded by many as "folk remedies" in the wound healing process and regenerative therapy.

Materials and methods

Patients and conventional therapy

The present research was carried out on a neutered male cat patient, aged one, European breed, weighing four kilograms and it was brought to the ALVET veterinary clinic, located in Cluj-Napoca, on the 11th of July 2018, with the following clinical symptoms: pain when handling the inguinal area, expressed by both aggressive behavior and vocalization, apathy, loss of appetite. According to its medical history, the patient suffered from a traumatic accident by hanging in a wire fence, which resulted in an open wound in the inguinal area.

The clinical exam emphasized a laceration in the inguinal area, which involved the whole tegument and subcutaneous tissue, while the abdominal wall remained intact; additionally, body temperature was measured and the patient did not present any fever. An immediate therapy was implemented on 11th of July and included general anesthesia, wound debridement, wound cleansing by using Ringer's solution, delayed primary closure, topical treatment by applying zinc oxide ("Protego" powder spray) in a thin layer once a day, for five days over the affected area.

The general anesthesia protocol consisted of premedication with Medetomidine (0.04 mg intravenously) and Butorphanol (0.8 mg intravenously), induction with Diazepam (0.8 mg intravenously) and Propofol (8 mg intravenously) and maintained anesthesia with isoflurane (1.5 %). Moreover, after five days of using Zinc oxide ("Protego" puder spray) as topical treatment, the wound did not show any signs of improvement and therefore, it was replaced with oxytetracycline chlorhydrate ("Neocaf" spray), which was applied once a day, for ten days. Furthermore, since the first day the patient was brought to the clinic, it has received Enrofloxacin 0.5 ml, subcutaneously, ("Enroxil" 5%) for seven days. After two weeks of clinical observations and implemented therapy, the wound did not suggest any signs of improvement and as a consequence, an alternative treatment was required. More importantly, before resorting to an alternative therapy, a blood sample was collected on EDTA anticoagulant, in order to evaluate the patient's health status through haematological tests. The haematological tests were performed within the Animal Physiology laboratory of the Faculty of Veterinary Medicine Cluj-Napoca, with the Abacus Junior Vet automatic analyzer, by using Diatron kits, which determined: red blood cells (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volum (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), thrombocytes (PLT), leukocytes (WBC), and leukocyte formula indices such as lymphocytes (LY), monocytes and eosinophils (MI), neutrophils (GR). There were also performed stained smears by using the Dia-quick panoptic method, for leukocyte formula in order to help diagnose any condition affecting white blood cells, such as infection or inflammation.

Cleaning and Dressing Application

The alternative therapy consisted of a dressing containing fir honeydew honey, propolis soft extract and thyme essential oil. The dressing has been applied topically once a day, in the morning, since 25th of July, on the previously cleaned wound with Ringer solution for 25 days, until 18th of August; the mixture was applied in a thick layer, which covered the entire wound area by using sterile spatula, and after that, a thin layer of micronized Aluminum ("Aluderm" spray) was pulverized on it, in order to maintain the dressing by avoiding its leakage and speed the wound healing process.

Wound Inspection

The wound was inspected daily since applying the dressing and any changes regarding its aspect was pointed out; moreover, the width of the laceration was measured with a wound measuring ruler in order to highlight any improvement of the wound status and pictures were taken on weekly basis.

Dressing's composition

The dressing was made by mixturing Romanian fir honeydew honey, propolis soft extract and several drops of thyme essential oil and it was provided by S.C. Apilife Ro SRL, located in Săliște, Sibiu county. Only natural, unprocessed and 100% λ -irradiated sterile fir honeydew honey was used in this study. The propolis soft extract was obtained from propolis tincture, after alcohol evaporation and it was incorporated in the fir honeydew honey together with several drops of thyme essential oil. The thyme essential oil was purchased from local commerce.

Evaluation of the dressing's bactericidal effect

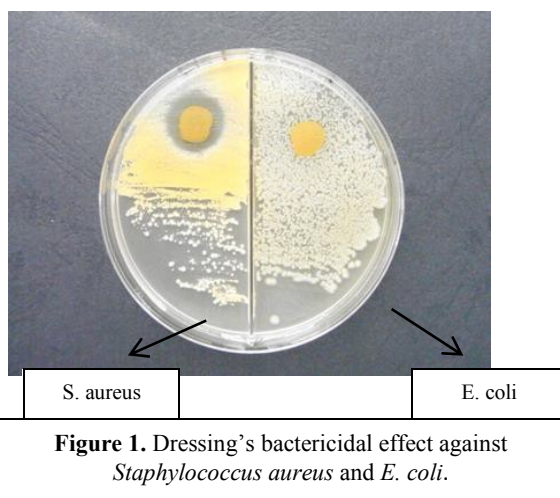
The two international reference strains (*Staphylococcus aureus* ATCC 6538P and *Escherichia coli* ATCC 25922) stored in soft agar collection were seeded on a sheep blood agar Petri plate and incubated for 24 hours at 37 ° C. From pure cultures, suspensions were made in sterile saline solution with a density equal to 0.5. The testing of the bactericidal activity of the dressing was performed by a diffusometric method similar to the antibiogram method. In this regard, a half of Mueller-Hinton medium Petri plate was seeded with 1.0 ml suspension of the *Staphylococcus aureus* strain ATCC 6538P and the second half of the suspension of *Escherichia coli* ATCC 25922 was deposited. After the surface of the medium culture was covered with the bacterial suspension, we removed the excess culture with the pipette with which it was deposited initially in the plate on the surface of the medium. A similar procedure was followed with the second reference strain, after which the Petri plate was held with the semi-open cover near the gas bulb in order to dry the surface of the environment. A sterile filter paper of 0.5 cm diameter was then deposited with an ophthalmic brush on each sector seeded with the international reference strain and 20 µl of the dressing sample was placed on the roundel. Then, the Petri plate was incubated for 24 hours at 37 ° C. The tests were carried out within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca.

Results and discussions

After two weeks of non-responding treatment, an alternative therapy was required regarding the patient's laceration wound but before this, haematological tests were performed in order to evaluate the health status. The haemoleucogram revealed that the majority of the parameters were within the physiological values, such as RBC ($9.71 \times 10^{12}/L$), Hb (14.9 g/dl), Hct (41.79%), MCV (43 fl), MCH (16.4 pg), MCHC (35 g/dl), PLT ($353 \times 10^9/L$), excepting WBC (white blood cells) and MI% (monocytes and eosinophils). It was registered a slight leukocytosis ($22.73 \times 10^9/L$; normal values: $5.50-19.50 \times 10^9/L$) and an increased MI% (6.3 %; normal values: 1.0-3.0 %), while the number of the other white cells were within the normal values.

The main disadvantage of the Abacus Junior Vet automatic analyzer was that it could not distinguish between monocytes and eosinophils (MI %), and therefore, a leukocyte formula was performed and it confirmed the monocytosis. Monocytosis often occurs during chronic inflammatory state (Ghergariu et al., 2000), which can be associated with the prolonged duration of the non-healing wound.

Before using the dressing consisting of Romanian fir honeydew honey, soft propolis extract and thyme essential oil as a topical treatment for the patient's wound, it was tested the bactericidal effect of the mixture against *Staphylococcus aureus* (ATCC 6538P) and *E. coli* (ATCC 25922). The results highlighted that the strain of *Staphylococcus aureus* ATCC 6538P was the only one sensitive to the action of the mixture, while the *E. coli* strain was resistant (Figure 1).



Numerous investigations demonstrated that honeydew honey can be applied topically, when it facilitates healing of different types of infected wounds or it can be used in order to promote the healing process in those wounds, which are infected and do not respond to the conventional therapy, such as antibiotics and antiseptics, including wounds infected with MRSA (methicillin-resistant *Staphylococcus aureus*) (Yao et al., 2011). Moreover, a stronger antimicrobial activity of propolis was noticed on *Staphylococcus aureus* (Krol et al. 1993, Fernandes Júnior et al., 2003, Sforcin et al. 2000). Several other studies showed that thyme essential oil exerts strong inhibitory action against resistant strains of *Streptococci*, *Pneumoniae*, *Staphylococcus aureus* and *Salmonella typhimurium* (Shin and Kim, 2005).

In this research, a topical treatment represented by a dressing consisting of honeydew honey, propolis soft extract and thyme essential oil contributed to the healing wound process in a cat, within 25 days. It is worth mentioning that the patient did not show any side effects, such as signs of allergies, pruritus, erythema after applying the dressing, but even more, the overall tolerance was good during the entire period of treatment.



Figure 2. Chronic non healing wound before applying the dressing topically (25th of July, 2018)

On the 25th of July, after two weeks of conventional therapy applied on the open chronic wound from the inguinal area, the cat was brought to Alvet Clinic. It was a laceration which did not show any signs of healing; it was surgically debrided and its diameter measured 2.5 cm (Figure 2).

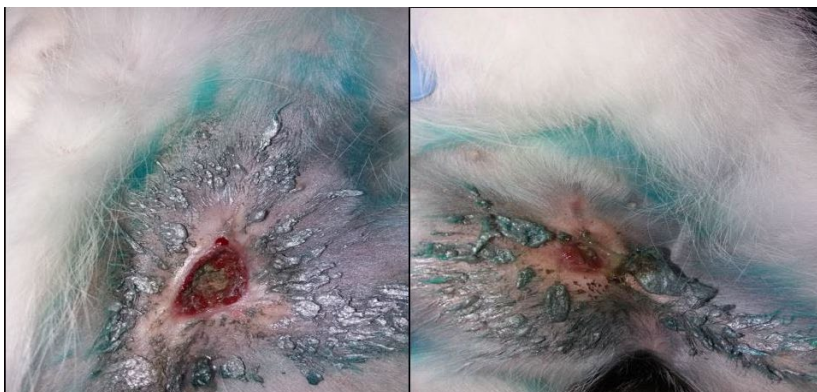


Figure 3. Chronic wound, after one week of applying the dressing topically (1st of August, 2018)

Figure 4. Chronic wound, after two weeks of applying the dressing topically (8th of August, 2018)

A week later, on 1st of August, the diameter of the wound decreased significantly, from 2.5 cm to 1.3 cm. Moreover, the granulation tissue covered the whole wound (Figure 3). Cats are known to produce significantly less granulation tissue than dogs, and the granulation tissue first appears on the wound edges and then slowly advances across the wound surface (Bohling and Henderson, 2006). On the 8th of August, the wound was notably improving and measuring 0.8 cm in diameter, epithelialization beginning to occur from periphery (Figure 4). On the 15th of August, the wound diameter has dramatically decreased over 3 weeks of topical treatment with honeydew honey, propolis extract and thyme essential oil. In addition to this, after 25 days, the wound healed completely without complication and with a very good cosmetic result (Figure 6).

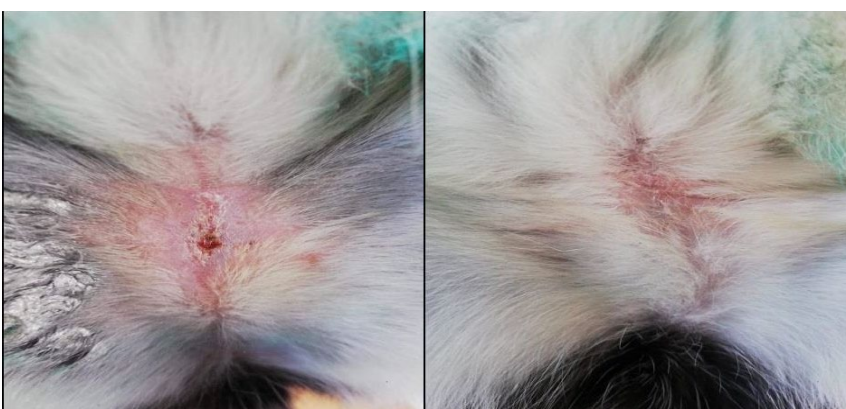


Figure 5. Chronic wound, after three weeks of applying the dressing topically (15th of August, 2018)

Figure 6. Healed wound, after 24 days of applying the dressing topically (18th of August, 2018)

The success of the implemented therapy can be associated with the valuable ingredients that the dressing used as topical treatment consists of. Honeydew honey may heal wounds through a combination of pathways.

It has been shown that honeydew honeys generate higher amounts of hydrogen peroxide, one of the major antibacterial components, than other honeys, including Manuka honey (Bucekova et al., 2014). Moreover, once wound exudate is removed, honey stimulates granulation, epithelialization and angiogenesis, which helps speed up the healing process (Wijesinghe et al., 2009). Furthermore, thanks to its osmotic properties, resulting from the high level of simple sugars (80%), honey possesses the amazing ability to debride necrotic tissue, which serves as a basic source for bacterial infection and related inflammation (Molan, 2009).

There is also evidence which suggests that thyme oil, when topically applied, increases collagen deposition, angiogenesis and keratinocyte migration in wound healing (Komarcevic, 2000). Biological activities of propolis on wound repair and tissue regeneration might be correlated to its antimicrobial, anti-inflammatory and immunomodulatory properties; additionally, the antimicrobial properties of propolis are essentially due to the flavonoid content and in particular to the presence of pinocembrin, galangin, and pinobanksin (Castaldo, 2002).

Our study showed that the healing process of a chronic traumatic laceration in a cat patient with the use of a dressing, consisting of honeydew honey, propolis soft extract and thyme essential oil can be effective in wound care. Moreover, the wound healing process resulted in a very good cosmetic appearance, for the owners' satisfaction.

Conclusions

On the basis of our results we conclude that the topical treatment of wounds with a dressing consisting of honeydew honey, soft propolis extract and thyme essential oil was successful in promoting proper wound closure of a complicated chronic wound. Furthermore, it had a positive impact on wound healing, deserving more than the clinicians' passing curiosity.

We also highly recommend that gamma-irradiated Romanian honeydew honey to be accepted by conventional medicine in order to be used for wound dressings without any reservations, due to its large proven efficacy in wounds therapy. We also advocate the idea that more randomized studies need to be done, in order to show the long-term outcome in acute and chronic open wound management in cats.

References

1. Bezerra A.M.F., Bezerra K.K.S, Albuquerque F.G.F., Fernandes Filho A., Casimiro G.S, Nunes E.M. (2015). Red propolis antifungal action on species of *Candida* of the oral cavity. *Int. Arch. Med.* 8.
2. Bohling, M. W., Henderson R. A. (2006). Differences in cutaneous wound healing between dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* 36, 687-692.
3. Bosio K., Avanzini C., D'avolio A., Ozino O., Savoia D. (2000). In vitro activity of propolis against *Streptococcus pyogenes*. *Lett. Appl. Microbiol.* 31 (2), 174–177.
4. Bozin B., Mimica-Dukic N., Simin N., Anackov G. (2006). Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J Agric Food Chem.* 54: 5, 1822-1828.
5. Bucekova M., Valachova I., Kohutova L., Prochazka E., Klaudivy J., Majtan J. (2014). Honeybee glucose oxidase- its expression in honeybee workers and comparative analyses of its content and H₂O₂-mediated antibacterial activity in natural honeys. *Naturwissenschaften*, 101, 661-670.
6. Castaldo S.C.F. (2002). Propolis, an old remedy used in modern medicine. *Fitoterapia*, 73:S1–6.

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7. Chirife J., Herszage L., Joseph A., Kohn E.S. (1983). In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. *Antimicrob. Agents Chemother.* 23: 766–773.
 8. Cushnie T.T., Lamb A.J. (2005). Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26 (5), 343–356.
 9. Fernandes Júnior A., Balestrin E.C.C., Cunha M.L.R.S. (2003). Anti-Staphylococcus aureus activity of bee propolis extracts prepared with different ethanol concentrations. *Rev Ciênc Farm* 24: 147-152
 10. Ghergariu S., Pop Al., Kadar I., Spînu Marina (2000). *Manual de laborator clinic veterinar*. Edit. All, București.
 11. Komarcevic A. (2000). The modern approach to wound treatment. *Med Pregl.*, 53: 7-8, 363-368.
 12. Krol W., Scheller S., Shani J., Pietsz G., Czuba Z. (1993). Synergistic effect of ethanolic extract of propolis and antibiotics on the growth of Staphylococcus aureus. *Arzneimittel-forsch* 43: 607-60.
 13. Kuropatnicki A.K., Szliszka E., Krol W. (2013). Historical aspects of propolis research in modern times. *Evid. Based Complement. Altern. Med.*
 14. Majtan J., Majtanova L., Bohova J., Majtan V. (2011). Honeydew honey as a potent antibacterial agent in eradication of multi-drug resistant *Stenotrophomonas maltophilia* isolates from cancer patients. *Phytother Res*, 25:584-7.
 15. Majtan J., Bohova J., Horniackova M., Kludiny J., Majtan V. (2014). Anti-biofilm effect of honey against wound pathogens *Proteus mirabilis* and *Enterobacter cloacae*. *Phytother. Res.*, 28:69–75.
 16. Martinotti S., Calabrese G., Ranzato E. (2015). Honey and Wound Healing: New solutions from an old remedy. In *Wound Healing: Cellular Mechanisms, Alternative Therapies and Clinical Outcomes*, Wade, L.E., Ed. Nova Publishers Inc., Hauppauge, NY.
 17. Molan P.C. (2002). Not all honeys are the same for wound healing. *Bull. Eur. Tissue. Rep.*, 9: 5–6.
 18. Molan P.C. (2006). The evidence supporting the use of honey as a wound dressing. *Int. J. Low Extremity Wounds*, 5: 40–55.
 19. Molan P. C. (2009). Debridement of wounds with honey. *J. Wound Technol.*, 5, 12–17.
 20. Qiao Z., Chen R. (1991). Isolation and identification of antibiotic constituents of propolis from Henan, *Zhongguo Zhong Yao Za Zhi* 16 (8) (1991) 481 in Chinese.
 21. Rodriguez Flores S., Escuredo O., Seijo C.M. (2015) Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. *Food Chem* 166(1):101–106.
 22. Sanz M.L., Gonzalez M., De Lorenzo C., Sanz J., Martinez-Castro I. (2005). A contribution to the differentiation between nectar honey and honeydew honey. *Food Chem* 91:313–317.
 23. Sforcin J.M., Fernandes Júnior A., Lopes C.A.M., Bankova V., Funari S.R.C. (2000). Seasonal effect on Brazilian propolis antibacterial activity. *J Ethnopharmacol* 73: 243-249.
 24. Shin S., Kim J.H. (2005). In vitro inhibitory activities of essential oils from two Korean thymus species against antibiotic-resistant pathogens. *Arch Pharm Res* 2005; 28: 8, 897-901
 25. Takzaree N., Hadjiakhondi A., Hassanzadeh G., Rouini M.R., Manayi A.(2016). Synergistic effect of honey and propolis on cutaneous wound healing in rats. *Acta Med. Iran.* 54 (4):233–239.
 26. Vica M.L., Glevitzky M., Dumitrel G.A., Junie L.M., Popa M. (2014). Antibacterial activity of different natural honeys from Transylvania, Romania. *J. Environ. Sci. Health.*, 49: 176–181.
 27. Wijesinghe M., Weatherall M., Perrin K., Beasley R. (2009). Honey in the treatment of burns: a systematic review and meta-analysis of its efficacy. *N. Z. Med. J.*, 122(1295), 47–60.
 28. Yao L. K., Razak S. L. A., Ismail N. (2011). Malaysian gelam honey reduces oxidative damage and modulates antioxidant enzyme activities in young and middle aged rats. *J Med Plant Res*, 5, 5618-5625.