International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 6 Issue 4, May-June 2022 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Invitro Evaluation of Antibacterial Activity of Honey against Wound Pathogen

J. Ishwarya, R. Ellammal

PG and Research Department of Microbiology,
Kamban College of Arts and Science for Women, Tiruvannamalai, Tamil Nadu, India

ABSTRACT

The medicinal importance of honey has been documented in the world's oldest medical literatures, and since the ancient times, it has been known to possess antimicrobial property as well as wound-healing activity. The healing property of honey is due to the fact that it offers antibacterial activity, maintains a moist wound condition, and its high viscosity helps to provide a protective barrier to prevent infection. Its immunomodulatory property is relevant to wound repair too. The antimicrobial activity in most honeys is due to the enzymatic production of hydrogen peroxide. However, another kind of honey, called non-peroxide honey (viz., manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked. Its mechanism may be related to the low pH level of honey and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes. The medical grade honeys have potent in vitro bactericidal activity against antibiotic-resistant bacteria causing several life-threatening infections to humans. Thus, identification and characterization of the active principle(s) may provide valuable information on the quality and possible therapeutic potential of honeys (against several health disorders of humans), and hence we discussed the medicinal property of honeys with emphasis on their antibacterial activities.

How to cite this paper: J. Ishwarya | R. Ellammal "Invitro Evaluation of Antibacterial Activity of Honey against Wound Pathogen" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-6 | Issue-4, June 2022, pp.955-959, URL: www.ijtsrd.com/papers/ijtsrd50210.pdf

IJTSRD50210

KEYWORDS: *Honey, Antibacterial activity, Wound healing property, Medical-grade honey, Medicinal property*

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey.

The use of traditional medicine to treat infection has been practiced since the origin of mankind, and honey produced by *Apis mellifera* (*A. mellifera*) is one of the oldest traditional medicines considered to be important in the treatment of several human ailments. Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria. In most ancient cultures honey has been used for both nutritional and medical purposes. The belief that honey is a nutrient, a drug and an ointment has been carried into our days, and thus, an alternative medicine branch, called apitherapy, has been developed in recent years, offering treatments based on honey and other bee products against many diseases including bacterial infections.

At present a number of honeys are sold with standardized levels of antibacterial activity. The *Leptospermum scoparium* (*L. scoparium*) honey,the best known of the honeys, has been reported to have an inhibitory effect on around 60 species of bacteria,

including aerobes and anaerobes, gram-positives and gram-negatives. Natural honey of other sources can vary as much as 100-fold in the potency of their antibacterial activities, which is due to hydrogen peroxide. In addition, honey is hygroscopic, which means that it can draw moisture out of the environment and dehydrate bacteria, and its high sugar content and low level pH can also prevent the microbes from growth.

Honey is a sweet, viscous food substance made by honey bees and some related insects, such as stingless bees. Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew), by regurgitation, enzymatic activity, and water evaporation. Honey bees store honey in wax structures called honeycombs, whereas stingless bees store honey in pots made of wax and resin. The variety of honey produced by honey bees (the genus *Apis*) is the best-known, due to its worldwide commercial production and human consumption. Honey is collected from wild bee colonies, or from hives of domesticated bees, a practice known as beekeeping or apiculture (meliponiculture in the case of stingless bees).

WOUNDS AND BURNS

Honey is a popular folk treatment for burns and other skin injuries. Preliminary evidence suggests that it aids in the healing of partial thickness burns 4–5 days faster than other dressings, and moderate evidence suggests that post-operative infections treated with honey heal faster and with fewer adverse events than with antiseptic and gauze. The evidence for the use of honey in various other wound treatments is of low quality, and firm conclusions cannot be drawn. Evidence does not support the use of honey-based products for the treatment of venous stasis ulcers or ingrown toenail. Several medical-grade honey products have been approved by the FDA for use in treating minor wounds and burns.

FOLK MEDICINE

In myths and folk medicine, honey was used both orally and topically to treat various ailments including gastric disturbances, ulcers, skin wounds, and skin burns by ancient Greeks and Egyptians, and in Ayurveda and traditional Chinese medicine.

MEDICINAL PROPERTY

Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the medical profession, particularly where conventional modern therapeutic agents fail. The first written reference to honey, a Sumerian tablet writing, dating back to 2100-2000 BC, mentions honey's use as a drug and an ointment. Aristotle (384-322 BC), when discussing different honeys, referred to pale honey as being "good as a salve for sore eyes and wounds". Manuka honey has been reported to exhibit antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Helicobacter pylori* (*H. pylori*) making this honey a promising functional food for the treatment of wounds or stomach ulcers.

The honey has been used from ancient times as a method of accelerating wound healing, and the potential of honey to assist with wound healing has been demonstrated repeatedly. Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds. The healing properties of honey can be ascribed to the fact that it offers antibacterial activity, maintains a moist wound environment that promotes healing, and has a high viscosity which helps to provide a protective barrier to prevent infection. There are many reports of honey being very effective as dressing of wounds, burns, skin ulcers and inflammations; the antibacterial properties of honey speed up the growth of new tissue to heal the wound. The medihoney and manuka honey have been shown to have *in vivo* activity and are suitable for the treatment of ulcers, infected wounds and burns.

The honey, when applied topically, rapidly clears wound infection to facilitate healing of deep surgical wounds with infection. The application of honey can promote the healing in infected wounds that do not respond to the conventional therapy, *i.e.*, antibiotics and antiseptics, including wounds infected with methicillin-resistant *S. aureus*. Moreover, it can be used on skin grafts and infected skin graft donor sites successfully.

Raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants. Clinical observations have been reported of reduced symptoms of inflammation when honey is applied to wounds. The removal of exudate in wounds dressed with honey is of help in managing inflamed wounds. It was concluded that proper characterization of the bioactivities of different honey types was required for a systematic and reliable comparison of their potential wound healing performance. Wounds infected with *Staphylococcus aureus* are quickly rendered sterile by honey. Different types of honey are available depending on the source of nectar used in its production. The source of the nectar determines the degree of antibacterial activity of the honey. The aim of the study was to determine the antibacterial activity of honey against wound pathogen.

Material and Methodology

Collection of honey

Three honey samples produced by three different species of honey-bees viz. A. mellifera, A. serena and A. dorsata were collected from different sources.

CONFIRMATORY TEST OF NATURAL (RAW) HONEY

A spoonful of honey was added to a glass of warm water, stirred slowly. This helped to find whether it dissolved in the water. Most raw honey sticks together and sunk as a solid lump or remains stuck as a lump on the spoon. A fire was set to a candle wick dipped in honey to check for added water in the honey which might prevented the honey from burning.

PREPARATION OF HONEY CONCENTRATION

Natural (raw) and processed honey was used. Different concentrations of each honey contributing 25% v/v, 50% v/v, 75% v/v and 100%v/v were made in sterile distilled water. This was done by dissolving the respective volumes of honey into corresponding volumes of sterile distilled water

COLLECTION OF PUS AND SWAB SAMPLE OF INFECTED WOUNDS

The samples were taken from patients visiting private clinics. The samples were collected by swabbing the surface of an infected wound by sterile swab and moistened the swab by placing it on transport media, transported to the Microbiology laboratory and processed immediately.

PROCESSING OF SAMPLE IDENTIFICATION OF TEST ORGANISMS

The pus and swab samples were inoculated on Mannitol Salt Agar (MSA) for the selective isolation of Staphylococcus aureus. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification by Gram staining, Catalase and Coagulase test of the organisms subcultured on Nutrient Agar.

ANTIBIOTIC SENSITIVITY TEST OF THE ISOLATED S. AUREUS

The broth cultures of S. aureus of 0.5 Mc Farland Turbidity standards were inoculated by swabbing on MHA. The refrigerated antibiotic discs were kept at room temperature for about 30 minutes before use. Antibiotic discs of Methicillin (5 mcg), Tetracyclin (30 mcg), Ciprofloxacin (5 mcg), Vancomycin (30 mcg) and Co-trimoxazole (25 mcg) were used. An antibiotic sensitivity test was performed using the modified Kirby-Bauer Disc Diffusion technique.

ASSAY OF HONEY AGAINST S. AUREUS

Crude, unprocessed and undiluted honey samples from A. mellifera, A. serena and A. dorsata were taken for the study. Antibacterial activity was analysed by the agar well diffusion technique on MuellerHinton Agar (MHA). The broth cultures of S. aureus of 0.5 Mc Farland Turbidity standards were inoculated by swabbing on MHA, and four wells were made with the help of sterile cork borer of 5mm diameter. 50 µL of three honey samples were dispensed in three different wells and the same volume of sterile water in one well. The plates were incubated overnight at 37^{o} C aerobically after complete diffusion of honey under refrigerated condition. After overnight incubation, the zones of inhibition on MHA plates around the wells were observed, and the diameters of the inhibition zones were measured.

RESULTS AND DISCUSSION

S. aureus was tested for the antibacterial activity of honey obtained from A. mellifera, A. serena and A. dorsata. In the present study, the means of a diameter of inhibition zones of 38 S. aureus isolates due to A. mellifera, A. serena and A. dorsata were found to be 13 mm, 8 mm and 22 mm respectively. Thus, honey obtained from A. dorsata (wild bee species) was found to be the most effective against S. aureus isolates from infected wounds. The past studies done for the antibacterial activity of honey were not found to be correlated with the bee-species, but with the type of honey sample and with the plant source from which nectar has been collected.

Antibiotic sensitivity test of the isolated S. aureus was also performed using the antibioticsMethicillin (5 mcg), Tetracyclin (30 mcg), Ciprofloxacin (5mcg), Vancomycin (30 mcg) and Co-trimoxazole (25 mcg). The most effective antibiotic against S. aureus tested was found to be Vancomycin with 100% efficacy. 57.9% of the isolates were sensitive to Tetracyclin, 42.1% of the isolates were sensitive to Ciprofloxacin, 34.2% were sensitive to Methicillin, and only 15.8% were sensitive to Co-trimoxazole. The antibiotic sensitivity pattern of the isolated S. aureus showed that most of the isolates were sensitive to vancomycin and tetracycline, but with other antibiotics, a considerable number of the isolates were resistant too. The effectiveness of the honey sample against the antibiotic-resistant organisms helps to treat the open wounds due to burning, abrasions, incision infected with S. aureus.

The lowest concentration of sugar that prevents the growth of S. aureus has a water activity of 0.864, equivalent to a concentration of 29% (v/v)3. (Water activity is a measure of the consequential effect of the

average intermolecular forces between water molecules, being increased when water molecules become oriented on the surface of solute molecules. When numerous molecules are tied up in this way the water molecules are on average less free to act, e.g. to hydrate something, so the 'activity' is lower.) Granulated sugar packed in an abdominal wound becomes diluted by body fluids within 4h for the water activity to increase to 0.897, which will allow growth of S. aureus. This is equivalent to a concentration of honey of 22%. In the present study, both of the honeys inhibited S. aureus completely at much greater dilution. This is because their mode of action is not exclusively through their osmolarity. The lack of significant variance in the sensitivity of a large number of clinical isolates collected from a wide range of wounds indicates that there is no mechanism of resistance to either of the additional types of antibacterial activity in honey (phytochemical or hydrogen peroxide). This contrasts with the variations seen in staphylococcal sensitivity to antibiotics14. Thus, either of these two honeys might be an effective treatment for a wound infected with any strain of S. aureus. However, although their MIC values differed little in vitro, in vivo the hydrogen peroxide produced in mixed pasture honey would be partly inactivated by the catalase in tissues and blood15 and manuka honey with its non-peroxide antibacterial activity is likely to be more effective. Their relative merits need to be tested in clinical trials.

CONCLUSION

Honey obtained from wild species of bee might be useful for the local application in the treatment of wounds infected by S. aureus. In this study it has been found that *Staphylococcus aureus* can be identified using Gram staining and simple biochemical test such as catalase, coagulase and oxidase tests. Finding of this study indicated that honey is active against *Staphylococcus* isolated from infected wound. The antibacterial activity of honey is largely due to certain antibacterial properties it possessed. These include high osmotic pressure, low water activity, low PH and possession of certain Gluco-oxidase enzymes which produce aseptic substances such as Hydrogen peroxide. It is concluded that honey and stem bark extract of *Vitex doniana* can be used as a therapy for wound infection.

BIBILIOGRAPHY

[1] Ahmed I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multi-drug resistance human pathogens. J Ethnopharmacol 74(2): 113-123.

[2] Ali M, Yahaya A, Zage AU, Yusuf ZM (2017) *in-vitro* Antibacterial Activity and Phytochemical Screening of Psidium guajava on Some Enteric Bacterial Isolates of Public Health Importance. Journal of Advances in Medical and Pharmaceutical Sciences 12(3): 1-7.

[3] Antibacterial Activity of Honey against Methicillin-Resistant Staphylococcus aureus: A Laboratory-Based Experimental Study Mohammedaman Mama ,1 Teklu Teshome,2 and Jafer Detamo3 Madda Walabu University, Goba Referral Hospital, Department of Medical Laboratory Science, Goba, Ethiopia Arba Minch University, College of Medicine and Health Sciences, Department of Biomedical Sciences, Arba Minch, Ethiopia Arba Minch University, College of Medicine and Health Sciences, Department of Pharmacy, Arba Minch, Ethiopia.

[4] Antibacterial Activity of Honey on Staphylococcus aureus Isolated from Wounds Anima Shrestha1* and Milan Kandel2 1Department of Microbiology, Tri-Chandra College, Tribhuvan University, Nepal 2 Department of Microbiology, St. Xavier's College, Tribhuvan University, Nepal Nepal Journal of Science and Technology (NJST) (2020), 19(1)

[5] Antimicrobial Activity of Honey Piotr Szweda May 31st, 2016Reviewed: November 30th, 2016Published: March 15th, 2017 DOI: 10.5772/67117

[6] Bilal AN, Molan PC, Sallal AK (1998) Antimicrobial activity of honey onselected microorganisms: A preliminary study. Biomed Res (India) 9: 51-54.

[7] Bogdanov, S., 1984. Characterization of antibacterial substances in honey, Lebensm, Wiss. u. Technol. 30: 748-753.

[8] Evaluation of antibacterial activity of honey against multidrug resistant bacteria in Ayder Referral and Teaching Hospital, Northern EthiopiaAraya Gebereyesus Wasihun1* and Berhe Gebreslassie Kasa2 Wasihun and Kasa SpringerPlus (2016) 5:842

[9] Fatemeh Ahmadi–Motamayel F, SeyedehSareHendi, Mohammad Yusof Alikhani, Zahra Khamverdi (2013) Antibacterial Activity of Honey on Cariogenic Bacteria. J Dent (Tehran) 10(1): 10-15.

[10] Halawani E, Shohayeb M (2011) Survey of the antibacterial activity of Saudi and some international honeys. J MicrobiolAntimicrob 3(4): 94-101.

[11] Holt JG, Krieg NR, Sneath PA, Stanley JT, Williams ST (1994) Bergey's manual of systematic bacteriology. (9th edn), Williams & Wilkins Co. Baltimore, Maryland, USA, p.786.

[12] Honey: its medicinal property and antibacterial activity Manisha Deb Manisha Deb Mandal and Shyamapada Mandal /Asian Pac J Trop Biomed 2011; 1(2): 154-160

[13] Hyungjae L, Churey JJ, Worobo RW (2008) Antimicrobial activity of bacterial isolates from different floral sources of honey. Int J Food Microbiol 126(1-2): 240-244.

[14] Irish J, Carter D, Blair S (2005) Honey kills some of our most dangerous microbial enemies. In Proceedings of the 39th Congress of the International Federation of Beekeepers, Dublin, Ireland, pp. 21–26.

[15] JN Ramalivhana CL Obi A Samie BC Iweriebor P Uaboi-Egbenni JE Idiaghe MNB Momba DOI: 10.5897/AJB11.892

[16] Joshua B, Keshu ND (2015) Comparing the Antibacterial and Functional Properties of Cameroonian and Manuka Honeys for Potential Wound Healing-Have We Come Full Cycle in Dealing with Antibiotic Resistance?. Molecules 20(9): 16068-16084.

[17] Maeda Y, Loughrey A, Earle JA, Millar BC, Rao JR, et al. (2008) Antibacterial activity of honey against community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Complement Ther Clin Pract 14(2): 77-82.

[18] Majtan J, Kaludiny J, Bohova J, Kohutova L, Dzurova M, et al. (2012) Methylglyoxal-induced modifications of significant Honeybee proteinous components in manuka Honey: possible therapeutics implications. Fitoterapia 83(4): 671-677.

[19] Molan P (2011) The evidence and the rationale for the use of honey as a wound dressing. Wound Practice and Research 19(4): 201-221.

[20] Molan, P.C.1992. The antibacterial activity of honey. Bee World. 73: 5-28, 59-76. Russel, K.M., P.C. Molan, A.L. Wilkins and P.T. Holland. 1990. Identification of some antibacterial constituents of New Zealand manuka honey. J. Agric. Food Chem. 38: 10-13.

[21] Rubin RH (2006) Surgical wound infection: epidemiology, pathogenesis, diagnosis and management. BMC Infect Dis 6: 171-172.

[22] SaadAlmasaudi The antibacterial activities of honey Saudi Journal of Biological Sciences Volume 28, Issue 4, April 2021, Pages 2188-2196

[23] Selcuk, H. and Nevin, K., 2002. Investigation of antimicrobial effect of honey collected from various regions of turkey. Pakistan Journal of Biological Sciences. 5 (3): 325-328.

[24] Subrahmanyam, M., Hemmady, A.R. and Pawar, S.G. 2001. Antibacterial activity of honey on bacteria isolated from wounds. Annals of Bums and Fire Disasters. 14 (1), 198-201. Subrahmanyam, M., 1991.Topical application of honey in the treatment of burns. Br. J. Surg.78: 497-498.

[25] Weston R (2000) The contribution of catalase and other natural products to the antibacterial activity of honey: a review. Food Chem 71(2): 235- 239.