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A study on the effect of storage time on Antibacterial Activity of honey

Jovita Saldanha[1,*], Suhas V. Abhyankar[2], Shankar Srinivasan[3], Arvind M. Vartak[4]

[1]Research Officer, [2]Assistant Honorary, [3]Fellow, [4]HOD, Burns & Plastic Surgery, Bai Jerbai Wadia Hospital for Children, Mumbai, Maharashtra

***Corresponding Author:**
Email: joviorwill79@gmail.com

Abstract

It is an age old belief that honey improves with age. In our laboratory, we conducted a study on the antibacterial property of honey against burn wound isolates. Honey freshly obtained from forest and commercially available honey was put to test against burn wound isolates. The freshly obtained honey samples showed good antimicrobial effect against burn wound isolates, forest honey showing a better antibacterial effect than commercial products. However the same honey when stored for 1 year and further on for three years showed reduced antimicrobial effect. Hence we can conclude that freshly obtained honey has antimicrobial activity against more number of burn wound isolates as compared to older honey.

Antibacterial property of honey on burn wound isolates does reduce with age. The specific reason for this reduced antibacterial property with respect to age needs further analysis. However on the basis of our findings we can suggest that the antibacterial property of honey must be assayed before any therapeutic use.

Keywords: Honey, Burns wound isolates, Antibacterial property, Invitro.

Introduction

The use of natural products to enhance wound healing is a common practice in many parts of the world.(1) Honey is one such natural product that has gained a lot of importance. It has been used as a medicine since ancient times and is still used in folk medicines [2]. The use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times and it is gaining acceptance as an anti bacterial agent for the treatment of ulcers and bed sores and other surface infections resulting from burns and wounds.(3,4,5,6,7)

Infection is the commonest and most serious complication of burn injury. Sepsis accounts for 50-60% of deaths in burn patients despite improvement in antimicrobial therapy, therefore many medical practitioners have found interest in our age old method of use of honey for effective wound healing. The root cause of most of the complication post injury is infection leading to sepsis and finally death. To control this infection honey has played a pivotal role and many studies have proved that honey could considerably reduce wound infection.(8,9,10,11,12)

Our study looks into this anti microbial effect of honey with respect to its age. Does storing enhance the anti microbial effect of honey? For this an in vitro study was carried out using different types of honey stored for different time intervals.

Materials and Methods

In April 2010, honey of three different brands was procured from the market. Honey obtained directly from bee hive was collected in a bottle and brought to the laboratory and labeled as forest honey (this honey was not from any specific flower). These bottles were stored in the laboratory at room temperature (25^0C to 35^0C) as such for three years(till April 2013). The honey was tested on procuring for antibacterial activity, after an interval of one and three years by agar gel well diffusion method(13,14,15) against bacteria isolated from burn wounds of the patients during dressing in the burns operation theatre of Bai Jerbai Wadia hospital for children, Parel, Mumbai. 20 isolates of each organism namely *Pseudomonas, Klebsiella, Escherichia coli, Staphylococcus* and *Proteus* isolated from burn wounds were included in the study. These strains were preserved on nutrient agar slants and were regularly sub cultured. For the test, each microorganism was grown to a density comparable to 0.5 Mc-Farland standards(containing $1x10^8$cfu/ml) in brain heart infusion broth. 1ml of thesuspension was evenly spread on to pre-prepared sterile 100mm Mueller Hinton agar (MHA) plate. Wells of 6mm diameter were made into the agar using cork borer dipped in alcohol and sterilized. The wells are filled with 0.2ml of anti microbial solution (in this case honey). All plates were incubated at 37°C for 16 –18 hrs. After incubation the diameter of clear zones around the wells containing anti microbial was measured with a ruler. It was decided that a zone of inhibition around the agar well of > 1mm radius (i.e. a total zone diameter measurement of > 8mm) would be considered as test bacterial strain susceptibility to honey.(13,14,15) A negative and a positive control were run each time the honey was tested. Negative control was a pre-prepared sterile MHA plate incubated at 37°C to check for any contamination. A positive control was a sterile MHA plate on which the isolate was inoculated and incubated at 37˚C to check for growth of isolates on the media.

Results

The antibacterial action of honey against 20 burn wound isolates was considered. Number of isolates of each organism (*Pseudomonas, Klebsiella, Staphylococcus, Escherichia coli, Proteus*) that showed susceptibility to honey was calculated in percentage. The data is tabulated and graphically represented.

Table 1: Antibacterial effects of fresh honey against burn wound isolates

Organisms	Forest	Brand 1	Brand 2	Brand 3
Pseudomonas	100%	70%	70%	100%
Klebsiella	90%	70%	100%	100%
Staphylococcus	100%	90%	100%	60%
Escherichia coli	100%	70%	90%	70%
Proteus	90%	60%	50%	90%

Table 2: Antibacterial effect of forest honey on storage

Organisms	Fresh	1 Year Old	>3 Years Old April 2013 onwards
Pseudomonas	100%	40%	30%
Klebsiella	90%	40%	40%
Staphylococcus	100%	40%	40%
Escherichia coli	100%	50%	40%
Proteus	90%	40%	30%

Table 3: Antibacterial effect of brand 1 honey on storage

Organisms	Fresh	1 Year Old	>3 Years Old April 2013 onwards
Pseudomonas	70%	40%	20%
Klebsiella	70%	30%	20%
Staphylococcus	90%	30%	20%
Escherichia coli	70%	40%	30%
Proteus	60%	20%	20%

Table 4: Antibacterial effect of brand 2 honey on storage

Organisms	Fresh	1 Year Old	>3 Years Old April 2013 onwards
Pseudomonas	70%	30%	30%
Klebsiella	100%	20%	20%
Staphylococcus	100%	20%	20%
Escherichia coli	90%	40%	20%
Proteus	50%	20%	20%

Table 5: Antibacterial effect of brand 3 honey on storage

Brand 3			
Organisms	**Fresh**	**1 Year Old**	**>3 Years Old**
Pseudomonas	100%	40%	30%
Klebsiella	100%	40%	40%
Staphylococcus	60%	30%	30%
Escherichia coli	70%	20%	20%
Proteus	90%	20%	20%

As we analyze the results we see a marked decrease in percentage of isolates that show susceptibility to honey that is stored for a year. However, the decrease is not progressive with storage time. Honey stored for more than 3 years does not show further decrease in the number of isolates that are susceptible. Hence we can conclude that freshly obtained honey has antimicrobial activity against more number of burn wound isolates as compared to older honey.

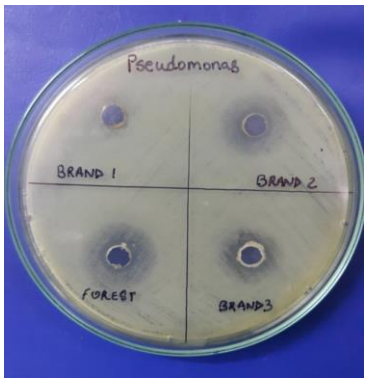


Fig. 1: Fresh honey from forest and from market showing zone of inhibition

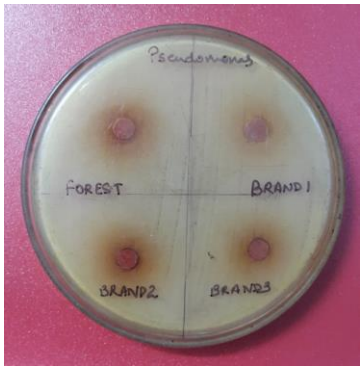
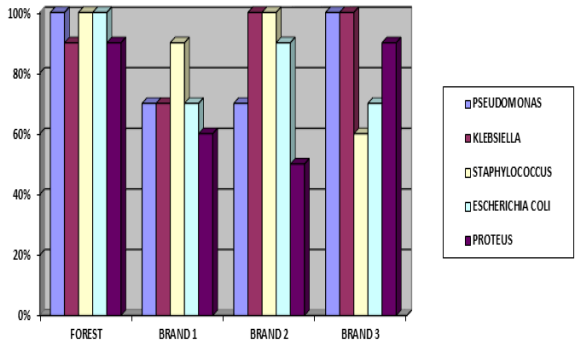
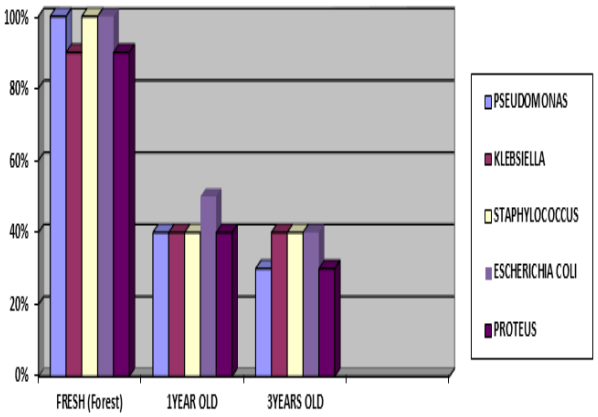


Fig. 2: Forest and market honey stored for 3years shows smaller or no zone of inhibition

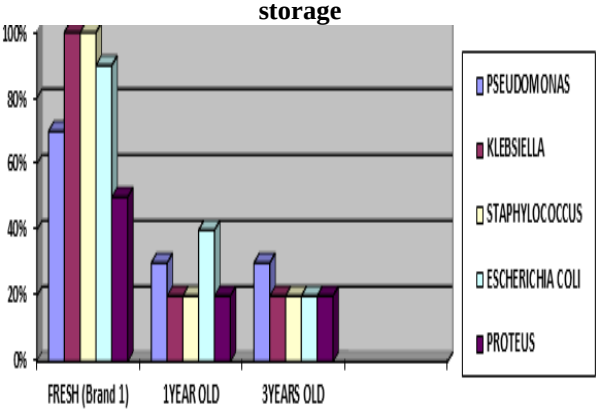
Graph 1: Antibacterial effects of fresh honey against burn wound isolates



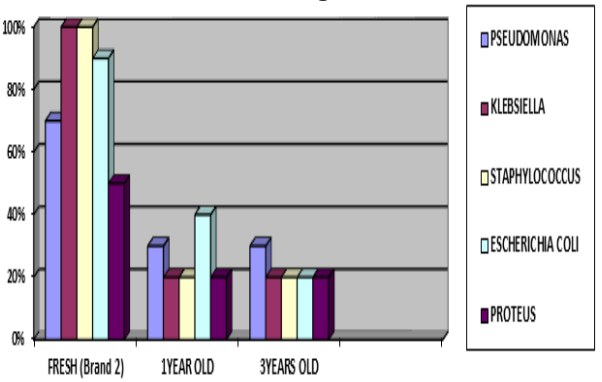
Graph 2: Antibacterial effect of forest honey on storage



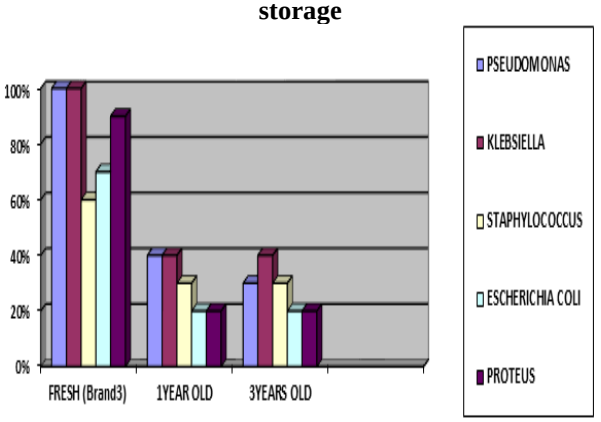
Graph 3: Antibacterial effect of brand 1 honey on storage



Graph 4: Antibacterial effect of brand 2 honey on storage



Graph 5: Antibacterial effect of brand 3 honey on storage



Discussion

Honey inhibits a broad spectrum of bacterial species. It is known to have bactericidal and bacteriostatic effect. It is known that honey has some antifungal property too.[16,2] Various reasons are put forth to explain this antibacterial effect of honey.[15,1] The viscous barrier that honey presents to wound invasion by microorganisms and to fluid loss is one of the reasons honey is considered as an effective topical agent in wound dressing.[17]

The osmotic effect due to its high sugar content (84%) is considered as another reasons for its antibacterial property. However on application of honey on wounds the wound exudates dilute its osmolarity yet honey continues to show anti microbial activity on wound isolate.

In our laboratory, we conducted a study on the antibacterial property of honey against burn wound isolates. Honey freshly obtained from forest and commercially available honey was put to test against burn wound isolates. These samples showed antimicrobial effect against greater number of burn wound isolates, forest honey showing a better effect than commercial products. However the same honey when stored for 1 year and more than three years showed antimicrobial effect against lesser number of burn wound isolates.

Few studies and literature[2,11,16] on honey states that the antimicrobial activity of honey is found to be better when honey is diluted. Findings went on to prove that there has to be some other property of honey which contributes to its antibacterial effect when diluted. It was later identified that honey gets a part of its antibacterial effect also due to the enzyme present in it. This antibacterial effect comes from the hydrogen peroxide produced enzymatically in honey. It is known that an enzyme called glucose oxidase is secreted from the hypopharyngeal gland of bee into the nectar and helps in the formation of honey from nectar. This enzyme converts glucose into gluconic acid and hydrogen peroxide in the presence of O_2 & H_2O.

Glucose + H_2O + O_2 glucose oxidase gluconic acid + H_20_2

Gluconic acid makes the honey acidic, thus adding to its antibacterial activity and also helps in preserving it. The H_20_2 also adds to the antibacterial activity to a larger extent. However H_20_2 is present only during the ripening of honey. Full strength honey has negligible levels of H_20_2 as glucose oxidase becomes inactive at a stage when acidity levels increase. So when honey is diluted the acidity decreases and the enzyme activity increases 2500 – 50000 times, thus giving a slow releasing antiseptic in the form of H_20_2 which is antibacterial but not tissue damaging.[16]

A solution of H_20_2 used as an antiseptic is likely to be far less effective than a "slow releasing preparation" in the form of honey.[16]

Hydrogen peroxide rapidly breaking down into water and oxygen and its production and decomposition are continuous. The H_20_2 concentration remains stable under a given set of conditions of temperature, sugar concentration etc. and is sufficiently high to give good protection against some harmful microorganisms by a biochemical mechanism which disrupts their metabolism. The same system is thought to operate when honey is diluted with water and for this reason honey has been successfully used as a microcidal wound dressing. This known fact about the anti bacterial action of H_20_2 could be one of the reasons for our invitro finding. However, there could be many other reasons for decrease in antibacterial property of honey. Exposure to light, heat and other factors are also of important consideration.[4]

Our in vitro study to a certain level supports the above claim as we have found that freshly obtained honey that is less viscous and less concentrated as compared to aged honey has a better antibacterial effect and that there is a considerable decrease in antibacterial property on storage at room temperature for a prolonged period.

The reason for this change will need to be further analyzed as there are many factors that are responsible for the antimicrobial activity of honey. The storage of honey has an effect on these factors hence the reduction in the susceptibility rate of burn wound isolates. This proves that antimicrobial property of honey in vitro does changes with age.

It is impossible to predict the antibacterial property of a particular honey without assessment, as there involves multiple factors that can cause variation in the extent of antibacterial activity. Hence it is recommended that honey obtained fresh, commercially or stored for a prolonged period should be assayed for its antibacterial activity before therapeutic use.

Conclusion

In Vitro results shows a marked decrease in percentage of isolates that show susceptibility to honey that is stored for a year. However, the decrease is not progressive with storage time. Honey stored for 3 years does not show a marked difference in the percentage of susceptibility when compared to results obtained after 1year. Hence we can conclude that freshly obtained honey has antimicrobial activity against more number of burn wound isolates as compared to older honey. It is an age old belief that honey improves with age but our study proves that the antibacterial property of honey on burn wound isolates does not improve with age. The specific reason for this reduced antibacterial property with respect to age needs further analysis. However on the basis of our findings we can suggest that the antibacterial property of honey must be assayed before any therapeutic use.

References

1. Subrahmanyam M.: Topical application of honey for burn wound treatment-An overview. Annals of burns and fire disasters. 2007: XX-n-3: 137-39.
2. Molan P.C.: The antibacterial activity of honey. Bee world, 1992:73(1):5-28.
3. Subrahmanyam M.: Topical application of honey in treatment of burns. Br. J. Surgery 1991:78:497-98.
4. Moore O.A., Smith L.A, Campbell F., Seers K., et al: Systematic review of the use of honey as a wound dressing. BMC Complementary and Alternative Medicine. 2001:1:2, http://www.biomedcentral.com/1472-6882/1/2.
5. Subrahmanyam M.: Honey-impregnated gauze versus amniotic membrane in the treatment of burns. Burns.1994:20:331-33.
6. Maghsoudi H, Salehi F, Khosrowshahi M.K, Baghaei M et. al: Comparison between topical honey and Mafenide acetate in treatment of burns wounds. Annals of burns and fire disaster. 2011:24:132-36.
7. Subrahmanyam. M, Hemmady A.R, Pawar S.G.: Multidrug-resistant staphylococcus aureus isolated from infected burns sensitive to honey. Annals of burns and fire disasters. 2003:16:192-93.
8. Abd-El Aal A.M, EL-Hadidy, EL- Mashad NB, Sebaie A.H.: Antimicrobial effect of bee honey in comparison to antibiotics on organisms isolated from infected burns. Annals of burns and fire disaster. 2007:20:83-88.
9. Subrahmanyam. M: Honey as a surgical dressing for burns and ulcers. Indian Journal of Surgery. 1993:55:468-73.
10. Subrahmanyam. M: Honey dressing for burns –An Appraisal. Annals of burns and fire disasters. 1996:9:33-35.

11. M. Subrahmanyam, Archan Hemmady, Pawar S.G.: Antibacterial activity of honey on bacteria isolated from wounds march 2001. Annals of burns and fire disaster 2001:14:22-24.
12. Subrahmanyam. M, Hemmady A.R, Pawar S.G.: The sensitivity to honey of multidrug-resistant pseudomonas aeruginosa from infected burns. Annals of burns and fire disasters 2003:16:84-86.
13. Holder I.A., Boyce S.T.: Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns. 1994:5:426-29.
14. P.D. Thomas, T.E. Taddonio, M.J. Tait, J.K. Prasad: Susceptibility of pseudomonas and staphylococcus wound isolates to topical antimicrobial agents: a 10year review and clinical evaluation. Burns 1989:15:190-92.
15. Osman O.F, Mansour I.S, El-Hakim S.: Honey compound for wound care: A preliminary report. Annals of burns and fire disasters. 2003:16:131-34.
16. Amy E. Jeffrey, Carlos M. Echazarreta: Medical uses of honey. Rev Biomed. 1996:7:43-49.
17. Subrahmanyam N.: Addition of antioxidants and polyethylene glycol 4000 enhances the healing property of honey in burns. Annals of burns and fire disasters. 1996:9:93-95.