

**ANTIBACTERIAL ACTIVITY OF HONEY AGAINST *STAPHYLOCOCCUS AUREUS* FROM INFECTED WOUNDS**

**Rajeswari, T.,<sup>1\*</sup> Anand Venugopal,<sup>2</sup> Viswanathan, C.,<sup>2</sup> Kishmu, L.,<sup>2</sup> Venil, C.K.,<sup>1</sup> Sasi kumar, J.M.<sup>2</sup>**

<sup>1</sup> Department of Microbiology, Karpagam University, Coimbatore – 641 021,  
Tamil Nadu, India

<sup>2</sup>Department of Industrial Biotechnology, Karpagam University, Coimbatore- 641 021,  
Tamil Nadu, India

[rajeswarithamarai@rediffmail.com](mailto:rajeswarithamarai@rediffmail.com)

**Summary**

The antibacterial activity of honey against infected wound mainly depends on the osmolarity of honey. The activity of honey against various microorganisms differs. Number of microorganisms isolated from wound was tested with honey [Nilgiris] using agar well diffusion method. Different concentration [5% - 50%] of honey was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. It was found that *Staphylococcus aureus* shows more sensitivity than *Pseudomonas aeruginosa* and *Escherichia coli* and the minimum inhibitory concentrations were found to be 25%, 35% and 40% respectively.

**Key words:** Honey, Antibacterial activity, Minimum Inhibitory Concentration, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*

**Introduction**

The medical value of honey has been known from ancient times. It has been known to be used for some respiratory diseases and also for healing of skin wounds. It has been proposed that the healing effect of honey could be due to various physical and chemical properties. The high osmolarity and acidity are among the physical characteristics that contribute to its antibacterial activity. Hydrogen peroxide, volatiles, organic acids, flavourous, beeswax, nectar, pollen and porpoise are important chemical factors that provide antibacterial properties to honey<sup>1</sup>. Chemical properties of honey give unique properties as a wound dressing; it has a rapid clearance of infections, rapid healing of wounds, rapid suppression of inflammation, minimisation of scarring, and stimulation of angiogenesis as well as tissue granulation and epithelium growth<sup>2</sup>.

Honey is increasingly being used in the management of infected wounds where conventional pharmaceutical products are failing, especially now that honey impregnated dressings are available; thus it is reasonable to consider prophylactic application of honey

inside the device exit site<sup>3</sup>. The activity of honey is high when it is used in pure form [undiluted]. But when used in dressings they become diluted to the point where its action ceases<sup>4</sup>.

The floral source of honey plays an important role in its biological properties. Honey from different areas/regions possesses different level of antimicrobial activity<sup>5</sup>. Different bacteria are responsible for wound contamination, wound colonization or clinical infection. Microorganisms such as *Staphylococcus aureus* (positive and negative), *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* are frequently isolated from skin wounds in humans and animals<sup>6</sup>. In the present work, the antimicrobial activity and minimum inhibitory concentration of honey from Nilgiris was studied. Only scanty research has been done using honey from Nilgiris.

### **Materials and Methods**

Pure honey sample was obtained from Nilgiris “The queen of hills”, Tamilnadu, India. Microorganisms such as *S. aureus*, *P. aeruginosa* and *E. coli* were brought from the microbiology laboratory of Karpagam University, Coimbatore, Tamilnadu, India. It was made sure that these organisms were isolated from wounds.

#### ***Test for susceptibility***

It is done using well diffusion method<sup>7</sup>. Plates of Muller Hinton agar medium were prepared and inoculated with the cultures. Wells were made in the gel. Added pure honey samples on respective wells and kept for incubation for 24 hrs at 37 °C and the results are noted down.

#### ***Determination of Minimum Inhibitory Concentration [MIC]***

Cultures those were taken above were used for finding the MIC by liquid diffusion method<sup>8</sup>. Honey samples were taken and added along with the media in 5%-50% in concentration and plated. Cultures were inoculated in the respective plates and incubated for 24 hrs at 37°C and the results were noted.

### **Results**

#### ***Zone formation in well diffusion plates***

Formation of zones after 24 hrs were noted and found that among the three isolates used *S. aureus* forms larger zone than other cultures and found to be the most sensitive. The relative zone formation and its calculations are shown in the Table 1.

#### ***Determining Minimum Inhibitory Concentration [MIC]***

The inoculated plates were noted after 24 hrs of incubation. The plate without honey allowed microorganisms to grow well where as in the case of plates containing honey the growth was found to be reduced. As there is increase in the percentage of honey, there occurs a decrease in the number of colonies. Minimum Inhibitory Concentrations (MIC) were found to be 25%, 35% and 40% for the isolates *S. aureus*, *P. aeruginosa* and *E. coli* respectively. MIC values are tabulated in the Table 2.

**Table 1: Antibacterial activity of honey against microorganisms**

S.No.	Microorganism	Zone formed (mm)	Standard mean (mm)	Standard error (mm)	Inhibitory Zone (mm)
1	<i>Staphylococcus aureus</i>	21	20.67	0.5574	20.67±0.5774
		21			
		20			
2	<i>Pseudomonas aeruginosa</i>	16	15.67	0.5574	15.67±0.5774
		15			
		16			
3	<i>Escherichia coli</i>	14	13.33	0.5574	13.33±0.5774
		13			
		13			

**Table 2: Minimum Inhibitory Concentration of honey against microorganisms**

S.No.	Organism used	Minimum Inhibitory Concentration
1	<i>Staphylococcus aureus</i>	25%
2	<i>Pseudomonas aeruginosa</i>	35%
3	<i>Escherichia coli</i>	40%

### Discussion

As per the result obtained in first test, *S. aureus* shows more sensitivity than other microorganisms taken for the test which were isolated from wounds. In earlier studies Molan *et al.* and colleagues had proven that *S. aureus* had showed more sensitivity to honey. The average zone formation in the case of *S. aureus* is 20.67±0.5774 where as in the case of *P. aeruginosa* and *E. coli* the zone formed is around 15.67±0.5774 and 13.33±0.5774 respectively showing the higher sensitivity of *S. aureus*.

In the case of MIC determination, the honey sample is used starting from 5% to 50%. As there is an increase in percentage of honey, the colony formation or growth is decreased. At a concentration of 25%, 35% and 40% the growth of microorganism *S. aureus*, *P.aeruginosa* and *E. coli* respectively were completely eradicated thus it is the minimum percentage of honey required to prevent the growth of these microorganisms for 48 hrs.

French *et al.* 1 found the MIC of honey to be 22% similar to the present study where it was found to be 25% which shows that *S. aureus* is more susceptible to honey samples as reported by many other researchers such as Cooper *et al*<sup>1</sup> who studied the antibacterial activity of honey against strains of *S. aureus* from infected wounds showing the minimum concentration of honey required against it. Simon *et al.*<sup>9</sup> has been the latest to explain the medical properties of honey for wound care.

French *et al.*<sup>1</sup> studied the minimum dilution of honey and found that typical honey are about eight times more potent against coagulase-negative *Staphylococci* than if bacterial inhibition were due to their osmolarity alone. Alandejani *et al.*<sup>10</sup> studied the efficiencies of honey on *S. aureus* and *P. aeruginosa* biofilms and found that honey was effective in killing 100% of the isolates in the planktonic form. The bactericidal rates for the sida and Manuka honeys against *MSSA*, *MRSA* and *P. aeruginosa* biofilms were 63%-82% and 73%-91% respectively.

Alnaqdy *et al.*<sup>11</sup> studied the antibacterial activity of honey from Oman and South Africa and found that *S. aureus* shows more susceptibility towards all types of honey. This is also a supporting factor for the results obtained in the present study. Malaysian Journal of pharmaceutical sciences studied the local honey of Malaysia for their antimicrobial treatment against certain bacterial cultures particularly *Staphylococcus* species and found to be very effective and also studied their physiochemical properties which could influence their antibacterial susceptibility<sup>12</sup>.

Antibacterial activity of honey produced by honey bees (*Apis mellifera*) on bacterial species isolated from infected wound was reported by Andargachew *et al.*<sup>13</sup> proving that *S. aureus* are more sensitive than other species.

From the present study, the antibacterial activity of honey showed highest activity against *Staphylococcus aureus* than *E. coli* and *P. aeruginosa*. The zone formed is about  $20.67 \pm 0.5774$  and so found that the honey is very effective and specific in its action and the Minimum Inhibitory Concentration (MIC) was found to be 25% which is also considered to be more effective, thus concluding the possible effectiveness of using honey for medical purposes.

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