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Assessment of antibacterial potential of natural and commercial honey samples against wound isolates.

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Abstract

Background: Antibiotic resistance has surged the development and exploration of new, improved and effective natural products from plants and other sources like honey, which has been rediscovered as a therapy for wounds, both in-vitro studies and in clinical trials. It has been observed to exert bacteriostatic and bactericidal potentials against pathogenic bacteria, including drug-resistant strains. The following study aimed to isolate and identify bacteria from wound infections and to investigate the antibacterial activity of honey samples; natural and commercial honey, against common clinical wound Pathogens.

Methodology: Bacteria from wound samples of patients with injured legs were isolated using differential and selective agars, while the antibacterial activity of natural and commercial honey samples was determined by agar well diffusion method and Minimum Inhibitory Concentrations (MIC).

Results: The results indicated that natural honey has more potential to exert antibacterial activity with an 18-30 mm zone of inhibition, which was significantly higher as compared to commercial honey with a 13-23 mm zone of inhibition. MIC of natural honey was visible in most of the test organisms at 25 µg/ml as compared to commercial honey with only Bacillus sp and Staphylococcus sp2 at 50 µg/ml while Staphylococcus sp1 at 25 µg/ml.

Conclusion: The results proposes due to a significant difference in the antibacterial activity of natural and commercial honey, these findings would help experts from the health sciences in the selection of the type of honey as an apitherapy for wound care and management.

Keywords

Honey, Antibacterial Activity, Drug-Resistant, Minimum Inhibitory Concentration, Agar Well Diffusion Method, Apitherapy.

Introduction

Natural honey is famous for its nutraceutical properties, as it is mainly composed of sugars, including fructose, glucose, sucrose, and maltose. However, oligosaccharides, proteins, minerals, vitamins, polyphenols and other trace elements may also be present. Pharmacologic effects of honey, i.e. antimicrobial, immunomodulatory, prebiotic, antinematodal, anti-inflammatory, and antinociceptive activities, have been reported in various studies with a bacteriostatic and bactericidal effect against resistant pathogens[1-3]. It has been used for medicinal purposes for many years, which has been confirmed by many clinical studies for skin burns, ulcers and wound[4-8]. It boosts the immune system and stimulates regeneration of wounds. Investigations into the microbial flora of wounds began in the late 19th century. Since then, there have been various improvements made in techniques in order to facilitate the recovery, identification and enumeration of a wide variety of microbial species. Most wounds are colonized with relatively stable polymicrobial communities, often without signs of infection[9, 10]. However, potential pathogens may be present that can disrupt the balance between host responses and microorganism[11]. Wound infection causes damaging effects to patients with increased pain, discomfort and inconvenience and could be fatal if not treated properly on time. Disruption in the balance between complex host and microbial interplay affects the healing process and might increase the hospital stays and treatment costs[12]. Antimicrobial agents are important in reducing the global burden of infectious diseases[13, 14]. However, the occurrence of drug-resistance has reduced the development of antibiotics and poses a big challenge as few pharmaceutical companies remain active in this area[15]. The failure of such antibiotics has surged the development and exploration of new, improved and effective natural products from plants and other sources[16, 17]. Antibacterial activity of honey was first recognized in 1892; however, due to lack of scientific support, it has limited use in modern medicine[18], which has lately been rediscovered as a therapy for wounds[19]. Interest in this approach stems partly from the emergence of antibiotic-resistant pathogens. Antimicrobial properties of honey have been reported in various studies, but the mechanisms by which it acts are not completely understood.

Strong solutions of honey and sugar pastes are thought to inhibit microbial growth because of their high osmolarity. Honey exerts its antimicrobial potential either through hydrogen peroxide and other enzymatic activity or through flavonoids present in the honey of floral source. Discovery of antibiotics had declined the trend of using folk medicine, but antibiotic resistance has resurrected folk medicinal usage both in-vitro studies and in clinical trials[20]. Hence the following study aimed to isolate and identify bacteria from wound infections and to investigate the antibacterial activity of honey against common clinical wound Pathogens.

Methodology

The study was conducted at Jinnah University for women, Department of Microbiology. 20 Wound samples from patients with injured legs were collected using sterile swab stick from different hospitals in Karachi. Written permission in the form of informed consent was taken from individual participants. No personal identity of participants was revealed. No participant was forced to take part in research work. All the information was treated with confidentiality.

Isolation & Identification

Differential and selective agars, i.e. Eosin Methylene Blue (EMB), Mannitol Salt Agar (MSA), Pseudo agar and Blood agar, were used for the isolation of bacteria. However, for the identification of isolates, preliminary screening methods were used, including gram staining TSI, SUGAR TEST, CATALASE and Sensitivity Testing.

Antibacterial Activity of Honey

Two samples of honey, i.e. commercially prepared and natural honey were used. Antibacterial activity was determined using the following methods;

Agar Well Diffusion Method

- Honey samples were preheated in order to reduce the Viscosity.

- Muller Hinton Agar (MHA) was used for sensitivity testing.
- Test organisms were inoculated on the MHA using the spread plate method separately.
- Wells were made on each plate using a sterile borer.
- 50 µl of the honey samples were placed in each well, and the plates were allowed to stay for a few minutes for pre diffusion to take place, followed by incubation of 24-48 hrs at 37°C.
- The zones of inhibition were measured with the use of a caliper/ruler.

MIC (Minimum Inhibitory Concentration)

MICs of honey for the wound isolates were determined using the broth dilution method.

- A stock solution of 50% honey was prepared in sterile de-ionized water.
- 2 ml of nutrient broth was pipetted into six tubes.
- Subsequently, 2 ml stock solution was transferred to the tube with nutrient broth to prepare a twofold serial dilution.
- 100 µl of an overnight culture of test organisms was added in each tube.
- After 24 hrs incubation, each tube was examined for the presence and absence of turbidity to indicate the growth of the microorganism.

Result

Among the isolates obtained, five were observed to be sensitive to honey samples. The antibacterial activity of honey samples is elaborated in Tables 2 and 3, whereas biochemical identification is presented in Table 1.

Table 1: Biochemical Identification of Isolates

Strain	Gram Stain	Sugar test				Catalase
		Glucose	Sucrose	Mannose	Lactose	
Bacillus sp.	Gram +ve rods	+ve	+ve	+ve	-ve	+ve
Staphylococcus sp1	Gram +ve cocci (clusters)	+ve	+ve	+ve	+ve	+ve
Staphylococcus sp2	Gram +ve cocci (clusters)	+ve	+ve	+ve	+ve	+ve
Strain	**Gram Stain**	**TSI**				-
		Butt	**Slant**	**Gas**	**H2S**	
E.coli	Gram -ve rods	Acid	Acid	+ve	-ve	+ve
Pseudomonas sp.	Gram -ve rods	Alkaline	Alkaline	-ve	-ve	+ve

Table 2: Agar Well Diffusion Method

Commercial HONEY		Natural HONEY	
Test organisms	**Antibacterial activity (zone of inhibition)**	**Test organisms**	**Antibacterial activity (zone of inhibition)**
Bacillus sp.	23 mm	**Bacillus sp.**	30 mm
Staphylococcus sp1	15 mm	**Staphylococcus sp1**	20 mm
Staphylococcus sp2	13 mm	**Staphylococcus sp2**	18 mm
E. coli	0 mm	**E.coli**	20 mm
Pseudomonas sp.	0 mm	**Pseudomonas sp.**	18 mm

Table 3: MIC (Minimum Inhibitory Concentration)

Commercial HONEY							Natural HONEY						
Test organisms	**MIC (µg/ml)**						**Test organisms**	**MIC (µg/ml)**					
	100	**50**	**25**	**12.5**	**6.25**	**3.125**		**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Bacillus sp.	-	-	+	++	++	++	**Bacillus sp.**	-	-	+	++	++	++
Staphylococcus sp1	-	-	-	+	++	++	**Staphylococcus sp1**	-	-	-	++	++	++
Staphylococcus sp2	-	-	+	+	++	++	**Staphylococcus sp2**	-	-	-	++	++	++
E. coli	+	+	++	++	++	++	**E. coli**	-	-	-	++	++	++
Pseudomonas sp.	+	+	++	++	++	++	**Pseudomonas sp.**	-	-	-	++	++	++

*(++) High turbidity (+) low turbidity (-) No turbidity

*The pattern of sensitivity was determined using different concentrations of honey. Tubes with less turbidity and no visible growth of microorganisms were considered as the minimum inhibitory concentrations.

Discussion

The rapid surge of Pathogenic and resistant microorganisms with only a few effective antibiotics and an increase in disease burden have shifted the interests towards 'alternative medicine' such as natural remedies, especially towards apitherapy[21, 22]. Honey is known for its regenerative properties, with no scar formation. Hygroscopicity, hypertonicity, lower pH and complex chemical composition are the main factors that participate in exerting its activity. It serves as broad-spectrum antimicrobial agents. Recently, 60 species were identified to be sensitive with honey[21, 23] where pure honey was reported to be bactericidal to many pathogenic microorganisms, including Salmonella, Shigella, Escherichia coli, Vibrio cholera[21].

In the following study, two samples of honey were tested to compare their antimicrobial activity against the bacterial isolates obtained from wound infection. Agar well diffusion method with commercial honey showed antibacterial activity against most of the isolates with a zone of inhibition between 13-23 mm, whereas natural honey was effective against all the isolates with 18-30 mm zone of inhibition as presented in table 2. However, according to Khalil et al., all undiluted honey samples (100%) showed significant antibacterial activity against the test strains[24]. Similarly, Hamza et al., in 2015, demonstrated undiluted honey with 25.38 mm zone of inhibition against S. aureus[25], which is in contrast to our results with the larger zone (18-20 mm) observed from natural honey against S. aureus strain. While in 2019, Bunza reported the significant antibacterial activity of honey at 100% and 50% with S. aureus, P. aeruginosa and E.coli test organisms[26]. More recently, Ifra et al., have demonstrated in 2020 that the antibacterial activity of honey depends on concentration as well as the nature of the test organisms by using different samples of honey tested at three different concentrations against wound isolates[27].

The antibacterial activity of honey samples was further evaluated through MIC at concentrations[3]. 125-100 µg/ml, as shown in table 3. Natural honey showed significant MIC at 25 µg/ml, visible in most of the test organisms as compare to commercial honey with only Bacillus sp and Staphylococcus sp2 with MIC at 50 µg/ml while Staphylococcus sp1 at 25 µg/ml. Considering the resistant pattern of Staphylococcus[28,29], these results demonstrate that staphylococcus is more susceptible to honey than other test isolates, which is similar to the results presented by Halima et al., in 2020 that Staphylococcus aureus was most sensitive organism isolated with MIC consistent at 20%[30]. Similarly, Molanaei et al., tested Methicillin-resistant staphylococcus strains against the honey samples and demonstrated the MIC of resistant strains at < 8% concentration. Therefore following results suggest that honey is highly potent against the pathogenic bacteria, especially against antibiotic-resistant staphylococcus[31]. Thus natural honey is an essential antibacterial agent that could inhibit the resistant bacteria at low concentration. Further, it can be concluded from these results that in comparison with commercial honey, natural honey has more potency for antibacterial activity. However, variations in the antibacterial activity of honey depend on various factors, including the plant source, environment; even honey collected from a single location can have variation in antibacterial activity and the concentration of honey used, higher the concentration, greater the antibacterial activity[32, 33].

Due to the lack of funding and financial constraints, standard controls were not used. A short monitoring period has also limited further molecular testing of the isolates and wound application to monitor the regenerative property. Therefore further research with a longer time duration is required to overcome these limitations.

Conclusion

Overall these finding suggests that there is a significant difference in the antibacterial activity of natural and commercial honey with distinct osmolality, pH, peroxide and non-peroxide components that possibly exert antibacterial effects against the wound isolates. As a rediscovered alternate medicine, with no toxicity and low cost, it needs further molecular testing of the isolates and characterization of the active bactericidal components to monitor the regenerative property. These findings would help health professionals in the assortment of the type of honey as an apitherapy, inpatient care and management of wounds.

Conflicts of Interest

None.

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