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Antibacterial Activity of Honey against Staphylococcus aureus and Escherichia Coli Isolated from Infected Wound.

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ABSTRACT: The development of wound infection has destructive effect on patients by causing pain, discomfort which can lead to life threatening conditions or even death. Antibiotics were emerged as a medical break in the 20th century due to its efficacy in the treatment of infections. However, the emergence of multiple antibiotic resistance(MAR) bacterial strains including Staphylococcus aureus and Escherichia coli are implicated in wounds. This study carried out to analyse the antibacterial activity of honey against Staphylococcus aureus and Escherichia coli isolated from infected wounds. Different concentrations of honey (20, 50, 150 and 200% v/v) was tested against the isolated bacteria using agar well diffusion methods to determine the antibacterial activity. The higher activity was observed in Staphylococcus aureus (27 mm) than Escherichia coli (16 mm). Based on the results of this study, it is concluded that honey has very potent antibacterial activity for treatment of pathogens that infect wounds

Keywords: Antibacterial activity, Honey, Staphylococcus aureus, Escherichia coli, Wound.

I. INTRODUCTION

The antibacterial activity of honey refers to some bee products , presence of "inhibin" which acts as an antibacterial factor other than H_2O_2 , several factors such as osmotic properties of honey which is saturated solution of sugars , 84% being a mixture of fructose and glucose by osmotic effect of dilute solutions of honey depends on the species of bacteria [1]. Wound infection causes great distress in terms of associated mortality and morbidity , increased length of hospital stays, profound discomfort and significant increase in healthcare cost . Infection in a wound delay healing and may cause wound break down, herniation of the wound and complete wound [2]. Most commonly used honey in ancient times is Algeria honey. Honey has been used as a medicine since ancient times [3]. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentration of this compound in different honeys result in their varying antimicrobial effects [4]. Due to lack of adequate scientific research and documentation the medicinal properties of Algeria honey still remains in dark. More recently, honey have been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positive and gram negatives [5]. Anti-infective drugs (antimicrobial agents) are critically important in reducing the global burden of infectious diseases [6].Honey has been useful in the treatment of surgical wounds, burns and ulcers and the antibacterial and antifungal properties of honey are well documented [7]. The antibacterial activity of honey was first recognized in 1892; however, it has a limited use in modern medicine due to lack of scientific support [8] .It is also known to show antimicrobial activity against several Gram positive and Gram negative bacteria [9].

II. METHODS

Collection of Honey:

Crude, Unprocessed and undiluted honey sample was taken for study. The honey was obtained from the market was stored in a in a clean and closed polyethylene flasks at 20-25^{0}C until required for analysis. The honey was mixed with distilled water to produce various concentrations viz., 20%, 50%, 150%, 200%.

Collection of pus and swab sample of infected wounds:

The samples were taken from patients who visit the outpatient area of the tertiary care Hospital. The samples were taken before the treatment of the infection with antibiotic. The samples were collected by swabbing the surface of an infected wound by sterile swab and moistened

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the swab by placing it in transport media [10, 11] for processing.

Isolation and identification of test organisms:

The swabs were inoculated on Blood agar, MacConkey agar and incubated at 37^0 for 24-48 hrs. The isolates were identified based on colony characteristics after which pure colonies were sub cultured on blood agar, chocolate agar, Mannitol salt agar, Mac Conkey agar. Further, confirmation of isolates was done by standard bacteriological techniques namely Gram staining, Catalase test, Coagulase test, Oxidase test as described by Cheesbrough, 2000.

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 [12].

Preparation of different concentration of honey:

Natural (raw) and processed honey was used. Different concentrations of each honey contributing 20% v/v, 50% v/v, 150% v/v and 200%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.

Phytochemical tests

Fresh honey was subjected to standard phytochemical analyses using standard procedure in order to find out the presence of various phytoconstituents [13].

Antibacterial Activity Test:

The antibacterial activity of honey against the isolated pathogens was tested using a well diffusion method (Kirby –Bauer method) [10]. Muller Hinton agar plates, prepared according to manufacturer's instruction were used. Each plate was inoculated with each isolate and evenly streaked out. Wells of 6mm in diameter were cork-borer. Aseptically, each respective well was filled with different concentration of the honey using a sterile dropper. The plates were then incubated at 37^0 for 24 hrs. Some procedure was followed in plate where penicillin disc of 6mm in diameter was used as incubation; the plates were examined for clear area around the wells, indicating the zone of inhibition. These areas were measured in diameter of inhibition of zone produced by the honey [12].

III. RESULTS

Isolation and Identification of bacterial pathogens

The bacterial isolates were screened and identified using the morphological features of each organism. Based on the gram staining, E.coli was gram negative organism confirmed with the appearance of pink color colonies and S.aureus was gram positive with purple colored colonies under microscopic observation. E.coli colonies were observed as rod- shaped and arrangement was single or in pairs. S.aureus colonies were observed as cocci and arranged in cluster form. Based on hanging drop method, E coli was observed as motile with flagellar motility while S.aureus was observed as non motile organisms. Morphological and biochemical characterization of isolated bacterial pathogens was shown in Table 1 and 2 respectively

TABLE 1: Morphological features of bacterial spp isolated in the study:

S. No	Morphological characteristics	Escherichia coli	Staphylococcus aureus
1	Shape	Rods	Cocci
2.	Gram staining	Negative	Positive
3.	Arrangement	Single or Pairs	Cluster
4.	Motility	Motile	Non motile

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Table 2: Biochemical characterization of isolated pathogens:

S. NO	BACTERIA	E.coli	Staphylococcus aureus
1.	Catalase	+	+
2	Oxidase	-	-
3	Indole	+	-
4	MR	+	+
5	Urease	-	+
6.	VP	-	+
7.	Coagulase	-	+
8.	TSI	A/A	A/A

Phytochemical screening

Honey was subjected to systemic phytochemical screening by aqueous extraction. Honey was found to contain carbohydrates, glycosides, alkaloids, saponins, flavonoids, tannins etc., (Table: 3). The presence of glycosides has been used for over two centuries as stimulants in cases of cardiac failure

Table 3: Phytochemical analysis of Honey:

Phytochemical Compound	Status
Carbohydrates	+
Glycosides	+
Alkaloids	+
Saponins	+
Flavonoids	+
Anthraquinones	+
Tannins	+
Reducing sugars	+

+ : Present

Antibacterial activity of honey on isolated pathogens:

The antibacterial activity of honey represented in the Table 4. Honey showed highest inhibition zone 27mm for S .aureus in 150% concentration and 16 mm for E coli for 200% concentration . The antibacterial activity of honey was represented in Table 4. Statistical analysis of antibacterial activity of honey was shown in Table 5. For Staphyloccus aureus, highest mean values was observed in 200% followed by 150%, 50%. Least mean value 8.7 was observed in 20% concentration. For E.coli, highest mean value was observed in 200% followed by 150%, 50%. Least mean value 8.7 was observed in 20% concentration.

Table 4: Antibacterial effect on honey in different concentration

S.No	Concentration of honey	Zone of inhibition (mm)	
		E .coli	S .aureus
1	20%	8	8
2.	50%	9	13
3.	150%	14	27

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4,	200%	16	22

Table 5: Statistical analysis of Antibacterial effect on honey in different concentration

S.No	Concentration of honey	Mean ± SD of Zone of inhibition	
		E .coli	S .aureus
1	20%	8±0.9	8.7±0.8
2.	50%	9±0.5	14.8±2.4
3.	150%	14±0.8	25.6±2.1
4,	200%	16±1.1	21.3±3.6

IV. DISCUSSION

Recent researches have shown that honey has an antibacterial effect on pathogenic bacteria of the gastrointestinal tract, urinary tract as well as wound infection [14]. In this study, honey samples showed the antibacterial activity against Staphylococcus aureus and E coli. Higher activity was shown for Staphylococcus aureus compared to E coli. The antibacterial efficacy of honey is enhanced by the presence of hydrogen peroxide, an important enzyme with antibacterial activity, which is also an oxidizing agent. Other enzymes produced in honey include glucose Oxidase, though not activated in undiluted honey [15] but activated when honey is diluted where it react with the endogenous glucose to produce hydrogen peroxide . Honey shows antibacterial effect, literature has shown that not all honey samples have the same degree of antibacterial activity against the same type of bacteria. This is due to differences in osmolarity, viscosity, hydrogen peroxide content as well as protein content [16]. S. aureus is a Gram-positive bacterium which is a major pathogen implicated in skin infections such as impetigo, furuncles, boils, sties, pustules, burns and wounds. Antibiotic –resistant strains S.aureus are the major cause of infections especially in a hospital setting [17] that were fully sensitive to penicillin now developed resistance to methicillin, and the other latest ones resort antibiotics [18]. Honey is also rich in phenolic compounds which might contribute to its antibacterial activity. These compounds, regarded as non peroxidase constituents of honey [19] along with flavonoids have been reported to enhance the antibacterial activity of honey.

V. CONCLUSION

This study found that honey is active against Staphylococcus aureus and Escherichia coli isolated from wounds as it has a very potent antibacterial activity for treatment of pathogens that infect wounds. It has been known for its antimicrobial potential, showing a broad spectrum of potential against microorganisms including bacteria. As such, honey can be a possible alternative antibacterial agent with promising therapeutic potential in the medical setting. Moreover, the pharmacological, standardization and clinical evaluation on the effect of honey are essential before using it as a preventive and curative measure to common diseases related to the test organisms. Therefore, the antibacterial activity of honey against clinical bacterial isolates indicates the usefulness of the honey in clinical practice against bacterial infections.

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