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Antimicrobial Properties of Different Kinds of Honey and its Benefits

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Abstract:

Honey is a well-known ancient remedy for the treatment of infected wounds, surgical wounds, burns, skin/peptic ulcers, inflammations etc. It is highly popular for its antimicrobial, antiseptic, antioxidants and immunomodulatory roles. The healing property of honey provides antibacterial activity and wound repair as well. Honey is extremely viscous in nature to prevent further infection. The antimicrobial property is coming due to the enzymatic production of hydrogen peroxide. The hygroscopic nature of honey plays an anti-bacterial role. It also has been reported in many journals that honey is described as reservoir of microorganism as it contains bacteria, fungi, yeast, moulds etc. The main aim of this research work is to study antimicrobial properties of different kinds of honey by preparing LB plates with different honey samples to check the microbial growth and then the characterization of the different microorganisms by the following methods of gram staining, disk diffusion methods, SEM analysis, graphical analysis etc. An ample of literature search was done on the very interesting topic of honey using Books, Scientific Journals, Google Search. Relevant Scientific Journals were collected to gather the concepts on the antimicrobial properties as well as the microbial contents of honey and the inferences of these. The development process of honey, preservation of honey maintain the presence of microorganisms. Most of them are in inactive forms and hardly surviving within the honey as it has some properties like hygroscopicity, hyperosmolarity, acidity, hydrogen peroxide contents, antibiotics properties, wound-healing properties etc. Finally this work will conclude with the study of microorganisms present within the honey, how this honey becomes beneficial to treat selective wounds and its antimicrobial roles as well.

Keywords: Honey, Glucose Oxidase, Gluconic Acid, Non-peroxide Honey, Medihoney, Manuka Honey, Immunomodulatory Property, Wound Repair, Wound Healing, Antibacterial Activity, Antiseptic Property, Antimicrobial Property, Antioxidant, Hygroscopicity, Nectar, Pathogens, MRSA, VRE.

1. Introduction:

In various research journals, honey was described as one of the world's oldest natural medicinal substance. From long time back or since the origin of mankind, honey is used as traditional medicine and it is produced by *Apismelifera* [1, 2]. It is purely natural sweetener, produced by honey bees from nectar, blossoms, or from the secretion of the living parts of plants or excretions of plants, which are collected and stored by honey bees in the honeycomb [1]. Honey production starts with collected nectar, pollen from flowers but nectar is only used to prepare honey and it is a sugar rich liquid component produced by plants in glands called nectarins or nectaries, either within flower to attract pollinating animals by which extrafloralnectaries which provide nutrient source to animal mutualists for herbivore protection. Nectar contains water with sugar (25-70%). It is sucked by the inserted proboscis of honey bees inside the flowers nectary and it goes through the oesophagus to the thorax and finally to the abdomen. Pollen is transported to pollen busket of hive whereas nectar reaches stomach. Nectar is placed into the different wax cells of honeycomb. Within few days, these cells get covered with a layer of wax which is removed later when bees want to eat honey and the bees produce Invertase enzyme to break sucrose molecule into glucose (31.3%) and fructose (38.2%) by evaporating excess water to make the honey more concentrated with approximately 83% sugar and 17% water and this concentrated honey is suitable for yeast or other microbial growth [3]. The other sugars within the honey include disaccharides such as maltose, isomaltose, sucrose or few oligosaccharides. The organic acid, Gluconic acid constitutes 0.57% of honey, which is a byproduct of enzymatic digestion of glucose and it is important to maintain acidity (pH 3.2-4.5) and taste [4]. Some minerals (0.17%) are also present such as potassium, calcium, copper, iron, phosphorus, manganese, nitrogenous compounds, and vitamin C, B (thiamine), B2 complex like riboflavin, nicotinic acid, B6 pantothenic acid along with some enzymes like invertase (saccharase), diastase (amylase), glucose oxidase and these play an important role in

the formation of honey.Honey is extremely viscous in nature to prevent further infection [5].For hygroscopic nature of honey, it can draw moisture out of the environment and it dehydrates bacteria ultimately and honey has also high sugar content and low pHlevel to prevent microbial growth. The surface tension of honey with high viscosity is responsible for the foaming characteristics of honey. The liquid honey colour varies from watery clear and colourless to dark amber to black due to different dilutions or concentrations of caramelized sugar, different botanical origin, age, storage conditions. Its transparency depends upon the quantity of suspended particles like pollen [6, 7].

2. Microbes in Honey:

Which microorganism can tolerate concentrated sugar, low pH or acidic environment, hygroscopicity, and other antimicrobial characters, only those microorganisms can survive within honey. The key sources of microbial contamination in honey including pollen, digestive tracts of honeybees, dust, air particles, dirt and flowers. Microbes found in honeycomb are bacteria, fungi, yeast, moulds etc. coming from bees, raw materials (nectar), or other external sources. Larvae are inoculated by feeding of nectar and pollen by worker honeybees though it was initially sterile [8]. Sackelt reported *Bacillus, Micrococcus, Saccharomyces* species were isolated from honeycomb, adult honeybees and faeces of larvae [9]. The intestinal microbial loads of bees contain 1% yeast, 27% Gram-positive bacteria including *Bacillus, Bacteridium, Streptococcus, Clostridium* spp., 70% Gram-negative bacteria including *Achromobacter, Citrobacter, Enterobacter, Erwinia, Escherichia coli, Flavobacterium, Proteus, Klebsiella, Pseudomonas* [10].*Bacillus* sp. are the most prevalent followed by Gram-variable pleomorphic bacteria. Mould, Actinomycetes, Gram-negative rods (i.e. *Enterobacteriaceae*) and yeast are isolated whereas *Streptomyces* spp. are recovered from larvae. *Bacillus, Enterobacteriaceae, Penicillium, Aspergillus, Torulopsis*spp. are coming from pollen, flowers, hives. Even aerobic spore forming *Bacillus* are mostly present on external surface, crop, or intestine of honey bees [11, 12, 13, 14]. The primary sources of sugar tolerant yeast are flowers and soil but the secondary sources of microbial contamination in honey are human, equipment, containers, wind, dust etc. Possible transmission routes into extracted honey including air (in the house or while the honey was packed), food handlers (from skin infections, sneezing or faecal contamination) [13].Mostly found

microorganisms in honey are tabulated in table 1 [11]. Most of the bacteria or other microbes are in dormant state and unable to reproduce because of antibacterial activity of honey. Honey sometimes acts as a carrier of typhoid, fever, dysentery and various diarrhoea infections [9].

Table 1: Microorganisms found in Honey

Bacteria	Yeasts	Moulds
Alcaligens	*Ascosphaera*	*Asperhillus*
Achromobater	*Debaromyces*	*Alihia*
Bacillus	*Hansenula*	*Bettsia alvei*
Bacteridium	*Lipomyces*	*Cephalosporium*
Brevibacterium	*Nematospora*	*Chaetomium*
Citrobacter	*Oosporidium*	*Coniothecium*
Clostridium	*Pichia*	*Hormiscium*
Enterobacter	*Saccharomyces*	*Peronsporoceae*
Escherichia coli	*Schizosaccharomyces*	*Peyronelia*
Erwinia	*Trichosporium*	*Triposporium*
Flavobacterium	*Torula*	*Urediancеae*
Klebsiella	*Torulopsis*	*Ustilaginaceae*
Micrococcus	*Zygasaccharomyces*	
Neisseria		
Pseudomonas		
Xanthomonas		

3. Medicinal Properties of Honey:

Honey is a well-known ancient remedy for the treatment of infected wounds, surgical wounds, burns, skin/peptic ulcers, inflammations even it can work very well when conventional modern therapeutic agents fail. It was first reported that honey was used as drug or ointment during 2100-2000BC.Manuka honey (non-peroxide based) has been reported as antimicrobial agent against pathogenic bacteria such as *Staphylococcus aureus*, *Helicobacter pylori* and this is used for the treatment of wounds or stomach ulcers [15]. Honey is used for the treatment of wound healing due to its antibacterial healing property [16, 17, 18].Even high viscous nature of honey helps to create a protective barrier to prevent infections [5].*In vivo* activity and treatment of ulcers, infected wounds and burns as well were reported for the Medihoney and Manuka honey [5, 19]. The topical application of honey rapidly clears the wound infection to facilitate healing of deep surgical wounds with infection [20]. The honey is useful for the healing in infected wounds that do not respond to the conventional therapy i.e. antibiotics and antiseptics [20], including wounds infected with methicillin-resistant *S. aureus*(MRSA) [21, 22]. The Manuka, jelly bush and pasture honeys canstimulate the monocytes, the precursors of

macrophages, to secrete TNF-α[23, 24]and the glycosylated proteins can induce TNF-α secretion by macrophages, and this cytokine is known to induce the mechanism of wound repairing. So, the immunomodulatory property is essential for wound repairing. From traditional folklore, honey is used in the treatment of peptic ulcers and gastritis and it also acts as anti-inflammatory agent [25, 26]. Raw honey also contains some antioxidant compounds such as flavonoids and other polyphenols [27]. Clinical observations have been reported of reduced the inflammation by applying honey on wounds. The removal of exudate in wounds dressed with honey to manage inflamed wounds.

4. Antimicrobial Properties of Honey:

It was proved in research that Manuka honey (*L. scoparium*)was demonstrated to be effective against several human pathogens, including *E. coli, Enterobacteraerogens, Salmonella typhimurium, S. aureus* [5, 28] and honey is also effective against methicillin-resistant *S. aureus*(MRSA), β-*haemolytic streptococci*and vancomycin resistant*Enterococci*(VRE) [29, 30].Manuka honey (*L. scoparium*) is the best honey and it was reported to have an inhibitory effect against 60 species of bacteria, including aerobes and anaerobes, and Gram-positive and Gram-negative as well [31]. Even Manuka honey has enough antimicrobial activity, local production or availability, greater selectivity against medically important organisms [5]. More susceptibility with antibacterial potency of honey is located against *S. aureus, Pseudomonas aeruginosa, Enterococcus*species [32, 33]. Minimal inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent for microbial inhibition. The MICs of Tualang honey ranges from 8.75-25% whereas Manuka honey has 8.75-20% against many pathogenic Gram-positive and Gram-negative bacteria [34]. The honey has some beneficial roles to attribute its antibacterial property with regards to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H2O2) and non-peroxide components, i.e., the presence of phytochemical components like methylglyoxal (MGO) [35, 36]. The antimicrobial agent in honey is hydrogen peroxide (H2O2) whose concentration is determined by relative levels of the enzyme, glucose oxidase, synthesized by the bee and catalase originating from flower pollen [37].This hydrogen peroxide is produced enzymatically in honey. The glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the

nectar to make honey from the nectar. The hydrogen peroxide and acidity produced by the reaction: Glucose+H2O+O2 - Gluconic acid + H2O2. Due to acidic nature of undiluted honey, it plays significant antibacterial factor against *Salmonella*spp, *E. coli*, *Pseudomonus aeruginosa*, *Streptococcus pyogenes*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Plesiomonasshigelloides*, *Aeromonashydrophila*, *Shigellaboydi*, *Clostridium jejuni*, *Aspergillus niger*, *Penicilliumchrysogenum*, *Candida albicans*, *Staphylococcus aureus* [38, 39, 40, 41].Honey contains acidic properties of gluconic acid and antiseptic properties of hydrogen peroxide (H2O2) [42]. Finally, higher the concentration of honey results greater the usefulness as an antibacterial agent [43].

5. Methodology:

Natural Method of Extraction of Honey from Beehive:

To extract honey, wax layer on the cells has to be removed first by the help of sharp knife, then it has been warmed in hot water. The combs are inserted into centrifuge and honey is carefully removed or honey is sometimes removed by pressure [44].

Isolation of different kinds of bacteria using non-selective agar (LB Agar) and selective MacConkey agar with honey sample:

Honey samples are usually microbiologically stable and can be transported and stored at ambient temperature. These samples should be protected against moisture and excessive heat [45].Sample unit must be greater than the required for analysis. The selected analytical unit of honey is 25g. Then the disinfection is required for the area outside of the packaging with 70% Ethanol and sample honey is removed from the jar aseptically.

Now, non-selective LB agar with 25g of honey is prepared for the purpose of isolation of different kinds of bacteria. 25g of honey, 25g LB agar powder and 1Lt. distilled water or deionized water are mixed and homogenized to prepare LB agar then it is serial diluted (10^-1, 10^-2, 10^-3) and the mixtures are boiled to dissolve the agar and sterilized by autoclaving at 15psi, 121°C for 15 min. Then different plates are prepared and incubated at 37°C for 24 hours overnight by adjusting pH 6.8-6.9 and finally streaking of LB agar plates is done with pure or mixed bacterial culture and these plates are incubated at 37°C for 24-48

hours overnight to observe colony morphology.

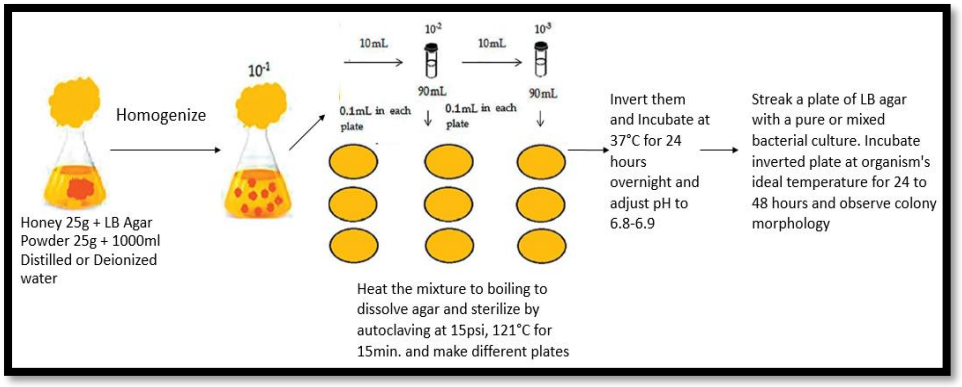

Fig. 1: Process Flow for LB agar plate preparation with honey sample and streaking the plates with pure or mixed bacterial culture

Similarly, selective MacConkey agar with 25g of honey is prepared for the purpose of isolation of different kinds of gram-negative bacteria. 25g of honey, 25g MacConkey agar powder and 1Lt. distilled water or deionized water are mixed and homogenized to prepare MacConkey agar then it is serial diluted (10^-1, 10^-2, 10^-3) and the mixtures are boiled to dissolve the agar and sterilized by autoclaving at 15psi, 121°C for 15 min. Then different plates are prepared and incubated at 37°C for 24 hours overnight by adjusting pH 6.8-6.9 and finally streaking of MacConkey agar plates is done with pure or mixed bacterial culture and these plates are incubated at 37°C for 24-48 hours overnight to observe colony morphology.

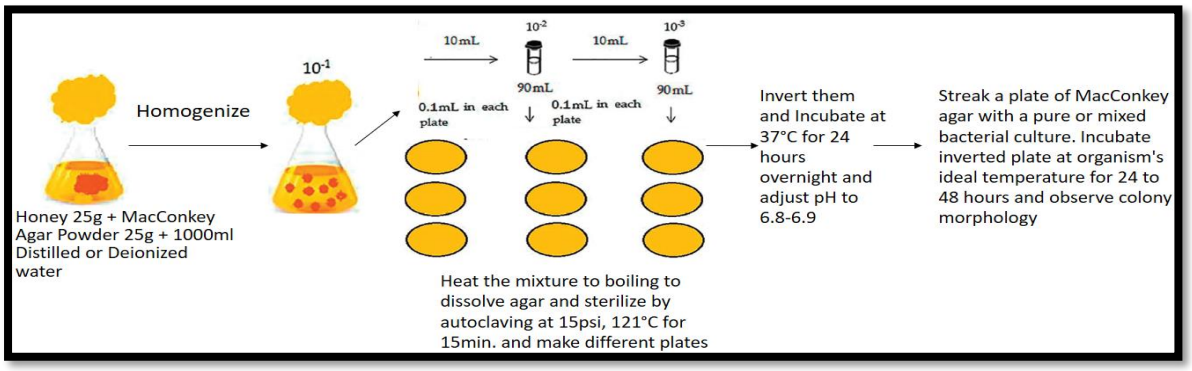

Fig. 2: Process Flow for MacConkey agar plate preparation with honey sample and streaking the plates with pure or mixed bacterial culture

6. Characterization of isolated bacteria with gram staining:

For gram staining,

1. Place a small drop of the culture on the labelled glass slide.

2. Spread the sample over a large surface with a pipette tip to form a thin film.

3. Allow the suspension to completely air dry.

4.To fix the microbes to the slide, hold the slide example side up, by its edge and immediately pass the slide across a Bunsen burner fire 10-20 times. Ensure the slide isn't overheated each time.

5.Cover the whole area of microscopic organisms with Gram Crystal Violet and leave at room temperature. Flush the slide for 5 seconds under sluggish running water utilizing a wash bottle. The example ought to seem blue-violet when seen with the unaided eye.

6.Cover the microorganisms with the Gram Iodine and leave at room temperature for 1 moment. Flush the slide for 5 seconds under sluggish running water then, at that point, promptly continue to the following stage. Now the example should in any case be blue-violet.

7. Add the Decolourizer Solution drop-wise until the blue-violet colour is no longer visualized on the sample.

8. Rinse the slide for 5 seconds under sluggish running water utilizing a wash bottle.

9.Cover the microscopic organisms with the Gram Safranin and leave at room temperature for 1 moment. Wash the slide for 5 seconds under sluggish running water to eliminate any overabundance color.

10. Stain the slide delicately with spongy paper or permit it to air dry prior to survey under a splendid field magnifying lens. 600 x amplification is satisfactory for survey microbes on the slide. Notice the focal point of the slide where microbes has been treated with all Gram Stains. An oil drenching objective might be utilized for high amplification. A drop of oil can be put straightforwardly on the slide [46-48].

7. Microscopy of Honey:

According to the method of the Association of Official Analytical Chemistry AOAC. The method is based on dissolving 100 g of the honey sample in 200 mL of distilled water which is further heated and acidified with 5 ml of nitric acid (HNO3) at 6 M concentration. Filter the sample in a Buchner funnel and mark four quadrants on filter paper. Analyse using a stereoscopic microscope with a total multiplication of 100× and finally confirm the type of sediment between slide and cover slip under an optical microscope with a multiplication of 100–400× [49,50].

8. Conclusion:

Honey is an ancient remedy from long years back but it is now rediscovered for various medicinal properties. But honey can get contaminated by human activities

including equipment, containers, wind, dust etc. and all the microbes present in honey are in dormant state. Non-spore forming bacteria in vegetative forms can't survive in honey whereas spore forming bacteria can't transit into vegetative forms and stay in honey and supportive of inhibitory role of honey on microorganisms. Honey is applied to wounds by diluting its initial high osmolarity and other properties like hygroscopicity, healing property, antibacterial activity etc. can inhibit the growth of microorganisms. Before commencing to the treatment of wound healing, microorganisms of honey should be isolated and their sensitivity should be assessed carefully. Chronic wound infection don't respond to antibiotic therapy but honey plays a promising role. Manuka honey is renowned globally because of its antibacterial role. Even honey plays an effective antimicrobial properties against antibiotic-resistant organisms such as MRSA, MDR etc. for the treatment of burn wounds, nosocomial infections etc. Nowadays, some standardised honeys such as Manuka honey (*L. scoparium*), Tualang honey (*Koompassiaexcelsa*) play proper antibacterial activities. The medical grade honeys like Revamil, Medihoney play topical antibacterial role because of its broad spectrum bactericidal activity, and these are applied for the treatment of topical infections caused by antibiotic resistant as well as antibiotic sensitive bacteria. These are used for therapeutic usage. Therefore it is required to study more and more natural honey for their antimicrobial activities.

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