

## Inhibitory Effect of Pure Honey on Microorganisms Isolated from Wound

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**Abstract:** The study aimed at investigating the effectiveness of pure honey against some isolated microorganisms from untreated infected wounds. Also, to compare with the effectiveness of various antibiotics. The isolates are four bacteria and two fungi. The bacteria are: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and the fungi are: *Blastomyces dermatitidis* and *Candida albicans*. All the microorganisms were cleared off with an excellent clear zone of inhibition including the problematic one in wound healing (*Pseudomonas aeruginosa*) when compared with the antibiotic sensitivity patterns in which majority are resistant. The results from this study showed the following microbial counts: Fungi; Wound A ( $2.67 \times 10^5$  cfu/ml), Wound B ( $3.33 \times 10^5$  cfu/ml). Bacteria: Wound A ( $4 \times 10^5$  cfu/ml), Wound B ( $4.33 \times 10^5$  cfu/ml).

**Key words:** Honey, Wound, inhibition

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### I. Introduction

Wounds are injuries to the body tissues caused by physical trauma or disease processes including surgery, diabetes, burns, punctures, gunshots, laceration, bites, bed sores and broken bone (1). From microbiology perspective, the primary function of normal intact skin is to control microbial populations that live on the surface and to prevent underlying tissues from becoming and invaded by potential pathogens. Exposure of subcutaneous tissue, following a loss of skin integrity (i.e. a wound) provides a moist, warm and nutritious environment that is conducive for microbial colonization and proliferation (2). Wounds are divided into two: acute wound are caused by external damage to intact skin and includes surgical wounds, bites, burns, minor cuts and abrasions and more severe traumatic wounds such as laceration and those caused by crush or gunshot injuries (3). Irrespective of the nature of the cutaneous injury, acute wound are expected to heal within a predictable time frame, although the treatment required to facilitate healing will vary according to the type, site and depth of a wound. Chronic wounds are caused by endogeneous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue (3). Pathophysiological abnormalities that may predispose to the formation of chronic wounds such as ulcers, foot ulcers, and pressure sores, including compromised perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus. Advanced age, Obesity, poor nutrition and immune suppression associated with disease (e.g AIDS) or drugs (e.g chemotherapy or radiation therapy) may also exacerbate chronic ulceration. Many of these predisposing factors impair blood flow, resulting in local hypoxia that may decrease leucocyte bactericidal action by affecting oxidant-processing enzymes such as myeloperoxidase.

The sources of microorganisms in wounds are:

- \* The environment (exogenous microorganisms in air or those introduced by traumatic injury).
- \* The surrounding skin (involving members of skin microflora such as *Staphylococcus epidermidis*, micrococci, skin diptheroids and propionibacteria).
- \* Endogeneous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosae). So also the normal microfloral of the gut, the oral cavity, and vagina are abundant source of microorganism (4).

In a study designed to investigate the bacteriology of non-surgical wound infections, a retrospective review of seven hundred and fifty cases of non-surgical wound infections were conducted between September, 2002 and February, 2005 at the University College Hospital, Ibadan, Nigeria. A total number of 871 bacteria and seven fungal isolates were obtained from these wound cultures. In 477 (70.3%) cases, cultures were monomicrobial and 2002(29.8%) polymicrobial. *Staphylococcus aureus* (38%) was the predominant pathogen, followed *Pseudomonas aeruginosa* (18.7%), *Klebsiella species*, *Escherichia coli* (10.6%), *Proteus species* (7.4%), *Staphylococcus epidermidis* (4.4%), *Streptococcus species* (1.6%), *Enterococcus faecalis* (1.4%) and *Candida albicans* (0.8%) (5)

Honey is the normal sweet substance produced by honey bees from nectar or blossoms or from the secretion of living parts of plants or excretions of plants, which honey bees collect, transform, and combine with

specific substances of their own to ripen and mature (6). It is also defined as the nectar and saccharine exudation of plants, gathered, modified and stored as honey in the honey comb by honey bees *Apis mellifera* (7). Honey is an ancient remedy for the treatment of infected wounds, which has recently been rediscovered by the medicinal profession, particularly where conventional modern therapeutic agents are failing (8) (9). There are now many published reports describing the effectiveness of honey in rapidly clearing infection from wounds, with no adverse effects to slow the healing process. There is also some evidence to suggest that honey may actively promote healing. In laboratory studies, it has been shown to have antimicrobial action against a broad spectrum of bacteria and fungi. Remarkable among the bacteria is *Pseudomonas aeruginosa*, a notorious organism in the resistance to antimicrobial compounds (8) (9). Honey is commonly used as a base for ointment and has sugar. Honey is commonly used as a base for ointment and has successfully been applied in surgical dressing to avoid septic infections (10). The current prevalence of the therapeutic use of ancient remedies, including honey (11) strong solution of honey or sugar pastes inhibits microbial growth due to high osmolarity but when used as dressings, this action ceases. But such wounds are rapidly rendered sterile by honey, because of its additional antimicrobial activity (12).

Honey is produced from many floral sources and its antimicrobial activities vary markedly with its origin and processing (13). This variation can be due to the difference in the enzymatic action and in the presence of additional antibacterial components derived from floral source (14). Honey primarily contains sugar and water. Sugar accounts for 95 – 99% of honey dry matter, majority of these are simple sugars, fructose (38.2%) and glucose (31.3%), which represents 85 – 95% of total sugars. These are “simple” sugars, 6-carbon sugars that are readily absorbed by the body (7). Other sugars include disaccharide such as maltose, sucrose and isomaltose, few oligosaccharides are also present.

Water is the second most important component of honey. Its content is critical, since it affects the storage of honey. The final water content depends on numerous environmental factors during production such as weather and humidity inside the hives, but also on nectar conditions and treatment of honey during extraction and storage (7). Organic acids constitute 0.57% of honey and include gluconic acid, which is a bi-product of enzymatic digestion of glucose. The organic acids are responsible for the acidity of honey and contribute largely to its characteristic taste (7). Minerals are present in honey in very small quantities (0.17%) with potassium as the most abundant, others are calcium, copper, iron, manganese, and phosphorus. Nitrogenous components among which the enzymes originate from salivary secretion of the worker honey bees are present. The main enzymes in honey are invertase (saccharase), diastase (amylase) and glucose oxidase (7). In 50AD, Dioscorides described honey as being “good for all rotten and hollow ulcers”. Honey has been reported to have inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives (15). An antifungal action has also been observed for some years and species of *Aspergillus* and *penicillium*, as well as common dermatophytes. (16). Pure honey has been shown to be bactericidal to many pathogenic microorganisms including *Salmonella spp*, *Shigella spp*, other enteropathogenic like *Escherichia coli*, *Vibrio cholera* and other gram-negative and gram-positive organisms (17) (18). High antimicrobial activity is as a result of osmotic effect, acidity, hydrogen peroxide and phytochemical factors (19). The clearing of infection seen when honey is applied to a wound may reflect more than just antibacterial properties. Recent research shows that proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentration as low as 0.1%; and phagocytes are activated by honey at concentration as low as 0.1%. Honey (at a concentration of 1%) also stimulate monocytes in cell culture to release cytokines, tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activated immune response to infection (20). The osmotic effect of honey has been described by (21). Honey is a super saturated solution sugars, 84% being a mixture of fructose and glucose. The strong interaction of these sugars molecules will leave very few of the water molecules available for microorganisms. The free water is measured as water activity ( $a_w$ ). Mean values of honey have been reported from 0.562 to 0.62. Many species of bacteria are completely inhibited if water activity is in the range of 0.94 to 0.99. These values correspond to solutions of a typical honey ( $a_w$  of 0.6 undiluted) of concentrations from 12% down to 2%(v/v). On the other hand, some species have their maximum rate of growth when, the ( $a_w$ ) is 0.99. so, inhibition by the osmotic (water drawing) effect of diluted solution of honey obviously depend on the species of bacteria (21). Honey is characteristically acidic with pH of between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens (22). The minimum pH values for grow of some common pathogenic species are: *Escherichia coli* (4.3), *Pseudomonas aeruginosa* (4.4), *Streptococcus pyogenes* (4.5). Thus, in the undiluted honey, the acidity is a significant antibacterial factor (23). Hydrogen peroxide is produced enzymatically in honey. The glucose oxidase enzyme is secreted from hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar. The hydrogen peroxide and acidity produced by the reaction;  $\text{Glucose} + \text{H}_2\text{O} \rightarrow \text{Gluconic acid} + \text{H}_2\text{O} + \text{O}_2$  servr to preserve the honey. On dilution of honey, the activity increases by a factor of 2,500 to 50,000, thus giving “slow-release” antiseptics at a level which is antibacterial but not tissue damage (24). Other workers (25) have however shown a reduction in antibacterial activity of honey on dilution to four times.

## II. Materials and Methods

### Collection of Samples

Untreated and infected wound samples were collected from two patients at Baptist Hospital, Ejigbo Local Government Area of Osun State, Nigeria. The samples were collected using needle aspiration method. Undiluted pure honey was bought from Masifa-Ejigbo Local Government Area of Osun State, Nigeria.

### Isolation

A total of six isolates were isolated from the wound samples. Four bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*. And two fungi; *Candida albicans*, *Blastomyces dermatitidis*. Bacteria isolation was done using standard microbiological technique and the identification was in reference to Bergey's manual of determinative Bacteriology (26). The fungi isolates were identified using standard microbiological simple staining technique.

### Inhibitory Effect of Honey

All materials used including the glass wares were appropriately sterilized. Inhibitory effect of pure honey against the isolates was determined by agar diffusion method. Double strength nutrient agar and potato dextrose agar were used for the study, measured out into 15ml aliquots and autoclaved. To prepare the plates, it was melted and maintained at temperature of 45°C until poured. This was done using pour plate method in which small colonies of each isolated microorganisms are made into suspension with 1ml of sterile water in test tubes. 1ml of each suspension was dispensed into sterile petridishes, after which the melted and sterilized nutrient medium and potato dextrose medium maintained at 45°C are poured (15 aliquot) into the respective plates. The plates were allowed to set well and then made in each plate using a sterilized cock borer. Into each of the wells 0.2milliliter of pure undiluted honey was introduced and the plates were incubated accordingly. After 24hours of bacteria incubation and 72hours of fungi incubation, clear zone of inhibition was observed in each of the plates. The antibiotic – sensitivity patterns of the isolates were studied by Kirby Bauer's disc; perfloxacin 10µg, Gentamycin 10µg, Ampiclox 30µg, Zinacef 20µg, Amoxicillin 30µg, Rocephin 25µg, Ciproflxacin 10µg, Streptomycin 30µg, Septrin 30µg, Erythromycin 10µg as per the Clinical and Laboratory Standards Institute (CLSI) standards.

## III. Results and Discussion

**Table 1: Fungi Count**

Wound	1 <sup>st</sup> Plate	2 <sup>nd</sup> Plate	3 <sup>rd</sup> Plate	Mean
A	2 x 10 <sup>-5</sup> cfu/ml	4 x 10 <sup>-5</sup> cfu/ml	2 x 10 <sup>-5</sup> cfu/ml	2.67 x 10 <sup>-5</sup> cfu/ml
B	3 x 10 <sup>-5</sup> cfu/ml	3 x 10 <sup>-5</sup> cfu/ml	4 x 10 <sup>-5</sup> cfu/ml	3.33 x 10 <sup>-5</sup> cfu/ml

**Table 2: Bacteria Count**

Wound	1 <sup>st</sup> Plate	2 <sup>nd</sup> Plate	3 <sup>rd</sup> Plate	Mean
A	5 x 10 <sup>-5</sup> cfu/ml	3 x 10 <sup>-5</sup> cfu/ml	4 x 10 <sup>-5</sup> cfu/ml	4 x 10 <sup>-5</sup> cfu/ml
B	3 x 10 <sup>-5</sup> cfu/ml	6 x 10 <sup>-5</sup> cfu/ml	4 x 10 <sup>-5</sup> cfu/ml	4.33 x 10 <sup>-5</sup> cfu/ml

**Table 3: Antibiotic Sensitivity Test Result**

Antibiotics	Concentration	S. aureus	P. aeruginosa	E. coli	S. Epidermidis	C. albicans	B. dermatitidis
Perfloxacin	10µg	S	R	S	S	R	R
Gentamycin	10µg	S	R	S	S	R	R
Ampiclox	30µg	R	R	R	R	R	S
Zinacef	20µg	R	R	R	R	R	R
Amoxicillin	30µg	R	R	R	R	R	R
Rocephin	25µg	R	R	R	S	R	R
Ciprofloxacin	10µg	R	R	S	S	R	R
Streptomycin	30µg	R	R	S	R	R	R
Septrin	30µg	S	R	S	S	R	R
Erythromycin	10µg	S	R	S	S	R	R

KEY:

S - Susceptible

R – Resistant

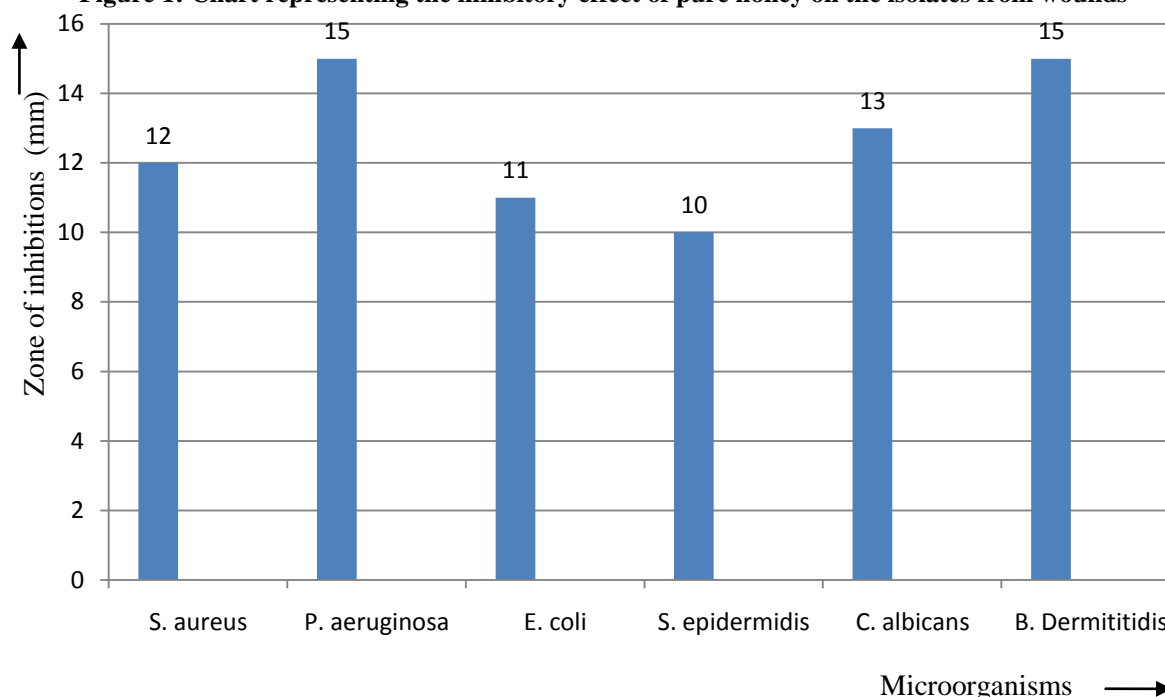
**Table 4: Inhibitory effect of pure honey on the isolates using gel diffusion method**

Test organisms	Dose	Inhibitory Effect	Zones of Inhibition
<i>S. aureus</i>	0.2ml	S	12mm
<i>P. aeruginosa</i>	0.2ml	S	15mm
<i>E.coli</i>	0.2ml	S	11mm
<i>S.epidermidis</i>	0.2ml	S	10mm
<i>Candida albicans</i>	0.2ml	S	13mm
<i>B. dermatitidis</i>	0.2ml	S	15mm

KEY:

S – Susceptible

**Figure 1: Chart representing the inhibitory effect of pure honey on the isolates from wounds**



The present study shows the bacteriacidal and fungicidal activities of pure honey against the wounds isolates; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans*, *Blastomyces dermatitidis* in a number of ways.

Considering the result obtained in table 4 where all the microorganism isolated showed a considerable clear zone of inhibition, this shows that they are susceptible to pure honey compared to antibiotic sensitivity test, where no single antibiotic shows inhibitory effect against at least four isolates, including those organisms which are detrimental to wound healing (e.g; *S. aureus*, *P. aeruginosa*, and coliform bacteria (27). All these were cleared off by pure honey. To *E. Coli*, *S. epidemidis*, *S. aureus* especially *P. aeruginosa* which is resistant to all the antibiotics but excellently cleared off by the action of pure honey is due to disruption of the cell membrane potentials and blocking of adenosine triphosphate (28).

Moreover, the fungi isolates; *Blastomyces dermatitidis* and *Candida albicans* in table 4 showed an excellent susceptibility result if compared with the antibiotic sensitivity disk test in table 3 where *Candida albicans* resist all the antibiotics and only ciprofloxacin are effective against *Blastomyces dermatitidis*. The fungicidal effect or action of pure honey in this study corerelates with the findings of (29) that *Candida albicans* strains are sensitive to honey.

In this study, *Pseudomonas aeruginosa* happened to be the microorganism which is greatly susceptible to antimicrobial action of pure honey followed by *Blastomyces dermatitidis*, a fungus (yeast like), next is *Candida albicans*, followed by *Staphylococcus aureus* and *Escherichia Coli* and the least susceptible is *Staphylococcus epidermidis*.

Besides its antimicrobial properties, honey can clear infection in a number of ways in vivo, like boosting the immune system, anti-inflammatory and anti-oxidant activities and via stimulation of cell growth (30).

### III. Conclusion

Findings in this study suggest that pure honey gotten from Western part of Nigeria in Masifa-Ejigbo Local Government Area of Osun state is useful in wound treatment as it has inhibited some microorganisms which are detrimental to wound healing.

The mechanisms of antibacterial action of pure honey in this study are suggested to be the following, although still remain speculative; High sugar concentration, low pH, hydrogen peroxide generation, proteinaceous compounds, or other unidentified components present in the honey. Shrinkage and disruption of the bacteria may be due to its osmotic effect, low pH and also due to the presence of antimicrobial substance such as inhibine.

Finally, antifungal mechanisms of the honey can be attributed to its low water activity and low pH, which may not encourage the proliferation and survival of fungi.

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