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Conventional Use of Honey as Antibacterial Agent

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Abstract
Background: Honey has since been found to possess antibacterial property and is therefore employed for wound therapy. The current problems with conventional antibacterial agents, led to the choice of honey as well as other natural products by the populace, in the treatment of bacterial infections. The present study evaluates the antibacterial spectrum and efficacy of honey and compared same with tetracycline and ciprofloxacin.
Methods: Different concentrations (12.5, 25.0, 50.0 and 100.0 %) of honey were studied in - vitro using *Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis, Klebsiella sp., Proteus mirabilis, Pseudomonas aeruginosa*, and *Escherichia coli.*
Results: The data obtained showed a dose dependent inhibitory action of honey, except with *Streptococcus faecalis* where there was no growth inhibition. The minimum inhibitory concentration (MIC) of honey presented *Staphylococcus albus* as the most susceptible organism and *Escherichia coli*, the least. While ciprofloxacin (2.0 mg/ml) exerted a greater potency than honey, tetracycline was found to be less potent than 100% concentration of honey, except with *Escherichia coli.*
Conclusion: The antibacterial action of honey was observed with 50% as well as the neat concentration. However, ciprofloxacin exhibited a greater potency and efficacy as well as a broader spectrum than honey, which shows that where a broad spectrum antibacterial is required, the conventional drugs, especially the newer ones are preferred to honey.

Key Words: Honey, natural products, antimicrobial agent

Résumé
Introduction : Depuis bien longtemps, on disait que le miel possède des vertus antibactériens et donc on l'utilisait pour la thérapie des blessures. Des problèmes actuels liés aux agents conventionnels antibactérien, a provoqué le choix du miel de même que d'autres produits naturels par le peuple, dans la prise en charge des infections bactériennes. Cette étude fait une évaluation du spectre antibactérien et l'efficacité du miel par rapport au tétracycline et ciproflocine.
Méthode : Des concentrations diverses (12,5 ; 25,0 ; 50,0 et 100,0%) du miel ont été étudiés in-vitro à travers l'utilisation du staphylococcus aureus, staphylococcus albus, streptococcus faecalis, klebsiella sp., proteus mirabilis, pseudomonas aeruginose, et escherichia coli.
Résultats : Les données obtenues avaient montré une action inhibiteur d'une dose dépendante du miel à l'exception du S. faecalis là où il n'y avait aucune inhibition de croissance. La concentration inhibiteur minimum (CIM) du miel a présenté S. albus comme un organisme le plus susceptible et E. coli le moins, Tandis que ciprofloxacine (2.0mg/ml) a donné une plus grande efficacité que du miel, tétracycline était notée d'avoir le moindre efficacité que 100% concentration du miel à l'exception du E. Coli.
Conclusion : L'action antibactérienne du miel était notée avec 50% de même que la concentration ingénieuse. Toutefois, la ciprofloxacine a donné une plus grande efficacité de même que un large spectre plus que du miel qui montre que là où un très grand spectre antibactérien est exigé, des drogues conventionnelles, des nouvelles drogues en particulier sont préférés au miel.

Mot clés: Miel, produit naturel, agent antimicrobien

Introduction

Honey has been used for the treatment of infected wounds hundreds of years ago, even before the discovery of bacteria as causes of infections.[1] In 50 A.D, Dioscorides described honey as being effective for all rotten and hollow ulcers. [1] The bactericidal action of pure honey on many pathogenic organisms including enteropathogens such as *Salmonella species, Shigella species, Escherichia coli* and other gram negative organisms has also been reported. [2] Furthermore, honey has been employed to shorten the duration of diarrhoea in patients with bactericidal gastro-enteritis due to bacterial infection as well as applied to heal wounds like the conventional antibiotics and antiseptics. [3-5]

The current antibiotic – resistant microbial species, for example, *Pseudomonas* and *Klebsiella species* resistance to gentamicin, amikacin and ceftazidine, [6] as well as toxicity to conventional therapy among other factors, have led to resurgence of ancient remedies. Honey has been employed by individuals in this environment for its numerous therapeutic benefits, since as a natural product it produces very few adverse effects. [7-9] *Of* utmost importance to the authors is its antimicrobial actions.

The present study was designed to screen for the antimicrobial spectrum and efficacy of a type of honey obtained from Ogun State, in Nigeria, using a few selected gram negative and gram positive bacteria and to also compare its effect with standard drugs, in order to justify its replacement of conventional drugs by some indigenes. The physical, chemical and biological properties of honey have already been documented. [10-13]

Materials and Methods

Source and dilution of honey

The honey used in this study was obtained from Ogun State in Nigeria. It was diluted in sterile distilled water to different concentrations of 12.5%, 25.0%, 50.0% (v/v). 100.0% honey was referred to as 'neat'.

Source and dilution of standard drugs

A concentration of 0.2% ciprofloxacin, 2 mg/ml (ampoule) marketed as Cifran (Fidson Drugs Nig. Ltd) was obtained for use. Tetracycline (Tetracap, Fidson Drugs Nig. Ltd) 250 mg was emptied into 10 ml sterile distilled water to make up the stock solution of 25 mg/ml. 10ug/ml was used for gram positive while 50ug/ml was employed for gram negative bacteria.

Organisms

Standard isolates and strains of micro-organisms namely: *Escherichia coli, Staphylococcus aureus, Streptococcus faecalis, Staphylococcus albus, Klebsiella sp., Proteus mirabilis, Peudomonas aeruginosa and Candida albicans* were employed for both sensitivity tests and the determination of minimum inhibitory concentration (MIC).

In-vitro demonstration of antimicrobial activity (Sensitivity tests)

The method employed [14, 15] has been widely used for antimicrobial susceptibility testing. Mueller Hinton and MacConkey agars were prepared according to the manufacturer's instructions. Pure culture of micro-organisms was grown on nutrient agar. Five colonies of each organism were picked using an inoculation loop into the Mueller Hinton broth (Oxoid, England) incubated for 4 h at 37°C, diluted with sterile saline to a density visually equivalent to 10^6 cfu/ml, which corresponded to MacFarland standard. The suspension was then seeded evenly onto the surface of Mueller Hinton agar plates (Oxoid, England) in triplicates with a sterile swab. Using a sterile 6 mm diameter cock borer, 5 wells were cut in the agar to which appropriate concentrations of honey were added, as well as the standard drugs, ciprofloxacin and tetracycline separately, which served as the controls. The plates were incubated at 37°C for 48 h under aerobic condition and were thereafter examined at 24 h for zone of inhibition and again at 48 h. *C. albicans* was grown on Sabouraud dextrose medium (Oxoid, England) and processed as above.

Minimum inhibitory concentration (Broth dilution method)

Appropriate volumes of honey was added to sterile tubes containing 10.0 ml of Mueller Hinton broth (Oxoid, England) to give a final concentrations of 512mg/ml, 256mg/ml, 128mg/ml, 64mg/ml, 32mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 2mg/ml and 1mg/ml. Using a volumetric pipette, 50µl of the test bacteria and fungal broth cultures were added into each of the tubes. The tubes containing the bacterial cultures were incubated at 37°C for 24 h, while the candida culture was incubated at 37°C for 48 h, after which they were read macroscopically to determine the lowest concentration of the test honey that did not permit any visible growth when compared with that of the control.

Two controls were employed; one was a row of positive control tubes containing only the growth medium and each of the micro-organisms, while the other was a negative control which consisted of a row of tubes containing different concentrations of honey with no organism. The minimum bactericidal concentration (MBC) was also determined.

Phytochemical analysis of honey

Phytochemical tests [16] were carried out on pure honey. A few millimeter sample of honey was mixed with aqueous sulphuric acid and benzene to test for presence of anthraquinone glycosides. Also, Mayer and Dragendorff's reagents were employed to test for presence of alkaloids. With the addition of gelatin and ferric chloride solution, test for tannins was conducted. Keller-keliani and Legal tests were carried out for cardiac glycosides, froth and haemolysis tests for saponins and 1%aluminium chloride solution in methanol was used to investigate flavonoids. Lastly, Benedict's and Fehling's solutions were added to determine presence of reducing sugars.

Results

The data obtained showed inhibitory effects of honey at 50 and 100% concentrations on the various investigated microorganisms as being dose-dependent (Table 1). However, both 50% and 100% honey did not produce any inhibition of *S. faecalis*.

In all cases of micro-organisms tested, the neat concentration of honey produced a greater inhibition than tetracycline except for *E. coli*, whereas, ciprofloxacin at the employed concentration of 0.2% produced a greater inhibition than honey. The data on MIC suggest that the most susceptible micro-organism to honey is *S. albus* and the order of susceptibility is *S. albus* > *S. aureus* = *P. aeruginosa* >*Klebsiella*=*Proteus* > *E. coli* (Table 2). *Candida albican* was not susceptible to honey at all concentrations tested.

The pure honey was found to contain alkaloids, anthraquinone glycosides, cardiac glycosides, flavonoids, saponins, tannins and reducing compounds.

Table 1: Antibacterial activities of different concentrations of honey compared with ciprofloxacin and tetracycline

Organisms	Inhibition zone diameters (mm, mean values, N=3)			
	Honey 100 %	Honey 50 %	Tetracycline 2.5%	Ciprofloxacin 0.2%
Staphylococcus albus	14.3	12.0	0	27.0
Staphylococcus aureus	11.0	0	10.0	30.0
Klebsiella species	12.3	8.0	6.0	29.7
Escherichia coli	13.3	0	16.0	27.5
Streptococcus faecalis	0	0	0	30.0
Pseudomonas aeruginosa	8.0	7.0	8.0	37.0
Proteus mirabilis	12.0	0	0	37.0

Table 2: Antibacterial activity of honey using the broth dilution method

Organisms	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus albus	8.0	16.0
Staphylococcus aureus	32.0	64.0
Klebsiella species	128.0	128.0
Escherichia coli	128.0	256.0
Streptococcus faecalis	Undetermined	Undetermined
Pseudomonas aeruginosa	64.0	64.0
Proteus mirabilis	128.0	128.0

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Discussion

Honey has been used to treat infections in a wide range of wound types, including leg ulcers, boils, pilonidal sinuses, and infected wounds from lower limb surgery.[17]

The present study showed that 100% honey inhibited growth of all the employed bacteria, both gram negative and gram positive in a dose- dependent manner, except with *S. faecalis* where there was no inhibition at all. While ciprofloxacin (0.2%) was found to be more potent and more efficacious than honey, tetracycline at the concentration of 2.5% employed in this study was less potent except with *E. coli* where it was superior to honey. *(*Table 1). Furthermore, *S. albus* was the most susceptible to honey as indicated by the clear zone of inhibition obtained at 100% (v/v) as well as the MIC value. This result compares well with the antibacterial action of manuka honey except that the latter had a greater potency.[18]

The following mechanisms have been suggested to explain the antimicrobial actions of honey;

1. Presence of an 'inhibine', factor in honey, which is hydrogen peroxide. [13, 19] Hydrogen peroxide is a well-known antimicrobial agent and its harmful effects when added in isolation is not noticeable with honey since the latter sequesters and inactivates the free iron which catalyses formation of oxygen free radicals produced by hydrogen peroxide. Its antioxidant components help to mop up free radicals. [9]
2. Osmotic property: Honey being a super-saturated sugar exerts an osmotic pressure which makes little or no water available for the micro-organisms to survive. [20]
3. Stimulation of lymphocytic and phagocytic activity. [21, 22] Recent studies showed that the 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 also activated by honey at such low concentrations. Furthermore, honey stimulates monocytes in cell culture to release cytokines, tumour necrosis factor (TNF) – alpha, interleukines (IL) -1 and (IL) –6, which stimulate the immune response to infection. In addition, the glucose content of honey and the acidic pH (typically between 3 and 4) may assist in the bacterial destroying action of macrophages.

4. Non- peroxide component: Among these are complex phenols and organic acids often referred to as flavonoids. [23]

While it could be said from the fore going that honey, when used *in vivo* might produce a greater effect than the *in-vitro* study, the antimicrobial profile might compare favourably with the present observation. However, the study has clearly demonstrated that honey might not adequately proffer a total solution to the current problems facing bacterial chemotherapy. Users therefore need to be enlightened that honey, being a natural product with very few side effects may not necessarily be superior to conventional therapies. The latter should be employed where necessary without skepticism.

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