Original article

Evaluation of antimicrobial effect of natural honey on some enteric pathogens

ADEMOKOYA A. A[1]

[1]Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Corresponding author: A. A Ademokoya E-mail: a.ademokoya@yahoo.com

Abstract

Objective: In search of appropriate treatment for most diseases caused by the intestinal pathogens due to antibiotic drug resistance by these organisms, this study is therefore designed to evaluate the antimicrobial effect of natural honey on some enteric pathogens.

Methods: The enteric pathogens used in this study were isolated from the stool samples of apparently healthy students of Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria. Natural honey on the other hand was purchased in Okusa market in Akungba Akoko. The honey was diluted using two different solvents namely; water and ethanol and was made in different concentration with ethanol and water respectively. One hundred stool samples (100) were collected from both males and females gender. The enteric pathogens were isolated using standard microbiology techniques.

Results: Out of the hundred samples analyzed, the following pathogens were recovered: *Escherichia coli, Salmonella typhi, Klebsiella pneumonae, Shigella dysenterae, Campylobacter jejuni* and *Helicobacter pylori.* The sensitivity patterns of the pathogen to the natural honey were also investigated. The pathogens were sensitive to different concentrations of the test honey in an increasing order as follows: *Klebsiella pneumonae, Helicobacter pylori* and *Campylobacter jejuni* (100 %), *Escherichia coli*, *Salmonella typhi and Shigella dysenterae* (85%). Statistical analysis showed a significant difference between natural honeys diluted with water and the one diluted with ethanol (P<0.5%).

Conclusion: Natural honey has been found to inhibit some intestinal pathogens with highest sensitivity impact on *Helicobacter pylori*, *Campylobacter jejuni* and *Klebsiella pneumonae*. Moreover, this study also shows that natural honey with different dilutions is more potent than the crude- undiluted natural honey for prophylaxis against infections caused by some enteric pathogens.

Key words: *Enteric pathogens, Natural honey, antimicrobial sensitivity*.

Introduction

Honey is a by-product of flower nectar and the upper aero-digestive tract of the honey bee which is concentrated through dehydration process inside the bee hive (12, 9). Honey is a natural product that has been widely used for its therapeutic effects (6). It has been reported to contain about 200 substances (12, 11). According to this latter author, honey is composed primarily of fructose and glucose but also contains fructose-oligosaccharides. In addition to these, honey also contains many amino acid, vitamins, minerals and enzymes (2). In the report of (2), the composition of honey varies depending on the plants on which the bee feeds. However, almost all natural honey contains flavonoides (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hespetin), phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acid), ascorbic acid, tocopherols, catalase, superoxide dismutase, reduced glutathione, Millard reaction products and peptides. Most of those compound work together to provide a synergistic anti-oxidant effect (2, 4).

Honey has a limited use in modern medicine because of lack of scientific support (12). From time immemorial, it has been observed that honey can be used to overcome liver, cardiovascular and gastro-intestinal disorder (12). Natural honey exhibits bactericidal activity against many organisms including *Salmonella*, *Shigella*, *Escherichia coli*, *Helicobacter pylori* e.t.c. (3, 8).

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Moreover, this latter author reported that, honey has anti-inflammatory effect in human after ingestion and has been shown to prevent reactive oxygen species (ROS)-induced low density lipoprotein (LDL) oxidation. In view of this numerous medicinal significance of natural honey, this study was carried out to further evaluate the antibacterial activity of natural honey mixed with other solvents precisely water and certain percentages of ethanol.

Methodology

Isolation of enteric pathogens

The enteric pathogens used in this study include, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonae*, *Campylobacter* jejuni, *Shigella dysenterae*, *Helicobacter pylori* and *Pseudomonad aureginosa*. They were isolated and identified using standard microbiological methods. One hundred stool samples were collected from both males and females apparently health students of Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria. The samples were brought to the university Microbiology Teaching Laboratory for investigation. The stool samples were cultured on MacConkey agar (a differential medium for enteric organisms), Eosin Methylene blue agar (a differential agar for *Escherichia coli*), selenite broth and desoxycholate medium. Furthermore, biochemical test which included the following: Methyl red test, Vosges Proskauer test (MRVP), Indole test, catalase test, oxidase test among others were done to confirm the identity of the pathogens according to the method of (10, 7).

Preparation of natural honey

The natural honey used for this investigation was purchased in local market in Akungba town, Akoko, Ondo State. The honey was subjected to different dilutions using ethanol and water as solvents. The dilution with ethanol ranged from: (2% ethanol + natural honey), (3% ethanol + natural honey), (4% ethanol + natural honey), (5% ethanol + natural honey), (6% ethanol + natural honey) and (7% ethanol + natural honey). On the other hand, the dilution with water was carried out as follows: 1ml of water in 5ml of natural honey, 2ml of water in 5ml of natural honey, 3ml of water in 5ml of natural honey e.t.c. The antimicrobial sensitivity pattern of the pathogens to different concentration of the honey was done using agar diffusion technique according to the method of (1). The broth culture of the pathogens was prepared overnight, incubated and inoculated aseptically in sterile plates of Mueller Hinton agar, each pathogen was seeded on different agar plate. Cork-borer was used to make a well inside the agar; different dilution of the honey was filled into the wells and incubated overnight. Zones of inhibition of the pathogens to different grades of the honey were measure in millimeter according to the method of (1). Control using the crude honey, water and ethanol separately was also set up.

Results

Table 1: Antibiogram of different concentration of ethanol in natural honey against some enteric pathogen

Pathogens	2%E+NH	3%E+NH	4%E+NH	5%E+NH	6%E+NH	7%E+NH	Crude honey
E.coli	5	6	8	12	15	20	18
Salmonella	0	6	9	10	18	20	16
Shigella	5	7	8	10	12	15	14
Klebsiella	10	20	25	28	32	35	30
Helicobacter	0	10	12	16	18	22	20
Campylobacter	6	8	10	14	16	18	16

Legend: E+NH = Ethanol plus Natural Honey. 10mm zone of inhibition is sensitive according to (5).

Table 2: Antibiogram of different percentage of water in natural honey against some enteric pathogen

Pathogens	1ml of H_2O+ ^NH	2ml of H_2O+ ^NH	3ml of H_2O+ ^NH	4ml of H_2O+ ^NH	5ml of H_2O+ ^NH	6ml of H_2O+ ^NH	Crude honey
E.coli	12	25	35	15	10	5	18
Salmonella	10	22	29	18	12	6	16
Shigella	11	23	30	20	15	8	14
Klebsiella	15	28	36	30	18	10	30
Helicobacter	12	20	25	21	19	11	20
Campylobacter	15	30	35	32	23	16	16

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Legend: H_2O+NH = Water plus Natural Honey, ^NH = 5ml of Natural Honey,
10mm zone of inhibition is sensitive according to (5)

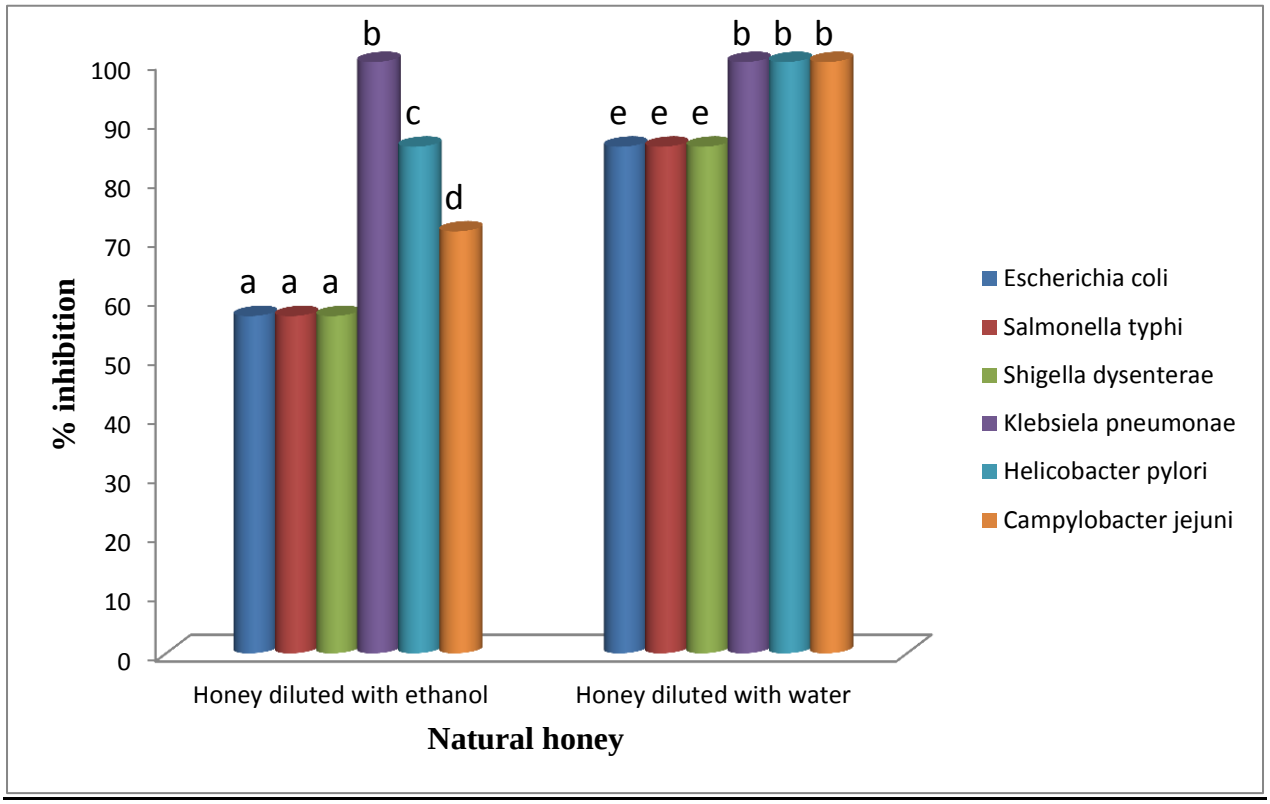


Figure 1: Percentage sensitivity of some enteric pathogens against different concentrations of natural honey
Mean with different alphabets outside end of the cylinders are significantly different P<0.5

Discusion

The evaluation of antimicrobial effect of natural honey on some enteric pathogens was carried out in this study. Although there have been several reports on the antimicrobial effect of honey on pathogens generally, but in this study different approaches were taken to investigate the antibacterial property of this natural substance. These included the choice of intestinal pathogens, the use of Gram negative pathogens, and addition of universal solvent-water which according to (7, 9), forms 75% human body weight. Moreover, the use of ethanol to dilute the honey- ethanol is one of the end products of sugar metabolism by microorganisms which subsequently inhibit their growth at stationary phase (5, 3). In this study, the results shows honey diluted with water had more percentage sensitivity than honey diluted with ethanol. This probably could be due to the hydrophilic nature of microbial cells toward water utilization which invariably permitted the active substances in the natural honey already dissolved in the water body to penetrate the cells of the microorganisms and inhibited them. On the other hand, the low sensitivity of honey diluted with ethanol could probably be due to inability of active substances in honey to act synergistically but antagonistically with the ethanol. In addition to this, since ethanol has antimicrobial property, the porin proteins in microorganism's cells could have prevented the absorption of the active substances dissolved in the ethanol solvent to penetrate and inhibit the pathogens.

In this study, the following organisms; Klebsiella pneumonae, Helicobacter pylori and Campylobacter jejuni were more sensitive to natural honey than *Escherichia coli*, *Salmonella typhi* and *Shigella dysenterae* in both dilutions-water and ethanol but the sensitivity obtained in honey diluted with water was higher than that of honey diluted with ethanol, this could probably be due to species or genus differences which made the sensitive organisms inhibited, and the less sensitive ones thwart the inhibitory property of the natural honey. In addition to this, the permeability

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differences of microbial cells also could be responsible, microbial cells are more permeable to water than to ethanol (11, 8). In this assay, it was discovered that the sensitivity of the honey was highest at the average dilution in both solvents used than the initial and the final dilutions. This discrepancy could probably be due to the proportion of the active substances that was abounded in the average dilution than at any point of the dilutions. This study reveals that intestinal pathogens are sensitive to natural honey with various degree of sensitivity depending on: the solvent used; the concentration of the honey and the purity of the natural honey because majority of honey sold in the market now-a-day is adulterated.

Conclusion

Natural honey has been found to inhibit some intestinal pathogens with highest sensitivity impact on *Helicobacter pylori*, *Campylobacter jejuni* and *Klebsiella pneumonae*. However, natural honey diluted moderately with water is more potent against those pathogens; therefore, natural honey could be adopted for prophylaxis against infections caused by those pathogens

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