

## Antibacterial Activity of Eucalyptus Honey Of Libyan against Multidrug Resistant Bacteria (MDR)

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### Abstract

The use of honey as a traditional remedy for microbial infections dates back to ancient times [1]. The aim of this study was to evaluate the antibacterial activity of Eucalyptus honey of Libyan against multidrug resistant bacteria (MDR) by the method of agar well diffusion. Different concentrations (50.75, 80 and 100%) of honey sample were checked for their antimicrobial activities, using some medically important micro-organisms including *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Acinetobacter sp* and *Staphylococcus aureus*. The mean inhibition zone produced by honey when applied to gram-negative bacteria and gram-positive bacteria was significantly higher than that of the antibiotic used.

**Keyword:** Antibacterial; Honey; Micro-organisms

### Introduction

Honey is the natural sweet substance from nectar or from the secretions of the living parts or excretions of plants which the honey bees collect and store in the honey [2]. Traditional importance and use of honey as therapeutics has been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago [3]. Secretions of the living parts or excretions of plants which the honey bees collect and store in the honey [2]. Traditional importance and use of honey as therapeutics has been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago [3].

Because honey inherits plants properties, its color, aroma, flavor, density, and physical and chemical properties depend on the flowers used by bees, although weather conditions as well as processing also influences its composition and properties [4]. As a result, the nutritional values and profiles vary accordingly and can thus influence the value of a specific honey for health promoting purposes [5]. The natural honey has been reported to contain about 200 substances.

The composition of honey is mainly sugars and water. The other constituents of honey are amino acids, antibiotic rich inhibitor, proteins, phenol antioxidants, and micronutrients [6]. The sugars in honey are sweeter and give more energy than artificial sweeteners [6]. These substances are of nutritional and health importance. Some of the vitamins found in honey include ascorbic acid, pantothenic acid, niacin and riboflavin along with minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc [7-8].

The main honey plants in Libya including; *Acacia spp*, *Pinus spp*, *Cupressus spp*, *Thymus vulgaris*, *Lantana camara*, *Hisbiscusrosa-sinensis*, *Eucalyptus cawaldulensis*, *Medicago sativa* and many wild plants [9]. Of those types, *Eucalyptus honey* (*Eucalyptus gonphocephala*), is one of the main honeys produced and consumed in Libya especially in the north where its extensive trees flowering in November and December. Since, there is a few or rare scientific information published worldwide about Libyan honey, this work aimed to determine the main quality criteria of Libyan Eucalyptus honey via the evaluate the antibacterial activity of Eucalyptus honey of Libyan against bacteria.

## Materials and Methods

### Bacteria

The antibacterial properties of three honeys were tested against five bacterial isolates i.e., three reference strains, *Escherichia coli* ATCC 13353, *Enterococcus faecalis* ATCC 51299 and *Staphylococcus aureus* NTCC 12493 and two were obtained from patients in an laboratory of microbiology in Banghazi medical center, *Klebsiella pneumonia* and *Acinetobacter sp.*

### Honey

Honey sample were used in this study obtained from apiary of the Umm Al-Qura. The honey sample was diluted by distilled water to 25, 50, 75, and 90%.

### Antibiotic

Four antibiotic discs were selected for both gram-negative and gram-positive bacteria including;

### Susceptibility testing of honey

A screening assay using well diffusion [10], Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100µl bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 Mc Farland in sterile saline) over the entire surface of the plate. After inoculation 9mm diameter wells were cut into the surface of the agar using a sterile cork borer. Different concentrations (25, 50, 75, and 90%) were added to the wells. Plates were incubated at 37°C for 24 h. Control wells contained distilled water. Zones of inhibition were measured by using ruler. The diameter of zones was recorded. Each assay was carried out in triplicate.

## Results

Honey sample showed marked inhibition of growth. The results of the assay of antibacterial activity of the honey sample with four concentrations (25, 50, 75 and 90% v/v) used in this study are shown in Tables (1), Figure (1). The present work is an attempt to verify the role of Libyan honey as an antibacterial agent and compared with the antibacterial activities of antibiotics. In this study, the antibacterial activities of honey were assayed separately against five species of human pathogenic bacteria using the Hole-pale Diffusion assay.

**Tables 1** demonstrate the effect of Eucalyptus honey on pathogenic bacteria based on the zone of clearing that was produced. All the different concentrations of honey showed antibacterial activity a large when tested against the multi-drug resistant bacteria (MDR). The zones of inhibition ranged from 9-31mm.

Honey sample showed marked inhibition of growth on *E.coli* ATCC 13353 the maximum inhibition zone was shown at concentration of 90% as 31mm, which reduce to 21mm at 25%. Also the table showed similar effect on *S.aureus* NTCC 12493 grow with inhibition zone at concentration of 90% as 31mm, and the inhibition zone reduce to 20mm at 25% also, Eucalyptus honey prevented the growth of *K.pneumonia* with a mean of inhibition zone equal to 29mm in diameter at 90% concentration *acinetobacter* showed. While Eucalyptus honey prevented the growth of *Enterococcus faecalis* ATCC 51299 with a mean of inhibition zone equal to 27mm in diameter at 90% concentration which reduce to 16mm at 25%. *Acinetobacter sp* a little less inhibition zone with honey sample. These were 20mm at 90% and 9 mm at 25% concentration (Figure 2).

The antibacterial activity of Eucalyptus honey was higher than antibacterial activity for Antibiotic (Tables2), Figure (2 and 3). The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low pH [11], and the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey [12].

Bacteria Types	Concentrations			
	%25	50%	75%	90%
	Mean Of Inhibition Zone For Three Recurrences (Mm)			
1- <i>Escherichia coli</i> ATCC 13353	21	27	30	31
2- <i>Staphylococcus aureus</i> NTCC 12493	20	26	28	31
3- <i>Enterococcus faecalis</i> ATCC 51299	16	22	23	27
4- <i>Acinetobacter</i> sp.	9	13	19	20
5- <i>Klebsilla pneumoniae</i>	23	26	28	29

Tables 1: Antibacterial activity (mm) of Eucalyptus honey at different concentrations against bacteria types (MDR).

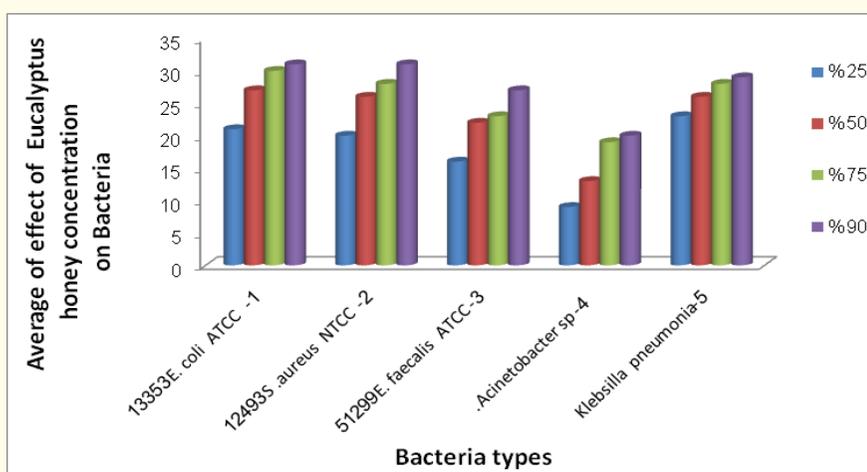
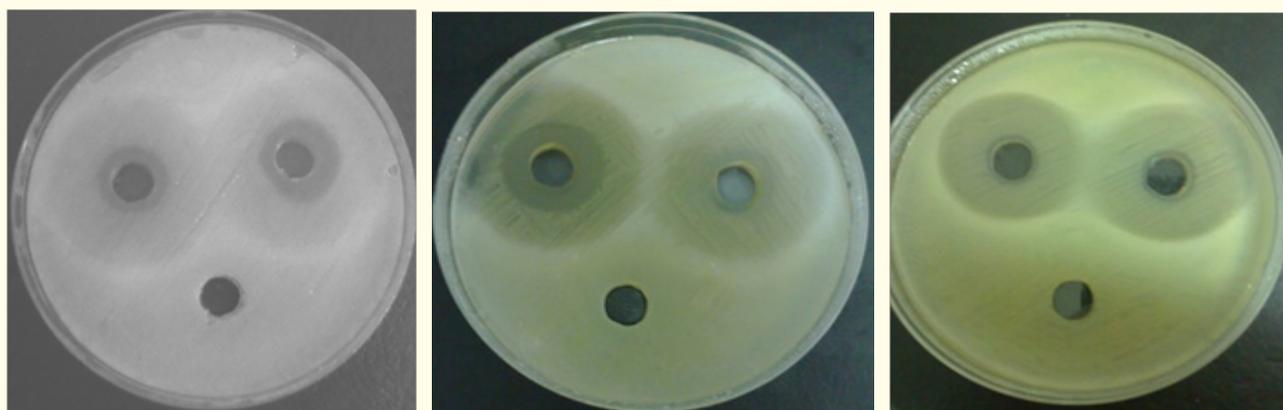
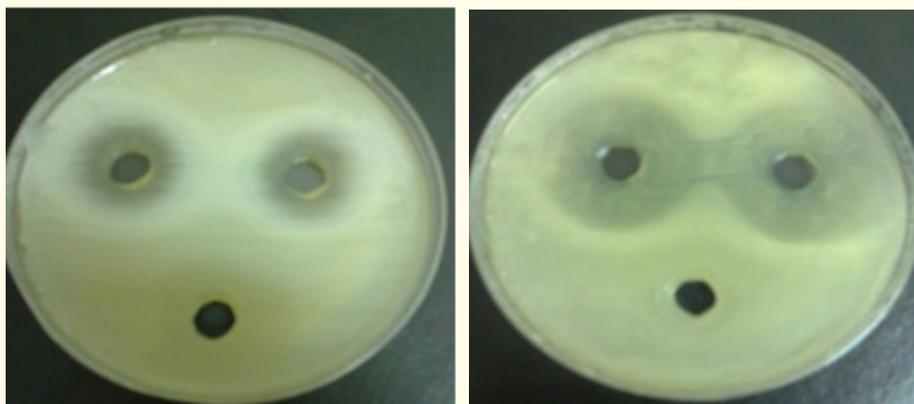


Figure 1: Mean inhibition zone of Eucalyptus honey at different concentrations against bacteria types (MDR).





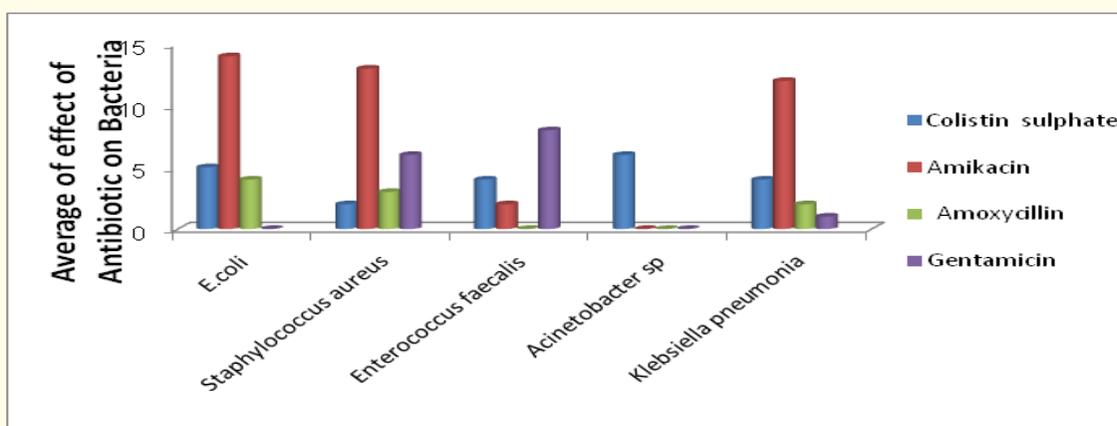
D:75% (*Acinetobacter* sp) E: 25% (*K.pneumonia*)

**Figure 2:** The inhibition zone by using (A:75%, B:90%, C:50%, D:75% and E: 50% ) concentration of Eucalyptus honey on bacteria types.

Antibiotic	Colistinsulphate	Amikacin	Amoxycillin	Gentamicin
1- <i>Escherichia coli</i> ATCC 13353	R	I	R	R
2- <i>Staphylococcus aureus</i> NTCC 12493	R	R	R	R
3- <i>Enterococcus faecalis</i> ATCC 51299	R	R	R	R
4- <i>Acinetobacter</i> sp.	R	R	R	R
5- <i>Klebsilla pneumonia</i>	R	R	R	R

**Tables 2:** Effect of Antibiotic on Bacteria (MDR).

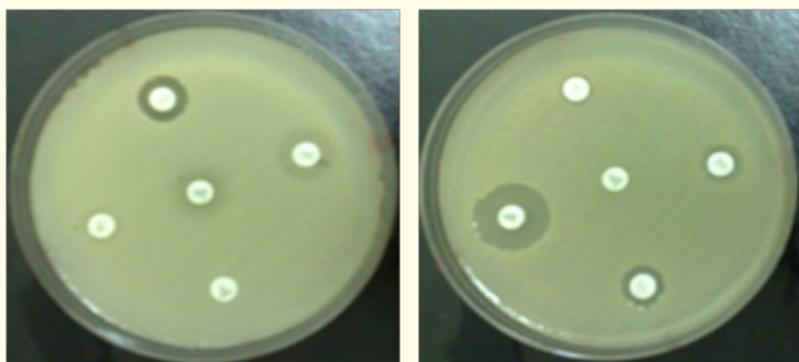
R= Resistant  
I= Intermediate



**Figure 3:** Mean inhibition zone of types of Antibiotic on Bacteria.



A: *E.coli* B: *S.aureus* C: *E.faecalis*



D: *Acinetobacter species* E: *K.pneumonia*

**Figure 4:** The inhibition zone by effect of antibiotic on bacteria types.

## Discussion

This study was undertaken to investigate *in vitro* antimicrobial activity of honey against certain microbial isolates. In the study, honey sample showed the antimicrobial activity and our result were in agreement with Willix, *et al.* [13] (1992) who found that honey inhibited the growth of *S.aureus* and *E.coli* and also in agreement with Bilal, *et al.* [14] (1998) who found honey exhibited a fairly good antimicrobial activity against both Gram-negative and positive bacteria and a remarkable activity was observed with *S.aureus*.

Eucalyptus honey had the highest antibacterial activity bactericidal was against *E.coli*, this result was in consistent with Chauhan, *et al.* [15] (2010) who reported that the most susceptible bacteria included *E.coli*. Al-Namma [16] (2009) also observed that honey has a greater inhibitory effect on Gram negative bacteria *E.coli*, *S. typhi* and *Paeruginosa* were more susceptible than other test organisms. Results showed that the Libyan (Eucalyptus honey) had great activity antibacterial against all bacteria tested.

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