

Antibacterial Activity of Eucalyptus Honey of Libyan Against Multi Drug Resistant Bacteria (MDR)

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Abstract: The use of honey as a traditional remedy for microbial infections dates back to ancient times. The aim of this study was to evaluate the antibacterial activity of Eucalyptus honey of Libyan against multi drug resistant bacteria (MDR) by the method of agar well diffusion. Different concentrations (50.75, 80 and 100%) of honey sample where 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.

Kew words: 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 [1]. Traditional importance and use of honey as therapeutics has been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago [2]. Secretions of the living parts or excretions of plants which the honey bees collect and store in the honey [1]. 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 [3]. As a result, the nutritional values and profiles vary accordingly and can thus influence the value of a specific honey for health promoting purposes [4].

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 inihbine, proteins, phenol antioxidants and micronutrients [5]. The sugars in honey are sweeter and give more energy than artificial sweeteners [5, 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*, *Hisbiscus rosa-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 it's extensive trees flowering in November and December. Since, there is a few or rare scientific information published worldwide about Libyan honey, therefore, this work was mainly 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 a laboratory of microbiology in Benghazi 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.

Susceptibility Testing of Honey: A screening assay using well diffusion [10]. Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse

100 µl bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation 9 mm diameter wells were cut into the surface of the agar using a sterile cork borer. Different concentrations viz., 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 i.e. 25, 50, 75 and 90% v/v used in this study are shown in Table 1 & Fig. 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.

Table 1 demonstrates 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.

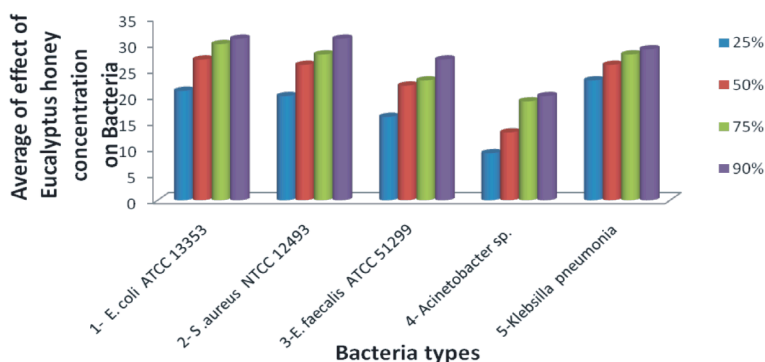


Fig. 1: Mean inhibition zone of Eucalyptus honey at different concentrations against bacteria types (MDR)

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

Bacteria types	Concentrations			
	25%	50%	75%	90%
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 sp.</i>	9	13	19	20
5- <i>Klebsiella pneumonia</i>	23	26	28	29

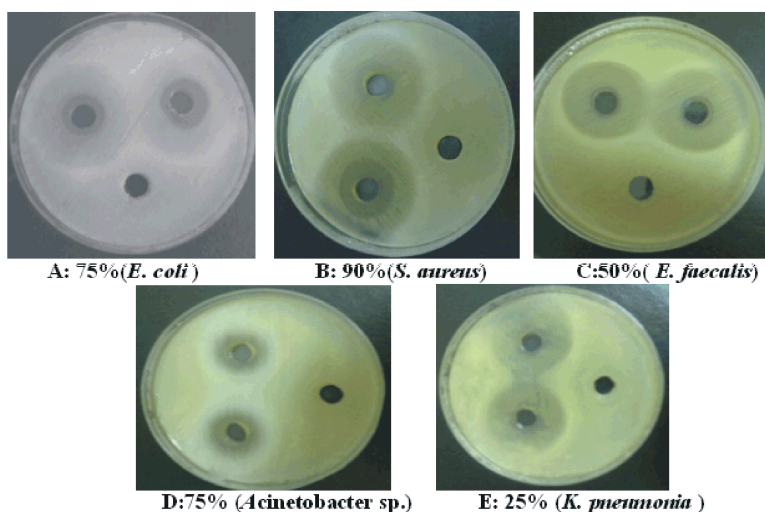


Fig. 2: The inhibition zone by using (A:75%, B:90%, C:50%, D:75% and E: 50%) concentration of Eucalyptus honey on bacteria types.

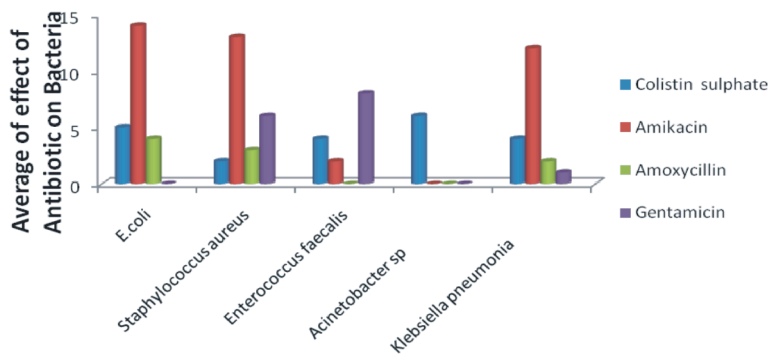


Fig. 3: Mean inhibition zone of types of antibiotic on bacteria.

Table 2: Effect of antibiotic on bacteria (MDR)

Bacteria types	Antibiotic			
	Colistin sulphate	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>Klebsiella pneumonia</i>	R	R	R	R

R= Resistant

I= Intermediate

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

The antibacterial activity of Eucalyptus honey was higher than antibacterial activity for Antibiotic (Table 2 & Fig. 2, 3). The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low

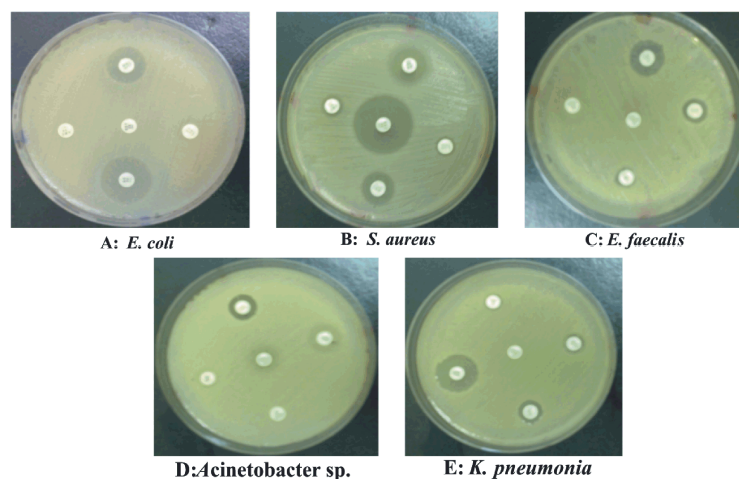


Fig. 4: The inhibition zone by effect of antibiotic on *bacteria types*

pH [11] and the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey [12].

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 [13] who found that honey inhibited the growth of *S. aureus* and *E. coli* and also in agreement with [14] 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 [15] who reported that the most susceptible bacteria included *E. coli*. [16] also observed that honey has a greater inhibitory effect on Gram negative bacteria. *E. coli*, *S. typhi* and *P. aeruginosa* 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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