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Research Article

Antibacterial Activity of Honey and *Acacia nilotica* Extract against Aerobic Bacteria Isolated from Diabetic Wound

Zobida Abd Elgadir Ahmed[1], Tawsul Mohammed Eltayeb[1], Hind Aljaili Mohamed[1] and Naser Eldin Bilal Mohmed[2]*

[1]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan*

[2]*Professor of Microbiology, Department of Microbiology, Faculty of Medical Laboratory Science, University of Khartoum, Sudan*

***Corresponding Author:** Naser Eldin Bilal Mohmed, Professor of Microbiology, Department of Microbiology, Faculty of Medical Laboratory Science, University of Khartoum, Sudan.

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Abstract

Background: This experimental analytical study was conducted at Jabir Abu Alizz Center to determine the antibacterial activity of honey and aqueous extract of Acacia nolitica against aerobic bacterial isolated from diabetic infected wound.

Materials and Methods: One hundred and fifty bacterial isolates including: *Pseudomonas aeruginosa, Klebsiella* species, *Staphylococcus aureus, Escherichia coli* and *Proteus* species and standard organisms(*S. aureus, Escherichia coli* and *Pseudomonas aeruginosa*) were studied to assess the antibacterial activity of honey and *Acacia nilotica* carried out by cup plate agar diffusion on Mueller-Hinton agar (MHA). The activity of honey and aqueous extract of *Acacia nilotica* was compared with some reference antibiotics including oxacillin and vancomycin for Gram positive bacteria and imipenem and amikacin for Gram negative bacteria.

Result: The study showed that the honey and *Acacia nilotica* exhibited antibacterial activity against standard and clinical isolates. Their antibacterial activities decreases when diluted.

Conclusion: Honey and *Acacia nilotica* exhibit antibacterial activity with varying degrees in all isolates according to concentration.

Keywords: *Honey; Acacia nilotica; Inhibition Zone; Susceptibility; Antimicrobial Activity*

Introduction

Honey has been used since ancient times in many cultures as an effective remedy [1], cures bacterial infections [2], and has antimicrobial activity against a wide range of bacterial and fungal species [3]. It's widely used as a topical antibacterial agent for treatment of wound, burns and skin ulcers [4].

The stem bark extract of *Acacia nilotica* the plant possessed the active compound (terpenoids, tannins, alkaloids, saponins, glycosides, phenol, resinol, eosin, steroids terpenes, phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, catechin, epi-gallacatechin-7-gallate epi gallocatechin-5 7-digallate) [5].

Diabetic wound infection: Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity which provides a moist, warm, and nutritive environment that is conducive for microbial colonization and proliferation [6]. Elbagir., *et al.* stated that diabetes is becoming more popular in developing countries like Sudan [7]. People with diabetes have a 12 - 25% life time risk of developing foot ulceration [8].

Materials and Methods

Standard bacterial control strains: *Staphylococcus aureus* ATCC: 29213, *Escherichia coli* ATCC: 25922, *Pseudomonas aeruginosa* ATCC: 27853 were tested for their susceptibility to reference antibiotics, honey and aqueous extracts of *Acacia nilotica*.

Sampling

A total of 150 isolates from diabetic wound swabs were collected from Jabir Abu Alizz Center.

Honey "honey kingdom" and *Acacia nilotica* were collected from the market.

Aqueous extract of the stem bark of the *Acacia nilotica*

A 1000g of the stem bark of the *Acacia nilotica* was air-dried; ground into powder using an electric blender. The blended material was transferred into a beaker containing 3000 ml of distilled water at ambient temperature (28 ± 2°C). The mixture was extracted by agitation for 3 hours and allowed to soak for 3 consecutive days.

Preparation of dilutions of honey

The honey sample was considered as 100% (stock), and then the dilutions were performed as follow:

- 10 ml of the honey stock was dissolved in 10 ml of sterile distilled water-50%
- 4 ml of the honey stock was dissolved in 16 ml of sterile distilled water-20%.

Sterilization of *Acacia nilotica* aqueous extract

The bottles of aqueous extract of *Acacia nilotica* were sterilized by autoclave at 121°C for 15 minutes after preparation of sample dilution (100%, 50%, 20%).

Preparation of standard bacterial suspension

10 ml normal saline were placed in test tubes and sterilized in an autoclave at 121°C for 15 minute, a full loop of purified bacteria was inoculated in sterile normal saline and compared with 0.5 McFarland standard.

In vitro testing of honey and aqueous extract of *Acacia nilotica* for antibacterial activity

The cup plate method [9] was adopted with some minor modification to assess the bacterial activity of the prepared extracts and honey dilution. Two ml of bacterial suspension (standards and clinical isolates) were taken with disposable syringe and added to twenty ml of molten Mueller-Hinton broth medium mixed, allowed to set and solidify for few minutes. Wells were made using sterile corn borer of 5 mm diameter. Alternated cups were filled with 0.1 ml of sterilized reference antibiotics, *Acacia nilotica* extract and diluted honey using sterile disposable syringes, allowed to diffuse at room temperature for 15 minutes then the plates were incubated in an incubator in upright position at 37°C for 18 hours [10]. The diameters of the resultant growth inhibition zones of *Acacia nilotica* and honey in both controls and clinical isolates were measured by a ruler and the mean values were obtained (result).

Two reference antibiotics vancomycin and oxacillin for Gram positive bacteria and amikacin and imipenem for Gram negative bacteria were tested against the controls and clinical isolates.

Result

Of the 150 clinical isolates, 59 were *Pseudomonas aeruginosa*, 39 *S. aureus*, 26 *E. coli*, 24 *Klebsiella* species and 2 were *Proteus* species. The effectivity of honey tested against standard organisms begin from 20% concentration in *S. aureus*, while in *E. coli* and *Pseudomonas aeruginosa* from 50% concentration. 20% concentration of *Acacia nilotica* was effective against all standard organisms.

The Susceptibility of control strains against reference antibiotics shown in table 1 below.

Controls	Vancomycin	Oxacillin	Amikacin	Imipenem
Staphylococcus aureus ATCC: 29213	Susceptible	Susceptible	-	-
Escherichia coli ATCC: 25922	-	-	Susceptible	Susceptible
Pseudomonas aeruginosa ATCC: 27853	-	-	Susceptible	Susceptible

The percentage of Susceptibility of clinical isolates against reference antibiotics shown in table 2 below.

Clinical isolate	Antibiotic	Susceptible	Resistant
Staphylococcus aureus	VA	73.3%	26.7%
	OX	6.5%	93.5%
Escherichia coli	AK	73.7%	26.3%
	IMP	94.4%	6.5%
Klebsiella species	AK	88.2%	11.8%
	IMP	100%	0%
Proteus species	AK	50%	50%
	IMP	100%	0%
Pseudomonas aeruginosa	AK	85.7%	14.3%
	IMP	92.5%	7.5%

VA: Vancomycin (concentration= 30 µg*); OX: Oxacillin (concentration= 5 µg); AK: Amikacin (concentration= 30 µg); IPM: Imipenem (concentration= 10* µg)

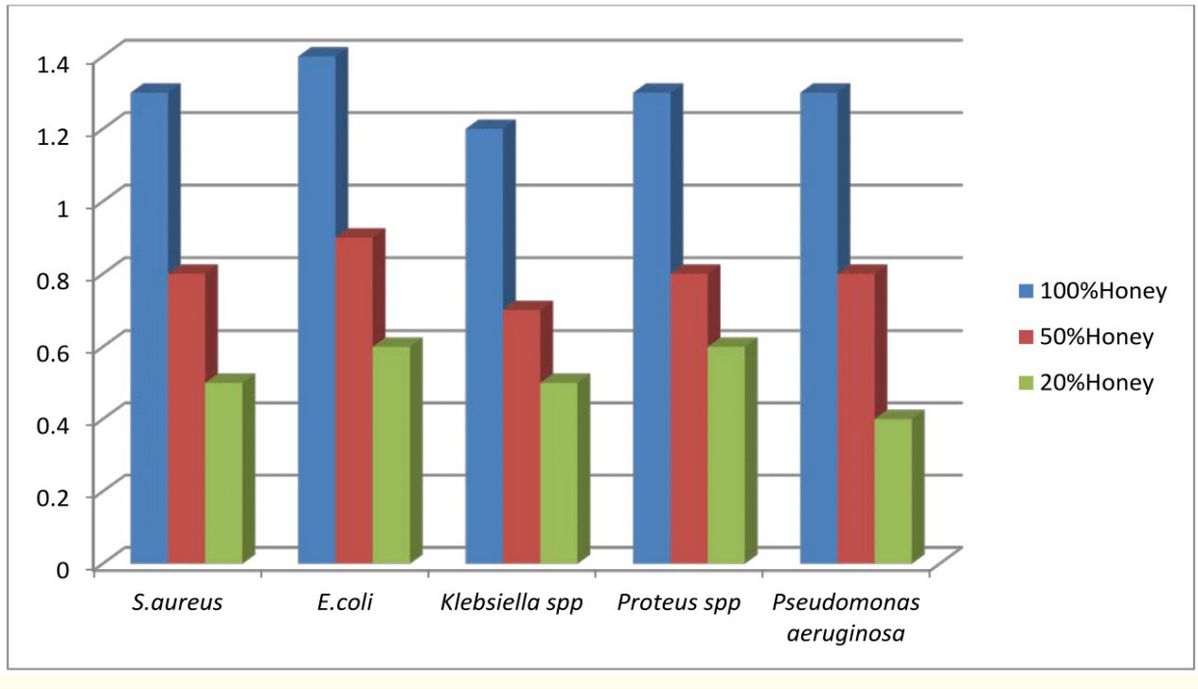

Figure 1: *The mean of inhibition zone diameter (mm) of different concentration honey against clinical isolates.*

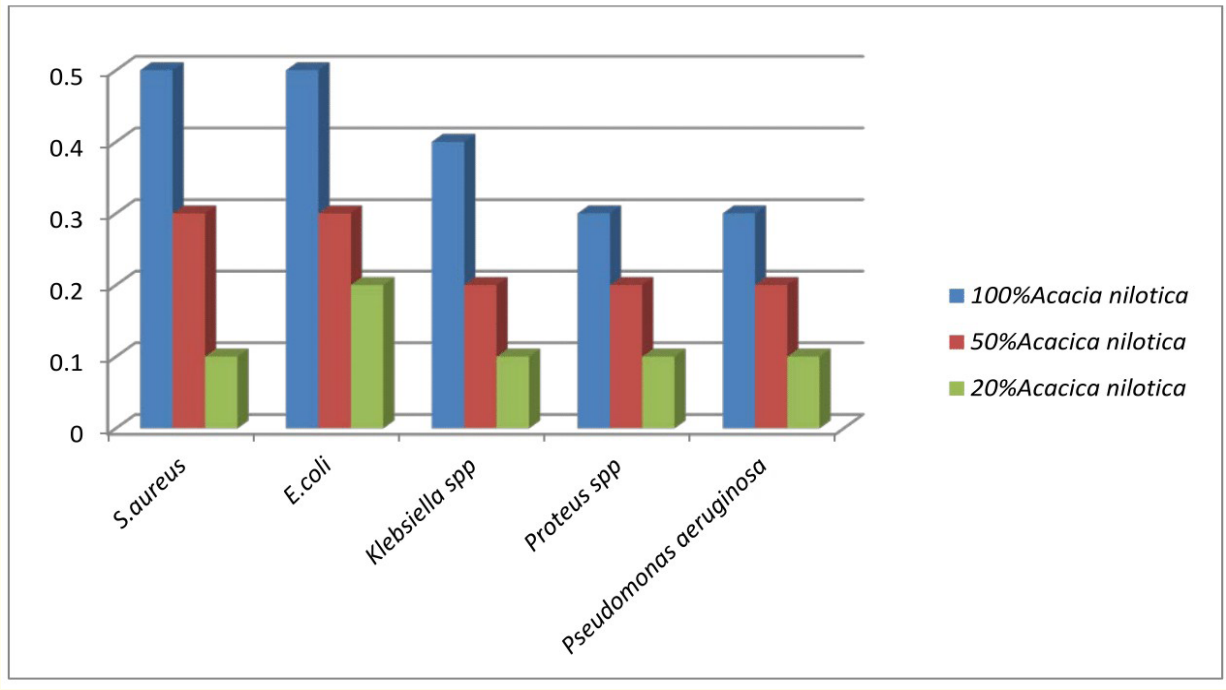

Figure 2: *The mean of inhibition zone diameter (mm) of different concentration Acacia nilotica against clinical isolates.*

Discussion

The finding of our study as regards the antibacterial activity of honey was in agreement with several studies. In 2010 Nur Azida proved that both Gram-positive and Gram-negative bacteria isolated were completely inhibited by the honey tested [11]. In 2009 Alandejani T conducted a study and proved that 100% of the isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were effectively inhibited by honey [12]. Hyungjaer., *et al*. in 2007 reported that 92.5% of bacterial isolates of different sources was inhibited by honey [13]. Also in 2004, Patricia reported that the bactericidal activity of honey against pathogenic bacteria was studied; showing that 93% of bacteria were inhibited by tested honey [14].

In this study the antibacterial activity of honey and aqueous extract of *Acacia nilotica* at 100% concentration inhibit all clinical isolates while at 50% concentration inhibit *E. coli, Klebsiella* species and *Proteus* species (100%), *Pseudomonas aeruginosa* (98.3%). *S. aureus* was 97.4% susceptible at 50% concentration of honey and 94.9% at 50% concentration of *Acacia nilotica*.

20%concentration of honey inhibit *E. coli, Proteus* species, completely, 92.2% of *Pseudomonas aeruginosa*, 91.7% of *Klebsiella* species, and 89.7% of *S. aureus*, while 20% concentration of *Acacia nilotica* inhibit *Proteus species* (100%), *E. coli* (96.2%), *Klebsiella* species (91.7%), *S. aureus* (84.6%) and *Pseudomonas aeruginosa* (83.1%).

The inhibition zone of honey against clinical isolates was border than reference antibiotics in all isolates, while aqueous extraction of *Acacia nilotica* gave smallest zone of inhibition.

Conclusion

Honey and aqueous extract of *Acacia nilotica* had antibacterial activity, but with varying degree of effectiveness against all clinical isolates and standard organisms. Honey had more potential antibacterial activity than aqueous extract of *Acacia nilotica*. The effectiveness of *Acacia nilotica* increases with repeated autoclaving.

Recommendation

1. Further in depth studies of active ingredient of *Acacia nilotica* to determine the active component responsible for antibacterial activity.
2. Confirmatory experimental *in vivo* to evaluate antibacterial activity of honey and *Acacia nilotica*.
3. Determination of Minimum Inhibitory Concentration (MIC).
4. Test the effectivity of honey and *Acacia nilotica* against anaerobic organism isolated from diabetic wound.

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