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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF CERTAIN IRRADIATED AND NON- IRRADIATED EGYPTIAN HONEY BEES' PRODUCTS

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Abstract

With the rise in prevalence of antibiotic-resistant bacteria, honey is increasingly evaluated for its antibacterial activity. In this study, 25 Egyptian honey samples of different types, companies and locations were used. From each sample, numbers of aerobic bacteria were determined using the aerobic plate count technique, and identified using the gram stain technique, biochemical tests and the GEN III Microstation^{TM} semi-automated ID system. The antimicrobial activity of non-irradiated and irradiated honey samples at different gamma radiation lethal dose levels (0.5, 1, 3, 5, 7, 10 and 15kGy) of different concentrations (100%, 75%, 50% and 25%) were investigated against 6 pathogenic microbial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Klebsiella pneumonia* and *Candida albicans*), using agar well-diffusion method. Also, the antimicrobial activity of some commonly- used antibiotic discs (Amoxicillin/ Clavulinic acid, Amikacin, Cefotaxime, Ciprofloxacin, Sulfamethoxazole/ Trimethoprim, Tobramycin, Tetracycline and Nystatin) against the tested pathogenic microorganisms were determined using the disc-diffusion method. The findings indicate that the antimicrobial activity of the irradiated honey samples were greater than that of both the non-irradiated honey samples and most of the antibiotic discs. The results also showed that most of the honey samples at 100% (v/v) concentration inhibit the growth of all the tested bacteria.

Keywords: Honey, Antimicrobial activity, Gamma irradiation, Antibiotics, Pathogenic microorganisms.

Introduction

Apitherapy (the medical use of honey bee products) has recently become the focus of attention as a form of folk and preventive medicine for treating certain conditions and diseases, as well as promoting overall health and well-being [(31)].

Honey is a thick, sweet liquid made by bees from the nectar of flowers through being gathered, modified and stored in the honey combs by the honey bees [(6)]. It is a mixture of fructose (average 38.4%), glucose (average 30.3%), sucrose (average 1.3%), and other carbohydrates (about 12%), minerals (average 0.169%) and proteins (169mg/100g), with a water content of about 17.2% [(35)]. It is known to be rich in both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acids, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins [(20)].

Honey has been used in the treatment of different diseases as long as 200 years. The use of honey as a medicine has continued into present-day medicine [(14)]. The medicinal properties of honey have been reported and documented by bee keepers

and medical practitioners [29]. It has been shown to reduce the risk of heart diseases, cancer, cataracts and inflammatory processes [5].

It is well-established that natural unheated honey inhibits a broad spectrum of bacterial species and is effective against antibiotic-resistant bacterial pathogens, oral bacteria as well as food-spoilage bacteria [29].

It has been also reported to have an inhibitory effort to around to 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives [24]. Thus, it has a bactericidal and bacteriostatic effect against various types of gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus pyogenes* and *Salmonella typhi*[15].

Bee honey is usually contaminated with numerous microorganisms, among these osmophilic yeast predominates, mainly the strains of *Saccharomyces*, *Schizosaccharomyces* and *Torula*. In bee honey, aerobic *Bacillus* and anaerobic *Clostridium* spores as well as spores and small fragments of moulds may appear [16].

Honey has two sources of contamination with microorganisms: primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar; secondary sources are those arising from honey manipulation by people, they include air, food handlers, cross contamination, equipment and buildings [10].

The gamma irradiation process seems to be a good alternative to pasteurization as it avoids heating. Gamma radiation applied on seven honey samples was found to decrease the amount of aerobic and anaerobic bacteria and fungi [23].

However, there is little information in the literature about the effect of radiation on the antimicrobial activity of honey against certain pathogenic microorganisms. This study aims to assess the changes in the antimicrobial activity of 25 Egyptian honey samples. These samples were irradiated at different radiation dose levels ranged from 0.5 kGy to 25kGy and the antimicrobial susceptibility of honey before and after radiation was measured along with commonly-used antibiotic discs.

Materials and Methods

Honey Samples

Twenty five different honey samples were obtained in between 2010 and 2013. Out of theses twenty five honey samples, four different honey samples were obtained from the Ministry of Agriculture along with twenty one different honey samples obtained from the local market of different five different companies and localities in Egypt. The investigated samples were two sweet marjoram, four citrus, two red Korean ginseng, four black seed, four clover flower, two royal jelly, one ginseng, one extra and one as a mixture of clover flower, sweet marjoram, anise, sesame and sunflower.

Microbial Counts:

Total aerobic bacterial counts:

The spread plate count technique was used [26], 1ml of each honey sample was mixed with 1ml of the sterile tween-saline solution (0.9% w/v NaCl in distilled water containing 0.1% v/v tween 80, adjusted at pH 7) on a vortex (type paramix II

No. 65, West Germany) for 3min, and 2-fold serial dilutions were made in the same diluent. Then, 0.1ml was taken from each suitable dilution and inoculated onto 20ml sterile nutrient agar in sterile petri-dishes.

The inverted plates were incubated at 37 ˚C for 24hrs. then, suitable dilutions were counted and the results were recorded. The microbial count was expressed as colony-forming unit per ml of honey (cfu/ml).

Isolation and Identification of the bacterial contaminants:

The bacterial strainswere isolated from the different honey samples according to their morphological characters, spread on nutrient agar plates for purification then kept on nutrient agar slants at 4˚C and then identified according to [4] and [9] involving the following steps: gram-stain, biochemical tests and using GEN III Microstation^{TM} semi-automated ID system (Biolog Inc., Hayward, CA, USA).

Gamma irradiation studies:

Gamma irradiation facility:

Cobalt-60 (Co^{60}) 220 Gamma Cell, Canada, Ltd., located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt have been utilized as radiation resources**.** The dose rate was 2.903kGy/hr. at the time of experiments.

Determination of the lethal radiation dose levels:

To determine the lethal radiation dose level for each honey sample. Aliquot of 1ml of each honey sample in sterile test tubes were exposed to gamma radiation doses of 0.5, 1, 3, 5, 7, 10, 15, 20, 25 and 30kGy. After irradiation, each irradiated sample was suspendedin 1ml sterile tween-saline solution and shaked-well on the vortex for 3min. From each irradiated honey sample; then 0.1ml was inoculated onto 20ml sterile nutrient agar plates and incubated at 35±2˚C for 24-48hrs. The radiation dose at which no bacterial growth detected was determined as the lethal dose.

Antimicrobial sensitivity tests[8]:

The test microorganisms for the antimicrobial tests:

The pathogenic clinical strains used as test organisms for the antimicrobial sensitivity tests, for both honey samples (non-irradiated and irradiated) and antibiotic discs, were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter* species and *Candida albicans*. They were obtained from different clinical samples (blood, urine and vaginal infections) from patients who admitted El-Demerdash Hospital, Ain Shams University, Cairo, Egypt. The bacterial strains and *Candida albicans* were kept on slants of nutrient agar and Sabouraud's agar, respectively at 4 ˚C.

Inoculum preparation:

All the tested pathogenic microorganisms were sub-cultured onto nutrient agar and Sabouraud's agar for bacterial and fungal growth, respectively. Inoculums were prepared by picking distinct colonies from a 24-hour culture, then suspended and vortexed for 15 sec. and its turbidity was adjusted with a spectrophotometer to adjust the transmittance to that produced by a 0.5 MacFarland standard at 530nm wavelength ($5X10^6$ cfu/ml).

Inoculation of tested plates:

Within 15min. after adjusting the turbidity of the inoculums' suspensions, the dried surface of each sterile Muller-Hinton agar plates (4mm depth) was inoculated by evenly spreading the swab containing the microbial suspension over the entire agar surface. The plates were then left to dry for 15min. before applying the different concentrations of either non-irradiated and irradiated honey samples or the antibiotic discs.

Antimicrobial assay of non-irradiated and irradiated honey samples[11]:

Non-irradiated honey samples:

Agar well-diffusion method was used for determination of the antimicrobial sensitivity tests of the different honey samples. Different concentrations of each non-irradiated honey sample (v/v) were prepared in sterile dist. water to give final concentrations of 25, 50, 75 and 100%. Dist. water was used as a negative control to all experiments. Wells (0.6 mm in diameter) were cut from each inoculated agar plate using a sterile cork-porer. Then, 0.1ml of each concentration of each concentration of each honey sample was put in each well. Plates were then incubated at 37˚C for 24 hrs. and 28±2˚C for 48-72 hrs. for bacteria and fungi, respectively.

Irradiated honey samples:

The same above procedure was performed, but after irradiation of the different concentrations of each honey sample (25, 50, 75 and 100%) at the lethal dose levels of gamma radiation (0.5, 1, 3, 5, 7,10 and 15kGy).

Antimicrobial assay of the tested antibiotic discs [8]:

Antibiotic susceptibility for the pathogenic clinical microorganisms was detected; nine antibiotic discs (Oxoid) were tested (eight of them for the bacterial strains and one for *Candida albicans*): Amoxicillin/Clavulinic acid, Amikacin, Cefotaxime, Ciprofloxacin, Imipenem, Sulfamethoxazole/Trimethoprim, Tetracycline, Tobramycin and Nystatin.

The appropriate antimicrobial-impregnated discs were placed on the surface of the agar, using sterile forceps to dispense each antimicrobial discs one at a time. The discs were placed with gentle pressing to ensure complete contact with the agar surface.

Interpreting of the results:

Antimicrobial activity was evaluated by measuring the diameter of the clear inhibition zone formed around each well containing each concentration of both non-irradiated and irradiated honey samples; and each antibiotic disc (expressed in mm) after incubation. All the tests were performed in triplicate and the mean of the three readings was calculated and recorded.

Statistical Analysis:

Data analysis of the results was expressed as means ± standard deviation and differences between the antimicrobial activity of the non-irradiated and irradiated honey samples; and between the honey samples before and after exposure to gamma radiation were analyzed statistically using the t-test by SPSS V17. Differences were considered significant when $p<0.05$.

Results

Microbial counts and identification of the isolated bacteria:

In the present study, determination of the bacterial contaminants isolated from the different types of honey samples showed that the aerobic bacterial counts were within the satisfactory limit ($\leq 10^3$cfu/ml). The bacterial counts from the samples of code (A) range from $8.8X10^1$cfu/ml (red Korean ginseng honey) to $2.9X10^2$cfu/ml (sweet marjoram honey). The samples of code (B) range from $5.2\ X10^1$cfu/ml (red Korean ginseng honey) to $2.7X10^2$cfu/ml (clover flower honey). Of code (C) range from $5.0X10^1$cfu/ml (clover flower honey) to $3.2X10^2$cfu/ml (extra honey). Of code (D) range from $8.4X10^1$cfu/ml (black seed honey) to $1.5X10^2$cfu/ml (clover flower honey) and those of code (E) range from $1.3X10^2$cfu/ml (citrus honey) to $4.6X10^2$cfu/ml (commercial honey), as shown in Table (1).

A total of 96 bacterial isolates were isolated from the different honey samples as follows: 22 bacterial isolates from the honey samples of code (A), 20 bacterial isolates from the honey samples of code (B), 19 bacterial isolates from the honey samples of code (C), 15 bacterial isolates from the honey samples of code (D) and 20 bacterial isolates from the honey samples of code (E).

Also, the results of this study show that the isolated bacteria from the different honey samples were identified using the gram-stain method as gram-negative rods, gram-positive rods and gram-positive cocci.

Table (1): Determination of the bacterial counts, number and the microscopical identification of the bacterial contaminants isolated from the different honey sample:

Honey type	Sample-Company code	Bacterial count (cfu/ml)	No. of bacterial isolates	Bacterial identification
Sweet marjoram	1-A	$2.9X10^2$	5	G-ve rods
			3	G+ve cocci
Citrus	2-A	$1.9X10^2$	2	G+ve rods
Red Korean ginseng	3-A	$8.8X10^1$	2	G-ve rods
Black seed	4-A	$1.0x10^2$	3	G-ve rods
Clover flower	5-A	$1.3x10^2$	3	G-ve rods
			2	G+ve rods
Total	**5**		**22**	
Sweet marjoram	6-B	$1.6X10^2$	6	G-ve rods
			1	G+ve cocci
Citrus	7-B	$1.1X10^2$	3	G-ve rods
Red Korean ginseng	8-B	$5.2X10^1$	2	G+ve rods
Black seed		$7.3X10^1$	1	G-ve rods
	9-B		1	G+ve cocci
Clover flower	10-B	$2.7X10^2$	4	G-ve rods
			2	G+ve rods
Total	**5**		**20**	

Ginseng	11-C	$1.5X10^2$	5	G-ve rods
Royal jelly	12-C	$2.7X10^2$	3	G-ve rods
			2	G+ve cocci
Extra	13-C	$3.2X10^2$	2	G-ve rods
			2	G+ve rods
Black seed	14-C	$2.5X10^2$	3	G-ve rods
Clover flower	15-C	$5.0X10^1$	2	G-ve rods
Total	**5**		**19**	
Black seed	16-D	$8.4X10^1$	2	G-ve rods
Royal jelly	17-D	$8.6X10^1$	3	G-ve rods
Citrus	18-D	$1.3X10^2$	4	G-ve rods
			1	G+ve cocci
Clover flower	19-D	$1.5X10^2$	2	G-ve rods
			3	G+ve cocci
Total	**4**		**15**	
Citrus	20-E	$1.3X10^2$	4	G-ve rods
Mix. of Clover flower, Sweet marjoram, Anise, Sesame and Sunflower	21-E	$1.6X10^2$	3	G-ve rods
Commercial honey	22-E	$4.6X10^2$	1	G-ve rods
			2	G+ve rods
Commercial honey	23-E	$2.3X10^2$	2	G-ve rods
Commercial honey	24-E	$1.6X10^2$	3	G-ve rods
Commercial honey	25-E	$3.8X10^2$	5	G-ve rods
Total	**6**		**20**	
Total			**96**	**25**

(cfu): colony forming unit

It was also found that the gram-staining of the bacteria isolated from the different types of the honey samples were in the order of gram-negative rods (representing 74%) > gram-positive rods (representing 14.5%) > gram-positive cocci (representing 11.5%) (Table, 2).

Table (2): Evaluation of the bacterial contaminants isolated from the different honey samples:

Company code	No. of samples	No. of bacterial isolates	Bacterial identification	%
A	5	22	G-ve rods G+ve rods G+ve cocci	59% 27% 14%
B	5	20	G-ve rods G+ve rods G+ve cocci	70% 20% 10%
C	5	19	G-ve rods G+ve rods G+ve cocci	79% 10.5% 10.5%
D	4	15	G-ve rods G+ve cocci	73% 27%
E	6	20	G-ve rods G+ve rods	90% 10%
Total	**25**	**96**	**G-ve rods** **G+ve rods** **G+ve cocci**	**74%** **14.5%** **11.5%**

The isolated bacterial contaminants were then identified using the GEN III Microstation^{TM} semi-automated ID system, and the results revealed that the gram-negative rods were both lactose ferementers (*Serratia marcescens*, *S. liquefaciens*, *Klebsiella pneumoniae*, *K. oxytoca*, *E. coli*, *Citrobacter freundii*, *C. diversus*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Erwinia amylovora*, and *Achromobacter* sp.) and non-lactose fermenters (*Pseudomonas aeruginosa*, *P. fluorescens*, *Acinetobacter baumannii*, and *Flavobacterium* sp.).

While the gram-positive rods were identified as members of the genus *Bacillus* spore-formers (*Bacillus cereus*, *B. megaterium*, *B. subtilis*, and *B. pumilus*) and *Lactobacillus* non-spore formers (*L. acidophilus*, and *L. casei*). For the gram-negative cocci, they were identified as catalase-positive *Staphylococci* (*S. aureus*) and catalase-negative *Micrococcus* sapecies (*M. roseus*, and *M. luteus*).

Gamma irradiation studies

Normal honey must lack pathogenic microorganisms or microorganisms that produce enteric illness [(30)].

In this investigation, the gamma irradiation decreases the number of aerobic bacteria at low dose levels. In which the lethal dose levels for the different honey samples range from 0.5 to 15kGy, as shown in Table (3). The dose level 0.5kGy was found to be the lethal dose for 10 honey samples, 3kGy for 1 honey sample, 5kGy for 2 honey samples, 7kGy for 7 honey samples, 10kGy for 4 honey samples and 15kGy for 1 honey sample.

Table (3): Detection of the lethal dose levels after exposure of the different types of honey samples to gamma radiation:

Honey type	Company code	Sample No.	Lethal dose (kGy)	No. of samples
Citrus	A	2	0.5	10
Citrus	B	7		
Red Korean ginseng	B	8		
Clover flower	B	10		
Royal jelly	C	12		
Black seed	D	16		
Citrus	D	18		
Mix. of Clover flower, Sweet marjoram, Anise, Sesame and Sunflower	E	21		
Commercial honey	E	22		
Commercial honey	E	24		
Citrus	E	20	3	1
Ginseng	C	11	5	2
Clover flower	D	19		
Sweet marjoram	A	1	7	7
Black seed	A	4		
Clover flower	A	5		
Sweet marjoram	B	6		
Black seed	B	9		
Extra	C	13		
Royal jelly	D	17		
Red Korean ginseng	A	3	10	4
Black seed	C	14		
Commercial honey	E	23		
Commercial honey	E	25		
Clover flower	C	15	15	1

Antimicrobial assay of non-irradiated and irradiated honey samples:

The different honeysamples vary in their potency of the antimicrobial activity. According to [26], the antibacterial activity was classified as: not sensitive (<8 mm); sensitive (8 to 14mm); very sensitive (15 to 19mm) and extremely sensitive (>20mm).

Table (4) shows that the antimicrobial activity of the honey samples against the pathogenic microorganisms decreased upon dilution. Thus, the undiluted non-irradiated honey (100% concentration) showed inhibition zones of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* sp., *Klebsiella pneumonia* and *Candida albicans* ranged from 29-41mm, 23-34mm, 10-33mm, 26-40mm, 20-38mm and 10-42mm, respectively. While, the 25% concentrations showed inhibition of growth ranged from 11-26mm, 12-25mm, 8-22mm, 15-27mm, 8-23mm and 11-28mm, respectively.

In the present study, it was found that the antimicrobial activity of the irradiated honey samples increased upon exposure to the different gamma radiation lethal dose levels (0.5, 3, 5, 7, 10 and 15kGy), as shown in tables (5-7).

Thus the undiluted irradiated honey (100% concentration) showed zones of inhibition of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* sp., *Klebsiella pneumonia* and *Candida albicans* ranged from 34-48mm, 11-39mm, 12-40mm, 30-44mm, 15-40mm and 15-49mm, respectively. While, the 25% concentrations showed zones of inhibition ranged from 21-32mm, 6-30mm, 18-30mm, 16-33mm, 19-30mm and 17-34mm, respectively.

Table (4): Antimicrobial activity of different concentrations of non-irradiated honey samples against certain pathogenic microorganisms:

M.O.	Range of inhibition zones (mm)			
	Non- irradiated			
	100%	**75%**	**50%**	**25%**
S. aureus	29-40	23-37	16-31	11-26
E.coli	23-34	18-31	15-27	12-25
P. aeruginosa	10-33	6-31	8-27	8-22
Enterobacter sp.	26-40	21-36	14-31	15-27
K. pneumonia	20-38	10-34	11-29	8-23
C. albicans	10-42	8-39	16-34	11-28

Table (5): Antimicrobial activity of different concentrations of honey samples irradiated at 0.5 and 3kGy against certain pathogenic microorganisms:

M.O.	Range of inhibition zones (mm) of irradiated honeys							
	0.5kGy				3kGy			
	100%	**75%**	**50%**	**25%**	**100%**	**75%**	**50%**	**25%**
S. aureus	36-45	30-42	30-38	26-32	39	32	28	25
E.coli	11-37	10-35	8-32	6-28	38	33	30	27
P. aeruginosa	12-39	10-35	21-33	18-30	39	34	30	28
Enterobacter sp.	35-44	31-39	31-36	27-33	40	38	32	30
K. pneumonia	26-38	24-32	21-30	19-28	29	26	24	22
C. albicans	15-48	12-40	20-36	17-32	R	R	R	R

Table (6): Antimicrobial activity of different concentrations of honey samples irradiated at 5 and 7kGy against certain pathogenic microorganisms:

M.O.	Range of inhibition zones (mm) of irradiated honeys							
	5kGy				7kGy			
	100%	**75%**	**50%**	**25%**	**100%**	**75%**	**50%**	**25%**
S. aureus	36-37	33-34	26-32	21-24	36-48	30-36	24-31	22-28
E.coli	32-34	29-30	25-28	22-24	27-34	24-31	22-28	18-27
P. aeruginosa	14-17	11-15	8-10	R	15-34	17-29	16-23	21-23
Enterobacter sp.	31-32	30	27-28	25-26	30-40	24-34	21-30	16-28
K. pneumonia	24	22	27-28	R	28-40	26-33	22-34	20-30
C. albicans	35-37	32-35	29-33	27-29	32-38	29-35	27-34	22-33

Table (7): Antimicrobial activity of different concentrations of honey samples irradiated at 10 and 15kGy against certain pathogenic microorganisms:

M.O.	Range of inhibition zones (mm) of irradiated honeys							
	10kGy				15kGy			
	100%	75%	50%	25%	100%	75%	50%	25%
S. aureus	34-48	28-44	26-40	25-30	44	41	36	31
E.coli	29-39	27-36	25-32	25-30	32	27	25	21
P. aeruginosa	17-34	14-30	13-21	12	38	33	30	20
Enterobacter sp.	30-44	28-40	26-36	25-33	38	35	32	29
K. pneumonia	15-24	12-22	12	R	R	R	R	R
C. albicans	38-46	36-42	32-40	25-34	49	41	38	31

Antimicrobial assay using antibiotic discs:

In this study, susceptibility of the pathogenic microorganisms was illustrated in Table (8). It was found that *Staphylococcus aureus* was the most susceptible bacteria, while *Pseudomonas aeruginosa*was the least one to most of the antibiotic discs used. While,*Candida albicans* was found to be resistant to Nystatin.

Also, it was shown that the antibiotic discs showed variable antimicrobial activity against the tested pathogenic microorganisms in the order of Ciprofloxacin> Amikacin> Tetracycline> Amoxicillin/Clavulinic acid> Tobramycin> Imipenem> Cefotaxime> Sulfamethoxazole/Trimethoprim.

The mean inhibition zones (in mm) of the non-irradiated honey samples (undiluted), when applied on the different pathogenic microorganisms, was higher than that given by any of the different antibiotic discs except for *Pseudomonas aeruginosa* with CIP and *Klebsiella pneumonia* with AMC and CIP.

While, the mean inhibition zones (in mm) of the irradiated honey samples (undiluted), when applied on the different pathogenic microorganisms, was also higher than that given by the different antibiotic discs except for *Pseudomonas aeruginosa* with CIP.

Table (8): Susceptibility of certain pathogenic microorganisms to some antibiotic discs:

Antibiotics / Bacteria	Inhibition zones (mm)								
	AMC	AK	CTX	IpM	CIP	SXT	TOB	TE	NS
Staphylococcus aureus	21(S)	18(S)	23(S)		23(S)	21(S)	11(R)	19(S)	
Escherichia coli	12(R)	20(S)	22(I)		22(S)	10(R)	18(S)	21(S)	
Pseudomonas aeruginosa	0(R)		18(I)	21(S)	36(S)	0(R)	22(S)	0(R)	
Enterobacter sp.	21(S)	18(S)	0(R)		22(S)	0(R)	11(R)	27(S)	
Klebsiella pneumonia	27(S)	25(S)	21(I)		30(S)	25(S)	14(I)	24(S)	
Candida albicans									0(R)

(S): sensitive (I): Intermediate (R): Resistant

Statistical analysis:

Statistical analysis of the antimicrobial activity of the non-irradiated and irradiated honey samples:

The statistical analysis of the data obtained from the T-test for the non-irradiated honey samples show that the differences between the various honey samples were statistically significant ($p<0.05$) against all the pathogenic microorganisms. Also, the differences between the undiluted honey samples irradiated at 0.5 and 7kGy were statistically significant ($p<0.0.5$) against the pathogenic microorganisms.

For the undiluted honey samples irradiated at 5kGy, the differences were statistically significant against *S. aureus*, *E. coli* and *C. albicans* ($p<0.05$). While the undiluted honey samples irradiated at 10.0kGy, the differences were statistically significant against *E. coli*, *P. aeruginosa* and *K. pneumoniae* ($p<0.05$).

Statistical analysis of the antimicrobial activity between the honey samples before and after exposure to gamma radiation:

The results of the T-test show that the differences between the undiluted honey samples irradiated at 0.5kGy and 7kGy were statistically significant against all the pathogenic microorganisms tested ($P<0.05$).

For the undiluted honey samples irradiated at 5kGy, the differences between the honey samples were statistically significant against *S. aureus*, *E. coli* and *C. albicans*. While those undiluted honey samples irradiated at 10kGy, the differences were statistically significant against *E. coli*, *P. aeruginosa* and *K. pneumoniae* ($p<0.05$).

Discussion:

The contamination of honey with fungi or bacteria indicates inadequate hygiene conditions during collection, manipulation, processing and storage [(11)]. It wasdetected that the satisfactory aerobic colony count for honey should not exceed ($\leq 10^3$cfu/g) [(7)].

In this investigation, the data show that the tested honey samples were contaminated with bacteria only and that the bacterial count was within the satisfactory limit rangedfrom $5.0X10^1$cfu/ml (red Korean ginseng honey 15-C) to$4.6X10^2$cfu/ml (commercial honey 22-E).

In consistence with this study, many scientists indicate that the total aerobic viable count in honey can vary from zero to tens of thousands per gram [(13)] and [(17)].

A total of 70 honey samples obtained from different parts of Argentina were tested. It was found that the total viable count of bacteria did not exceed $1.0X10^3$cfu/g in any sample[(13)]. Also, it was found that viable count of bacteria of tested Moroccan honey samples varied between $1.0X10^1$ and $1.0X10^2$cfu/g[(22)].

While,[(28)] found that the mean value of aerobic bacteria in the tested Nigerian honey samples was above the limit of detection, ranged from $1.0X10^3$ to $5.0X10^3$cfu/g.Also, [(32)] stated that the mean of total number of aerobic bacteria of 109 uni-floral and multi-foral honey samples purchased from Polish apiaries varied from $1.9X10^1$ to $4.6X10^3$cfu/g depending on the type of honey.

In the present study, the microscopical examination of the 96 bacterial isolates from the various honey samples in this study show that they were either gram-negative rods (74%), gram-positive rods (14.5%) or gram-positive cocci (11.5%).

These results were consistent with the study of [16] who found that the four bacterial isolates recovered from six different honey samples produced by three native bee species in Northern Thailand were either, gram-negative or gram-variable, short rod bacteria, which is a typical character of acetic acid bacteria.

The results of the present study were also consistent with those obtained by [22] who examined honey samples from Morocco and detected the presence of *Bacillus* to have the highest occurrence. The species of *Bacillus* were identified as *B. cereus*, *B. megaterium*, *B. polymyxa*, *B. lichenformis*, and *B. firmus*.

Four species of bacteria from honey samples marketed in six states in Southwestern Nigeria were detected. These bacterial species were found to be *Klebsiella edwardsii*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*[2].

The gamma irradiation studies in this study show that the lethal doses for the bacterial contamination among the different honey samples tested ranged from 0.5 to 15kGy.

It was reported that the risk analysis recommended for the management of the risk of contamination in honey, each consignment of imported honey must be gamma irradiated with 15kGy [37].

According to [23], the irradiation process decreases in honeys the number of aerobic bacteria and fungi (yeasts and moulds) as well as the number of anaerobic spores of *Clostridium* by 98.1% at radiation dose of 10kGy.

The results of the present study are also consistent with [33]who detected that a dose level 15kGy of gamma radiation was sufficient for complete decontamination of honey including spores, thus improving its microbial safety without affecting the quality attributes.

The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature (pH being 3.2-4.5), endogenous hydrogen peroxide content [12]; inhibine, which acts as an antibacterial factor other than H_2O_2 [26] and its phytochemical nature [15].

The results of the antimicrobial sensitivity testing of the honey samples using the agar well-diffusion method clearly show that both the undiluted non-irradiated and irradiated honey samples have the potential to be used as antimicrobial agent to prevent and control infections with *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* sp., *Klebsiella pneumonia* and *Candida albicans*.

The antibacterial activity of four concentrations (10, 5, 2.5 and 1%w/v) of 13 honeys was compared using the standard well-diffusion method. The results showed that all the tested honeys had an inhibitory effect on the growth of *E. coli* and *P. aeruginosa*[36].

Besides, different Nigerian honey samples were examinedagainst *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*. The tested pathogens showed zone diameter inhibition with an average (5.3-22.6mm), (1.4-15.4mm), (4.4-13.5mm) and (9.1-17mm), respectively, and with honey concentrations of 80%-100%[3].

In consistent with the present study [26] and [27]found that the diameter of zone of the inhibition of four undiluted Algerian honey types ranged from 36-46mm for *S.*

aureus, 31-38mm for *E. coli*,26-30mm for *P. aeruginosa*, and 39-44mm for *Streptococcus pyogenes*.

Several authors reported antifungal efficacy of various honeys against clinical strains of *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, *Candida tropicalis* and *Candida kefyr* [12] and [19].

Also, [25]demonstrated that the range of the diameter zone of inhibition of the tested honeys was 7-23mm for *Rhodotorula* sp., while *Candida albicans* showed clearly resistance towards all the concentrations used (undiluted, 70%, 50%, 30% and 10%).

From the point of view of gamma irradiation, in agreement with our results, several investigators showed that there was no significant loss of antibacterial activity when the honey samples were gamma-irradiated up to 50kGy [34].

It was also reported that the gamma irradiation did not impair the antimicrobial properties of the honeys in the doses (5, 15 and 25kGy) against the clinical isolated microorganisms (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) but on the other hand, they did not differ significantly from both types (irradiated and non-irradiated) of honey against the control strains of the same microorganisms[14].

The presented results of the antimicrobial susceptibility testing of the tested pathogens against the antibiotic discs showed different responses to the used discs differ from susceptible, intermediate to resistant.

The inhibition zones of *Staphylococcus aureus* range from11-23mm, *Escherichia coli* range from 10-21mm, *Pseudomonas aeruginosa* range from 18-36mm, *Enterobacter sp.* range from11-27mm and for *Klebsiella pneumonia* range from14-30mm. while, *Candida albicans* showed resistance to Nystatin.

It was also found that the honey sample No. 23, code E (Mix. honey) showed the highest antimicrobial activity and the honey sample No. 2, code A (Citrus honey) showed the lowest antimicrobial activity against most of the tested pathogenic microorganisms. While, the other honey samples showed variable antimicrobial activity.

Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source [21] and [26].

In consistence with the present study, honey had a more inhibitory effect on gram-negative bacteria than Amoxicillin and Ceftriazone [1].

The inhibitory effect of honey on the growth of isolated gram-negative bacteria was evident, as the mean inhibition zone of honey was significantly higher than that of Amoxicillin/Clavulinic acid and ceftriaxone, but there was no significant increase when compared with Amikacin and Ciprofloxacin and it was similar to that of Imipenem [15].

Besides, itwas proved that the antibacterial potentials of honey, especially at 40%v/v on the gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*) isolated from infected wounds were better than Amoxicillin, Streptomycin, Chloramphenicol, Ceftriazone and Erythromycin[6].

In the present study, differences regarding inhibition of the tested pathogenic microorganisms were observed for both non-irradiated and irradiated honey samples.

The non-irradiated undiluted honey samples, along with honey samples irradiated at 0.5 and 7.0kGy exhibiting the largest inhibition to all strain. Those differences are statistically significant ($p<0.05$).

The undiluted honey samples irradiated at 5.0kGy showed the largest inhibition only to *S. aureus*, *E. coli* and *C. albicans* ($p<0.05$). While, the undiluted honey samples irradiated at 10.0kGy showed the largest inhibition only to *E. coli*, *P. aeruginosa* and *K. pneumonia* ($p<0.05$).

Conclusion:

Honey is a famous rediscovered remedy which is cheap and non-toxic. It showed high inhibitory effect on the growth of pathogenic gram-negative, gram-positive bacteria and fungi. Gamma irradiation not only imparts sterility to honey, but it also increases its antimicrobial activity. Thus, this study suggests that radiation treatment at different radiation dose levels is not only useful in sterilizing the honey but also in enhancing the antimicrobial activity of honey.

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الملخص العربي

تقييم النشاط ضد الميكروبي لأنواع معينة من عسل النحل المصري المشعع و غير المشعع

زينب الدمرداش البزة- سلوي سليم عفيفي- سحر محمد رمزي السيد –
دينا عبد العليم سليمان أبوالمجد

لقد زادت قيمة إستخدام العسل علي نحو متزايد لنشاطها المضاد للبكتيريا و ذلك نتيجة لإنتشار البكتيريا المقاومة للمضادات الحيوية. في هذا البحث تم دراسة أهمية النشاط الضد ميكروبي لعسل النحل المصري و علي ذلك فإنه قد تم دراسة 25 عينة عسل نحل من أنواع و شركات مختلفة تم تجميعها من مناطق متعددة و قد تم تعيين الأعداد الميكروبية الهوائية بإستخدام طريقة عد المستعمرات البكتيرية داخل الأطباق، كما تم تعريفها بإستخدام صبغة الجرام، الإختبارات البيوكيميائية، و الجيل الثالث من جهاز النعريف المصغر الشبه آلي. و قد تم دراسة النشاط ضد الميكروبي لعينات من العسل الشععة عند جرعات تشعيعية مختلفة تتراوح من 0.5 إلي 10 كيلوجراي وذلك عند التركيزات (25، 50، 75، 100%) ضد بعض الميكروبات الممرضة (المكورات العنقودية الذهبية، الزائفة الزنجارية، الأمعائية، الكلبسيلة الإلتهاب الرئوي، المبيضات البيض) المعزونلة من عينات طبية. كما تم دراسة النشاط الضد ميكروبي لأنواع مختلفة من أقراص المضادات الحيوية وهم (أموكسسيللين/كلافيولينك أسيد، أميكاسين، سيفوتاكسيم، سيبروفلوكساسين، سالفاميثوكسازول/ترايميثوبريم، توبراميسين، نستاتين) ضد نفس أنواع الميكروبات الممرضة التي تم إستخدامها مع العسل. و قد أوضحت الدراسة أن العسل المشعع الغير مخفف (100%) قد أعطي أعلي نشاط ضد ميكروبي.