

Egyptian Bee (*Apis Mellifera*) Propolis: A Promising Antibacterial Agent for Combating Antibiotic Resistance and Biofilm Formation of Multidrug-Resistant *Staphylococcus aureus*

Fatma Alzahraa Hareidy¹, Ahmed F. Azmy², Nesreen Mostafa Kamel³, Abeer Sayed Moawd⁴, Maha Eid Omran¹

¹Department of Microbiology and Immunology, Faculty of Pharmacy for girls, Al-Alzhar University, Cairo, Egypt

²Department of Microbiology and Immunology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt

³Department of Clinical and Chemical pathology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

⁴Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt

* Corresponding author: Fatma Alzahraa Hareidy, Email: FatmaAl-zahraaRabee.52@azhar.edu.eg

Email: fatmaalzahraamicro@yahoo.com, Tel: +2- 01067687508, +2- 01005726009

Table (S1): Mean values of inhibition zones diameters $\pm$ SD in disc diffusion method using MHA plates with and without PEE against *S. aureus* standard strains and four clinical isolates.

Antibiotic	Mean value in mm without PEE	Mean value in mm for UPE-PEE & synergism%		Mean value in mm for WN-PEE & Synergism%	
Cefoxitin	21.5 $\pm$ 7.49	24.66 $\pm$ 7.43	14.5%	30 $\pm$ 7.92	39.5%
Ciprofloxacin	29 $\pm$ 1.67	38.5 $\pm$ 3.49	32.5%	32 $\pm$ 13.57	10.5%
Amikacin	22.2 $\pm$ 3.57	35 $\pm$ 5.85	56.3%	34.83 $\pm$ 8.23	57%
Clindamycin	23.75 $\pm$ 6.96	33.2 $\pm$ 9.63	39.5%	27.8 $\pm$ 9.71	17%
Gentamycin	18.33 $\pm$ 7.78	23 $\pm$ 7.13	25.4%	22 $\pm$ 7.88	20%
Penicillin G	21.25 $\pm$ 7.54	32.5 $\pm$ 7.86	53%	28.75 $\pm$ 6.04	35%
Ampicillin	21.5 $\pm$ 88	32.75 $\pm$ 7.49	52.5%	28.75 $\pm$ 6.7	33.5%
Linezolid	36 $\pm$ 1.92	43.66 $\pm$ 4.91	21%	39.83 $\pm$ 2.64	10.5%

* UP-PEE*: Upper-Egypt propolis ethanolic extract

*WN-PEE**: West-Nile propolis ethanolic extract

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Table (S2): Classification of *S. aureus* isolates according to ability to form biofilm into strong and moderate biofilm producers

Isolate	OD _{Av} ± SD	OD _c	OD _T	Biofilm production Capability
<i>S. aureus</i> 65	0.645 ± 0.07	0.143	0.502	moderate
<i>S. aureus</i> 11	0.626 ± 0.09	0.143	0.483	moderate
<i>S. aureus</i> 15	0.697 ± 0.105	0.143	0.554	moderate
<i>S. aureus</i> 90	1.406 ± 0.25	0.143	1.263	strong
<i>S. aureus</i> 17	0.853 ± 0.072	0.143	0.710	strong
<i>S. aureus</i> 31	0.822 ± 0.094	0.143	0.679	strong
<i>S. aureus</i> c10	0.977 ± 0.23	0.143	0.834	strong
MRSA ATCC 4300	0.875 ± 0.14	0.143	0.732	strong
MSSA ATCC 25923	1.008 ± 0.33	0.143	0.865	strong

*OD_{Av} = optical density, OD_c= optical density of control. OD_T: optical density of tested isolate (all measured at 590 nm for formed biofilms).

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Table (S3): Comparison of Propolis extracts ability to reduce biofilm formation in *S. aureus* at relevant MIC.

M.O	UPE-PEE % Biofilm Formation Inhibition at MIC		Control of untreated isolate		WN-PEE % of Biofilm Formation Inhibition at MIC	
	%	OD± SD of treated test	OD±SD of untreated control		%	OD± SD of treated test
MSSA ATCC 25923	15 %	0.853 ± 0.1538	1.008667± 0.331197		29%	0.719 ±0.1896
MRSA ATCC 43300	25%	0.654 ± 0.0782	0.875167± 0.148269		32%	0.595± 0.1620
S31.O	15%	0.701± 0.2411	0.822667± 0.094534		39%	0.501±0.088
S11.O	13.5%	0.541 ± 0.096	0.626333± 0.09237		32.5%	0.423 ± 0.071
S65.O	14.5%	0.551 ± 0.0426	0.645333± 0.07098		28%	0.463 ± 0.0617
S90.O	27%	1.029± 0.2623	1.406833± 0.258965		25%	1.054 ± 0.2623
S17.O	24%	0.649 ± 0.1908	0.8535± 0.072122		44%	0.477 ± 0.0732
SC10.	33%	0.654 ± 0.2287	0.977167± 0.23203		43.5%	0.552 ± 0.0194
S15.O	25%	0.525 ± 0.0054	0.697667± 0.105858		49.5%	0.352 ± 0.0617

*OD=optical density measured at 590 nm for inhibited biofilms

* UP-PEE*: Upper-Egypt propolis ethanolic extract

*WN-PEE**: West-Nile propolis ethanolic extract

Table (S4): Percentage of biofilm eradication using different concentrations of PEE samples.

M.O	UPE-PEE % of Preformed Biofilm eradication		WN-PEE % of Preformed Biofilm eradication	
	¼ MIC	1/8 MIC	¼ MIC	1/8 MIC
MSSA ATCC 25923	97.47%	66.43%	91.94%	88.44%
MRSA ATCC 43300	94.42%	87.31%	91.45%	93.53%
S31.O	81.8%	86.3%	55.66%	63.41%
S65.O	83.21%	76.27%	80.20%	82.17%
S90.O	68.75%	12.72%	61.07%	64.87%
S17.O	58.43%	60.49%	31.27%	24.48%
S11.O	38.37%	18.91%	1.49%	NL
S.C10.	49.54%	28.37%	2.49%	NL
S15.O	35.71%	25.5%	2.5%	NL

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Table (S5): Average OD±SD values with standard deviation at different PEE concentrations.

M.O	UPE-PEE OD ±SD Preformed Biofilm Inhibition				Un-treated Control Isolate control	WN-PEE OD ±SD Preformed Biofilm Inhibition			
	¼ MIC		1/8 MIC			¼ MIC		1/8 MIC	
MSSA ATCC 25923	0.1133	±0.001	1.5043	±0.81	4.481	0.361	±0.225	0.518	±0.214
MRSA ATCC 43300	0.139	±0.008	0.3166	±0.07	2.492	0.2136	±0.019	0.161	±0.017
S31.O	0.13	±0.012	0.1366	±0.007	0.954±0.0032	0.4233	±0.025	0.349	±0.030
S65.O	0.1453	±0.02	0.2053	±0.023	0.864±0.0087	0.1713	±0.002	0.154	±0.013
S90.O	0.1406	±0.002	0.391	±0.147	0.448±0.0075	0.195	±0.019	0.176	±0.003
S17.O	0.202	±0.095	0.202	±0.095	0.486±0.012	0.334	±0.009	0.367	±0.036
S11.O	0.1143	±0.002	0.1503	±0.026	0.185±0.0064	0.213	±0.022	0.249	±0.025
SC10.	0.1123	±0.009	0.159	±0.008	0.222±0.0035	0.2676	±0.023	0.322	±0.028
S15.O	0.126	±0.015	0.146	±0.005	0.196±0.0053	0.1913	±0.006	0.263	±0.047

*OD=optical density measured at 590 nm for preformed biofilms.

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Table (S6): Effect of different PEE concentrations on adherence of *S. aureus* standard strains MRSA ATCC 4300 and MSSA ATCC 25923 as well as clinical isolates calculated as a percentage of adherence inhibition

M.O	UPE-PEE % Adherence Inhibition			WN-PEE % of Adherence Inhibition		
	MIC	1/4 MIC	1/8 MIC	MIC	1/4 MIC	1/8 MIC
MSSA ATCC 25923	63.52%	53.15%	46.01%	63.64%	53.26%	46.07%
MRSA ATCC 43300	56.86%	49.87%	38.89%	56.90%	49.74%	38.52%
S31.O	52.24%	45.94%	42.57%	52.133%	45.93%	42.52%
S65.O	46.33%	44.45%	41.62%	46.27%	44.24%	44.49%
S90.O	55.04%	54.12%	43.56%	55%	54.07%	43.45%
S17.O	89.80%	66.47%	53.59%	89.63%	66.27%	53.55%
SC10.	73.5%	18.04%	16.86%	73.5%	18.12%	16.80%
S15.O	56.50%	52.39%	44.32%	56.53%	52.44%	44.36%

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Table (S7): Relevant OD $\pm$ SD values of *S. aureus* standard strains ATCC 4300 and ATCC 25923 as well as clinical isolate adherence inhibition after treatment with different propolis ethanolic extract concentrations compared to untreated negative control of isolates.

M.O	Propolis concentrations	Control OD $\pm$ SD	UP-PEE OD $\pm$ SD		WN-PEE OD $\pm$ SD	
MSSA ATCC 25923	MIC		0.600	± 0.031	0.597	± 0.032
	1/4 MIC	1.045 $\pm$ 0.0785	0.921	± 0.133	0.917	± 0.132
	1/8 MIC		1.226	± 0.063	1.223	± 0.062
MRSA ATCC 43300	MIC		0.449	± 0.011	0.446	± 0.011
	1/4 MIC	0.590 $\pm$ 0.0943	0.595	± 0.058	0.595	± 0.058
	1/8 MIC		0.93	± 0.075	0.94	± 0.080
S31.O	MIC		0.414	0 $\pm$.0138	0.532	± 0.007
	1/4 MIC	0.453 $\pm$ 0.0156	0.533	± 0.007	0.415	± 0.014
	1/8 MIC		0.611	± 0.084	0.611	± 0.085
S65.O	MIC		0.469	± 0.075	0.469	0 $\pm$.0181
	1/4 MIC	0.405 $\pm$ 0.0834	0.506	± 0.052	0.509	± 0.055
	1/8 MIC		0.504	± 0.002	0.504	± 0.003
S90.O	MIC		0.343	± 0.025	0.342	± 0.028
	1/4 MIC	0.436 $\pm$ 0.115	0.356	± 0.028	0.355667	± 0.028
	1/8 MIC		0.544	± 0.0712	0.544	± 0.070
S17.O	MIC		0.69	± 0.016	0.68	± 0.0174
	1/4 MIC	1.356 $\pm$ 0.0682	0.154333	± 0.030	0.155	± 0.031
	1/8 MIC		1.177333	± 0.095	1.178	± 0.096
SC10.	MIC		0.30	± 0.020	0.299	± 0.021
	1/4 MIC	0.832 $\pm$ 0.147	0.377667	± 0.067	0.4116	± 0.031
	1/8 MIC		0.409333	± 0.032	0.3756	± 0.066
S15.O	MIC		0.742	± 0.065	0.741	± 0.066
	1/4 MIC	0.964 $\pm$ 0.0877	0.876	± 0.054	0.923	± 0.087
	1/8 MIC		1.211	± 0.101	1.209	± 0.104
S11.O	MIC		0.582	± 0.065	0.581333	± 0.067
	1/4 MIC	0.763 $\pm$ 0.0877	0.656	± 0.054	0.923	± 0.055
	1/8 MIC		1.011	± 0.1013	1.009	± 0.1081

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