



Research article

Antimicrobial activity of *Ziziphus mauritiana* and *Acacia nilotica* against multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae*

Abdul Rafay Rafiq^{1,2,*} and Areeba Ameen³

¹ Youth Affairs and Sports Department, Government of Punjab, Lahore, Pakistan

² Cell biology laboratory, Department of physiology, Government College University Faisalabad, Faisalabad, Pakistan

³ Department of bioinformatics and biotechnology, Government College University Faisalabad, Faisalabad, Pakistan

* **Correspondence:** Email: arafayrfq@gmail.com; Tel: +923277273464.

Abstract: Multidrug-resistant (MDR) strains such as *Escherichia coli* and *Klebsiella pneumoniae*, resistant to broad-spectrum antibiotics, are a modern global issue. Plant extracts offer promising antimicrobial activity for such pathogens. This study evaluated the efficacy of aqueous and hydro-ethanolic extracts from leaves and bark of two cheaply and easily available plants, i.e., *Ziziphus mauritiana* (ber) and *Acacia nilotica* (gum Arabic or Kikar), against MDR *E. coli* and *K. pneumoniae*. Antibiotic susceptibility testing confirmed resistance in both strains to all tested antibiotics (ampicillin, ciprofloxacin, streptomycin, and tetracycline), with inhibition zones ≤ 10 mm. Antimicrobial assays revealed that hydro-ethanolic extracts of *Z. mauritiana* leaves exhibited the highest activity, with inhibition zones of 20 mm (*E. coli*) and 19 mm (*K. pneumoniae*) at 100 mg/mL, outperforming aqueous extracts. The minimum inhibitory concentration (MIC) for all the tested extracts was notably low (6 mg/mL for *E. coli* and *K. pneumoniae*). Growth curve analysis demonstrated significant suppression of bacterial proliferation by all extracts at MIC, with optical density (OD₆₀₀) values remaining below 0.25 over 24 h compared to controls (OD₆₀₀ ≥ 2.20). Additionally, biofilm formation was completely inhibited at MIC as well as sub-inhibitory concentrations (sub-MIC) of $\frac{1}{2}$ MIC, highlighting that the antibiofilm activity of these extracts is not due to antimicrobial activity. Findings highlight the efficacy of extracts from different parts and solvents against MDR pathogens, suggesting their potential as an adjunct therapy. Findings suggest that hydro-ethanolic extracts perform significantly better than aqueous extracts, while no significant difference in antimicrobial activity was found in extracts from different parts of plants. Lastly, it was found that *Z. mauritiana* extracts have greater antimicrobial

potential than *A. nilotica* extracts.

Keywords: antimicrobial resistance; antibiofilm activity; *Klebsiella*; *Escherichia*; plant extract

Appendix

Table S1. Dilutions of stock solution (100 mg/mL) of each plant extract.

Target Conc. (mg/mL)	Volume of Stock (100 mg/mL) (μL)	Volume of TSB (μL)	Total Volume (μL)
12	120	880	1000
10	100	900	1000
8	80	920	1000
6	60	940	1000
4	40	960	1000

Table S2. Antimicrobial activity of *Acacia nilotica* and *Ziziphus mauritiana* extracts (leaves and bark) at different concentrations against *Escherichia coli*. Data represents mean optical density (OD₆₀₀) ± standard deviation, alongside T-statistic and P-values indicating significant differences from controls.

Plant	Part	Concentration (mg/mL)	Mean optical density (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	12	0.01 ± 0.001	−108.553	3.19	≤0.001
		10	0.01 ± 0.001	−111.068	3.92	≤0.001
		8	0.01 ± 0.001	−108.553	2.29	≤0.001
		6	0.01 ± 0.001	−111.068	2.73	≤0.001
		4	0.3 ± 0.004	−36.4969	2.37	≤0.001
	bark	12	0 ± 0.001	−110.669	3.75	≤0.001
		10	0 ± 0.001	−113.233	3.03	≤0.001
		8	0.01 ± 0.002	−104.716	2.08	≤0.001
		6	0.05 ± 0.002	−96.5507	2.94	≤0.001
		4	0.4 ± 0.003	−22.7104	3.52	≤0.001
<i>Ziziphus mauritiana</i>	leaves	12	0.01 ± 0.002	−104.716	2.87	≤0.001
		10	0.01 ± 0.001	−111.068	2.61	≤0.001
		8	0.02 ± 0.003	−92.8725	3.42	≤0.001
		6	0.03 ± 0.003	−91.0262	3.15	≤0.001
		4	0.4 ± 0.002	−23.9691	3.09	≤0.001
	bark	12	0.01 ± 0.002	−99.9688	2.45	≤0.001
		10	0.01 ± 0.001	−111.068	3.58	≤0.001
		8	0.01 ± 0.002	−99.9688	3.98	≤0.001
		6	0.05 ± 0.003	−87.3334	3.66	≤0.001
		4	0.3 ± 0.003	−38.8193	2.69	≤0.001

Continued on next page

Plant	Part	Concentration (mg/mL)	Mean optical density (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
Growth/positive Bacteria)	control	(TSB +	0.52 ± 0.005			
Solvent control Bacteria)	(TSB + DMSO<1% +		0.50 ± 0.008			
Sterility/negative Control	(TSB only)		0.02 ± 0.001			

Table S3. Antimicrobial activity of *Acacia nilotica* and *Ziziphus mauritiana* extracts (leaves and bark) at different concentrations against *Klebsiella pneumoniae*. Data represents mean optical density (OD₆₀₀) ± standard deviation, alongside T-statistic and P-values indicating significant differences from controls.

Plant	Part	Concentration (mg/mL)	Mean optical density (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	12	0.01 ± 0.002	−27.3454	3.12	≤0.001
		10	0.02 ± 0.001	−26.9228	3.92	≤0.001
		8	0.02 ± 0	−26.935	2.29	≤0.001
		6	0.01 ± 0.001	−27.4445	2.73	≤0.001
		4	0.2 ± 0.003	−17.3433	2.37	≤0.001
	bark	12	0 ± 0.001	−27.9663	3.71	≤0.001
		10	0.01 ± 0.002	−27.3454	3.03	≤0.001
		8	0.01 ± 0	−27.457	2.08	≤0.001
		6	0.01 ± 0.002	−27.3454	2.94	≤0.001
		4	0.4 ± 0.004	−6.9462	3.52	≤0.01
<i>Ziziphus mauritiana</i>	leaves	12	0.02 ± 0.001	−26.9228	2.87	≤0.001
		10	0.01 ± 0.001	−27.4445	2.61	≤0.001
		8	0.01 ± 0	−27.457	3.42	≤0.001
		6	0.01 ± 0.001	−27.4445	3.15	≤0.001
		4	0.4 ± 0.003	−6.98585	3.09	≤0.001
	bark	12	0.01 ± 0.002	−27.3454	2.45	≤0.001
		10	0.01 ± 0.002	−27.3454	3.58	≤0.001
		8	0.01 ± 0	−27.457	3.98	≤0.001
		6	0.01 ± 0.002	−27.3454	3.66	≤0.001
		4	0.5 ± 0.002	−1.86568	2.69	0.199
Growth/positive Bacteria)	control	(TSB +	0.52 ± 0.005			
Solvent control Bacteria)	(TSB + DMSO<1% +		0.50 ± 0.008			
Sterility/negative Control	(TSB only)		0.02 ± 0.001			

Table S4. Biofilm inhibition by *Acacia nilotica* and *Ziziphus mauritiana* extracts against *Escherichia coli*. Values represent mean biofilm absorbance (OD₅₇₀) ± standard deviation, with corresponding T-statistics and P-values.

Plant	Part	Concentration	Mean optical density (OD ₅₇₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	MIC	0.01 ± 0.007	−57.1309	3.35	≤0.001
		½MIC	0.1 ± 0.001	−56.0031	3.22	≤0.001
		¼ MIC	2.53 ± 0.023	−56.4638	3.95	≤0.001
	bark	MIC	0.01 ± 0.0012	−57.2189	3.72	≤0.001
		½MIC	0.002 ± 0.001	−56.7441	3.73	≤0.001
		¼ MIC	2.52 ± 0.023	−56.1504	3.01	≤0.001
<i>Ziziphus mauritiana</i>	leaves	MIC	0.02 ± 0.003	−56.9775	2.88	≤0.001
		½MIC	0.03 ± 0.001	−56.0176	2.89	≤0.001
		¼ MIC	2.59 ± 0.024	−56.114	2.63	≤0.001
	bark	MIC	0.02 ± 0.0013	−56.9922	2.59	≤0.001
		½MIC	0.03 ± 0.001	−56.8596	2.47	≤0.001
		¼ MIC	2.56 ± 0.017	−56.1996	3.56	≤0.001
Growth/positive control (LB + Bacteria)			2.53 ± 0.044			
Sterility/negative Control (LB only)			0.008 ± 0.006			

Table S5. Biofilm inhibition by *Acacia nilotica* and *Ziziphus mauritiana* extracts against *Klebsiella pneumoniae*. Values represent mean biofilm absorbance (OD₅₇₀) ± standard deviation, with corresponding T-statistics and P-values.

Plant	Part	Concentration	Mean optical density (OD ₅₇₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	MIC	0.02 ± 0.0016	−41.6348	3.11	≤0.001
		½MIC	0.01 ± 0.001	−41.8345	3.25	≤0.001
		¼ MIC	3.28 ± 0.004	−41.7295	3.88	≤0.001
	bark	MIC	0.02 ± 0.0015	−41.6419	3.68	≤0.001
		½MIC	0.02 ± 0.001	−41.7346	3.7	≤0.001
		¼ MIC	3.31 ± 0.004	−41.8234	3.01	≤0.001
<i>Ziziphus mauritiana</i>	leaves	MIC	0.01 ± 0.001	−41.7657	2.85	≤0.001
		½MIC	0.03 ± 0.001	−41.7469	2.98	≤0.001
		¼ MIC	3.24±0.004	−41.7656	2.65	≤0.001
	bark	MIC	0.01 ± 0.0003	−41.7586	2.24	≤0.001
		½MIC	0.03 ± 0.001	−41.8545	2.55	≤0.001
		¼ MIC	3.22 ± 0.003	−41.6956	3.45	≤0.001
Growth/positive control (LB + Bacteria)			3.2 ± 0.76			
Sterility/negative Control (LB only)			0.014 ± 0.007			

Table S6. Growth kinetics of *Escherichia coli* under treatment with leaves and bark extracts of *Acacia nilotica* and *Ziziphus mauritiana* compared to LB broth controls over 24 hours. Values represent optical density (OD₆₀₀) measurements at each time point, demonstrating the inhibitory effects of the extracts on bacterial growth.

Time (Hours)	Plants				
	Control LB (OD ₆₀₀)	<i>Acacia nilotica</i> Leaves (OD ₆₀₀)	Bark (OD ₆₀₀)	<i>Ziziphus mauritiana</i> Leaves (OD ₆₀₀)	Bark (OD ₆₀₀)
0	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001
1	0.08 ± 0.001	0.06 ± 0.001	0.07 ± 0.001	0.06 ± 0.001	0.07 ± 0.001
2	0.15 ± 0.002	0.07 ± 0.001	0.09 ± 0.001	0.08 ± 0.001	0.08 ± 0.001
3	0.3 ± 0.003	0.09 ± 0.001	0.11 ± 0.001	0.1 ± 0.001	0.1 ± 0.001
4	0.6 ± 0.007	0.12 ± 0.001	0.13 ± 0.002	0.14 ± 0.002	0.12 ± 0.001
5	1 ± 0.012	0.15 ± 0.002	0.15 ± 0.002	0.17 ± 0.002	0.14 ± 0.002
6	1.5 ± 0.017	0.18 ± 0.002	0.17 ± 0.002	0.2 ± 0.002	0.16 ± 0.002
7	1.8 ± 0.021	0.2 ± 0.002	0.18 ± 0.002	0.22 ± 0.002	0.17 ± 0.002
8	2 ± 0.023	0.21 ± 0.002	0.19 ± 0.002	0.23 ± 0.003	0.18 ± 0.002
9	2.1 ± 0.024	0.22 ± 0.002	0.2 ± 0.002	0.24 ± 0.003	0.19 ± 0.002
10	2.15 ± 0.025	0.22 ± 0.002	0.2 ± 0.002	0.24 ± 0.003	0.19 ± 0.002
11	2.2 ± 0.025	0.22 ± 0.002	0.2 ± 0.002	0.24 ± 0.003	0.19 ± 0.002
12	2.2 ± 0.023	0.22 ± 0.002	0.2 ± 0.002	0.24 ± 0.003	0.19 ± 0.002
13	2.2 ± 0.023	0.22 ± 0.002	0.2 ± 0.002	0.24 ± 0.003	0.19 ± 0.002
14	2.2 ± 0.023	0.22 ± 0.002	0.2 ± 0.002	0.24 ± 0.003	0.19 ± 0.002
15	2.15 ± 0.025	0.21 ± 0.002	0.19 ± 0.002	0.23 ± 0.003	0.18 ± 0.002
16	2.1 ± 0.024	0.2 ± 0.002	0.18 ± 0.002	0.22 ± 0.002	0.17 ± 0.002
17	2.05 ± 0.024	0.19 ± 0.002	0.17 ± 0.002	0.21 ± 0.002	0.16 ± 0.002
18	2 ± 0.023	0.18 ± 0.002	0.16 ± 0.002	0.2 ± 0.002	0.15 ± 0.002
19	1.95 ± 0.023	0.17 ± 0.002	0.15 ± 0.002	0.19 ± 0.002	0.14 ± 0.002
20	1.9 ± 0.022	0.16 ± 0.002	0.14 ± 0.002	0.18 ± 0.002	0.13 ± 0.002
21	1.85 ± 0.021	0.15 ± 0.002	0.13 ± 0.002	0.17 ± 0.002	0.12 ± 0.001
22	1.8 ± 0.021	0.14 ± 0.002	0.12 ± 0.001	0.16 ± 0.002	0.11 ± 0.001
23	1.75 ± 0.02	0.13 ± 0.002	0.11 ± 0.001	0.15 ± 0.002	0.1 ± 0.001
24	1.7 ± 0.02	0.12 ± 0.001	0.1 ± 0.001	0.14 ± 0.002	0.09 ± 0.001

Table S7. Growth kinetics of *Klebsiella pneumoniae* under treatment with leaves and bark extracts of *Acacia nilotica* and *Ziziphus mauritiana* compared to LB broth controls over 24 hours. Values represent optical density (OD₆₀₀) measurements at each time point, demonstrating the inhibitory effects of the extracts on bacterial growth.

Time (Hours)	Plants				
	Control LB (OD ₆₀₀)	<i>Acacia nilotica</i> Leaves (OD ₆₀₀)	Bark (OD ₆₀₀)	<i>Ziziphus mauritiana</i> Leaves (OD ₆₀₀)	Bark (OD ₆₀₀)
0	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001
1	0.09 ± 0.001	0.06 ± 0.001	0.06 ± 0.001	0.07 ± 0.001	0.07 ± 0.001
2	0.18 ± 0.002	0.08 ± 0.001	0.08 ± 0.001	0.09 ± 0.001	0.09 ± 0.001
3	0.35 ± 0.003	0.1 ± 0.001	0.1 ± 0.001	0.11 ± 0.001	0.11 ± 0.001
4	0.7 ± 0.007	0.13 ± 0.002	0.12 ± 0.001	0.15 ± 0.002	0.14 ± 0.002
5	1.2 ± 0.012	0.16 ± 0.002	0.14 ± 0.002	0.18 ± 0.002	0.16 ± 0.002
6	1.8 ± 0.017	0.19 ± 0.002	0.16 ± 0.002	0.21 ± 0.002	0.18 ± 0.002
7	2.1 ± 0.024	0.21 ± 0.002	0.17 ± 0.002	0.23 ± 0.003	0.19 ± 0.002
8	2.2 ± 0.025	0.22 ± 0.002	0.18 ± 0.002	0.24 ± 0.003	0.2 ± 0.002
9	2.25 ± 0.026	0.23 ± 0.003	0.19 ± 0.002	0.25 ± 0.003	0.21 ± 0.002
10	2.28 ± 0.028	0.23 ± 0.003	0.19 ± 0.002	0.25 ± 0.003	0.21 ± 0.002
11	2.3 ± 0.023	0.23 ± 0.003	0.19 ± 0.002	0.25 ± 0.003	0.21 ± 0.002
12	2.3 ± 0.023	0.23 ± 0.003	0.19 ± 0.002	0.25 ± 0.003	0.21 ± 0.002
13	2.3 ± 0.023	0.23 ± 0.003	0.19 ± 0.002	0.25 ± 0.003	0.21 ± 0.002
14	2.3 ± 0.023	0.23 ± 0.003	0.19 ± 0.002	0.25 ± 0.003	0.21 ± 0.002
15	2.25 ± 0.026	0.22 ± 0.002	0.18 ± 0.002	0.24 ± 0.003	0.2 ± 0.002
16	2.2 ± 0.025	0.21 ± 0.002	0.17 ± 0.002	0.23 ± 0.003	0.19 ± 0.002
17	2.15 ± 0.025	0.2 ± 0.002	0.16 ± 0.002	0.22 ± 0.002	0.18 ± 0.002
18	2.1 ± 0.024	0.19 ± 0.002	0.15 ± 0.002	0.21 ± 0.002	0.17 ± 0.002
19	2.05 ± 0.024	0.18 ± 0.002	0.14 ± 0.002	0.2 ± 0.002	0.16 ± 0.002
20	2 ± 0.023	0.17 ± 0.002	0.13 ± 0.002	0.19 ± 0.002	0.15 ± 0.002
21	1.95 ± 0.023	0.16 ± 0.002	0.12 ± 0.001	0.18 ± 0.002	0.14 ± 0.002
22	1.9 ± 0.022	0.15 ± 0.002	0.11 ± 0.001	0.17 ± 0.002	0.13 ± 0.002
23	1.85 ± 0.021	0.14 ± 0.002	0.1 ± 0.001	0.16 ± 0.002	0.12 ± 0.001
24	1.8 ± 0.021	0.13 ± 0.002	0.09 ± 0.001	0.15 ± 0.002	0.11 ± 0.001

Table S8. Evaluation of the antimicrobial activity of selected plant extracts against *Escherichia coli* based on maximum absorbance (11 h). Results from Welch's t-test comparing the mean maximum absorbance (OD₆₀₀) of extract-treated groups with the control (untreated bacteria) are shown. The T-statistic quantifies the difference, and the P-value indicates statistical significance.

Plant	Part	Mean Max Absorbance (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	0.22	−85.31	3.28	≤0.0001
	bark	0.2	−86.17	2.75	≤0.0001
<i>Ziziphus mauritiana</i>	leaves	0.24	−84.22	3.91	≤0.0001
	bark	0.19	−86.6	3.63	≤0.0001
LB (Control)		2.2			

Table S9. Inhibition of *Escherichia coli* by plant extracts. Final mean absorbance readings (24 h) for extract-treated and control groups are presented, along with the results of Welch's t-test (T-statistic and P-value) and the calculated percentage inhibition (Degree of Reduction). Statistical significance is indicated by P-values.

Plant	Part	Mean Absorbance (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	0.12	−80.3505	3.17	≤0.0001
	bark	0.1	−81.3676	2.91	≤0.0001
<i>Ziziphus mauritiana</i>	leaves	0.14	−79.163	3.82	≤0.0001
	bark	0.09	−81.8762	3.55	≤0.0001
LB (Control)		1.7			

Table S10. Evaluation of the antimicrobial activity of selected plant extracts against *K. pneumoniae* based on maximum absorbance (11h). Results from Welch's t-test comparing the mean maximum absorbance of extract-treated groups with the control (untreated bacteria) are shown. The T-statistic quantifies the difference, and the P-value indicates statistical significance.

Plant	Part	Mean Max Absorbance (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	0.23	−88.94	2.51	≤0.0001
	bark	0.19	−90.91	2.19	≤0.0001
<i>Ziziphus mauritiana</i>	leaves	0.25	−88.08	3.87	≤0.0001
	bark	0.21	−90.05	3.49	≤0.0001
LB (Control)		2.3			

Table S11. Inhibition of *Klebsiella pneumoniae* by plant extracts. Final mean absorbance readings (24 h) for extract-treated and control groups are presented, along with the results of Welch's t-test (T-statistic and P-value) and the calculated percentage inhibition (Degree of Reduction). Statistical significance is indicated by P-values.

Plant	Part	Mean Absorbance (OD ₆₀₀)	T-statistic	Degree of Freedom (df)	P-value
<i>Acacia nilotica</i>	leaves	0.13	−80.0704	2.68	≤0.0001
	bark	0.09	−82.1457	3.05	≤0.0001
<i>Ziziphus mauritiana</i>	leaves	0.15	−79.1114	3.94	≤0.0001
	bark	0.11	−81.185	3.41	≤0.0001
LB (Control)		1.8			



AIMS Press

© 2025 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>)