Supporting Information

Bio-Based Nanocomposites Derived from Tannery Waste for Antimicrobial Coatings Functional Leather: Circular Valorization of Chrome Shaving Dust

Sharmin Akter Liza,[a] Md. Abdulla-Al-Mamun,*[a] Md. Nazmul Islam,[a] Md. Rezaul Karim Rana,[b] and Md. Shariful Islam[c]

[a] Institute of Leather Engineering and Technology, University of Dhaka, 44-50 Hazaribagh, Dhaka-1209, Bangladesh

[b] Molecular Biology Laboratory, Dept. of Biochemistry and Molecular Biology, Faculty of Biological Sciences, University of Dhaka, Dhaka 1000, Bangladesh

[c] Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Table S1: Amino Acid Analysis of protein

No.	Compound Name	Amount (mg/g)	Amount (g/100g protein)	% of Amino acid
1	Aspartic Acid	34.12	3.412	8
2	Threonine	7.54	0.754	2
3	Serine	12.312	1.2312	3
4	Glutamic Acid	60.116	6.0116	14
5	Glycine	134.829	13.4829	31
6	Alanine	56.87	5.687	13
7	Cystine	14.645	1.4645	3
8	Valine	12.51	1.251	3
9	Methionine	1.075	0.1075	0
10	Isoleucine	5.756	0.5756	1
11	Leucine	11.381	1.1381	3
12	Tyrosine	1.746	0.1746	0
13	Phenylalanine	7.421	0.7421	2
14	Histidine	6.817	0.6817	2
15	Lysine	21.081	2.1081	5
16	Arginine	40.148	4.0148	9
17	Proline	97.133	9.7133	23
	Total	428.368		
	Proline	97.133		18
	Total	525.501		

Table S2: Results of dry rub fastness test

No of cycle	Grey Scale Rating					Standard value
	Sample 1	Sample-2	Sample-3	Sample-4	Sample-5	
08	5	5	5	5	5	After 1024 cycles, greyscale ratings should be between 5-3
16	5	5	5	5	5	
32	5	5	5	5	5	
64	5	5	5	5	5	
128	5	5	5	5	5	
256	5	5	5	5	5	
512	4	4	4/5	4/5	5	
1024	4/5	4/5	4/5	4/5	5	

Table S3: Results of wet rub fastness test

No of cycle	Greyscale rating					Standard value
	Sample 1	Sample-2	Sample-3	Sample-4	Sample-5	
08	5	5	5	5	5	After 512 cycles, greyscale ratings should be between 5-3
16	5	5	5	5	5	
32	5	5	5	5	5	
64	5	5	5	5	5	
128	5	4/5	4/5	4/5	5	
256	4/5	4/5	4/5	4/5	5	
512	4/5	4	4/5	4/5	4/5	

Table S4: Results of heat fastness of leather.

Temperature (°C)	Grey Scale Rating					Standard value
	Sample 1	Sample-2	Sample-3	Sample-4	Sample-5	
100	5	5	5	5	5	Greyscale ratings should be

125	5	5	5	5	5	between 5-3
150	5	5	5	5	4/5	
175	4/5	4	4/5	4/5	4/5	
200	4	4	4	4/5	4	

Table S5: Results of water vapor permeability.

Samples	Sample 1	Sample-2	Sample-3	Sample-4	Sample-5	Standard value
Water vapor permeability $(mg/cm^2\text{-}hr)$	10	4	4	7.90	2.3	Minimum 0.8 $mg/cm^2\text{-}hr$ for shoe upper

Table S6: Results for bond strength of the sample.

Samples	Sample 1	Sample-2	Sample-3	Sample-4	Sample-5	Standard value
Bond strength (g)	275	275	300	275	325	Minimum 250 g

Table S7: Zone of inhibition (ZOI) values for the four nanocomposite samples were compared against the known antibiotic positive control and the negative control to evaluate antimicrobial activity based on **Fig. S4**.

Bacterial	Replicate #	Protein-ZnO (S-1)	Ag-Protein (S-2)	PANI/Protein-ZnO(S-3)	PANI/Ag-Protein(S-4)	Negative Control (Protein, S-5)	Positive Control (PRL 100)
	1	19.3	17	14	12	0	21
B. cereus	2	20	16	15	13	0	22
	3	20.3	18	16	14	0	23

Comparison	p-values	Significant
Sample 1 vs Negative	0.000222326	Significant
Sample 1 vs Positive	0.046501126	Significant
Sample 2 vs Negative	0.001151411	Significant
Sample 2 vs Positive	0.003602233	Significant
Sample 3 vs Negative	0.001478197	Significant
Sample 3 vs Positive	0.001016663	Significant
Sample 4 vs Negative	0.00196657	Significant
Sample 4 vs Positive	0.000385068	Significant

Bacterial	Replicate	Protein-	Ag-	PANI/Protein-	PANI/Ag-	Negative	Positive

	#	ZnO (S-1)	Protein (S-2)	ZnO(S-3)	Protein(S-4)	Control (Protein, S-5)	Control (PRL 100)
	1	18	21.5	12	12	0	21
E. coli	2	19	22	12.5	13	0	22
	3	20	22.5	11.5	14	0	23

Comparison	p-values	Significant
Sample 1 vs Negative	0.000922084	Significant
Sample 1 vs Positive	0.021311641	Significant
Sample 2 vs Negative	0.000172132	Significant
Sample 2 vs Positive	1	Not Significant
Sample 3 vs Negative	0.000578202	Significant
Sample 3 vs Positive	0.000651453	Significant
Sample 4 vs Negative	0.00196657	Significant
Sample 4 vs Positive	0.000385068	Significant

Antibiotic	Bacteria	Comparison	p-values	Significant
PRL 100	B.cereus	Sample 1 vs Negative	0.000222326	Significant
		Sample 1 vs Positive	0.046501126	Significant
		Sample 2 vs Negative	0.001151411	Significant
		Sample 2 vs Positive	0.003602233	Significant
		Sample 3 vs Negative	0.001478197	Significant
		Sample 3 vs Positive	0.001016663	Significant
		Sample 4 vs Negative	0.00196657	Significant
		Sample 4 vs Positive	0.000385068	Significant

Antibiotic	Bacteria	Comparison	p-values	Significant
PRL 100	E.coli	Sample 1 vs Negative	0.000922084	Significant
		Sample 1 vs Positive	0.021311641	Significant
		Sample 2 vs Negative	0.000172132	Significant
		Sample 2 vs Positive	1	Not Significant

		Sample 3 vs Negative	0.000578202	Significant
		Sample 3 vs Positive	0.000651453	Significant
		Sample 4 vs Negative	0.00196657	Significant
		Sample 4 vs Positive	0.000385068	Significant

Antibiotic	Bacteria	Comparison	p-values	Significant
CIP	*E.coli*	Sample 1 vs Negative	0.000922084	Significant
		Sample 1 vs Positive	0.000175635	Significant
		Sample 2 vs Negative	0.000172132	Significant
		Sample 2 vs Positive	0.001245812	Significant
		Sample 3 vs Negative	0.000578202	Significant
		Sample 3 vs Positive	0.000116779	Significant
		Sample 4 vs Negative	0.00196657	Significant
		Sample 4 vs Positive	3.1443E-05	Significant

Antibiotic	Bacteria	Comparison	p-values	Significant
CIP	*B.cereus*	Sample 1 vs Negative	0.000222326	Significant
		Sample 1 vs Positive	0.000586774	Significant
		Sample 2 vs Negative	0.001151411	Significant
		Sample 2 vs Positive	9.09619E-05	Significant
		Sample 3 vs Negative	0.001478197	Significant
		Sample 3 vs Positive	5.16504E-05	Significant
		Sample 4 vs Negative	0.00196657	Significant

		Sample 4 vs Positive	3.1443E-05	Significant

Antibiotic	Bacteria	Comparison	p-values	Significant
AM	*B.cereus*	Sample 1 vs Negative	0.000222326	Significant
		Sample 1 vs Positive	6.40646E-06	Significant
		Sample 2 vs Negative	0.001151411	Significant
		Sample 2 vs Positive	0.000651453	Significant
		Sample 3 vs Negative	0.001478197	Significant
		Sample 3 vs Positive	0.001245812	Significant
		Sample 4 vs Negative	0.00196657	Significant
		Sample 4 vs Positive	0.002854418	Significant

Antibiotic	Bacteria	Comparison	p-values	Significant
AM	*E.coli*	Sample 1 vs Negative	0.000922084	Significant
		Sample 1 vs Positive	0.000339688	Significant
		Sample 2 vs Negative	0.000172132	Significant
		Sample 2 vs Positive	2.87419E-06	Significant
		Sample 3 vs Negative	0.000578202	Significant
		Sample 3 vs Positive	0.000175635	Significant
		Sample 4 vs Negative	0.00196657	Significant
		Sample 4 vs Positive	0.00226865	Significant

Table: ZOI (Zone of Inhibition) of different commercial antibiotics against gram-positive *B.cereus* and gram-negative *E.coli*

Bacteria	CIP	PRL 100	AM
B.cereus Zone of inhibition (mm)	31 ±1	22±1	7±0.5
E.Coli Zone of inhibition (mm)	30±1	22±1	6.5±0.5

Bacteria	CIP	PRL 100	AM
B.cereus Zone of inhibition (mm)	31 ±1	22±1	7±0.5
E.Coli Zone of inhibition (mm)	30±1	22±1	6.5±0.5
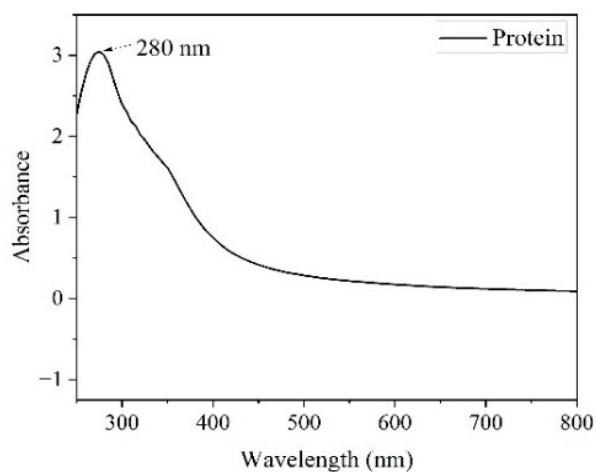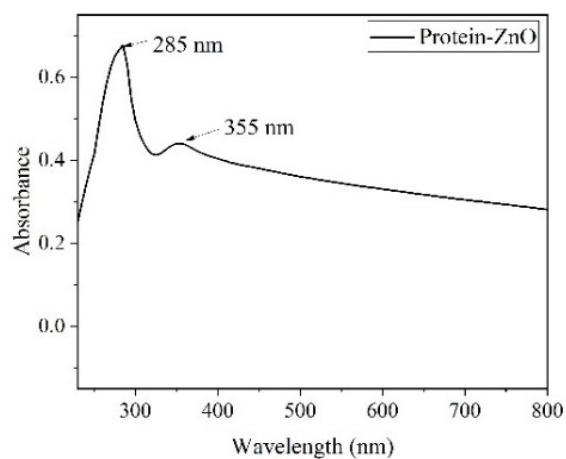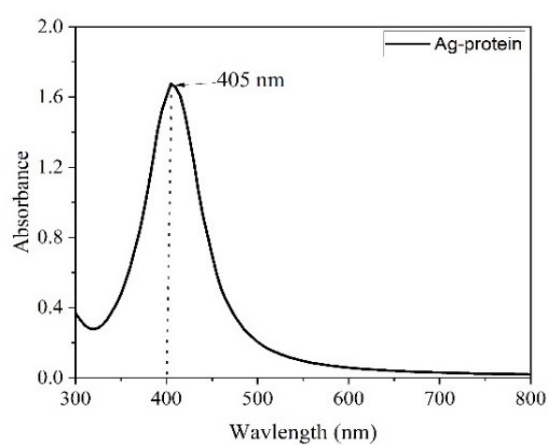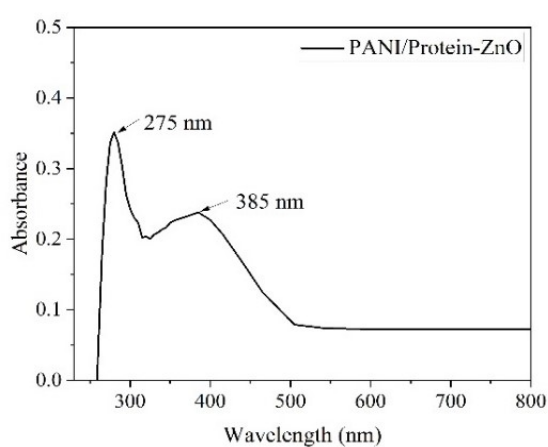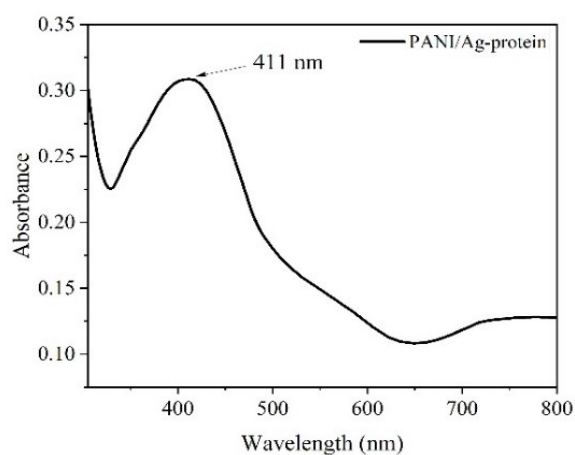
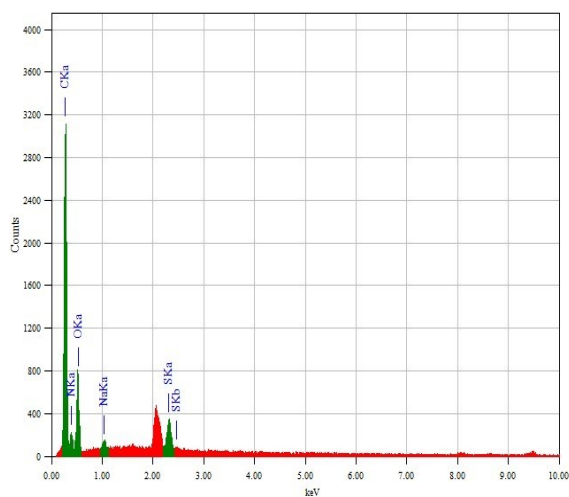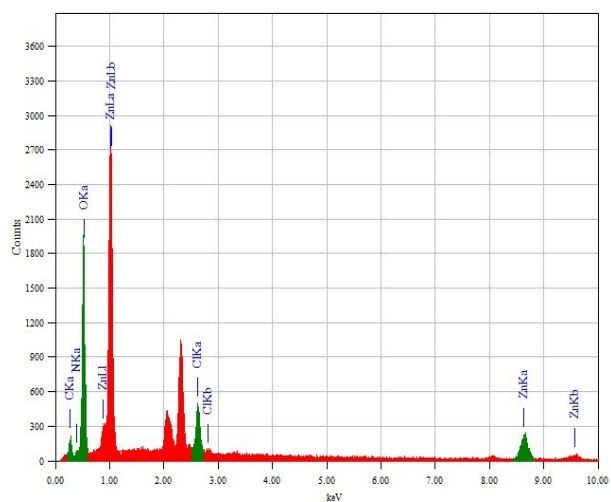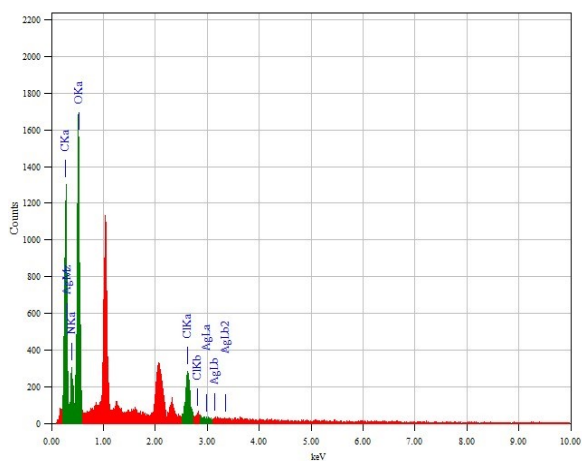**Fig. S1** UV vis spectrum of (a) Protein, (b) Protein-ZnO, (c) Ag-Protein, (d) PANI/Protein-ZnO, and (e) PANI/Ag-Protein.
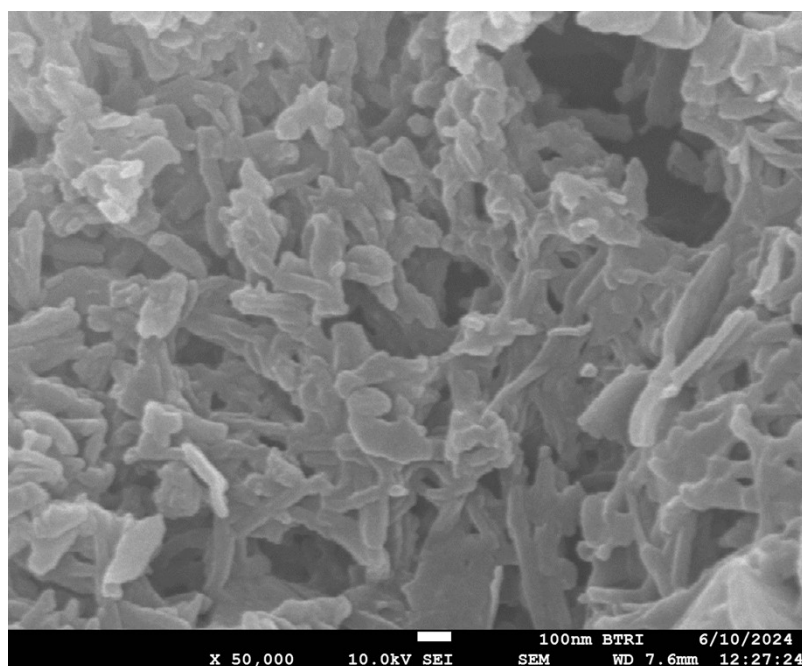
(a)
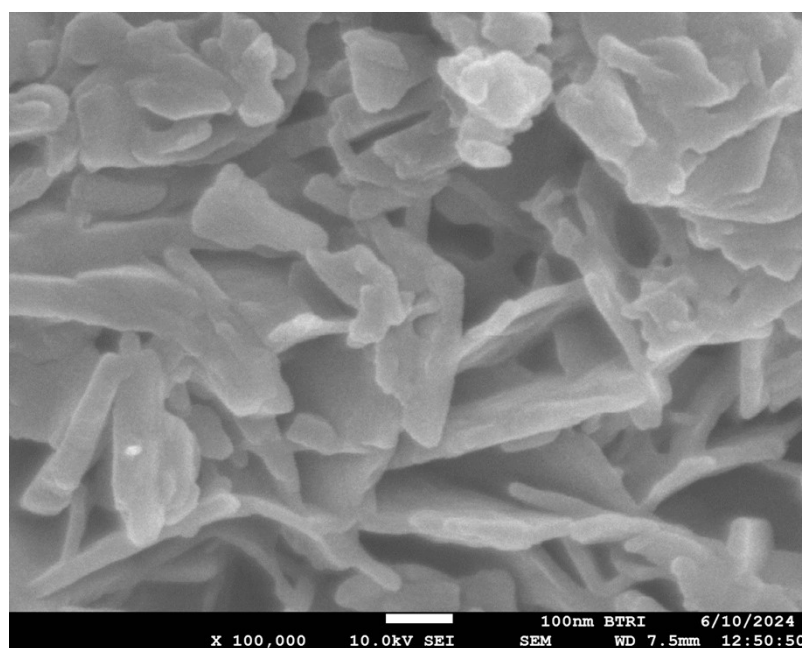
(b)

Fig. S2 FESEM analysis of (a) PANI/Protein-ZnO and (b) PANI/Ag-Protein.
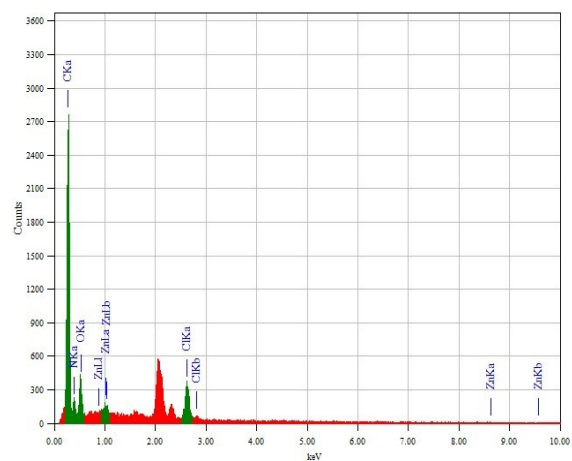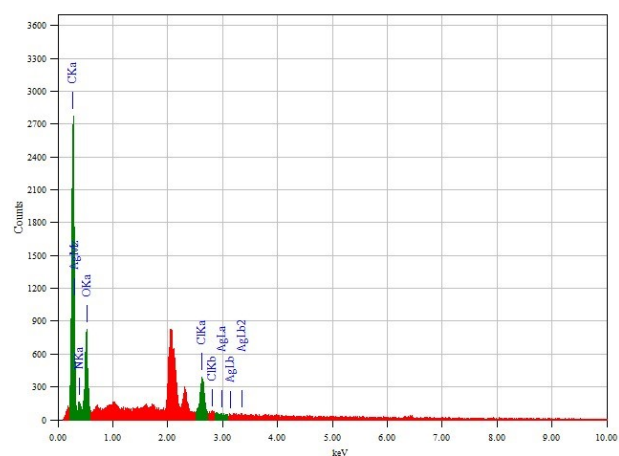
Fig. S3 EDX analysis of (a) Protein, (b) Protein-ZnO, (c) Ag-Protein, (d) PANI/Protein-ZnO, and (e) PANI/Ag-Protein.
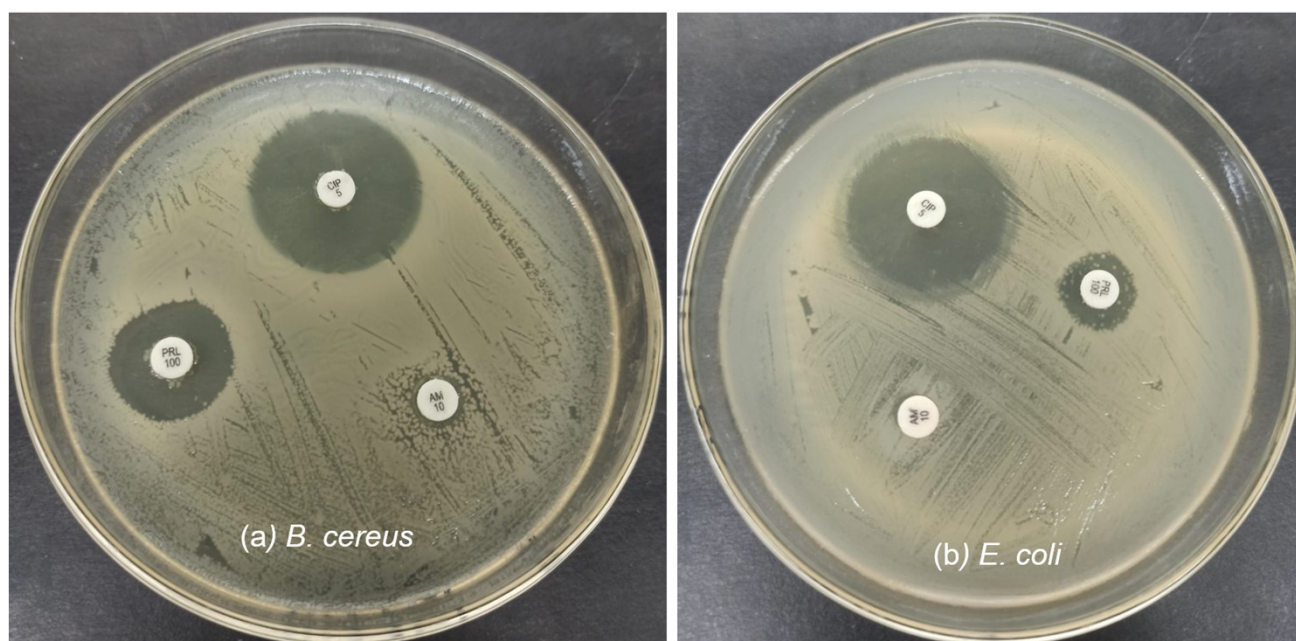
Fig. S4 Zone of inhibition (ZOI) of the known antibiotic against (a) *B. cereus* and (b) *E. coli*.
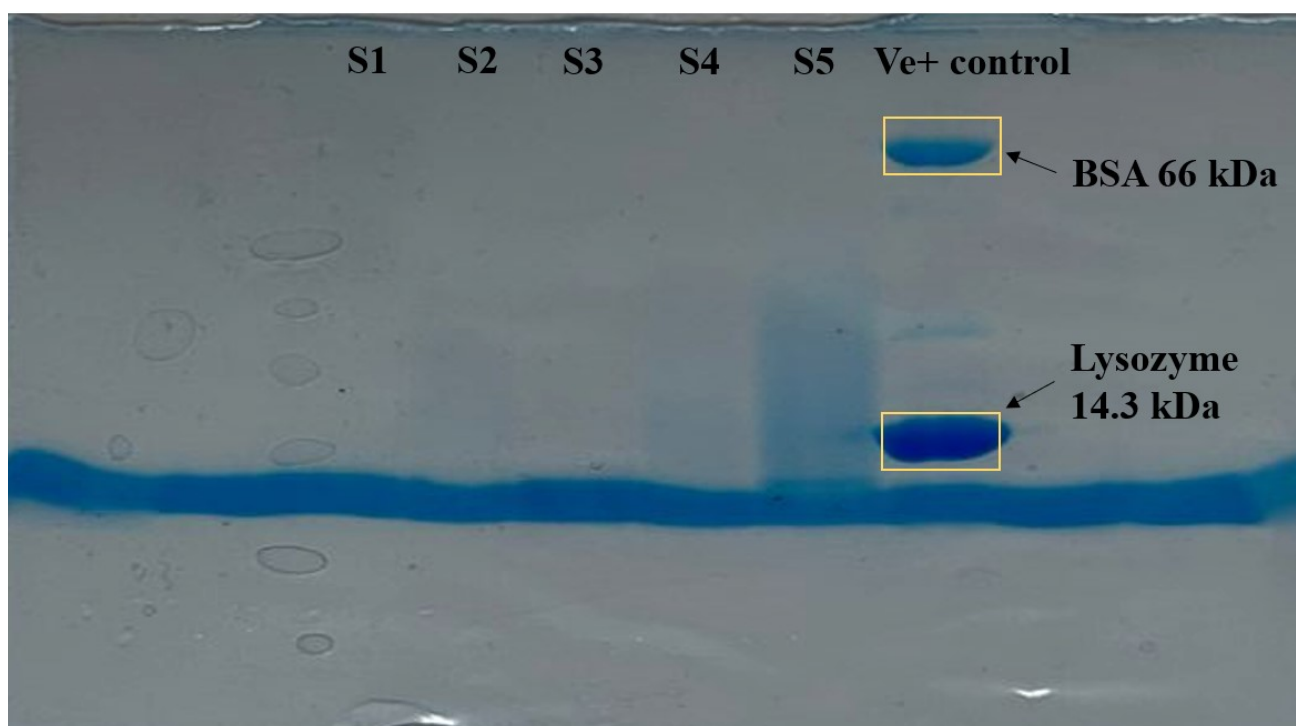
No visible protein bands were observed in S1-S4. S5 exhibited a smear instead of a distinct band.

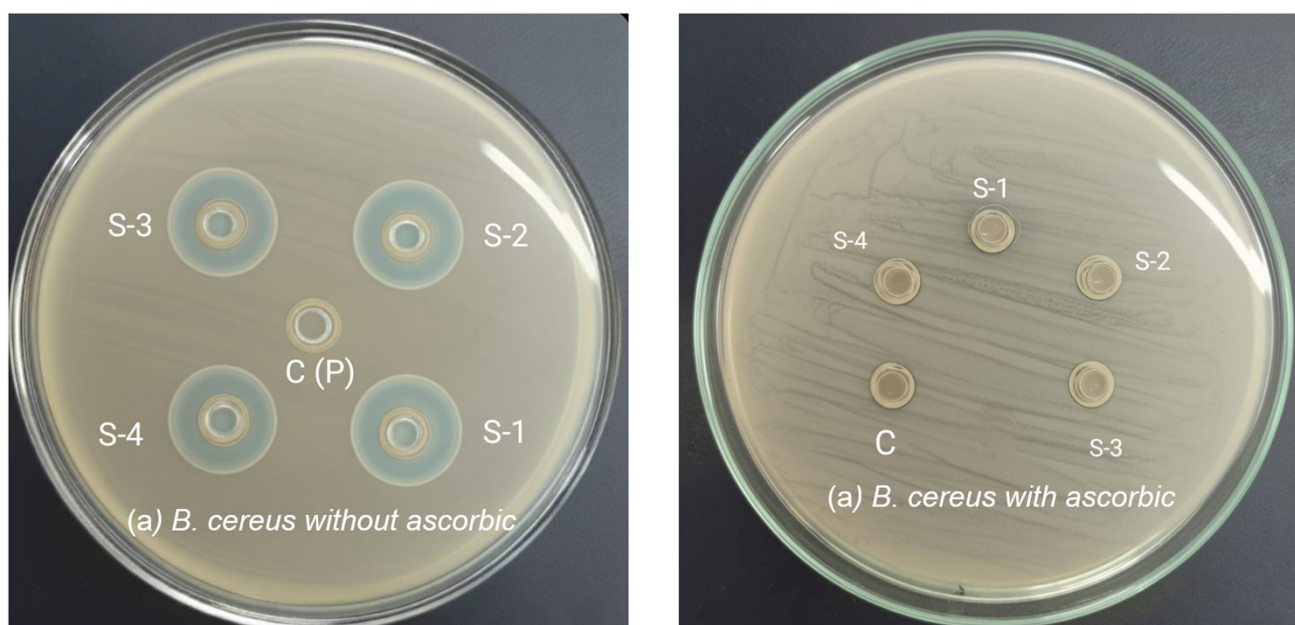**Fig. S5** Molecular weight distribution of S-1: Protein-ZnO, S-2: PANI/Protein-ZnO, S-3: PANI/Ag-protein, S-4: Ag-Protein, S-5: Protein hydrolysate from shaving dust.
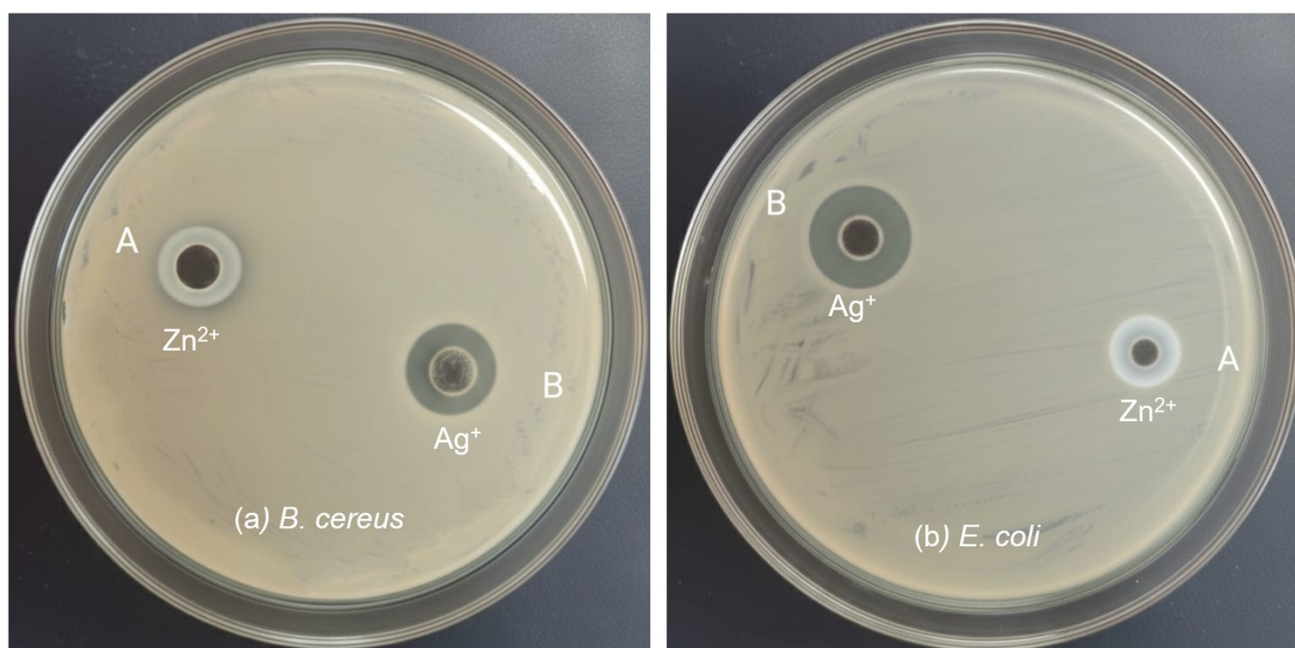
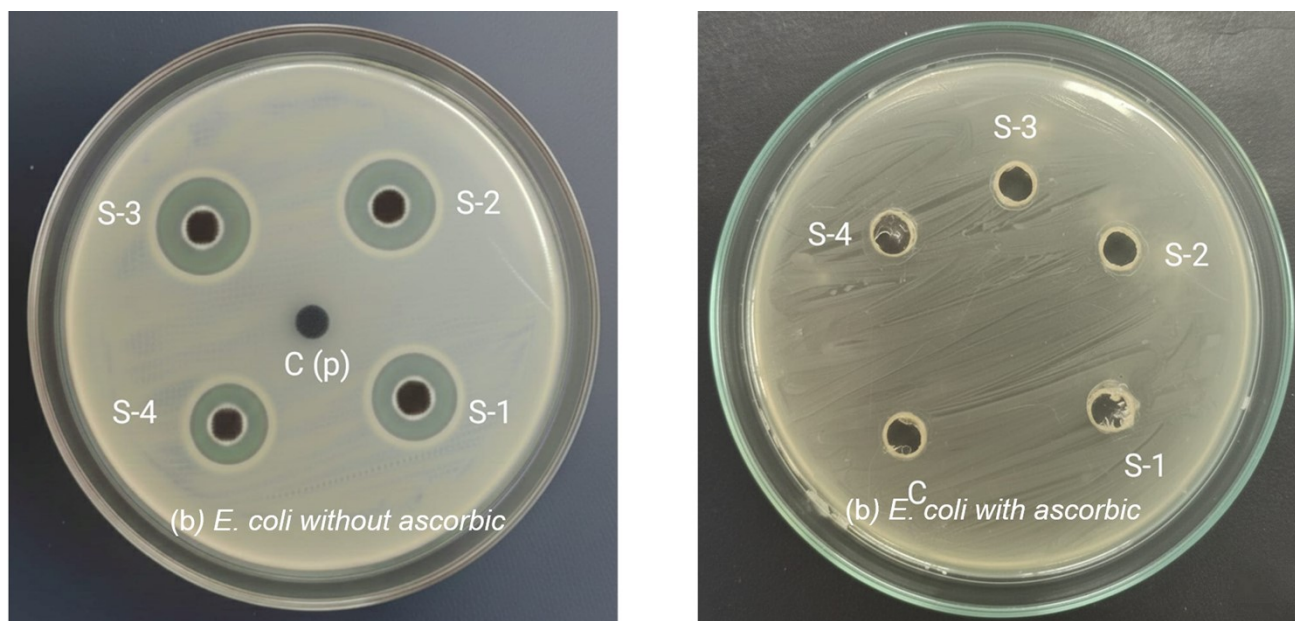**Fig. S6** Ion release test using synthetically prepared Zn²⁺ and Ag⁺ solutions (1 M).

Fig. S7 The effect of the antioxidant with/without ascorbic acid on the survival of (a) *B. Cereus* and (b) *E. coli*.
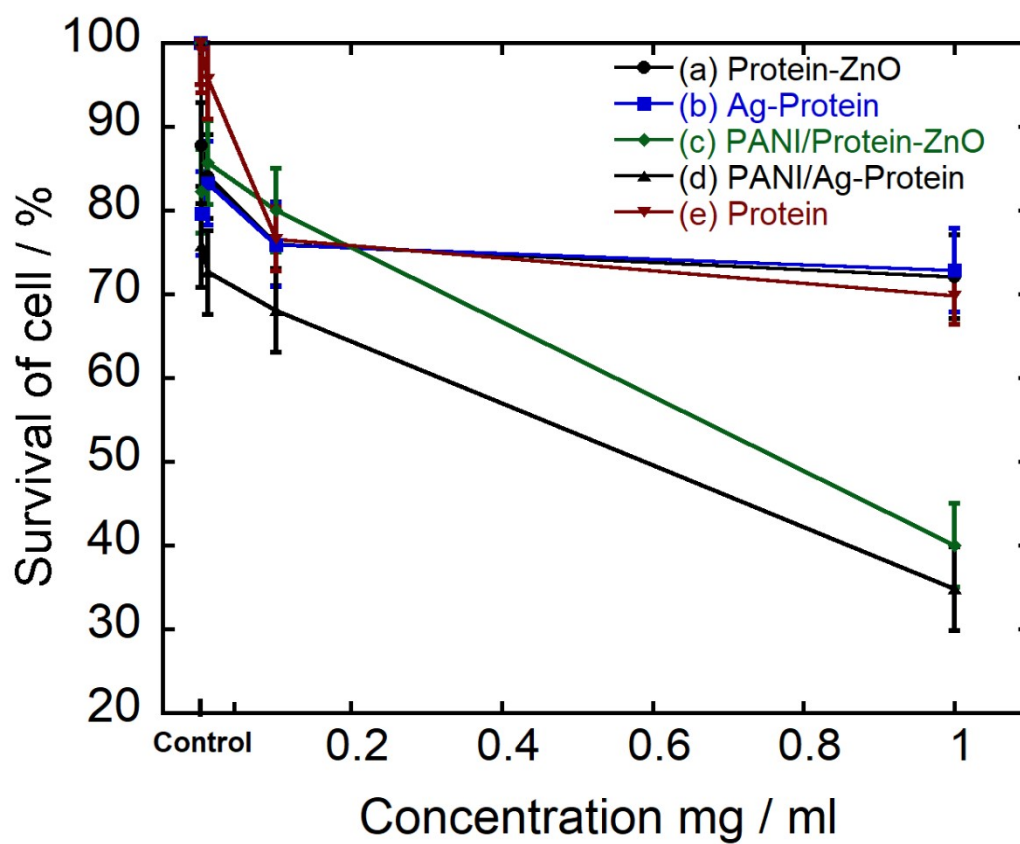

Fig. S8 Cytotoxicity analysis after 24 h incubation of (a) Protein-ZnO, (b) Ag-protein, (c) PANI/protein-ZnO, (d) PANI/Ag-protein, and (e) Protein composites against Vero cell line
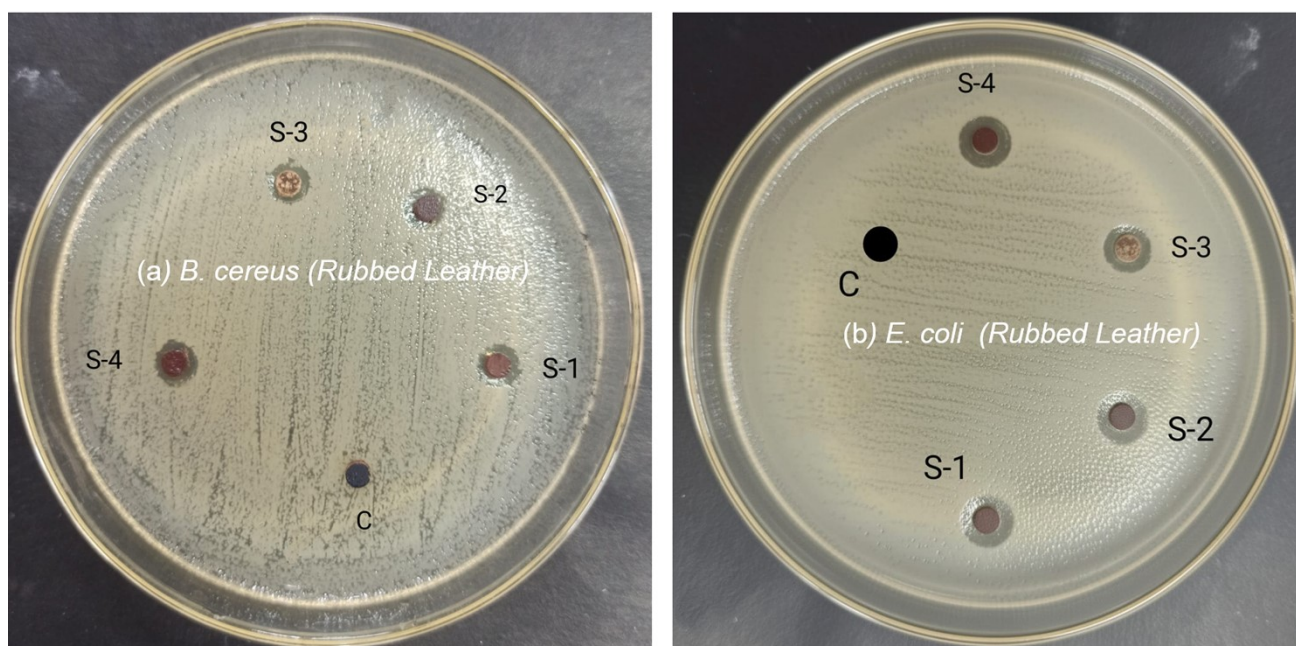
Fig. S9 Re-evaluation of the antimicrobial activity of rubbed leather (a) *B. cereus* (b) *E. coli*. S-1: Protein-ZnO, S-2: Ag-protein, S-3: PANI/Protein-ZnO, S-4: PANI/Ag-protein C: Conventional finished rubbed leather