Investigation of antibacterial activity and synergistic antibacterial potential of sericin protein extracts

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RESEARCH OBJECTIVES

Objectives

- Extraction of sericin (silk protein) from the silk cocoon.
- Study its potential applications such as antibacterial activity and synergistic antibacterial activity.

RESEARCH METHODOLOGY

Methodology

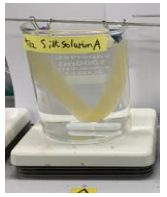
Silk Cocoon



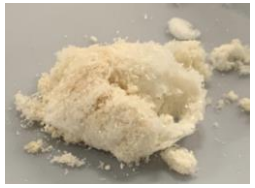
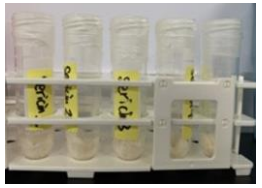
Silk cocoon from silkworm *Bombyx mori*

1. Extraction

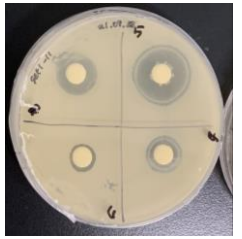

1) Purification



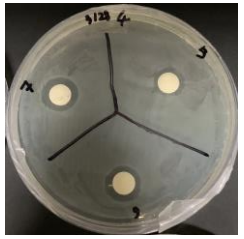

2) Lyophilization or Freeze drying

2. Antibacterial assay



3. Synergistic antibacterial assay



Extraction

- Silk cocoon dissolves into 0.2% sodium carbonate solution and autoclaves.
- Subsequently, it is filtered and concentrated to 1/3rd of its volume.

1) For purification, the solution dialysis for 48~72 hours using a membrane tubing and then store at −80℃ in a deep freezer.

2) The purified solution is subjected to a lyophilization for making sericin protein powder.

→ **The purpose of the extraction is to extract sericin protein from silk cocoon to make sericin protein powder and to determine the yield of the protein.**

Antibacterial activity

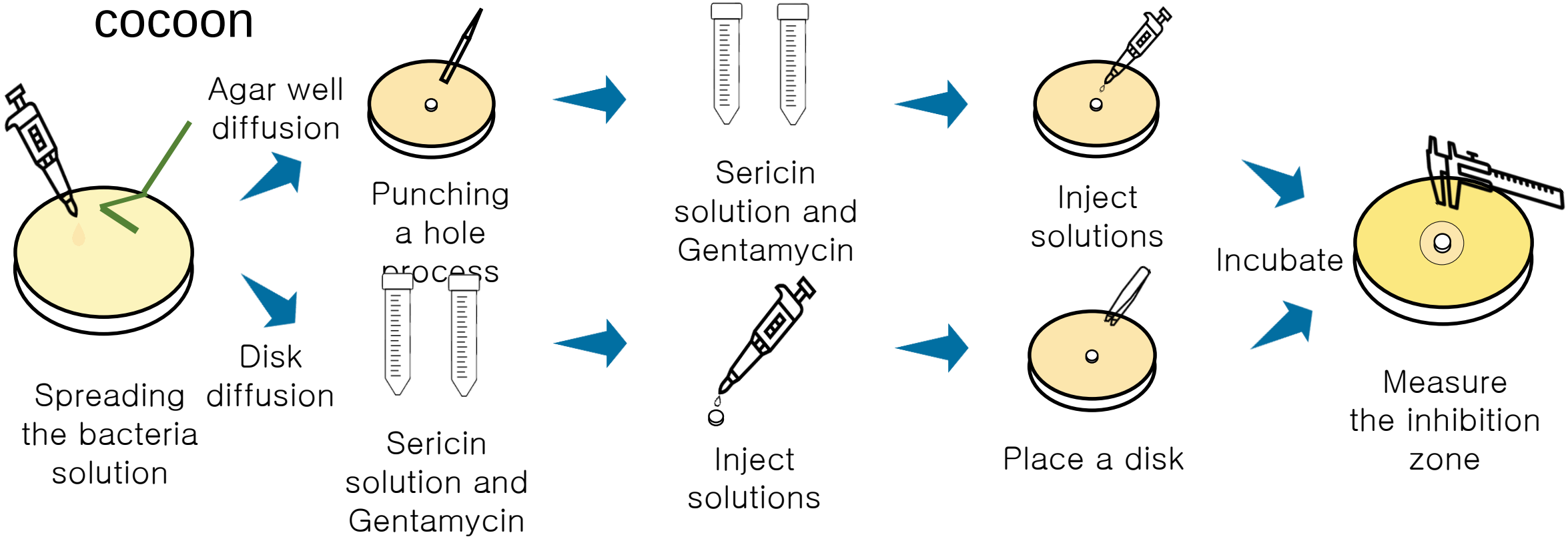
- The antibacterial activity of the sericin protein powder was evaluated by testing against a number of foodborne pathogenic bacteria including *Escherichia coli O157:H7 ATCC 23514, Enterococcus feacium DB01, Aeromonas hydrophila ATCC 7966, Salmonella enteritica KCCM 11806, Salmonella Typhimurium KCTC 1925, Shigella flexneri ATCC 12022, Pediococcus acnes ATCC 6919, Listeria monocytogenes ATCC 33090, Bacillus cereus KCTC 3624, Staphylococcus aureus ATCC 13565, Candida albicans ATCC 10231, Pseudomonas aeruginosa ATCC 27583*

❑ By using a standard agar well diffusion and disk diffusion assay.*Ref.

*Ref.: Heungsup Sung (2009). Clarithromycin and Amoxicillin Susceptibility Testing of Helicobacter pylori by Disk Diffusion Method, Korean J Clin Microbiol Vol. 12, No. 1, 2p

Antibacterial activity

- Overview of sericin antibacterial activity process from silk cocoon



Synergistic antibacterial activity

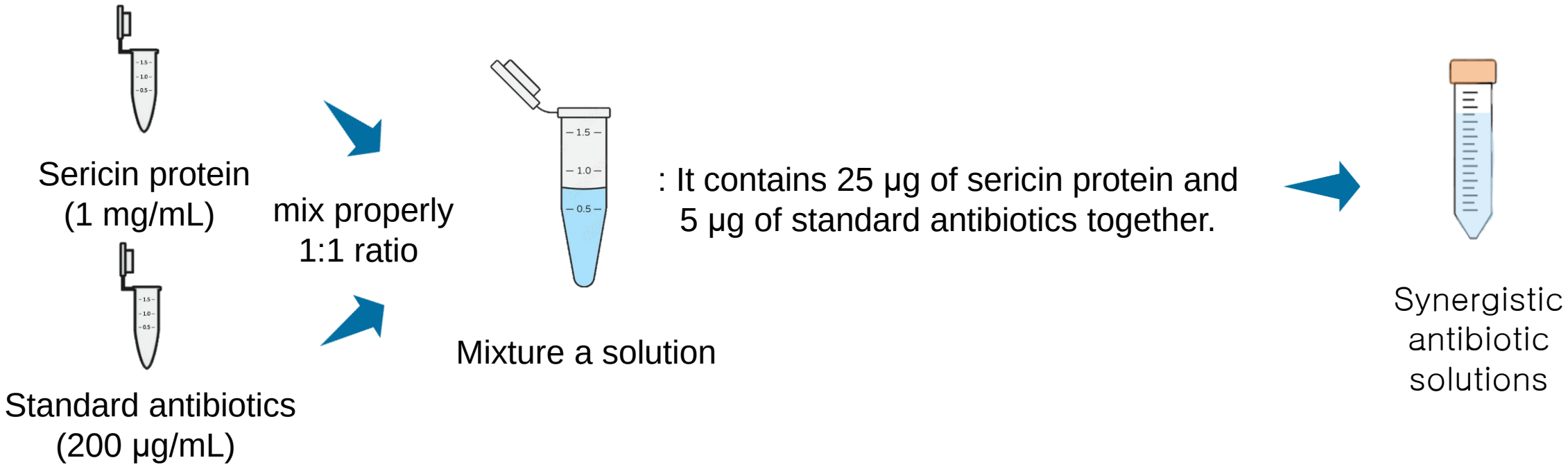
- The synergistic antibacterial potential of sericin with standard antibiotics, such as ampicillin, azithromycin, cephalexin, erythromycin, gentamycin, kanamycin, streptomycin as a standard antibiotics was determined against a number of foodborne pathogenic bacteria including *Escherichia coli O157:H7 ATCC 23514, Enterococcus feacium DB01, Aeromonas hydrophila ATCC 7966, Salmonella enteritica KCCM 11806, Salmonella Typhimurium KCTC 1925, Shigella flexneri ATCC 12022, Pediococcus acnes ATCC 6919, Listeria monocytogenes ATCC 33090, Bacillus cereus KCTC 3624, Staphylococcus aureus ATCC 13565, Candida albicans ATCC 10231, Pseudomonas aeruginosa ATCC 27583.*

- By using a synergistic antibacterial activity assay using both standard antibiotics agents and sericin protein.*Ref.

*Ref.: Patra Jayanta Kumar, Baek Kwang-Hyun (2017). Antibacterial Activity and Synergistic Antibacterial Potential of Biosynthesized Silver Nanoparticles against Foodborne Pathogenic Bacteria along with its Anticandidal and Antioxidant Effects, Frontiers in Microbiology Vol. 8

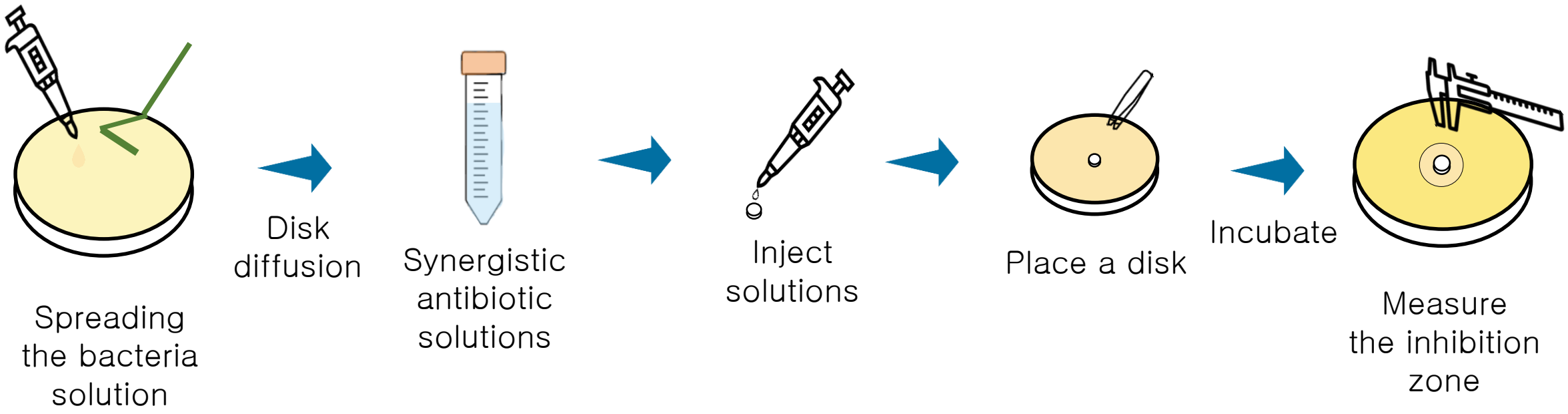
Synergistic antibacterial activity

- Pre-process with synergistic antibacterial activity from silk cocoon : overview of making synergistic antibiotic solutions



Synergistic antibacterial activity

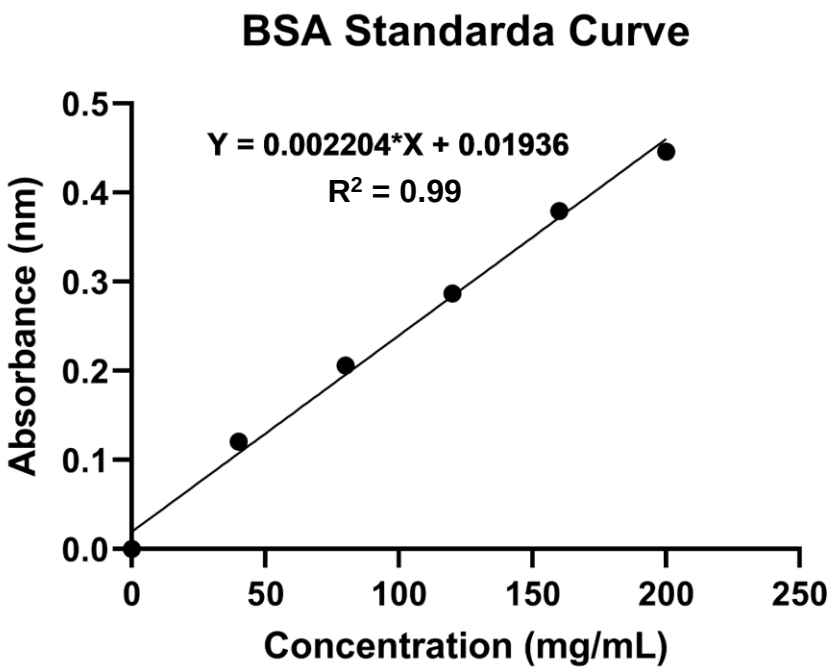
- Overview of synergistic antibacterial activity process using both antibiotic agents and sericin protein from silk cocoon

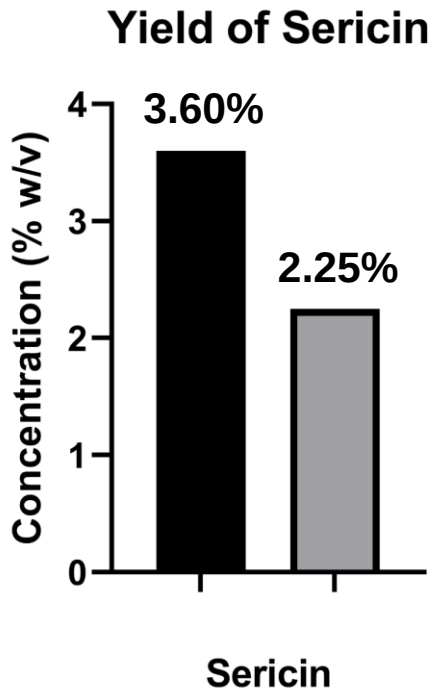


RESEARCH FINDINGS

Results

- The concentration of the extracted crude sericin solution (3.6%) and freeze-dried sericin solution (2.25%)





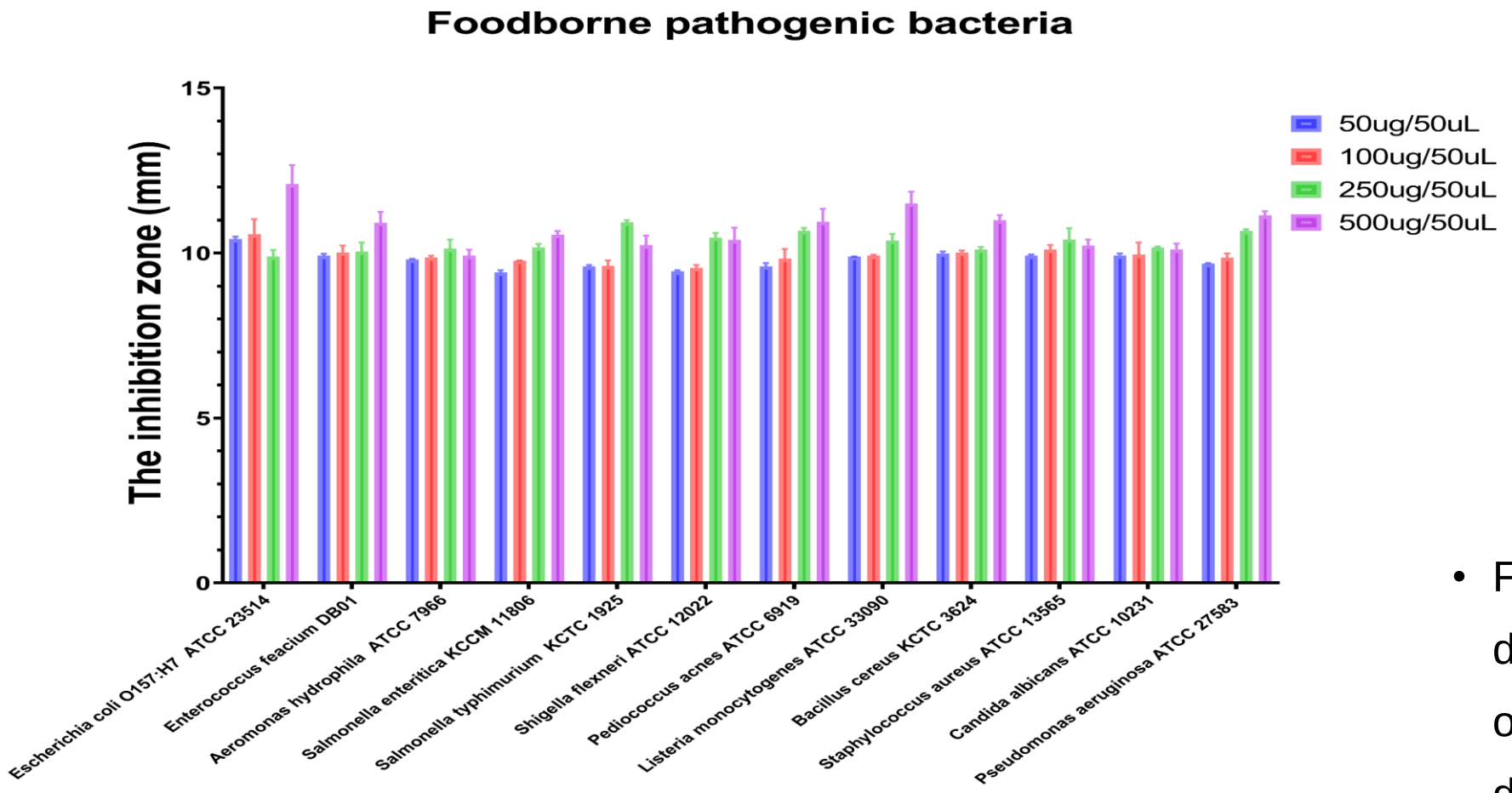
Results

Table 1: The Disk diffusion antibacterial assay of Sericin and standard Gentamicin.

Pathogenic bacteria sample	*Escherichia coli O157:H7 ATCC 23514*	*Enterococcus feacium DB01*	*Aeromonas hydrophila ATCC 7966*	*Salmonella enteritica KCCM 11806*	*Salmonella typhimurium KCTC 1925*	*Shigella flexneri ATCC 12022*	*Pediococcus acnes ATCC 6919*	*Listeria monocytogenes ATCC 33090*	*Bacillus cereus KCTC 3624*	*Stephylococcus aureus ATCC 13565*	*Candida albicans ATCC 10231*	*Pseudomonas aeruginosa ATCC 27583*
Gentamycin (10µg/disc)	12.51±0.31	0±0	13.33±0.4	11.67±0.12	13.25±0.26	11.93±0.15	0±0	12.62±0.15	13.35±0.46	0±0	0±0	0±0
Purified Sericin (50µg/disc)	10.3±0.2	10.93±0.39	10.89±0.09	11.06±0.13	11.77±0.08	10.45±0.11	10.87±0.21	9.11±0.06	9.1±0.32	0±0	8.97±0.11	0±0

The zone of inhibitions are presented in mean values (mm) ± standard deviation

Results



- Figure 1. The Agar well diffusion antibacterial activity of Freeze-dried Sericin at different concentration

Results

Table 2A: The synergistic antibacterial assay of standard antibiotic agents.

STD 5μg/disk	*Escherichia coli O157:H7 ATCC 23514*	*Enterococcus feacium DB01*	*Aeromonas hydrophila ATCC 7966*	*Salmonella enteritica KCCM 11806*	*Salmonella typhimurium KCTC 1925*	*Shigella flexneri ATCC 12022*	*Pediococcus acnes ATCC 6919*	*Listeria monocytogenes ATCC 33090*	*Bacillus cereus KCTC 3624*	*Staphylococcus aureus ATCC 13565*	*Candida albicans ATCC 10231*	*Pseudomonas aeruginosa ATCC 27583*
Ampicillin	12.01±0.77	10.21±0.06	11.22±0.03	12.63±0.18	13.3±0.3	13.09±0.13	9.03±0.26	11.35±0.07	12.82±0.17	12.06±0.2	11.83±0.08	13.32±0.45
Azithromycin	8.6±0.01	15.68±0.27	12.83±0.11	17.77±0.92	17.9±0.19	18.04±0.59	7.27±0.01	15.74±0.34	17.47±1.06	11.81±0.4	17.5±1.19	12.18±0.29
Cephalexin	9.96±0.18	11.54±0.52	11.69±0.02	13.63±0.25	14.14±0.33	11.2±0.18	8.52±0.08	12.05±0.19	13.83±0.34	13.98±0.31	11.37±0.1	11.62±0.13
Erythromycin	8.65±0.01	8.59±0.02	9.11±0.11	8.82±0.04	8.62±0.03	8.57±0.06	8.61±0.01	8.63±0.04	8.76±0.02	8.6±0.01	8.67±0.03	8.63±0.04
Gentamycin	10.24±0.3	9.73±0.12	10.14±0.28	9.85±0.16	10.26±0.05	10.68±0.04	11.05±0.29	10.58±0.08	10.51±0.13	11.01±0.22	10.46±0.13	9.01±0.07
Kenamycin	12.19±0.74	11.89±0.1	12.72±0.18	11.67±0.11	12.63±0.21	12.87±0.09	12.84±0.05	11.93±0.52	12.37±0.57	12.66±0.27	13.54±0.25	11.71±0.2
Streptomycin	13.05±0.34	13.46±0.39	13.38±0.45	13.38±0.56	12.41±0.09	13.53±0.25	13.51±0.01	12.46±0.15	13.7±0.27	13.28±0.13	13.68±0.27	11.97±0.2

The zone of inhibitions are presented in mean values (mm) ± standard deviation

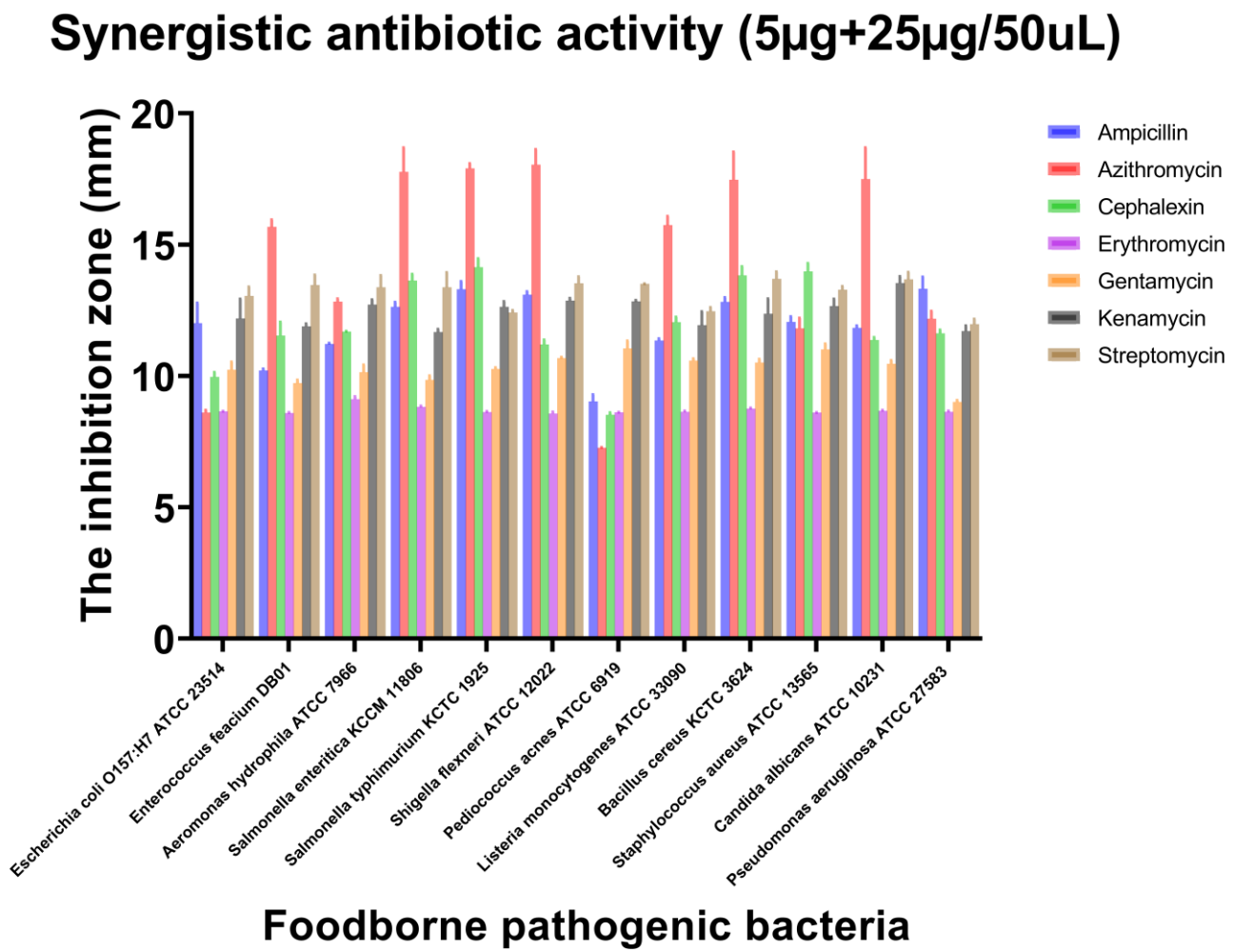
Results

Table 2B: The synergistic antibacterial assay of Sericin with standard antibiotic agents

5μg+25μg/disk	*Escherichia coli O157:H7 ATCC 23514*	*Enterococcus feacium DB01*	*Aeromonas hydrophila ATCC 7966*	*Salmonella enteritica KCCM 11806*	*Salmonella typhimurium KCTC 1925*	*Shigella flexneri ATCC 12022*	*Pediococcus acnes ATCC 6919*	*Listeria monocytogenes ATCC 33090*	*Bacillus cereus KCTC 3624*	*Staphylococcus aureus ATCC 13565*	*Candida albicans ATCC 10231*	*Pseudomonas aeruginosa ATCC 27583*
Ampicillin	19.74±0.03	16.21±0.12	21.18±0.18	22.21±0.2	26.59±0.74	25.74±0.53	24.71±1.1	24.26±0.3	25.2±0.38	22.53±1.02	18.55±0.19	19.25±0.15
Azithromycin	8.62±0.03	17.73±0.97	8.61±0.02	8.64±0	9.01±0.02	8.63±0.05	18.76±0.25	8.6±0.02	19.17±1.16	8.61±0.02	8.45±0.31	8.64±0.03
Cephalexin	14.12±0.08	13.76±0.09	12.83±0.13	10.98±0.03	14.09±0.02	13.27±0.09	13.75±0.43	12.18±0.47	14.07±0.35	11.89±0.12	13.63±0.19	10.26±0.21
Erythromycin	8.65±0.03	9.03±0.06	8.94±0.06	8.61±0.06	8.87±0.04	8.75±0.02	8.61±0.03	8.62±0.04	8.89±0.01	8.64±0.05	8.66±0.02	8.59±0.02
Gentamycin	10±0.05	9.78±0.21	9.5±0.08	11.79±0.22	11.19±0.17	11.48±0.52	13.06±1.48	11.34±0.15	8.63±0.06	13.67±0.14	11.64±0.23	10.58±0.03
Kenamycin	11.25±0.11	11.77±0.32	9.44±0.13	13.02±0.27	12.93±0.22	13.08±0.24	18.49±2.62	12.71±0.25	9.74±0.11	15.6±0.18	13.16±0.11	10.26±0.1
Streptomycin	13.35±0.27	12.68±0.56	10.7±0.37	13.66±1.02	13.59±0.19	13.36±0.52	14.68±0.34	14.42±0.29	9.23±0.22	15.17±0.31	14.5±0.16	11.11±0.17

The zone of inhibitions are presented in mean values (mm) ± standard deviation

Results



- The synergistic antibacterial activity of Sericin with standard antibiotic agents at 5μg+25μg/disk

SUMMARY/ CONCLUSIONS/ RECOMMENDATIONS

Summary/ Conclusions

- The sericin was extracted from the silk cocoon by the degumming method.
- The crude sericin was purified by dialysis and it added dialysis process to Lyophilization or Freeze drying process was the purified sericin.
- The protein concentration of the crude sericin was found out to be 3.60 % (W/V) whereas the protein concentration of the purified sericin powder was found out to be 2.25 % (W/V).
- The antibacterial activity of the purified sericin was evaluated against a number of foodborne pathogenic bacteria by agar well diffusion and disk diffusion method.
- The results showed that the sericin is highly effective against a number of pathogenic bacteria such as *E. coli, Salmonella* Typhimurium*, Shigella flexineri* etc.

Summary/ Conclusions

- The antibacterial potential of sericin is comparable with the standard antibiotic, Gentamycin.
- The sericin extract at 500µg/disc displayed highest inhibition zones against both the tested *Escherichia coli* (12.09 ± 0.58 mm) and the *Listeria monocytogenes* (11.51 ± 0.35 mm).
- Further, the sericin along with the standard antibiotics at (25µg+5µg)/disk respectively displayed significant antibacterial potential against all the tested foodborne pathogenic bacteria.
- 25µg of standard antibiotics and 5µg of the purified sericin are mixed for using as the synergistic antibacterial solution.
- The highest inhibition zone about the synergistic antibacterial activity of sericin with standard antibiotics is against the *Salmonella* Typhimurium (26.59 ± 0.74 mm).

ACKNOWLEDGMENTS

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Thank you for listening.