Karthikeyan et al | Article 9532
J Pure Appl Microbiol. 2024;18(3):2070-2084. doi: 10.22207/JPAM.18.3.57
Received: 06 May 2024 | Accepted: 02 August 2024
Published Online: 31 August 2024

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Caesalpinia bonducella Seeds Extracts are Non-toxic to the Gut Bacteria *Lactobacillus rhamnosus,* as Substantiated by *In vitro* and *In silico* Studies

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Abstract

The seed kernels of *Caesalpinia bonducella*, a traditional medicinal plant in India, are widely used to treat various disorders, including polycystic ovary syndrome. The seed kernel possesses anti-bacterial properties against many pathogenic bacteria. However, their impact on *Lactobacillus* spp., a prominent gram-positive gut bacterium, has not been studied till date. The present study employed both *in vitro* and *in silico* methods to illustrate the effect of seed extract of *C. bonducella* against *Lactobacillus rhamnosus* GG. For this, disc diffusion assay was performed with 100, 500, and 1000 µg/ml of aqueous and methanolic seed extract against *L. rhamnosus* and *E. coli,* and the zone of inhibition was measured. While both the extracts inhibited the growth of *E. coli*, it did not show any zone of inhibition against *L. rhamnosus*. The latter possess surface layer proteins, SlpX and SlpA, which prevented the influx of the phytocompounds of *C. bonducella*, as demonstrated by molecular docking using Autodock Vina. Docking results showed that the binding of the phytocompounds to the SlpX and SlpA proteins was not in the active pockets. These findings conclude that *C. bonducella* seed kernel extracts are safe against the gut bacteria *L. rhamnosus.*

Keywords: Antibacterial Activity, Molecular Docking, Polycystic Ovary Syndrome, Phytocompounds, Surface Layer Protein

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Citation: Karthikeyan M, Akshatha P, Mohideen HS, Usha B. *Caesalpinia bonducella* Seeds Extracts are Non-toxic to the Gut Bacteria *Lactobacillus rhamnosus,* as Substantiated by *In vitro* and *In silico* Studies. *J Pure Appl Microbiol.* 2024;18(3):2070-2084. doi: 10.22207/JPAM.18.3.57

INTRODUCTION

Caesalpinia bonduc (L.) Roxb., a traditional medicinal plant belonging to the family Caesalpiniaceae, is found in Africa and South Asia, particularly in the tropical regions of India. This plant possesses various secondary metabolites like alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids. Major phytocompounds found in the seed kernels are bonducellin, citrulline, α-caesalpin, β-caesalpin, γ-caesalpin, oleic acid, palmitic acid, stearic acid, aspartic acid, and arginine.[1-3] *C. bonducella* seed kernel (Figure 1) has numerous therapeutic properties, including anti-bacterial, anti-pyretic, anti-diuretic, anthelmintic, antioxidant, analgesic, anti-diarrhoeal, anti-anaphylactic, anti-asthmatic, anti-viral, anti-amoebic, and anti-cancer. It also possesses anti-diabetic, anti-inflammatory, anti-estrogenic, and anti-androgenic properties; hence, it is widely used in the treatment of polycystic ovary syndrome (PCOS) in Indian women.[4-9]

PCOS is an endocrine disorder that affects women of childbearing age.[10] Research on human trials has revealed that there is a significant change in the taxonomic diversity of gut bacteria in PCOS patients.[11] It has been shown that dysbiosis in the gut is directly related to PCOS and obesity.[12] Alternative medicine for PCOS is popular in India, and *C. bonducella* seed kernel with equal parts of pepper mixed with ghee or honey for 48 days is prescribed by Siddha practitioners. Although there is a plethora of evidence that *C. bonducella* seeds are toxic to human pathogens like *Staphylococcus aureus, Pseudomonas aeruginosa*, *Mycobacterium smegmatis, Salmonella typhi, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae*, and *Vibrio cholerae,*[13-15] its effect on probiotic gut bacteria has not been investigated.

The gut microbiota is a complex biological community, comprising more than thousand microbial species.[16] The microbiota in our gut typically sustains our health, which ensures resistance to pathogen colonization, produces short-chain fatty acids (SCFAs) that fuel epithelial cells, regulates gene expression, and produces vitamins and toxins, in addition to regulating cholesterol metabolism and bile deconjugation, all of which are vital functions for human existence.[17] The gut microbiota is composed primarily of the phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Among these phyla, 90% are composed of Bacteroidetes and Firmicutes. More than two hundred distinct genera comprise the Firmicutes phylum, including *Ruminicoccus, Bacillus, Lactobacillus,* and *Clostridium*.[18] The most significant probiotic bacteria in the gut microbiome belong to the genus *Lactobacillus*, which is a member of the phylum Firmicutes.

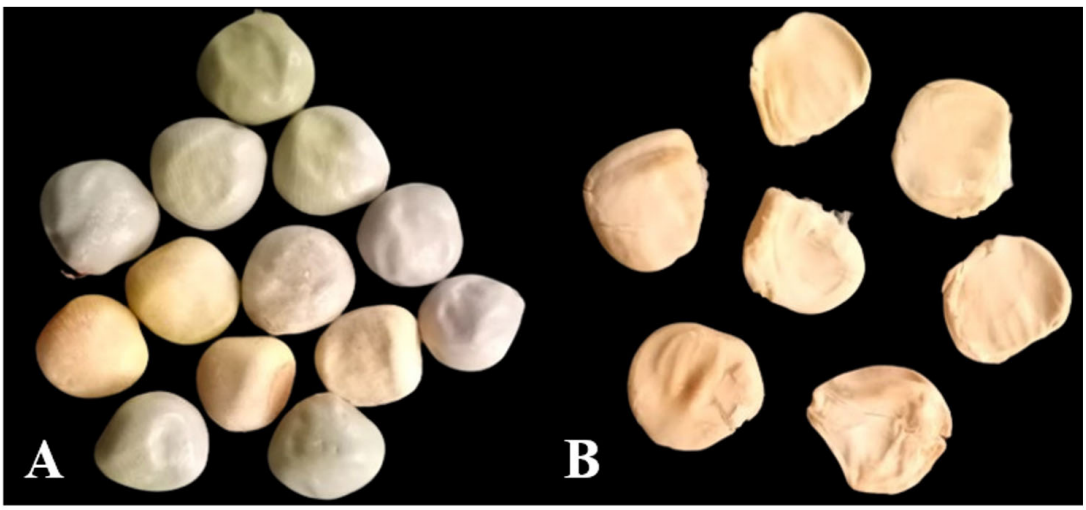


Figure 1. A) *Caesalpinia bonducella* seed; B) *Caesalpinia bonducella* seed kernels

Lactobacillus, a frequently consumed probiotic, makes up 6% of the total bacteria in the human duodenum and 0.3% in the human colon.[19] It kills the pathogens by producing lactic acid, acetic acid, propionic acid, bacteriocins, and reactive functional niches in the gut.[20]

Lactobacillus species also influence the functions and behaviors associated with the central nervous system, where species like *Lactobacillus helveticus* NS8 reduce depression, anxiety, and cognitive dysfunction and increase serotonin and neuropeptides in the hippocampus.[21] *Lactobacillus rhamnosus* regulates the GABA receptors that reduce anxiety and depression.[22] Dysbiosis in the gut microbiota results in the alteration of the beneficial and harmful bacterial ratio and is associated with many diseases and disorders, especially obesity, insulin resistance, and diabetes, that are directly associated with PCOS.[23] To balance gut barrier integrity and mucosal barrier defense and improve host immunological responses, these gut-dwelling *Lactobacillus* species converse not only with one another but also with the gut epithelial lining.[24] Hence, it is essential to know if the phytocompounds consumed for treating various disorders alter the gut bacterial community.

In this study, we investigated the effects of methanolic and aqueous extracts of *C. bonducella* seed kernels on *L. rhamnosus*, the most prevalent gut bacteria. Further, we performed molecular docking of the bioactive compounds in the seed kernel against the surface layer proteins of *Lactobacillus* spp*., (*SlpX and SlpA), as these proteins play a crucial role in protecting the bacteria from the outer environment.

MATERIALS AND METHODS

Bacterial culture

Two strains of bacteria were used in this research. *E. coli* TOP10 M15 was procured from the Microbial Type Culture Collection (MTCC). *L. rhamnosus* GG was isolated from kimchi (fortified food) by spread-plating the serially diluted food powder. Both cultures were validated using 16S rDNA sequencing. The forward primer -5'AGGCTACGCTAACCGATGTC 3' and reverse primer -5'AACGGCATACATTAACGCGC 3' were used in the 20 µL reaction containing 100 ng of isolated genomic DNA as a template, two primers (10 pmol each) described by Scarpellini *et al.*[25], 0.2 mM of dNTPs, 6.0 units of Taq polymerase, and 10 x buffer with 3 mM $MgCl_2$. The reaction protocol was: 94°C for 5 mins, 30 cycles of 94°C for 1 min, 58°C for 2 mins, 72°C for 2 min, and then 72°C for 10 mins, carried out in a thermal cycler (Aligent Sure cycler 8800). Purified PCR products of about 1500 bp were sequenced, and BLAST was performed to identify the strains. All strains were stored at -20°C in glycerol until use.

Composition of culture medium

E. coli TOP10 M15 was grown in Luria-Bertani broth (LB) agar composed of tryptone (10 g/L), yeast extract (5 g/L), sodium chloride (10 g/L), and agar (15 g/L) with a pH adjusted to 7.5 ± 0.2 and incubated at 37°C for 24 hours. *L. rhamnosus* GG was grown in De Man-Rogosa-Sharpe (MRS) agar, composed of proteose peptone (10 g/L), HM peptone B (10 g/L), yeast extract (5 g/L), dextrose (glucose) (20 g/L), polysorbate 80 (Tween 80) (1 g/L), ammonium citrate (2 g/L), sodium acetate (5 g/L), magnesium sulfate (0.1 g/L), manganese (II) sulfate (0.05 g/L), di-potassium hydrogen orthophosphate (2 g/L), agar (12 g/L), adjusted to pH 6.5 ± 0.2, and incubated at 37°C for 48 hours.

Seed extraction

Fresh seeds of *C. bonducella* Roxb. were collected from the National Institute of Siddha, Chennai, India (11° 15' 42.9984 N; 78° 23' 8.5668 E) and stored at 4°C. Prof. P. Jayaraman, a taxonomist from Presidency College in Chennai, India, validated the seeds. The collected seeds were shade-dried for 2 weeks, and the seed coats were broken to collect the seed kernels, which were shade-dried for 10 more days and pulverized into a fine powder. The powder was then air-dried for 30 minutes. About 5 g of the seed powder was measured and dissolved in 50 ml of methanol and water, sealed, and incubated for 12 hours at 28 ± 2°C in a shaking incubator at 125 rpm. After 12 hours, the entire mixture was filtered using Whatman filter paper grade 1 (90 mm), and the same process was repeated for 3 subsequent days to extract most of the active ingredients. The methanol and aqueous extracts were dried and stored at 4°C until further analysis.

Disc diffusion assay

The effect of methanol and aqueous crude extracts of the seed kernel (100, 500, and 1000 µg/ml) were screened against *L. rhamnosus* GG and *E. coli* using the disc diffusion method. Sterile discs (6 mm diameter, Himedia) were saturated with the above-mentioned concentrations of the extracts and placed on the MRS agar and LB agar medium, uniformly spread with *L. rhamnosus* GG, and *E. coli,* respectively. The inoculum was approximately 10^6 cfu/ml. A standard antibiotic (Ampicillin, 30 µg/disc) was imbued in the sterile disc and was used as a positive control. The plates were then incubated at 4°C for one hour, which is efficient for the diffusion of the test material. Then the plates were incubated at 37°C and checked for zones of inhibition after 24 and 48 hours.

Selection and preparation of target proteins

Surface layer proteins play a crucial role in the communication between the cell and the environment. Surface layer proteins SlpX and SlpA, which are essential for protecting *Lactobacillus spp*. against the external environment, were selected as target proteins for docking against the phytocompounds of *C. bonducella* seeds.

The three-dimensional structures of the surface layer proteins, SlpX (PDB ID: 7QFJ) with a resolution of 2.50Å determined with X-ray diffraction and SlpA (PDB ID: 7QFG) with a resolution of 1.65Å determined with X-ray diffraction were obtained from the protein data bank (PDB) (https://www.rcsb.org/). Any associated groups, including PO_4^{2-} and water molecules, were eliminated from the proteins in PyMol software, and the protein structures were saved in pdbqt format for docking.[26] The binding pockets of the proteins were predicted by an online tool CASTp (http://sts.bioe.uic.edu/castp/) by uploading the protein pdb files and setting the radius to 1.4Å.[27]

Selection and preparation of ligands

A comprehensive literature search was

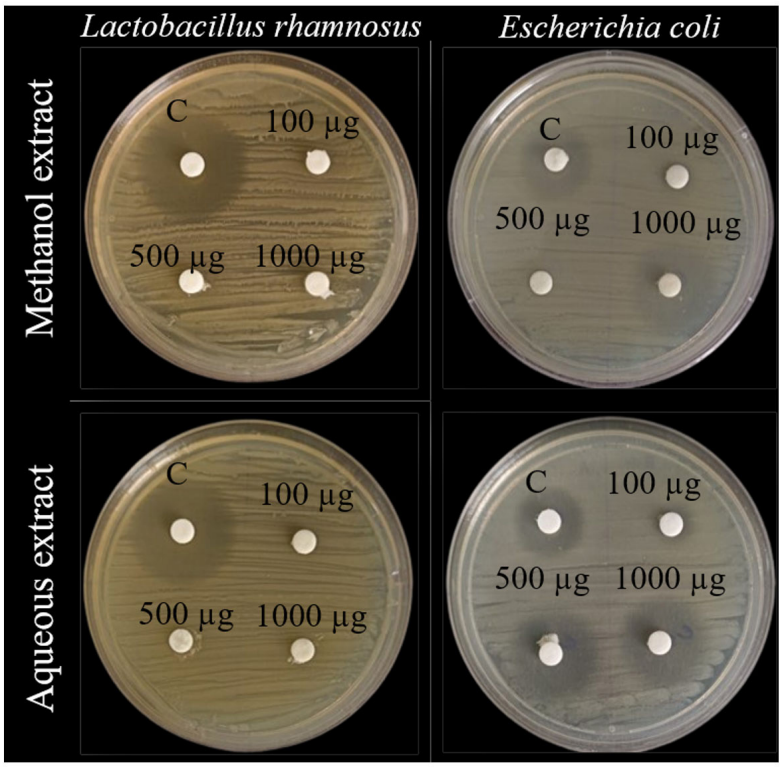


Figure 2. *In-vitro* disc diffusion assay for aqueous and methanol extracts of *Caesalpinia bonducella* seed kernel. The zones of inhibition are observed on the cultured plates

conducted to gain insights into the bioactive compounds that are found in *C. bonducella* seed kernels. All relevant information was gathered from well-known databases, such as PubMed, Scopus, Google Scholar, and EMBASE; associated articles were located by looking up their references. Bioactive compounds of *C. bonducella*, secondary metabolites, bonduc nut, LC-MS, GC-MS, and additional pertinent terms were the keywords used in the literature search. The search did not exclude any specific region, period, or language. The structures of the compounds retrieved from the literature were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The structures were uploaded to PyRx and converted from the spatial data file (SDF) to PDB format using Open Babel (https://www.cheminfo.org/), an open-source software. The 2D structure of the derived bioactive compounds was drawn using ChemSketch version 2022.1.2 (https://www.acdlabs.com/). The Universal Force Field was applied to all the selected ligands before the molecular docking procedure.

Molecular docking

For molecular docking, the National Biomedical Computation Resources PyRx open-source software for virtual screening, which incorporates Autodock Vina, was utilized. The Vina Wizard software was utilized to bind two different surface layer proteins (SlpA and SlpX) against 54 compounds from the seeds. The docking site of SlpA was defined at the active site with a grid box size of 80x80x80, a grid center of -26.055Å, -6.121Å, 33.527Å, grid space of 0.375Å. Similarly, for SlpX, a grid box size of 80x80x80, a grid center of 10.524Å, 0.441Å, 7.755Å, grid space of 0.375Å. The binding energy scores were used to predict the efficiency with which the compounds would interact with the target proteins.[26] Visualization of the docked protein-ligand complex was processed by PyMOL 2.3 software.

Table 1. Effect of the seed extracts against *E. coli* and *L. rhamnosus*

Sample	Concen. (µg/disc)	*E. coli* (mm)	*L. rhamnosus* (mm)
Ampicillin	30	16 ± 1	25 ± 2
Methanolic extract	100	-	-
	500	-	-
	1000	24 ± 1	-
Aqueous extract	100	10 ± 1	-
	500	22 ± 1	-
	1000	23 ± 2	-

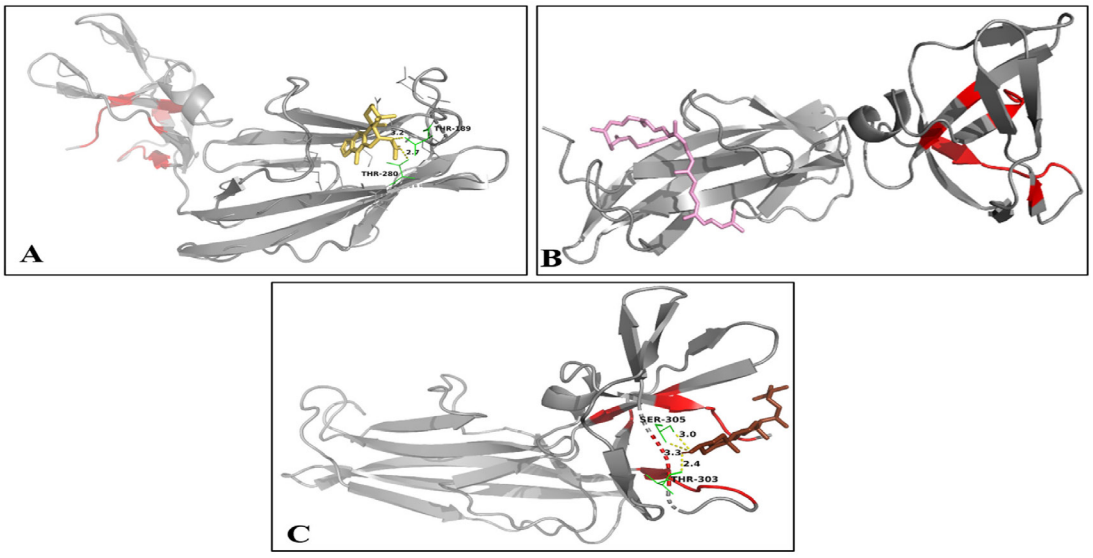


Figure 3. Binding of protein SlpX with compound A) Cassane furano-diterpene B) Lycopene C) Campesterol. (Red indicates active sites of protein; yellow indicates hydrogen bond; grey indicates the protein; yellow indicates Cassane furano-diterpene; pink indicates Lycopene; brown indicates Campesterol)

Table 2. The active pockets of the proteins SlpA and SlpX predicted with CASTp tool

Protein	Area (Å^2)	Volume (Å^3)	Binding pockets
SlpA (PDB:7QFG)	64.462	50.394	His323, Asn324, Ala325, Tyr326, Tyr328, Asn375, Ala377, Asn378, Gly415, Ala416
SlpX (PDB:7QFJ)	134.916	69.305	Gly294, Thr295, Leu296, Tyr297, Gly298, Asn299, Ile304, Val329, Ser342, Val344, Val359, Lys360, Thy361, Ser362

RESULTS AND DISCUSSION

C. bonducella has been widely used to manage disorders like PCOS, Diabetes mellitus, insulin resistance, bacterial infections, diarrhea, and urinary tract infections. Prior research has examined the seed kernel extract's ability to combat pathogenic bacteria, including gram-positive bacteria like *Bacillus subtilis*, as well as gram-negative bacteria such as *P. aeruginosa*, *E. coli*, and *P. mirabilis.*[28,29] However, the impact of the seed extract on the human gut bacteria has not been studied yet. Our study focused on the effect of *C. bonducella* seed against *L. rhamnosus* GG, the most prominent bacterium in the human gut.

The antibacterial assay was performed for *L. rhamnosus* GG and *E. coli* with 3 different concentrations of the methanolic and aqueous extracts of *C. bonducella* seed kernel (100, 500, and 1000 µg), with ampicillin (30 µg) as the positive control (Table 1). No zone of inhibition was observed for *L. rhamnosus* GG in both methanolic and aqueous extracts, whereas *E. coli* showed a distinct zone of inhibition for 1000 µg of methanolic extract and all 3 concentrations of aqueous extract, with an increase in the zone size in proportion to the concentration of the extracts (Figure 2). This states that growth of *L. rhamnosus* GG is not affected by both the seed extracts, whereas that of *E. coli* is affected.

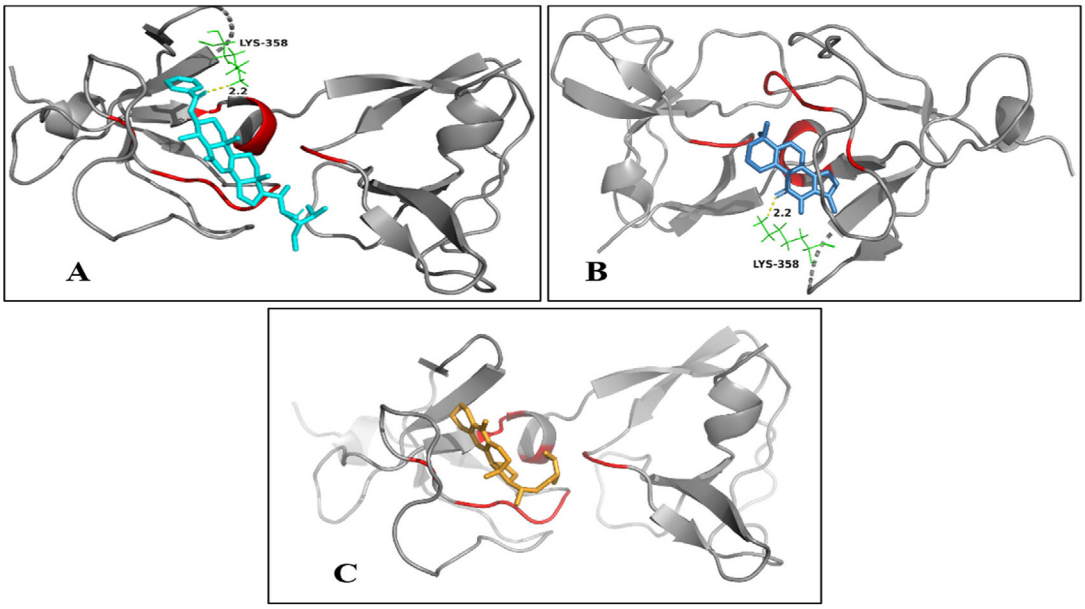
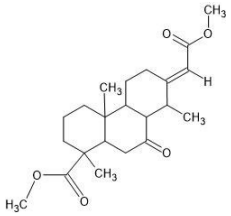
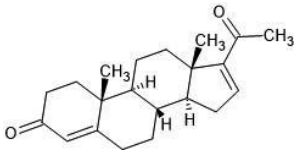
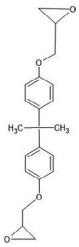
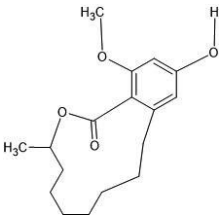
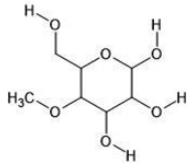
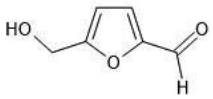
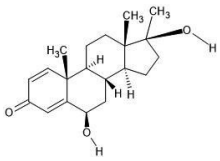
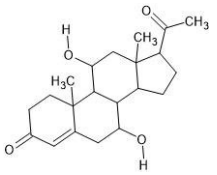
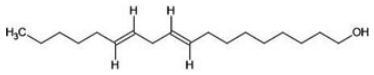
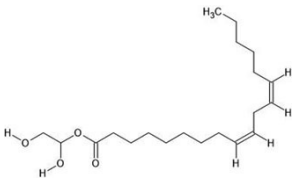
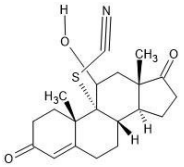
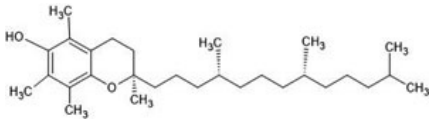
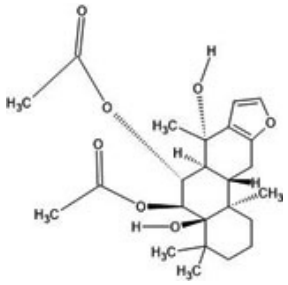
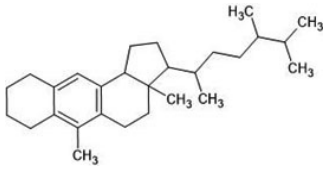
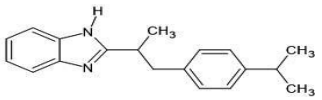
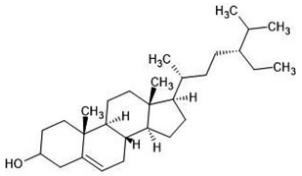
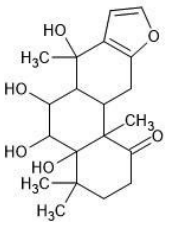
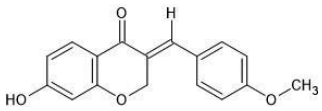
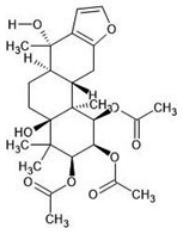
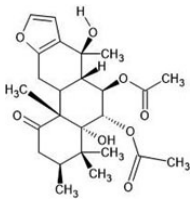
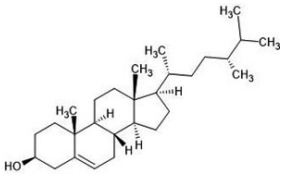
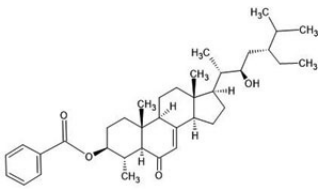
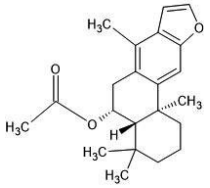
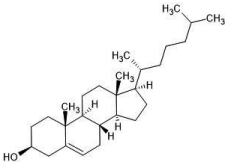


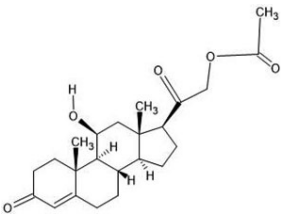
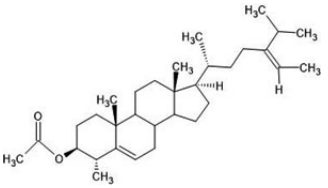
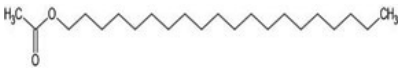
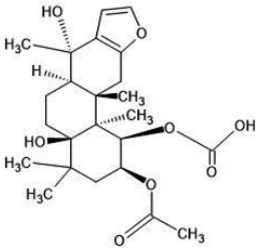
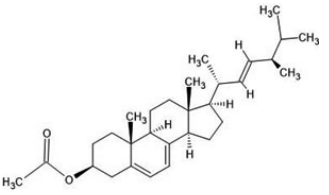
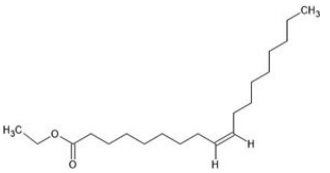
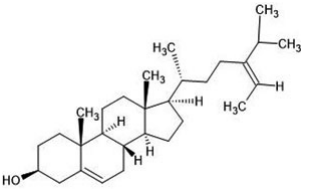
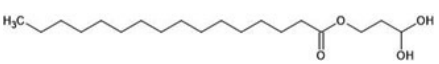
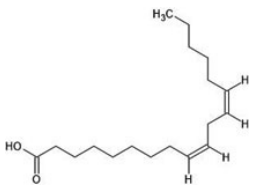
Figure 4. Binding of Protein SlpA with Compounds A) Carpesterol, B) Tanshinone IIA, and C) Anthraegostatrine. (Red indicates active sites of protein; yellow indicates hydrogen bond; gray indicates the protein; turquoise indicates carpesterol; blue indicates tanshinone IIA; orange indicates anthraegostatrine)

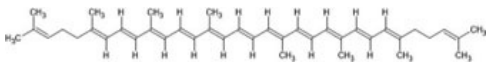
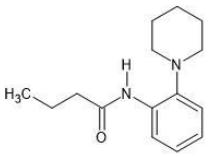
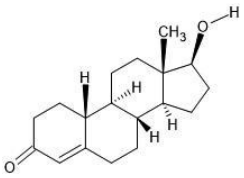
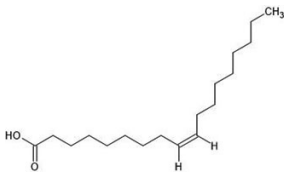
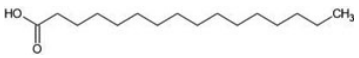
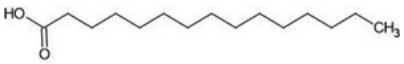
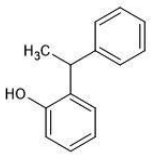
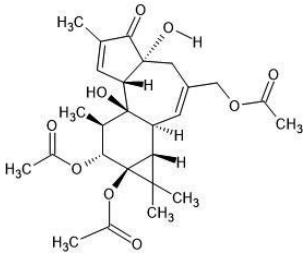
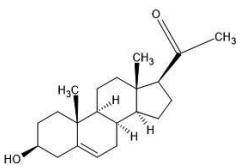
Table 3. Binding affinity of 54 phytocompounds of *Caesalpinia bonducella* seeds against SlpX and SlpA

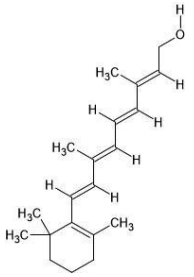
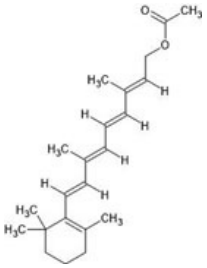
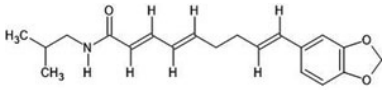
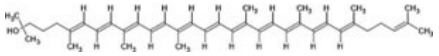
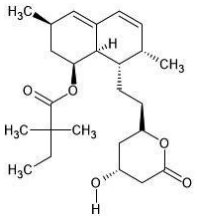
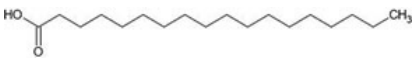
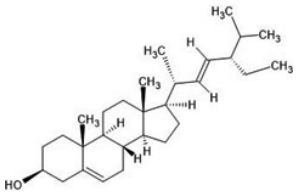
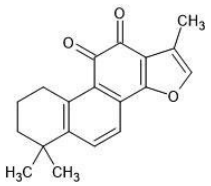
No.	Compound Name	Structure	Binding affinity SlpX	SlpA
1.	1-undecanol (8184)		-4.1	-4.3
2.	1-Phenanthrenecarboxylic acid tetradecahydro 7-(2-methoxy-2-oxoethylidene)-1, 4a,8-trimethyl-9-oxo-methyl ester (5379250)		-6.5	-4.5
3.	15,17,19,21-Hexatriacontatetrayne (537054)		-3.3	-4.1
4.	16-dehydroprogesterone (101964)		-6.3	-7.1
5.	2,4 bis (1 methyl 1 phenyl ethyl phenol (102877)		-6.4	-6.9
6.	2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13, 14-octahydro-6-oxabenzocyclododecen-5 -one (602765)		-6.7	-6.2
7.	4-o-methylmannose (345716)		-4.9	-4.9
8.	5-hydroxymethyl-furfural (237332)		-4.3	-4.5
9.	6b-Hydroxymethandienone (13241205)		-6.6	-7

10.	7,11-Dihydroxyprogesterone (539326)		-6.9	-6.6
11.	9,12-octadecadien-1-ol (5462912)		-3.9	-5
12.	9,12-Octadecadienoic acid (Z, Z) 2-hydroxy-1-(hydroxymethyl) ethyl ester (5365676)		-4.1	-4.4
13.	9,12-Octadecadienoic acid methyl ester (8203)		-4.5	-5.2
14.	Androst-4-en-11-ol-3,17-dione-9-thiocyanato- (91694000)		-6.7	-4.6
15.	Alpha-tocopherol (57393415)		-5	-6.4
16.	Alpha-caesalpin (21679154)		-6.4	-6.2
17.	Anthraegostatrine (313024)		-6.2	-7.3
18.	Benzimidazole 2-[1-(4-isopropylbenzyl) ethyl] (589515)		-6.6	-7.1
19.	Beta-sitosterol (222284)		-6.2	-6.2

20.	Beta-caesalpin (71440416)		-6.6	-6.5
21.	Bonducellin (14079439)		-6.1	-6.5
22.	Caesalpin F (101937720)		-5.7	-6.4
23.	Caesalpinin (15329770)		-6.3	-6.5
24.	Campesterol (173183)		-7	-6.7
25.	Carpesterol (21155918)		-6.7	-8.1
26.	Cassane furano-diterpenes (102286656)		-7.3	-7.2
27.	Cholesterol (5997)		-6	-6.6

28.	Corticosterone 21 acetate (255846)		-6.4	-6.3
29.	D5-avenasterol (91753899)		-6.5	-6.5
30.	Eicosyl acetate (110347)		-3.5	-3.7
31.	Epsilon-caesalpin (21679153)		-6.2	-7.2
32.	Ergosterol (6436903)		-6.9	-7
33.	Ethyl oleate (5363269)		-3.8	-4
34.	Fucosterol (5281328)		-6.7	-6.6
35.	Hexadecanoic_acid_2-hydroxy-1-(hydroxymethyl) _ethyl_ester (129853056)		-4.5	-4.1
36.	Linolei acid (5280450)		-4.7	-5.3

37.	Lycopene (446925)		-7.2	-6.7
38.	N-nonadecanol-1 (890281)		-4.4	-3.6
39.	Nandrolone (9904)		-6.5	-6.8
40.	Octacosane (12408)		-3.8	-3.3
41.	Oleic acid (445639)		-4.2	-4.2
42.	Palmitic acid (985)		-4.2	-4.5
43.	Pentadecanoic acid (13849)		-3.9	-3.8
44.	Phenol,2_(1_phenyl_ethyl) (95322)		-6.8	-7
45.	Phorbol 12,13,20 triacetate (499954)		-6	-6.4
46.	Pregnenolone (8955)		-6.2	-6.8

47.	Retinol (445354)		-6.2	-6.4
48.	Retinyl acetate (638034)		-6.3	-6.1
49.	Retrofractamide A (11012859)		-5.7	-6
50.	Rhodopin (5365880)		-5.1	-4.9
51.	Simvastatin (54454)		-6.6	-7.3
52.	Stearic acid (5281)		-4.1	-4.9
53.	Stigmasterol (5280794)		-6.4	-6.7
54.	Tanshinone IIA (164676)		-6.7	-7.8

The result shows that the seed kernel does not affect the growth of *L. rhamnosus* while inhibiting that of *E. coli.* We speculated that the exclusive surface layer proteins present in *Lactobacillus* species may be responsible for *L. rhamnosus* GG's protection against the phytocompounds. These surface layer proteins have been found in many *Lactobacillus* spp.,

including *L. brevis, L. buchneri, L. helveticus, L. hilgardii, L. acidophilus, L. amylovorus, L. crispatus, L. gallinarum,* and *L. rhamnosus.*[30,31] Numerous strains of *Lactobacillus* are capable of inhibiting pathogen adhesion to the intestinal epithelial cells and mucus with the help of their surface layer proteins, SlpX and SlpA, with molecular weights ranging from 30-130 kDa.[30,32] These proteins also assist these gut microbes to survive in a hostile gut environment that is rich in digestive enzymes and bile salts.[33]

In silico analyses were performed to investigate if the phytocompounds bind to the active binding pockets of the Slps. For this, the 3D structure of the two Slps were derived from the Protein Data Bank and was used to predict the binding pocket with the CASTp tool. The tool predicted 10 amino acid sites for SlpA protein and 14 amino acid sites for SlpX as binding pockets (Table 2). These two proteins were docked with the 54 phytocompounds in the seed kernel of *C. bonducella* that were retrieved from the previously reported literature and their binding affinity is listed in Table 3.

SlpA showed a higher binding affinity with compounds, including Carpesterol, tanshinone IIA, and anthraegostatrine, with a binding score of -8.1, -7.8, and -7.3 kcal/mol, respectively. The hydrogen bond interaction between the complex of both Carpesterol and tanshinone IIA with SlpA is at Lys358 with a length of 2.2Å, whereas anthraegostatrine did not show any hydrogen bond interaction with the protein (Figure 3). SlpX showed a higher binding affinity with compounds cassane furano-diterpenes, lycopene, and campesterol, with binding scores of -7.3, -7.2, and -7 kcal/mol, respectively. The hydrogen bond interaction between the complexes of cassane furano-diterpenes is with Thr189 and Thr280, with a length of 3.2Å and 2.7Å, respectively. Campesterol showed two hydrogen bond interactions at Thr303 with a length of 2.4Å and Ser305 with a length of 3.0Å, whereas lycopene had no hydrogen bond interaction with SlpX (Figure 4).

Although these compounds showed higher binding scores with the Slp proteins, their interaction with the Slp amino acid residues did not match the amino acid residues at the active binding pocket (Table 2). Thus, it shows that the phytocompounds do not bind tightly to the Slps and are hence prevented from entering the bacterial cell.

E. coli lacks these proteins and may be susceptible to the phytocompounds, thereby inhibiting its growth. Likewise, *Clostridium* spp*.,* having huge potential as a probiotic by producing metabolites including secondary bile acids, butyrate, acetic acid, and propionic acid, helps in strengthening and energizing intestinal epithelial cells and intestinal barrier,[34] and *Enterococcus* spp*.,* produces small peptides that are bacteriocin, having antimicrobial properties inhibiting the growth of *E. coli, P. aeruginosa*, *L. monocytogenes, and V. cholerae* in the human gut.[35] These *Clostridium* spp*.* and *Enterococcus* spp. families in the gut also possess Slp[35,36] that the seed's phytocompounds may not kill, and thus the gut microbiota will not be disturbed by consuming the seed kernel as a medicine. However, pathogenic bacteria like *S. aureus, P. aeruginosa, M. smegmatis, S. typhi, P. mirabilis, K. pneumoniae,* and *V. cholerae* lack Slps, making them vulnerable to and inhibited by the phytocompounds. Our finding put forth that *C. bonducella* seed extract affects those bacteria without Slp proteins and is safe against probiotic gut bacteria.

Limitations of this study

The study's scope is confined to only *L. rhamnosus*, hence, requires further validation with other gut bacteria to ascertain the safe use of *C. bonducella* seed for various disorders.

CONCLUSION

Caesalpinia bonducella seeds are used to treat various disorders, notably fever, edema, malaria, diabetes, and PCOS, and are shown to possess antibacterial properties against several pathogens. *Lactobacillus* is a prominent probiotic bacterium colonizing the human intestinal tract and plays a crucial role in preventing intestinal damage caused by certain bacterial infections. In our research, the impact of the seed extract of *C. bonducella* against *Lactobacillus rhamnosus* was tested where seed extract did not hinder the growth of the bacteria. Molecular docking of the surface layer proteins, SlpX and SlpA, to the phytocompounds of *C. bonducella* seed kernel,

revealed no binding to active binding pockets. This suggests that *C. bonducella* seed extracts are not toxic to *Lactobacillus* spp., which is prevalent in the gut microbiome.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BU contributed to the study's conception and design. AP and MK contributed equally to material preparation, data collection, and analysis. SMH performed molecular docking studies. AP and MK wrote the manuscript. BU edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

This research work was funded by the SRM Institute of Science and Technology, Kattankulathur, Chennai, India.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

REFERENCES

1. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Gomathi P, Rajeshwar Y. Antioxidant defense system induced by a methanol extract of Caesalpinia bonducella in rat liver. *J Pharmacol Sci.* 2005;43(5):411-419. doi: 10.1080/13880200590963439
2. Gaur RL, Sahoo MK, Dixit S, et al. Antifilarial activity of Caesalpinia bonducella against experimental filarial infections. *Ind J Med Res.* 2008;128:65-70
3. Lilaram, Ahmed RN. Effect of ethanolic seed extract of Caesalpinia bonducella on female reproductive system of albino rat: a focus on antifertility efficacy. *Asian Pacific J Trop Dis.* 2012;2(2):S957-S962. doi: 10.1016/S2222-1808(12)60300-4
4. Neogi NC, Nayak KP. Biological investigation of *Caesalpinia bonducella* Flem. *Indian J Pharmacol.* 1958;20:95-100. Accessed April 10, 2024.
5. Gayaraja S, Shinde S, Agarwal SL. Antiasthmatic properties of Caesalpinia bonduc leaves. *Indian J Pharmacol.* 1978;10:86-89. Accessed April 10, 2024.
6. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity: I. *Indian J. Exp. Biol.* 1968;6(4):232-247. https://pubmed.ncbi.nlm.nih.gov/5720682/. Accessed April 14, 2024.
7. Shukla S, Mehta A, Mehta P, Vyas SP, Shukla S, Bajpai VK. Studies on anti-inflammatory, antipyretic and analgesic properties of *Caesalpinia bonducella* F. seed oil in experimental animal models. *Food Chem Toxicol.* 2010;48(1):61-64. doi: 10.1016/j.fct.2009.09.015
8. Salunke KR, Ahmed RN, Marigoudar SR, Lilaram. Effect of graded doses of *Caesalpinia bonducella* seed extract on ovary and uterus in albino rats. *J Basic Clin Physiol Pharmacol.* 2011;22(1-2):49-53 doi: 10.1515/jbcpp.2011.006
9. Murugesan BM, Muralidharan P, Hari R. Effect of ethanolic seed extract of *Caesalpinia bonducella* on hormones in mifepristone induced PCOS rats. *J Appl Pharm Sci.* 2020;10(2),072-076. doi: 10.7324/JAPS.2020.102012
10. Sivasankari R, Usha B. Reshaping the gut microbiota through lifestyle interventions in women with PCOS: a review. *Indian J. Microbiol.* 2022;62(3),351-363. doi: 10.1007/s12088-022-01019-8
11. Rizk MG, Thackray VG. Intersection of polycystic ovary syndrome and the gut microbiome. *J Endocr Soc.* 2021;5(2):bvaa177. doi: 10.1210/jendso/bvaa177
12. Liu R, Zhang C, Shi Y, et al. Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. *Front Microbiol.* 2017;8:324. doi: 10.3389/fmicb.2017.00324
13. Billah MM, Islam R, Khatun H, et al. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of *Caesalpinia bonducella* (L.) Roxb leaves. *BMC Complement Altern Med.* 2013;13:1-7. doi: 10.1186/1472-6882-13-101
14. Kumar SRS, Shastri SL, Venkatesh R, Pradeepa K, Krishna V. Antibacterial potential of *Caesalpinia bonducella* extracts and their isolated phytoconstituents: *in vitro* and *in silico* analysis. *J Appl Biol Biotechnol*. 2019;7(3):41-46. doi: 10.7324/JABB.2019.70308
15. Arif T, Mandal TK, Kumar N, et al. *In vitro* and *in vivo* antimicrobial activities of seeds of *Caesalpinia bonduc* (Lin.) Roxb. *J Ethnopharmacol.* 2009;123(1):177-180. doi: 10.1016/j.jep.2009.02.040
16. Lagier JC, Khelaifia S, Alou MT, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol.* 20161(12):16203. doi: 10.1038/nmicrobiol.2016.203
17. Harmsen HJ, de Goffau MC. The human gut microbiota. Microbiota of the human body: Implications in health and disease. 2016;902:95-108. doi: 10.1007/978-3-319-31248-4_7
18. Rinninella E, Raoul P, Cintoni M, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms.* 2019;7(1),14. doi: 10.3390/microorganisms7010014
19. Huang R, Wu F, Zhou Q, et al. *Lactobacillus* and intestinal diseases: Mechanisms of action and clinical

applications. *Microbiol Res.* 2022;260:127019. doi: 10.1016/j.micres.2022.127019

20. Dempsey E, Corr SC. *Lactobacillus* spp. for gastrointestinal health: current and future perspectives. *Front Immunol.* 2022;13:840245. doi: 10.3389/fimmu.2022.840245
21. Desbonnet L, Clarke G, Traplin A, et al. Gut microbiota depletion from early adolescence in mice: Implications for brain and behavior. *Brain Behav Immun*. 2015;48:165-173. doi: 10.1016/j.bbi.2015.04.004
22. Liang S, Wang T, Hu X, et al. Administration of *Lactobacillus helveticus* NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neurosci J.* 2015;310:561-577. doi: 10.1016/j.neuroscience.2015.09.033
23. Yurtda G, Akdevelioglu Y. A new approach to polycystic ovary syndrome: the gut microbiota. *J Am Coll Nutr.* 2020;39(4):371-382. doi: 10.1080/07315724.2019.1657515
24. Martin R, Chamignon C, Mhedbi-Hajri N, et al. The potential probiotic *Lactobacillus rhamnosus* CNCM I-3690 strain protects the intestinal barrier by stimulating both mucus production and cytoprotective response. *Sci Rep.* 2019;9(1):5398. doi: 10.1038/s41598-019-41738-5
25. Liu W, Bao Q, Jirimupu, et al. Isolation and identification of lactic acid bacteria from Tarag in Eastern Inner Mongolia of China by 16S rRNA sequences and DGGE analysis. *Microbiol Res.* 2012 Jan 20;167(2):110-115. doi: 10.1016/j.micres.2011.05.001
26. Kandasamy V, Sathish S, Madhavan T, Balasundaram U. *In-Silico* Screening of Phytochemical Compounds in *Caesalpinia Bonducella* L. Seeds Against the Gene Targets of Ovarian Steroidogenesis Pathway. *J Microbiol Biotechnol Food Sci.* 2023;13(2):e6124-e6124. doi: 10.55251/jmbfs.6124
27. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res.* 2018;46(W1),W363-W367. doi: 10.1093/nar/gky473
28. Tambekar DH, Khante BS. Antibacterial properties of traditionally used medicinal plants for enteric infections by adivasi's (Bhumka) in melghat forest (Amravati District). *Int J Pharm Sci Res.* 2011;1(9):120-128. doi: 10.13040/IJPSR.0975-8232.1(9-S).120-28
29. Savaji P, Navanit S, Nayeem A. Phytochemical extraction and antibacterial studies of *Caesalpinia bonducella* seed extracts. *Mapana Journal of Sciences.* 2014;13(4):47-54. doi: 10.12723/mjs.31.5
30. Zhang W, Wang H, Liu J, Zhao Y, Gao K, Zhang J. Adhesive ability means inhibition activities for *Lactobacillus* against pathogens and S-layer protein plays an important role in adhesion. *Anaerobe.* 2013;22:97-103. doi: 10.1016/j.anaerobe.2013.06.005
31. Hynonen U, Palva A. *Lactobacillus* surface layer proteins: structure, function and applications. *Appl Microbiol Biotechnol.* 2013;97(12):5225-5243. doi: 10.1007/s00253-013-4962-2
32. Wakai T, Kano C, Karsens H, Kok J, Yamamoto N. Functional role of surface layer proteins of *Lactobacillus acidophilus* L-92 in stress tolerance and binding to host cell proteins. *Biosci Microbiota Food Health.* 2021;40(1),33-42. doi: 10.12938/bmfh.2020-005
33. Shi Z, Li X, Fan X, et al. The SlpX protein plays a crucial role in the intestinal juice tolerance of *Lactobacillus acidophilus* CICC6074. *Food Biosci.* 2024;59(4):103865. doi: 10.1016/j.fbio.2024.103865
34. Guo P, Zhang K, Ma X, He P. *Clostridium* species as probiotics: potentials and challenges. *J Animal Sci Biotechnol.* 2020;11:24. doi: 10.1186/s40104-019-0402-1
35. He Y, Xu X, Zhang F, et al. Anti-adhesion of probiotic *Enterococcus faecium* WEFA23 against five pathogens and the beneficial effect of its S-layer proteins against *Listeria monocytogenes*. *Can J Microbiol.* 2019;65(3):175-184. doi: 10.1139/cjm-2018-0031
36. Fagan RP, Fairweather NF. Biogenesis and functions of bacterial S-layers. *Nat Rev Microbiol.* 2014;12(3):211-222. doi: 10.1038/nrmicro3213