Research Article

Radhakrishnan Narayanaswamy*, Vasantha-Srinivasan Prabhakaran, Fatimah Oleyan Al-Otibi, Raedah Ibrahim Alharbi, Kalibulla Syed Ibrahim

Molecular docking analysis of subtilisin-like alkaline serine protease (SLASP) and laccase with natural biopolymers

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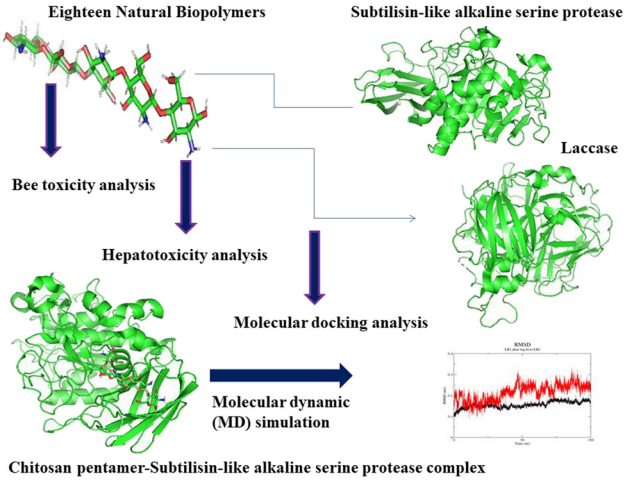
Abstract: Alkaline serine proteases (ASPs) and laccases (Lacs) have been reported to possess several industrial applications, particularly in the food, cosmetic, and leather industries. Thus, in the present study, eighteen natural biopolymers, including agar, agarose, alginate, kappa carrageenan, cellulose, chitosan pentamer, chitosan oligosaccharide, chondroitin sulfate, dextran, fucoidan, heparin, hyaluronan, lignin, mannan, pectin, phytic acid, pullulan and starch soluble, were studied for their docking behavior, such as subtilisin-like alkaline serine protease (SLASP) KP-43 and *Bacillus subtilis* laccase (Bs Lac), using the SwissDock method. Additionally, the toxicity toward honey bees and human liver was determined by utilizing the free Bee-Tox and pkCSM web servers, respectively. Bee-Tox analysis demonstrated that four ligands (namely, agar, cellulose, lignin, and pullulan) exhibited acute oral toxicity toward honey bees (*Apis mellifera*). The docking study revealed that chitosan pentamer and lignin exhibited maximum binding energies of −9.67 and −11.37 kcal/mol against the target proteins SLASP (KP-43) and Lac (Bs Lac), respectively. Interestingly, in the present study, agarose was shown to interact with the His68 and Ser255 amino acid residues of SLASP (KP-43) from KSM-KP43 *Bacillus* sp. Thus, the current investigation showed the potential of eighteen natural biopolymers as immobilizing agents to prepare ASP and Lac nanoparticles for biomedical applications.



Graphical abstract

Keywords: molecular docking, natural biopolymer, subtilisin-like alkaline serine protease, *Bacillus subtilis* laccase, chitosan, nanoparticles, MD simulation

*** Corresponding author: Radhakrishnan Narayanaswamy,** Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (Deemed to be University), Thandalam, Chennai, Tamil Nadu, 602 105, India, e-mail: radhakrishnann.smc@saveetha.com
Vasantha-Srinivasan Prabhakaran: Department of Bioinformatics, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (Deemed to be University), Thandalam, Chennai, Tamil Nadu, 602 105, India
Fatimah Oleyan Al-Otibi, Raedah Ibrahim Alharbi: Department of Botany and Microbiology, College of Science, King Saud University, Post Box – 22452, Riyadh, 11451, Saudi Arabia
Kalibulla Syed Ibrahim: Department of Biotechnology, PSG College of Arts and Science, Civil Aerodrome Post, Coimbatore, Tamil Nadu, 641 014, India
ORCID: Radhakrishnan Narayanaswamy 0000-0001-5259-761X

1 Introduction

Enzymes are proteins in nature that serve as biocatalysts by enhancing biochemical reactions in living organisms. For a long time, it has been utilized in the production of various products, such as beer, cheese, sourdough, vinegar, and wine, and in the manufacturing of materials, including indigo, leather, and linen. Enzymes from microbial sources are involved in various metabolic reactions and are utilized in different industrial applications. Currently, enzymes, especially those utilized in manufacturing, are hydrolytic in

nature and are utilized for the breakdown of large substances into smaller substances [1]. For instance, microbial proteases catalyze protein hydrolysis into polypeptides and oligopeptides along with amino acids (AAs). Proteases are classified into three types on the basis of pH: (i) acidic, (ii) neutral, and (iii) alkaline protease (AlP). AlP is a widely utilized industrial enzyme owing to its relatively high activity and high stability at basic pH [2]. Moreover, proteases alone contribute to approximately 60% of the global enzyme sales. Furthermore, *Bacillus* species serve as major enzyme producers [3]. Similarly, proteases are distinguished into four important groups on the basis of AA active site residues along with catalytic actions: (i) aspartic, (ii) cysteine, (iii) metallo, and (iv) serine proteases. Among them, serine proteases are a widespread group of proteolytic enzymes from microbial and animal sources [4]. Moreover, alkaline serine proteases (ASPs) are a widely utilized group of commercial manufacturing enzymes, especially in meat, detergent, and leather production. Furthermore, ASPs are recognized as microbial enzymes with greater market value (>35%) [5]. Macquarrie and Bacheva have reported that subtilisin immobilized in chitosan has shown high catalytic activity and good stability nature [6]. Eser and Aydemir have demonstrated that subtilisin immobilized in magnetic chitosan has been utilized for eco-friendly leather preparation process [7]. Shettar et al. have reported that subtilisin entrapped in calcium alginate beads has exhibited superior storage longevity, recyclability, and confirming its practical viability. Thus, in the present study, subtilisin-like alkaline serine protease (SLASP) from *Bacillus* sp. strain KSM-KP43 was chosen as the first enzyme target [8].

Laccases (Lacs) (copper-containing enzymes) are involved in (i) the biodegradation of organic pollutants, (ii) bioremediation, (iii) cosmetics, (iv) the food sector, (v) the paper and pulp sector, and (vi) synthetic chemistry applications. In recent years, the Lac enzyme has been shown to play a vital role in nanobiotechnology owing to its unique characteristic ability to catalyze electron transfer reactions (rxs) without any additional cofactors. Lac has been immobilized using different immobilization agents, such as carbon fibers, ceramics, and pyrolytic graphite [9]. Ping et al. have reported that Lac immobilized in alginate/gelatin mixed with polyethylene glycol has been applied in decolorization of reactive dyes [10]. Unal and Pazarlioglu have demonstrated that Lac immobilized in gelatin has been utilized for analysis of phenolic contents present in commercially prepared fruit juices [11]. Mogharabi et al. have reported that Lac immobilized in alginate-gelatin mixed gel has been used for the decolorization of synthetic dyes [12]. Rocasalbas et al. have demonstrated that Lac entrapped in chitosan/gelatin hydrogel has exhibited antibacterial activity against both *Pseudomonas aeruginosa* and *Staphylococcus aureus* [13]. Khalighi et al. have reported that Lac immobilized in sugar beet fiber (fibrex) gel has been applied in pharmaceutical and several other industrial sectors [14]. Harguindeguy et al. have demonstrated that Lac immobilized in gelatin has been employed as biocatalyst for waste water treatment process [15]. Therefore, in the present study, *Bacillus subtilis* laccase (Bs Lac) was selected as the second target enzyme.

Natural biopolymers are biologically acceptable substances or biomaterials that can be utilized as structures in tissue engineering applications and regenerative medicine. Natural biopolymers such as chitosan, collagen, fibrin, gelatin, and silk fibroin have been utilized as biocompatible scaffolds (platforms) to fulfill the relevant biological and mechanical properties required for tissue engineering and regenerative medicine applications [16]. Similarly, natural biopolymers such as (i) albumin (protein), (ii) alginate (polysaccharide), (iii) apoferritin (protein), (iv) beta-glucan (polysaccharide), (v) casein (protein), (vi) chitosan (polysaccharide), (vii) collagen (protein), (viii) cyclodextrin (oligosaccharide), (ix) dextran (polysaccharide), (x) fibrinogen (glycoprotein), (xi) fucoidan (polysaccharide), (xii) gelatin (protein), (xiii) heparin (polysaccharide), (xiv) hyaluronic acid (polysaccharide), (xv) lectin (glycoprotein), (xvi) mannan (polysaccharide), (xvii) pullulan (polysaccharide), (xviii) starch (polysaccharide), and (xix) zein (protein) have been commonly utilized for the preparation of nanoparticles [17].

The current investigation aims to impart an understanding of the mechanistic basics for the *in silico* binding of eighteen natural biopolymers with two target enzymes from *Bacillus* species. And, moreover, the present investigation objective was to reveal insights about the binding efficiency of the two target enzymes (SLASP and Bs Lac) with eighteen selected natural biopolymers which include agar, agarose, alginate, kappa carrageenan, cellulose, chitosan pentamer, chitosan oligosaccharide, chondroitin sulfate, dextran, fucoidan, heparin, hyaluronan, lignin, mannan, pectin, phytic acid, pullulan, and starch soluble by utilizing molecular docking approaches which in turn pave way for biomedical applications.

2 Materials and methods

2.1 Ligand preparation

The biochemical structures of selected ligands, namely, (i) agar (ID 71571511), (ii) agarose (ID 11966011), (iii) alginate (ID 131704328), iv) kappa carrageenan (ID 11966249), (v) cellulose (ID 14055602), (vi) chitosan pentamer* (0C09269), (vii) chitosan oligosaccharide (ID 3458144), (viii) chondroitin

sulfate (ID 24766), (ix) dextran (ID 4125253), (x) fucoidan (ID 129532628), (xi) heparin (ID 22833565), (xii) hyaluronan (ID 24759), (xiii) lignin (ID 73555271), (xiv) mannan (ID 25147451), (xv) pectin (441476), (xvi) phytic acid (ID 890), (xvii) pullulan (ID 131636581), and (xviii) starch soluble (ID 51003661), were downloaded from the PubChem (www.pubchem.com) and Biosynth* (www.biosynth.com) databases. These ligands were selected as adapted from previous research [18]. Mol2 files are given for each ligand (natural biopolymer), Chemistry at Harvard Macromolecular Mechanics (CHARMM) topology, parameters, and coordinates; files proceed spontaneously from the Merck Molecular Force Field (MMFF). These prepared structures were used for further study (SwissDock).

2.2 Prediction of bee toxicity

Bee-Tox (www.beetoxai.labmol.com.br) is a free web server based on artificial intelligence technology that is currently utilized to predict the acute "oral and contact" toxicity of ligands/chemicals to honey bees [19]. In the present research, the toxicity of 18 selected chemicals (natural biopolymers) toward honey bees (*Apis mellifera*) and human liver was assessed using the Bee-Tox (LabMol) and pkCSM (www.biosig.lab.uq.edu.au/pkcsm/) free web servers, respectively [20].

2.3 Identification and preparation of target enzymes

The three-dimensional structures of the SLASP from the KP-43 strains of the *Bacillus* species KSM-KP43 (Protein Data Bank [PDB] ID: 1WME) and Bs Lac (PDB ID: 3ZDW) were obtained from the PDB. The "A" chains of both enzymes were prepared separately by eliminating ligands and crystallographic water molecules (water deprived of hydrogen bonds), as observed by utilizing UCSF Chimera software [21].

2.4 Docking investigation

Docking investigations were carried out for eighteen selected natural biopolymers using the SwissDock online server. The SwissDock web server is executed with open-source technologies (Apache web server, Hypertext Preprocessor) and, moreover, it uses the EADock DSS engine. Calculations are currently accomplished on 32 computing nodes with Dual Xeon E5440 2.83 GHz and 16 GB of RAM, computing for a total of 256 CPU and 512Go of RAM, which pertain to the Molecular Modeling group of the Swiss Institute of Bioinformatics and are integrated into the Vital-IT cluster. The docking assays are performed in the Chemistry at Harvard Macromolecular Mechanics (CHARMM22/27) all-hydrogen force field [22]. Finally, interaction site investigation was carried out by using PyMOL software.

2.5 Molecular dynamics (MD) simulation

The MD simulation of the protein-ligand complex was performed with GROMACS using NMRbox virtual machines [23,24] simulation and OPLS-AA/M force field. The molecular topology file of the ligand compatible with the OPLS-AA/M force field was obtained from LigParGen web service [25]. The protein-ligand complex was solvated with Simple Point Charge water (SPC) and the complete system was neutralized with the addition of chlorine (Cl^- ions) by replacing the water molecules. The energy minimization of the system was performed with the steepest gradient algorithms (5,000 cycles), which was then followed by equilibration of the system using two consecutive NVT (constant number of particles (NPT), volume, and temperature) and (NPT, pressure, and temperature are all constant) runs. Finally, the complex was subjected to a 100 ns MD simulation. The simulation trajectories were then analyzed based on the root mean square deviations, the root mean square fluctuations, solvent accessible surface area (SASA), and radius of gyration [26].

3 Results and discussion

Proteases usually play a key role in enhancing wash performance parameters in the formulation of detergents [27]. These proteases (detergent) fit into the serine protease family, which cleaves peptide bonds in proteins, and they catalyze biochemical reactions using a catalytic triad covering histidine (His), aspartate (Asp), and serine (Ser). The presence of individual AAs (Asp–His–Ser) in the catalytic triad plays a specific role in the catalytic process [28]. The considerable production of microbial ASP on an industrial or large scale could be achieved by suitable downstream processing and extraction methods. However, in some cases, it is tedious and difficult to achieve good enzyme yield. The immobilization of ASPs on different nanomatrices or

Table 1: The "simplified molecular input line entry system" (SMILES) of eighteen selected natural biopolymers is shown

Ligand name	"Simplified molecular input line entry system" (SMILES)
Agar	"CC1C(C2C(C(O1)CO2)OC3C(C(C(C(O3)CO)O)OC)O)O"
Agarose	"C1C2C(C(O1)C(C(O2)O)O)OC3C(C(C(C(O3)CO)O)OC4C(C5C(C(O4)CO5)OC6C(C(C(C(O6)CO)O)O)O)O)O)O"
Alginate	"C1(C(C(OC(C1OC2C(C(C(C(O2)C(=O)O)P)O)O)C(=O)O)OP)O)O"
Kappa carrageenan	"C1C2C(C(O1)C(C(O2)O)O)OC3C(C(C(C(O3)CO)OS(=O)(=O)[O])OC4C(C5C(C(O4)CO5)OC6C(C(C(C(O6)CO)OS(=O)(=O)[O-])O)O)O)O"
Cellulose	"COC1C(OC(C(C1O)O)OC2C(OC(C(C2O)O)OC)CO)CO"
Chitosan pentamer	"Cl.Cl.Cl.Cl.Cl.NC1C(O)OC(CO)C(OC2OC(CO)C(OC3OC(CO)C(OC4OC(CO)C(OC5OC(CO)C(O)C(O)C5N)C(O)C4N)C(O)C3N)C(O)C2N)C1O"
Chitosan oligosaccharide	"C(C1C(C(C(C(O1)OC2C(OC(C(C2O)N)O)CO)N)O)O)O"
Chondroitin sulfate	"CC(=O)NC1C(C(C(OC1O)OS(=O)(=O)O)O)OC2C(C(C(C(O2)C(=O)O)O)O)O"
Dextran	"C(C1C(C(C(C(O1)OCC2C(C(C(C(O2)OCC(C(C(C(C=O)O)O)O)O)O)O)O)O)O)O)O"
Fucoidan	"CC1C(C(C(C(O1)C)OS(=O)(=O)O)O)OC"
Heparin	"CC(=O)NC1C(C(C(OC1OC2C(C(C(OC2C(=O)O)OC3C(C(C(OC3O)OC4C(C(C(OC4C(=O)O)O)OS(=O)(=O)O)O)OS(=O)(=O)O)CS(=O)(=O)O)O)O)COS(=O)(=O)O)O)O"
Hyaluronan	"CC(=O)NC1C(C(C(OC1O)CO)O)OC2C(C(C(C(O2)C(=O)O)OC3C(C(C(C(O3)CO)O)OC4C(C(C(C(O4)C(=O)O)O)O)O)NC(=O)C)O)O"
Lignin	"CC(C(C1=CC2=C(C(=C1)OC)OC(C2CO)C3=CC(=C(C=C3)OC(CO)C(C4=CC(=C(C=C4)O)OC)OC5=C(C=C(C=C5OC)C(C(CO)OC6=C(C=C(C=C6)C7C8COCC8C(O7)C9=CC(=C(C(=C9)OC)O)OC)OC)OC1=C(C=C(C=C1)C(C(CO)OC1=C(C=C(C=C1)C=CCO)OC)O)OC)O)OC)O)O"
Mannan	"C(C1C(C(C(C(O1)OC2C(OC(C(C2O)O)OC3C(OC(C(C3O)O)OC4C(OC(C(C4O)O)O)CO)CO)CO)O)O)O)O"
Pectin	"C1(C(C(OC(C1O)O)C(=O)O)O)O"
Phytic acid	"C1(C(C(C(C(C1OP(=O)(O)O)OP(=O)(O)O)OP(=O)(O)O)OP(=O)(O)O)OP(=O)(O)O)OP(=O)(O)O"
Pullulan	"COCC1C(C(C(C(O1)OC2C(OC(C(C2O)O)OC3C(OC(C(C3O)O)OC)CO)CO)O)O)O"
Starch soluble	"C(C1C(C(C(C(O1)OC2C(OC(C(C2O)O)O)CO)O)O)O)O"

nanoparticles will be able to resolve the abovementioned problems by (i) enhancing the product yield, (ii) improving the shelf life, (iii) preventing the agglutination, self-decomposition, and denaturation of ASPs, and (iv) ensuring the long-lasting shelf life/stability of immobilized ASPs over nanoparticles or nanomatrices [29].

Table 2: The honey bee (*A. mellifera*) and human liver toxicity analysis of eighteen selected natural biopolymers using Bee-Tox (LabMol) and pkCSM (Biosig Lab) free web servers

Ligand name	Acute oral toxicity (LD50$^{\diamond}$)	Acute contact toxicity	Hepatotoxicity
Agar	Toxic (7.37 µg/bee$^{\diamond}$)	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Agarose	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Alginate	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Kappa carrageenan	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Cellulose	Toxic (15.69 µg/bee$^{\diamond}$)	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Chitosan pentamer	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Chitosan oligosaccharide	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Chondroitin sulfate	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Dextran	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Fucoidan	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Heparin	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Hyaluronan	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Lignin	Toxic (13.14 µg/bee$^{\diamond}$)	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Mannan	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Pectin	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Phytic acid	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Pullulan	Toxic (24.58 µg/bee$^{\diamond}$)	NT$^{\blacksquare}$	NT$^{\blacksquare}$
Starch soluble	NT$^{\blacksquare}$	NT$^{\blacksquare}$	NT$^{\blacksquare}$

Note: LD50$^{\diamond}$ – expressed in micrograms/bee, NT$^{\blacksquare}$ – nontoxic.

Table 3: Interaction energy analysis of eighteen selected natural biopolymers with SLASP KP-43 from the *Bacillus* species KSM-KP43 using the SwissDock method

Ligand name	SwissDock interaction energy (−kcal/mol)	Interaction amino acid (AA) residue	Bond distance (Å)
Agar	7.17	Gly214	2.3
		Trp393	2.3 and 2.4
		Gly395	3.1
		Asn397	3.4
Agarose	9.52	His68	3.2
		Asn162	2.5, 3.1 and 3.3
		Glu163	1.8
		Ser239	2.2
		Trp241	3.3 and 3.4
		Ser255	3.2 and 3.4
Alginate	8.23	Asn383	2.4
		Asp394	1.9 and 3.4
Kappa carrageenan	8.82	Ala293	3.3
		Asp295	3.5
		Arg309	3.1
		Lys314	3.2 and 3.3
		Lys343	3.4
		Lys346	3.0
Cellulose	7.42	Asn405	2.1 and 3.0
Chitosan pentamer	9.67	Asp295	2.1
		Tyr300	3.4
		Tyr389	3.2
		Phe403	2.7
		Asn405	2.0, 3.1 and 3.5
		Ala406	2.4
Chitosan oligosaccharide	7.90	Gly214	2.2 and 2.3
		Asn383	2.7
		Trp393	2.2
		Asp394	2.0
		Gly395	3.0
		Asp397	3.4
Chondroitin sulfate	8.01	Leu104	3.2
		Ser129	2.7
		Gly131	3.2
		Asn162	3.1
		Glu163	1.9 and 1.9
		Ser171	2.0 and 3.5
Dextran	9.42	Asp295	1.8
		Lys346	3.3
		Phe385	2.0
		Tyr389	2.2, 3.0 and 3.4
		Phe403	2.0
		Asn405	3.4 and 3.4
		Ala406	2.9
Fucoidan	7.04	Asp384	2.5
		Thr386	3.2 and 3.5
Heparin	8.29	Tyr300	3.2
		Asn376	3.1
		Thr378	3.5 and 3.6
		Tyr380	2.0, 3.3 and 3.4
		Tyr389	3.0
		Asn405	3.4
		Ala406	2.2
		Glm408	3.2
Hyaluronan	8.38	Asp295	2.1
		Leu298	2.3
		Tyr380	2.9
		Phe403	2.1
		Asn405	3.3
Lignin	8.63	Lys343	3.0 and 3.3
		Asn434	2.0 and 2.9
Mannan	7.73	Tyr300	2.1
		Tyr380	2.0 and 3.0
		Phe403	2.2
		Asn405	3.4
		Gln408	3.1
Pectin	6.54	Leu364	1.9 and 2.0
		Asp367	1.9 and 3.3
		Asn420	3.3
Phytic acid	8.81	Asn383	1.9 and 3.2
		Phe385	3.1
		Trp393	2.1
		Asp394	2.0
		Asn397	3.3
Pullulan	8.03	Gly131	2.2
		Ala133	3.2 and 3.2
		Asn162	3.0, 3.2 and 3.3
		Glu163	2.1
		Ser171	3.4
Starch soluble	7.59	Gly214	1.8 and 2.2
		Asn383	2.0
		Asp384	2.1
		Phe385	3.0
		Thr386	2.5 and 3.3
		Asn397	3.1

Lac (a copper-containing enzyme) is an extensively studied enzyme worldwide. Moreover, Lac is recognized as a “green tool” owing to its potential to carry out catalytic reactions by utilizing molecular oxygen as a co-substrate [30].

Thus, natural biopolymers such as alginate, cellulose, chitosan pentamer, chitosan oligosaccharide, chondroitin sulfate, dextran, fucoidan, hyaluronan, mannan, pullulan, and starch soluble (as shown in Table 1) were chosen for

the present research to assess the behavior of docking on ASP and Lac.

Bee-Tox analysis demonstrated that four ligands (namely, agar, cellulose, lignin and pullulan) exhibited acute oral toxicity toward honey bees. However, none of the eighteen selected natural biopolymers (ligands) exhibited any acute contact toxicity toward honey bees. Similarly, none of the eighteen chosen natural biopolymers (ligands) have shown any hepatotoxicity activity (as shown in Table 2).

A numerous studies have been carried out to understand the binding efficiency of the modeled protease (enzyme), especially from *Bacillus* species with various substrates by molecular docking approaches [28,31]. The present docking investigation and binding energy [BE] (Table 3) showed that the chitosan pentamer exhibited the maximum BE (−9.67 kcal/mol); additionally, pectin had the least energy binding (−6.54 kcal/mol) against the targeted protein (SLASP KP-43). The BE analysis of the current investigation showed the following order: chitosan pentamer (−9.67 kcal/mol), <agarose (−9.52 kcal/mol), <dextran (−9.42 kcal/mol), <kappa carrageenan (−8.82 kcal/mol), <phytic acid (−8.81 kcal/mol), <lignin (−8.63 kcal/mol), <hyaluronan (−8.38 kcal/mol), <heparin (−8.29 kcal/mol), <alginate (−8.23 kcal/mol), <pullulan (−8.03 kcal/mol), <chondroitin sulfate (−8.01 kcal/mol), <chitosan oligosaccharide (−7.90 kcal/mol), <mannan (−7.73 kcal/mol), <starch soluble (−7.59 kcal/mol), <cellulose (−7.42 kcal/mol), <agar (−7.17 kcal/mol), fucoidan (−7.04 kcal/mol), and <pectin (−6.54 kcal/mol). Previously, we reported that natural biopolymers, including (i) alginate, (ii) albumin, (iii) apoferritin, (iv) beta-glucan, (v) chitosan, (vi) casein, (vii) cyclodextrin, (viii) collagen, (ix) dextra, (x) fucoidan, (xi) fibrinogen, (xii) gelatin, (xiii) hyaluronic acid, (xiv) heparin, (xv) lectin, (xvi) mannan, (xvii) pullulan, (xviii) starch, and (xix) zein, have been utilized as contrast agents for magnetic resonance (MR) imaging [17]. According to Rezakhani et al., the protease immobilized in alginate-chitosan beads (A–C) exhibited fairly stable and significant enzyme activity [32].

Alnoch et al. reported the use of biomaterials, including cellulose, agarose, chitin, cellulose, chitosan, and starch, for lipase immobilization [33]. Prokopijevic reported that natural biopolymers (alginate, cellulose, chitosan, collagen, xylan, and pectin) can be utilized as carriers for enzyme immobilization processes [34]. Sharma et al. reported the utilization of natural polysaccharides such as alginate, carrageenan, cellulose, chitin, pectin, and starch in various enzyme immobilization processes [35]. Similarly, Lyu et al. reported the use of natural polymers such as alginate, agarose (lyoxylagarose, amino-glyoxylagarose, carboxy-glyoxylagarose, and chelate-glyoxylagarose), cellulose, chitosan, dextran and pectin in various enzyme immobilization processes [36]. In recent years, computational techniques have been utilized for analyzing nanoparticle interactions with biomolecules, including enzymes [37]. Borkotoky et al. reported that heparin (mucopolysaccharide) and low molecular weight derivatives of heparin interact with the T7 RNA polymerase enzyme [38]. Narayanaswamy reported that

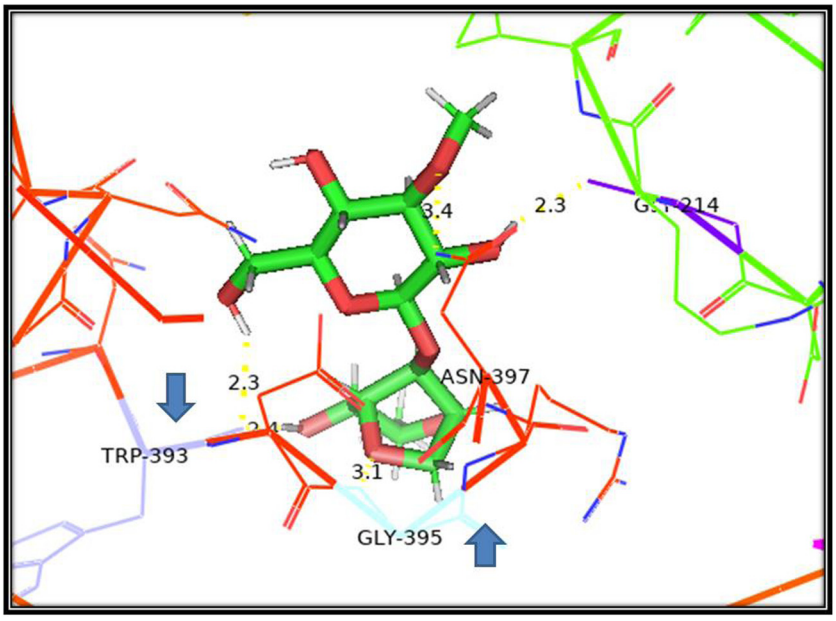


Figure 1: The interaction energy analysis of agar (a natural biopolymer) with the *Bacillus* species KSM-KP43 SLASP KP-43 was performed using the SwissDock method. Note: ↓ (down arrow) indicates the Trp393 amino acid residue, and ↑ (up arrow) indicates the Gly395 amino acid residue.

Table 4: The interaction energy analysis of eighteen selected natural biopolymers with Bs Lac using the SwissDock method

Ligand name	SwissDock interaction energy (−kcal/mol)	Interaction amino acid (AA) residue	Bond distance (Å)
Agar	7.36	Thr260	2.1
		Thr262	3.0
		Thr415	3.1
		Gly417	3.3
		His419	3.4
Agarose	8.58	Thr262	3.0
		Gly321	2.0
		Gly323	3.3
		Gly324	3.5
		Arg416	3.5
		Thr418	3.5
Alginate	8.04	Thr262	3.2
		Gly321	2.6
		Thr415	3.5
		Gly417	3.3
		Thr418	3.0 and 3.3
		His419	3.1
		His497	3.4
Kappa carrageenan	10.01	Thr262	3.0
		Gly321	3.2
		Arg416	2.9 and 3.1
		His497	3.1
Cellulose	7.48	Gly321	2.0
		Thr418	2.0, 2.0, 3.1 and 3.2
Chitosan pentamer	9.00	Ala227	2.3
Chitosan oligosaccharide	7.23	Gly323	3.4
		Thr418	1.9 and 2.1
Chondroitin sulfate	8.18	Thr260	3.0
		Thr262	3.0 and 3.3
		Gly321	2.2
		Thr415	3.4
		Thr418	2.9
		His419	2.2
Dextran	7.96	Gly323	3.4
		His497	3.2
Fucoidan	7.29	Thr415	3.1
		Gly417	3.2
		Thr418	2.0
		His419	2.1
Heparin	8.91	Thr260	2.7
		Thr262	2.9 and 3.4
		Gly323	3.4
		Gly417	3.0
		Thr418	3.3
Hyaluronan	9.25	Thr262	2.8
		Gly323	3.2
		His497	2.2 and 2.3
Lignin	11.37	Ala227	2.2
		Gly323	3.0
		Gly324	3.5
		Arg416	3.2
		Thr418	2.4, 3.1 and 3.1
Mannan	8.27	Thr260	2.5
		Thr262	3.4
		Gly323	3.1
		Gly376	2.4
		Thr418	2.7, 3.1 and 3.4
Pectin	6.50	Gly230	2.5
		Arg261	3.1
		Cys322	1.9
		Asp325	1.8
		Asn327	3.3
		Thr330	3.4
Phytic acid	8.41	Pro414	3.4
		Thr415	3.4
		Arg416	3.2 and 3.4
		Gly417	3.0
		His419	3.4
Pullulan	8.50	Gly417	3.3
		His419	3.2
Starch soluble	7.59	Thr260	2.0
		Thr262	3.1 and 3.3
		Thr418	2.5

alginate oligosaccharides (Alg2–Alg6) interact with bacterial collagenase enzymes [39]. Maksimenko et al. reported that glycosaminoglycan ligands, namely, chondroitin sulfate (trimer) and heparin (tetramer), interact with the enzyme bovine testicular hyaluronidase [40]. Franco and Mesa utilized an *in silico* approach to analyze the interactions of chitosan and carboxy methyl chitosan with the beta-galactosidase enzyme [41]. Omar et al. reported that biopolymers (such as cellulose, chitosan, gelatin, and starch), as well as their blends (such as chitosan/cellulose, chitosan/gelatin, and chitosan/starch), interact with the HIV protease enzyme [42]. Thus, the current investigation demonstrated the potential of eighteen selected natural biopolymers as immobilizing agents for the preparation of ASP nanoparticles.

Two ligands, namely, fucoidan and starch soluble, interact with the Asp384 and Thr386 amino acid residues (AAR) of the protein (SLASP KP-43), respectively. Similarly, three other ligands, agarose, chondroitin sulfate, and pullulan, interact with the AARs Asn162 and Glu163 of the protein (SLASP KP-43). Furthermore, four ligands (chitosan

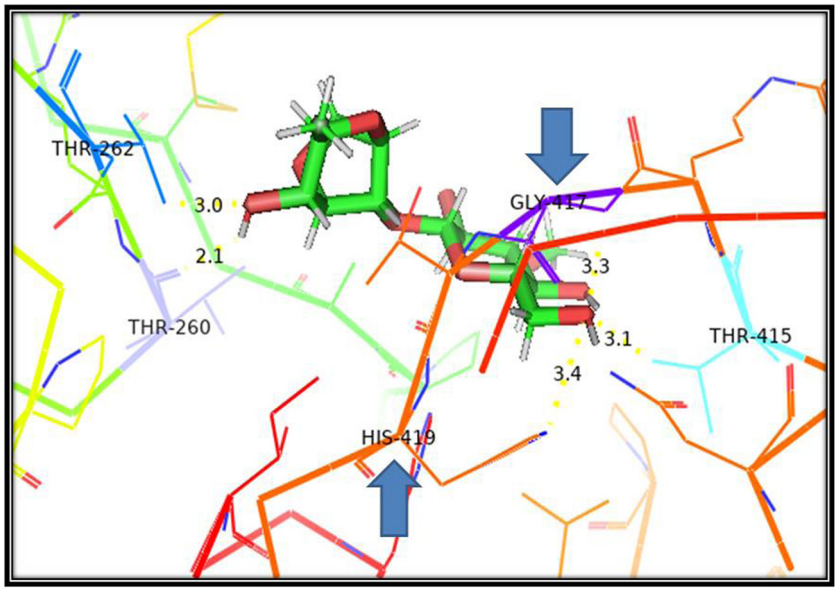


Figure 2: The interaction energy analysis of agar (a natural biopolymer) with Bs Lac using the SwissDock method. Note: ↓ (down arrow) indicates the Gly417 amino acid residue, and ↑ (up arrow) indicates the His419 amino acid residue.

pentamer, dextran, hyaluronan, and mannan) interact with the AAR Phe403 of the protein (SLASP KP-43). According to Nonaka et al., calcium ions (Ca^{2+}) assist in the thermal stability of subtilisins, and thus far, three calcium ion (Ca^{2+})-binding sites have been reported in the SLASP KP-43 [43]. Moreover, calcium-II and calcium-III ions existing in the C-terminal domain of KP-43 have been reported to stabilize a loop section (from aspartic acid 384 to asparagine 398) in the domain [43]. Interestingly, in the present study, nine ligands, namely, agar, alginate, chitosan pentamer, chitosan oligosaccharide, dextran, fucoidan, heparin, phytic acid, and starch soluble, exhibited AA interactions in the above calcium ion (Ca^{2+})-binding region, i.e., between aspartic acid 384 and asparagine 398 in the target protein. The N-domain of KP-43 has been reported to contain residues of the catalytic triad, such as His68, Ser255, and Asp30 [43,44]. Interestingly, in the present study, agarose showed AA interactions with both the His68 and Ser255 residues of SLASP KP-43 (as shown in Table 3). Okuda et al. have reported that the hydrophobicity of AAR (especially AARs like Tyr195, Phe192, Phe205, Phe226, and Trp241) in the flexible loop of KP-43 protease has altered catalytic activity towards a macromolecule substrate (casein) [45]. In the present study, agarose (natural biopolymer) has shown interaction with Trp241 AAR of SLAP KP-43. Mahmoud et al. have reported that AARs like Leu188, Ser361, Trp393, Thr419, and Asn420 have shown interaction with macromolecule substrate (casein) [46]. In the current investigation, agar (Figure 1), chitosan oligosaccharide and phytic acid (natural biopolymers) have exhibited interaction with Trp393 AAR of SLAP KP-43. Mahmoud et al. have demonstrated that the AARs namely Asp155, His187, and Ser384 as catalytic triad of AKD9 protease [46]. In the present investigation, fucoidan and starch soluble (natural biopolymers) have shown interaction with Asp384 AAR of SLAP KP-43, instead of Ser384 AAR of AKD9 protease.

The docking investigation and BE (Table 4) showed that lignin exhibited the highest BE (−11.37 kcal/mol); additionally, pectin had the lowest energy of binding (−6.50 kcal/mol) to the targeted enzyme (Bs Lac). The BE analysis of the current investigation showed the following order: lignin (−11.37 kcal/mol), <kappa carrageenan (−10.01 kcal/mol), <hyaluronan (−9.25 kcal/mol), <chitosan pentamer (−9.00 kcal/mol), <heparin (−8.91 kcal/mol), <agarose (−8.58 kcal/mol), <pullulan (−8.50 kcal/mol), <phytic acid (−8.41 kcal/mol), <mannan (−8.27 kcal/mol), <chondroitin sulfate (−8.18 kcal/mol), <alginate (−8.04 kcal/mol), <dextran (−7.96 kcal/mol), <starch soluble (−7.59 kcal/mol), <cellulose (−7.48 kcal/mol), <agar (−7.36 kcal/mol), <fucoidan (−7.29 kcal/mol), <chitosan oligosaccharide (−7.23 kcal/mol), and <pectin (−6.50 kcal/mol). Singh et al. utilized a docking method to predict the binding potential of seven pharmaceutical pollutants with both bacterial and fungal Lacs [47].

Rivera-Hoyos et al. have demonstrated that the AARs namely Pro226, Ala227, His319, Cys322, Gly323, Pro384, Arg416, Gly417, and His497 of Lac have exhibited interaction towards 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) substrate [48]. In the current investigation, chitosan pentamer and lignin have shown interaction with Ala227 AAR of Lac. Similarly, pectin (natural biopolymer) has exhibited interaction with Cys322 AAR of Bs Lac. Moreover, agarose, kappa

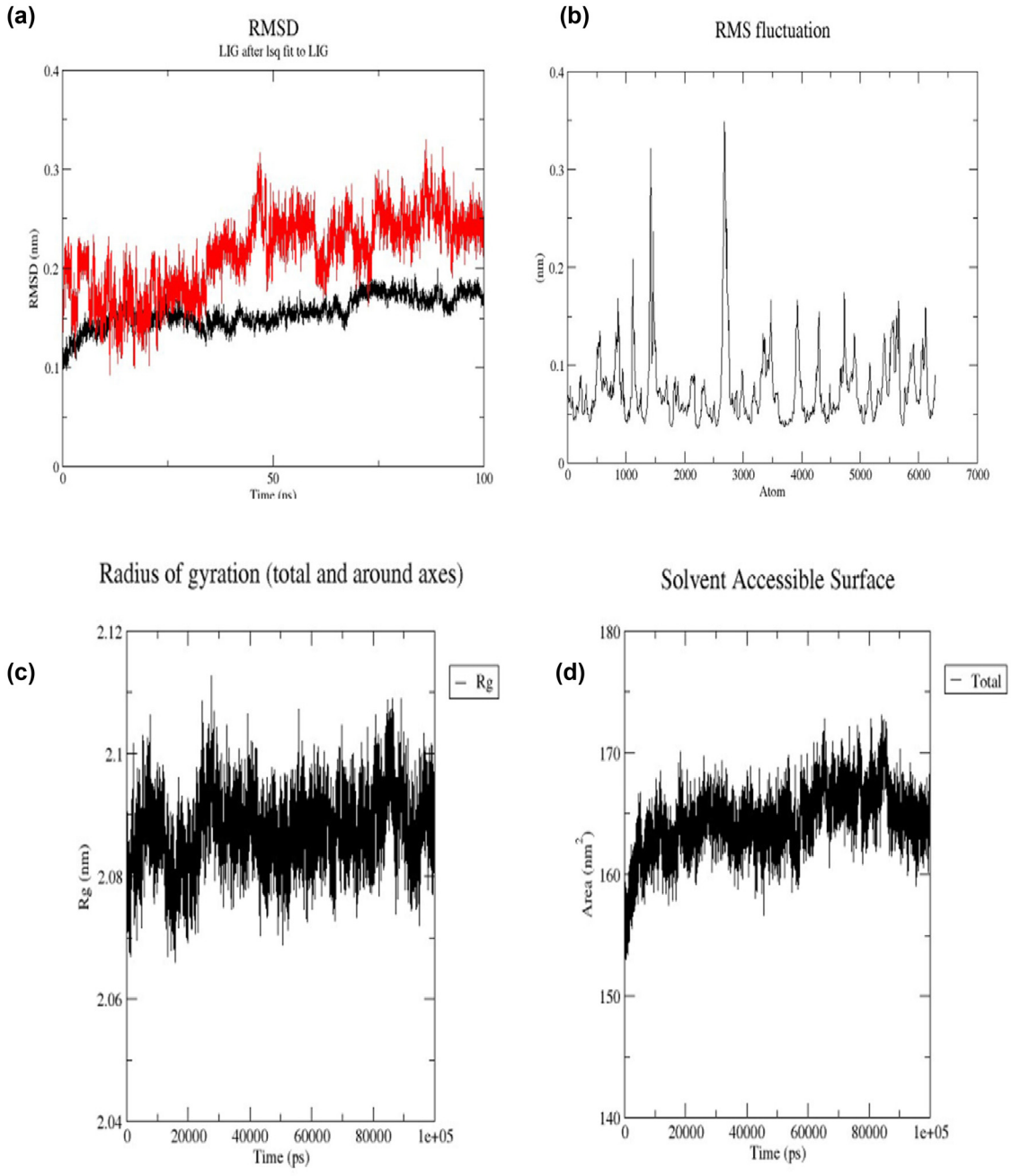


Figure 3: Represents the result of 100 nanoseconds (ns) MD simulation of SLASP KP-43 and chitosan pentamer complex (a) RMSD (root mean square deviation) values of docked complexes from C-α atoms. The structural changes of receptor protein by means of: (b) RMSF (root mean fluctuation), (c) R_g (radius of gyration), and (d) SASA respectively. Note: Red color plot in Figure 3a indicates the target protein (SLASP KP-43), similarly black color plot indicates the selected ligand (chitosan pentamer).

carrageenan, lignin, and phytic acid have shown interaction with Arg416 AAR of Bs Lac. Similarly, agar (Figure 2) alginate, fucoidan, heparin, phytic acid, and pullulan have exhibited interaction with Gly417 AAR of Lac. Furthermore, alginate, kappa carrageenan, dextran and hyaluronan (natural biopolymers) have shown interaction with His497 AAR of Bs Lac.

Xie et al. have demonstrated that the AARs namely Pro226, Pro384, Leu386, Thr415, Arg416, His419, Cys492, and His497 of Lac have exhibited interaction towards sinapic acid [SA] substrate [49]. In the current investigation, agar, alginate, chondroitin sulfate, fucoidan, and phytic acid have shown interaction with Thr415 AAR of Bs Lac.

Six ligands (agarose, chitosan oligosaccharide, heparin, hyaluronan, lignin and mannan) interact with the Gly323 AAR of the enzyme (Bs Lac). This finding was on par with a previous report in which Gly323 was reported as a binding pocket-1 AAR of Lac [50].

During the docking process, the interaction between the ligands and the protein are instantaneous and may be unstable. The MD simulation on the other hand provides us with information about the stability of the molecular interactions of the complexes over a period of time [51]. In this study, root-mean-square deviations (RMSD) of the C-alpha atoms of the complexes were evaluated with respect to the atoms in starting structures. However, it can be noted that the complex had lower RMSD ranges during the whole simulation periods (100 ns) which correlates with the complex's stability. Figure 3a illustrates the plotting of the RMSD values of Target (in red) and ligand (in black), indicating that the complexes were stabilized after 30 ns. Also, the root-mean-square fluctuations (RMSF) of the complexes were analyzed to understand the flexibility across the AARs of the complexes [51]. Figure 3b clearly indicates that almost every residue has a lower RMSF than 2.5 Å which defines the complex's stability. The stability of those complexes was further evaluated by plotting radius of gyration (R_g) to understand the mobility of the complexes where the higher R_g defines the higher flexibility [51]. The rise observed in the target is due to the higher flexibility of the target. The calculated R_g value over the simulation time scale is demonstrated in Figure 3c, where complexes exhibit a stable R_g profile across the simulation trajectories. Additionally, SASA predicts the surface area of a target protein that is accessible by the solvent molecules which is defined as the extent to which atoms on the surface of a protein can form contact with the solvent [52]. Generally, it is measured in squared nanometers (nm^2). Figure 3d shows the predicted SASA of the target (SLASP KP-43) which indicated lower fluctuations.

The present *in silico* analysis can be further confirmed by performing detailed *in vitro* analyses against the target enzymes, particularly for those natural biopolymers with the highest BE. Moreover, the natural biopolymer with the highest BE was not investigated by using a cell-based assay or an animal model for assessing their pharmacokinetic and toxicokinetic properties.

4 Conclusion

In the present study, all eighteen selected natural biopolymers were found to dock with both target proteins (SLASP KP-43 from the *Bacillus* species KSM-KP43 and Bs Lac). In the present study, nine ligands, namely, agar, alginate, chitosan pentamer, chitosan oligosaccharide, dextran, fucoidan, heparin, phytic acid, and starch soluble, were shown to participate in AA interactions between aspartic acid 384 and asparagine 398 in the target protein (SLASP). The MD simulation results reveal that SLASP KP-43 and chitosan pentamer complex remain stable in nature. Similarly, six ligands, namely, agarose, chitosan oligosaccharide, heparin, hyaluronan, lignin and mannan, interact with the glycine 323 position of the protein (Bs Lac). Thus, the current study demonstrated the potential of eighteen selected natural biopolymers as immobilizing agents to prepare SLASP and Lac nanoparticles. Furthermore, wet laboratory studies are required to improve our knowledge of eighteen selected natural biopolymers as immobilizing agents to prepare SLASP and Lac nanoparticles for biomedical applications.

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Conflict of interest: The authors declare that they have no conflicts of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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