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Inhibition of A*spergillus* VosA protein by lactic acid bacteria metabolites (*in silico* study)

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ABSTRACT: In this work, we performed an *in silico* study using 3D structure protein of VosA, and analyzed the protein interaction via molecular docking using PyRx to test the inhibition efficacy of 15 metabolites compounds produced by lactic acid bacteria in conidia germination protein of *Aspergillus*. The antifungal docking findings revealed that these compounds showed good interactions and binding affinity against the target involved in conidia germination. The highest binding energy (-6.3 kcal/mol) was given by stearic acid. This interaction is due to the residue amines Ser and Phe. Palmitic acid also showed a good binding affinity with -6 kcal/mol. Lactic acid has not the same efficiency as palmitic, and stearic acid, which represented a value of -3.6 kcal/mol, the values recorded by cytidine was from -5 kcal/mol, which was also important compared to oxalic and acetic acid.

Keywords: *In silico*; Docking; Antifungal; Fungi; Lactic acid bacteria.

1. INTRODUCTION

Aspergillus causes spoilage of food, and this food contamination by this fungus is a crucial matter that needed to be checked. Visible mould can be observed on common household vegetables and fruits, like tomatoes, grapes and onions [1, 2]. The fungus can give the spoilage food rise at its different developmental stages, both conidia and hyphae. Additionally, mycotoxins production, which is toxic secondary metabolites, can also affect food safety. Problems caused by *Aspergilli* can be minimized or stopped by biological, physical, or chemical methods [3, 4].

The group of lactic acid bacteria (LAB) occupies a central role in the fermentation processes, has been used for thousands of years to protect a range of foods, including bread, vegetables, and dairy products, against spoilage organisms, and has a long and safe history of application and consumption in the production of fermented foods and beverages. In recent years, there has been an increased emphasis on identifying novel LAB strains with antimicrobial activity for food bioprotection [5]. The most essential and best characterized antimicrobials substances produced by LAB are lactic and acetic acid. Other substances such as ethanol, aroma compounds, bacteriocins and exopolysaccharides played a vital role in microbial inhibition. In this

way, they enhance shelf life and microbial safety, improve texture and contribute to the end product's pleasant sensory profile [6].

Molecular docking has become the most widely used program that can be used to comprehend the interaction between a small molecule and a protein at the atomic level, which permits us to establish the behavior of small molecules in the binding site of target proteins as well as to interpret fundamental biochemical processes. The goal of molecular docking is to grant a prediction of the ligand-receptor complex structure using computation methods. The main two interrelated steps from which docking can be achieved are: by sampling conformations of the ligand in the active site of the protein and, next, ranking these conformations using a scoring function [7, 8].

Here, we aim to address the issue caused by fungi by using lactic acid bacteria metabolites as antifungal substances to reveal the action mode aginst VosA conidia protein of *Aspergillus*; this can be partially achieved through the use of molecular docking approach.

2. MATERIALS AND METHODS

2.1. Protein preparation

The PDB file of protein was downloaded from protein data bank (4N6Q: Crystal structure of VosA velvet domain of *Aspergillus nidulans*), and it was used for the molecular docking after water and ligand retrieved by discovery software, then the active site was detected by the website of CASTp (http://sts.bioe.uic.edu/castp/index.html?1bxw) and prepared to dock by chimera software,

2.2. Ligands preparation

For this study, we have selected 15 compounds produced by lactic acid bacteria previously reported to have antifungal activity against several fungi (the antifungal compounds and their first antifungal activity detection by LAB were reported in Table 1).

Table 1. First report of compounds as antifungal metabolites of LAB.

S.No.	Compounds	Antifungal LAB	References
1.	Acetic acid	*Pediococcus halophilus*	[10]
2.	Lactic acid		
3.	Cytidine	*Lactobacillus amylovorous*	[12]
4.	Decanoic acid	*Lactobacillus arizononas*	[22]
5.	Diacetyl	*Lactobacillus paralimentarius*	[13]
6.	Formic acid	*Lactobacillus sanfrancisco*	[18]
7.	Propionic acid		
8.	Valeric acid		
9.	Linoleic acid	*Lactobacillus plantarum*	[16]
10.	Malonic acid	*Lactobacillus feacalis*	[23]
11.	Oxalic acid		
12.	Oleic acid	*Lactobacillus plantarum*	[24]
13.	Palmitic acid		
14.	Stearic acid		
15.	Reuterin	*Lactobacillus reuteri*	[14]

PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to download the ligands 3D structure and then were loaded into the PyRx after SDF files converted to PDB files using OpenBabel software.

2.3. Molecular docking

The molecular docking was performed by using Autodock vina incorporated in PyRx virtual screening open source software. The protein and ligand molecules were loaded to the PyRx software, where they were converted to Pdbqt and selected under the vina wizard control. The binding energy value (kcal/mol) of the ligand-receptor interaction's best pose was obtained and viewed under the analysis results tab. The best interaction result was analyzed by the discovery studio visualize (DSV) version 2020 from BIOVIA to represent all bonding types and amino acid residues involved in the interaction.

3. RESULTS

All metabolites were able to bind the VosA protein, with binding energies between -2.3 to -6.3 kcal/mol. The residues that interact in the active site with different compounds showed different types of interactions, as shown in Table 2.

Table 2. Binding energy values of metabolites interacting with the conidia VosA protein of *Aspergillus.*

S.No.	Compounds	Binding energy (kcal/mol)
1.	Acetic acid	−2.3
2.	Lactic acid	−3.6
3.	Cytidine	−5.0
4.	Decanoic acid	−4.1
5.	Diacetyl	−3.0
6.	Formic acid	−2.3
7.	Propionic acid	−2.6
8.	Valeric acid	−3.3
9.	Linoleic acid	−4.1
10.	Malonic acid	−4.3
11.	Oxalic acid	−3.0
12.	Oleic acid	−4.1
13.	Palmitic acid	−6.0
14.	Stearic acid	−6.3
15.	Reuterin	−2.8

The binding interactions of the selected antifungal metabolites of LAB interacted with the conidia protein of *Aspergillus* were listed in Table 3. Figure 1a shows the interaction of acetic acid with conidia protein of *Aspergillus*. The conventional hydrogen bond was predicted between the acetic acid and Leu A: 77 with distance of 2.03 Å and the carbon-hydrogen interaction was obtained between the acetic acid and Pro a: 90 by distance of 3.57 Å (Figure 1). The binding energy score of acetic acid when interacted with conidia protein of *Aspergillus* was predicted as −2.3 kcal/mol, indicating that acetic acid activity is inferior to other molecules activity.

The binding energy score of the interaction of lactic acid with the conidia protein of *Aspergillus* was predicted as −3.6 kcal/mol. The lactic acid forms a conventional hydrogen bond with the residue Leu a: 77 and distance of 2.60 Å (Figure 2). The residue Ser a: 76 give a distance of 3.38 Å by carbon-hydrogen

interaction. It is important to note that the residue Leu a: 77 are predicted as an active site of the conidia protein of *Aspergillus* for the interaction with acetic acid, lactic acid, decanoic acid, diacetyl, formic acid, propionic acid, valeric acid and reuterin (Table 3).

Table 3. Antifungal lactic acid bacteria (LAB) metabolites compounds interaction with conidia VosA protein of *Aspergillus*.

Compounds	Residue	Distance	Interaction
Acetic acid	Leu a: 77	2.03	Conventional hydrogen bond
	Pro a: 90	3.57	Carbon hydrogen bond
Lactic acid	Leu a: 77	2.60	Conventional hydrogen bond
	Ser a: 76	3.38	Carbon hydrogen bond
	Ala a: 86	2.82	Unfavorable acceptor/acceptor, donor/donor
Cytidin	Ala a: 86	2.78	Conventional hydrogen bond
	Leu a: 77	1.00	Unfavorable acceptor/acceptor, donor/donor
	Ala a: 86	2.80	Pi-alkyl
	Pro a: 90	4.57	
	Ala a: 93	4.95	
Decanoic acid	Leu a: 77	2.22	Conventional hydrogen bond
		2.00	
	Ala a: 86	2.95	Unfavorable acceptor/acceptor, donor/donor
		1.90	
	Ala a: 93	4.34	Alkyl
	Leu a: 94	5.08	
	Pro a: 90	4.87	
		5.45	
Diacetyl	Leu a : 77	2.14	Conventional hydrogen bond
Formic acid	Ala a: 86	2.07	Conventional hydrogen bond
		2.63	
	Leu a: 77	2.01	
Propionic acid	Leu a: 77	1.96	Conventional hydrogen bond
	Ser a: 76	3.58	Carbon hydrogen bond
Valeric acid	Leu a: 77	3.28	Conventional hydrogen bond
		2.12	
	Pro a: 90	5.37	alkyl
	Leu a: 94	4.26	
	Ala a: 93	3.84	
Linoleic acid	Ser a: 74	2.51	Conventional hydrogen bond
	Pro a: 85	4.28	alkyl
	Pro a: 90	4.63	
		3.83	
		4.01	
	Ala a: 93	4.19	
	Leu a: 94	4.87	
Malonic acid	Ser a: 74	2.20	Conventional hydrogen bond
	Ser a: 134	2.11	
Oxalic acid	Ser a: 74	2.95	Conventional hydrogen bond
		3.38	
	Ser a: 76	3.10	
	Ser a: 134	2.08	
Oleic acid	Ser a: 76	2.95	Conventional hydrogen bond
	Leu a: 77	5.18	Alkyl
	Ala a: 93	4.85	
		3.59	
		4.49	
	Pro a: 90	5.36	
		4.92	
		5.31	
	Leu a: 94	5.41	
		5.09	
	Pro a: 85	4.30	

Compounds	Residue	Distance	Interaction
Palmitic acid	Ser a: 148	2.56	Conventional hydrogen bond
	Phe a: 136 Phe a: 145	4.63	pi-pi-stacked
		3.71	
	Phe a:136 Phe a:145	4.56	
		4.63	pi-pi-t-shaped
		3.71	
		4.56	
Stearic acid	Ser a: 134	2.15	Conventional hydrogen bond
	Phe a:145	4.59	Alkyl
	Phe a: 145	4.62	pi-pi-stacked
		3.67	
Reuterin	Leu a: 77	1.93	Conventional hydrogen bond
	Ala a: 86	2.17	Unfavorable acceptor/acceptor
	Leu a: 77	2.85	Donor/donor

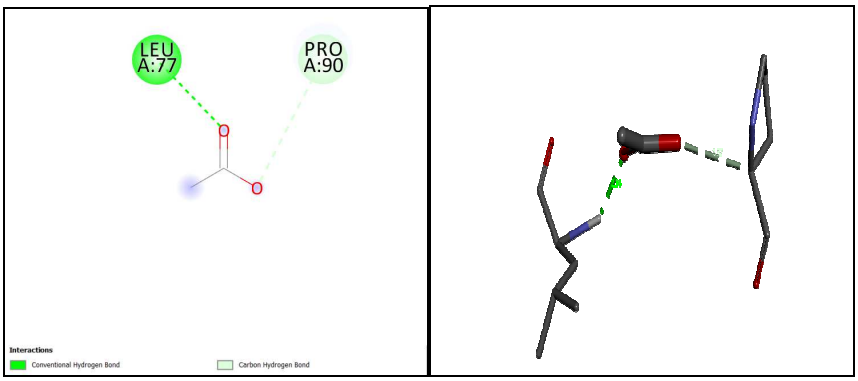

Figure 1. 2D and 3D VosA protein and acetic acid interaction.

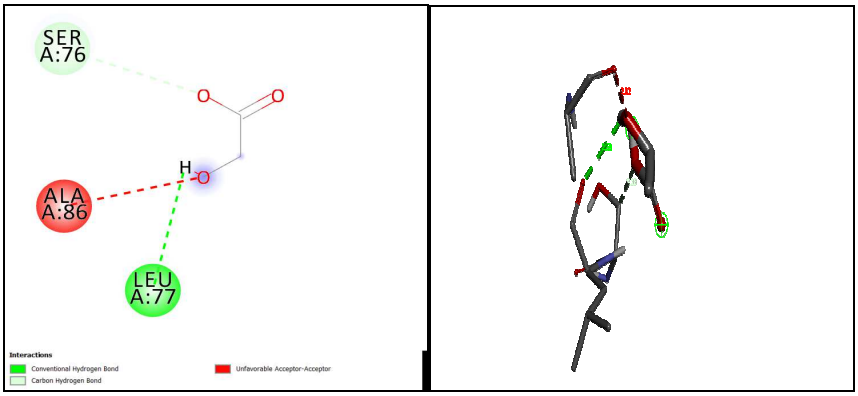

Figure 2. 2D and 3D VosA protein and lactic acid interaction.

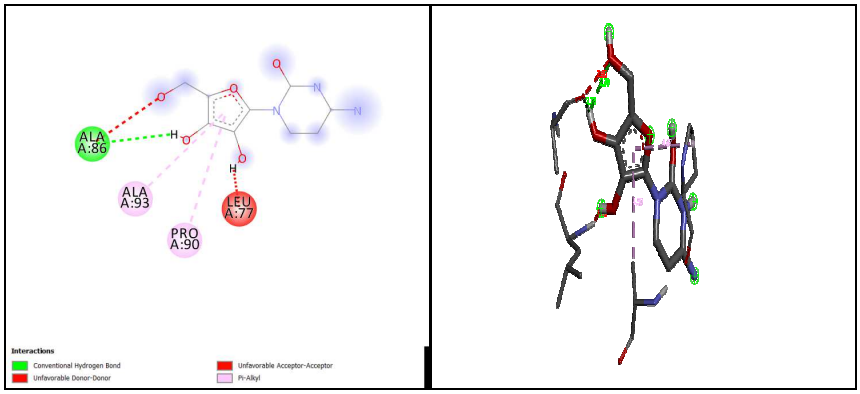

Figure 3. 2D and 3D VosA protein and cytidine interaction.

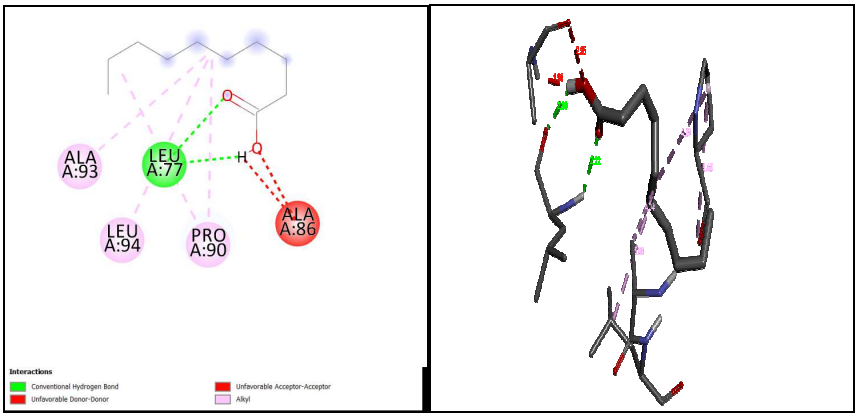

Figure 4. 2D and 3D VosA protein and decanoic acid interaction.

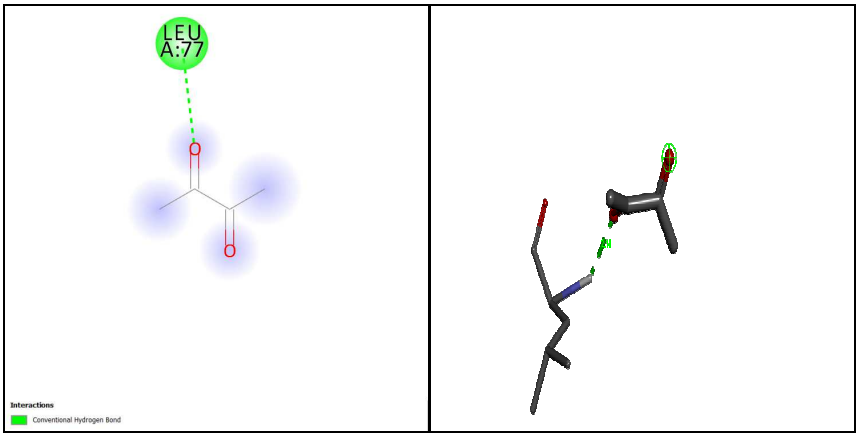

Figure 5. 2D and 3D VosA protein and diacetyl interaction.

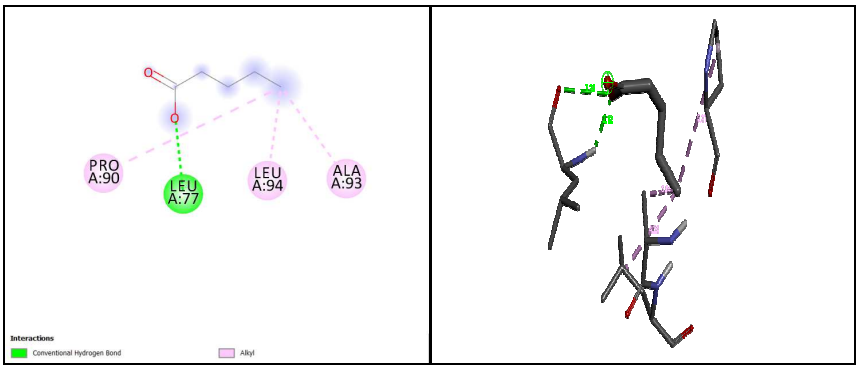

Figure 6. 2D and 3D VosA protein and valeric acid interaction.

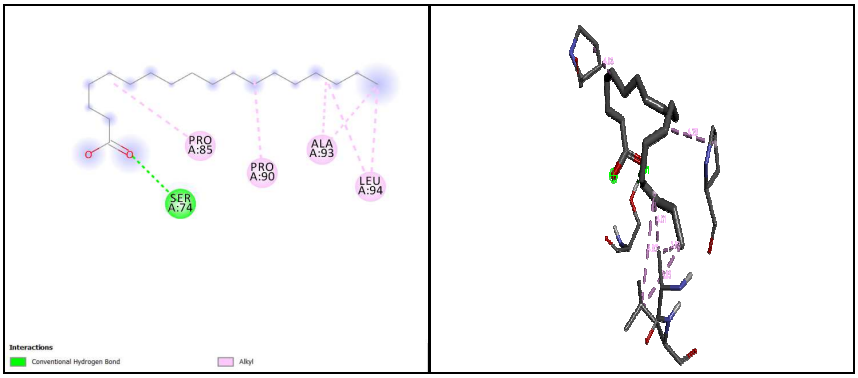

Figure 7. 2D and 3D VosA protein and linoleic acid interaction.

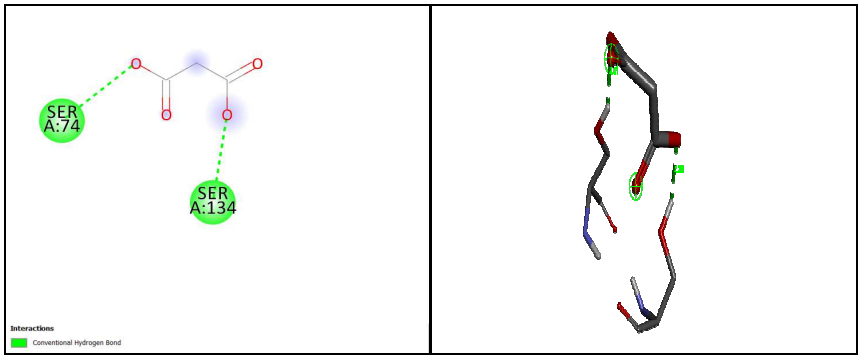

Figure 8. 2D and 3D VosA protein and malonic acid interaction.

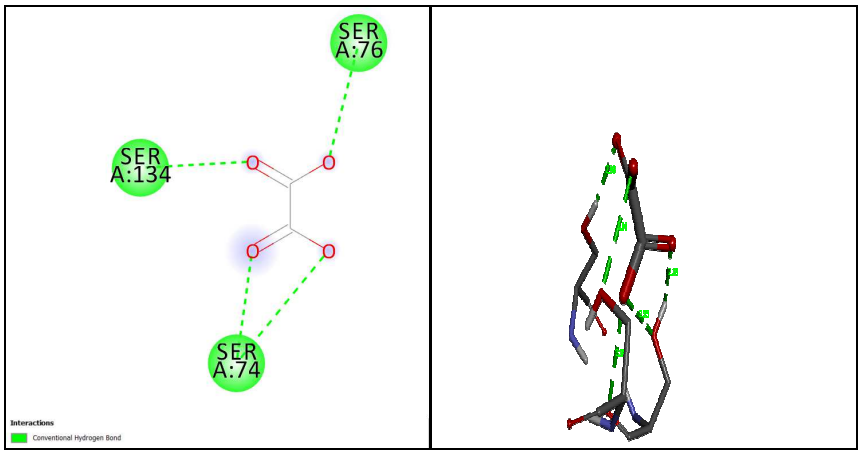

Figure 9. 2D and 3D VosA protein and oxalic acid interaction.

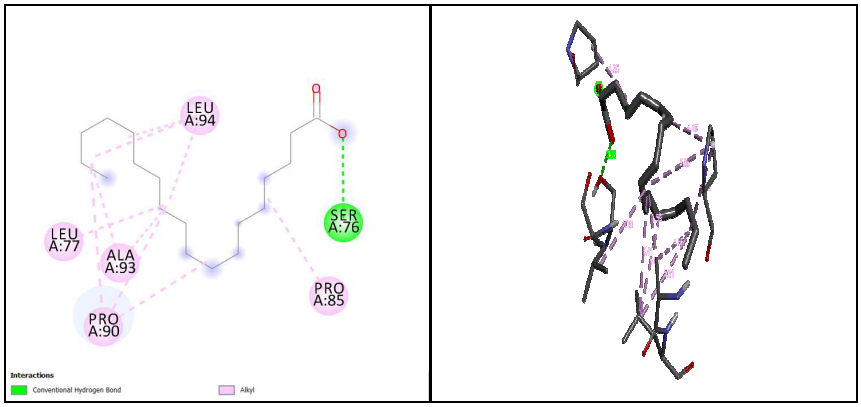

Figure 10. 2D and 3D VosA protein and oleic acid interaction.

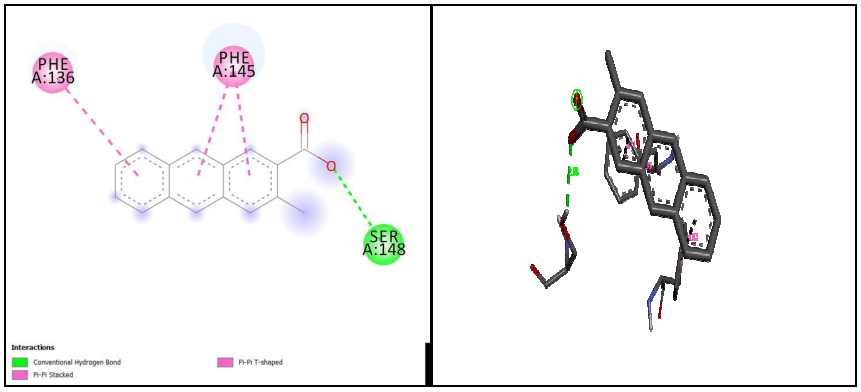

Figure 11. 2D and 3D VosA protein and palmitic acid interaction.

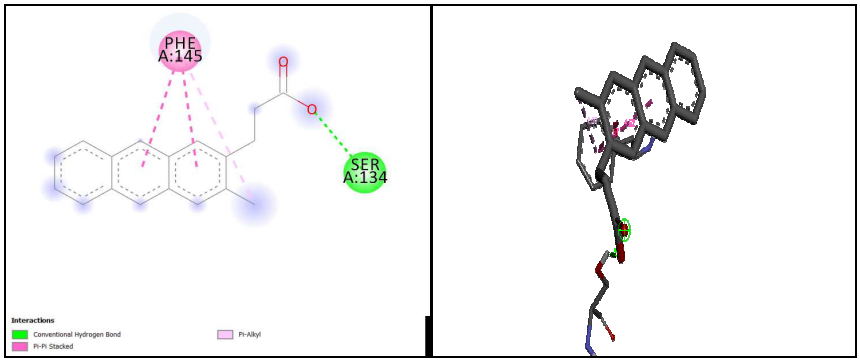

Figure 12. 2D and 3D VosA protein and stearic acid interaction.

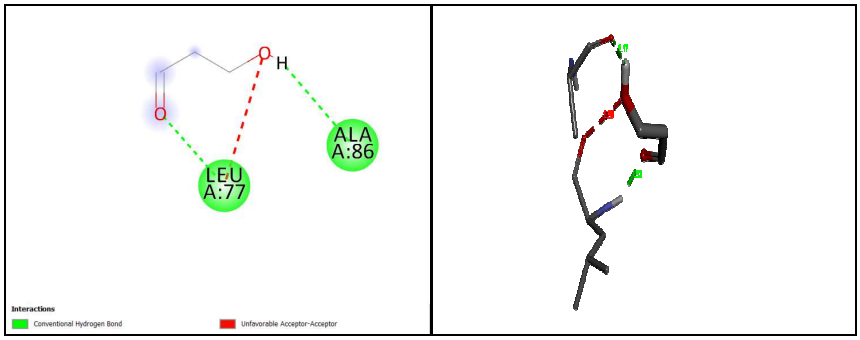

Figure 13. 2D and 3D VosA protein and reuterin interaction.

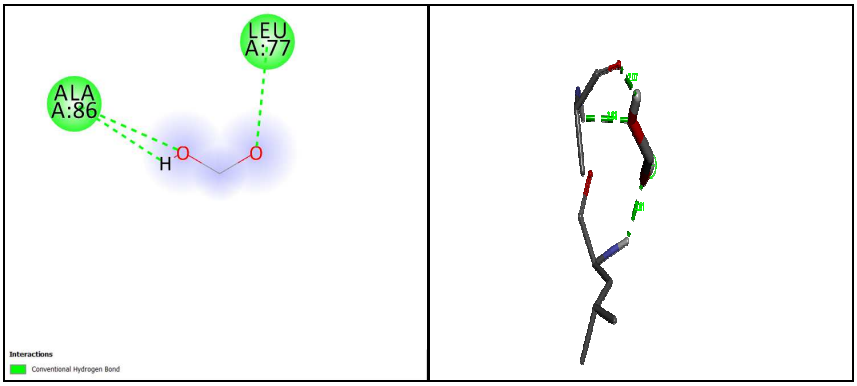

Figure 14. 2D and 3D VosA protein and formic acid interaction.

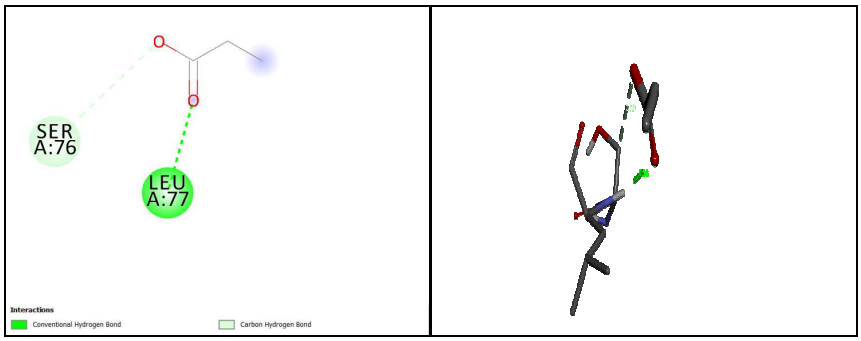

Figure 15. 2D and 3D VosA protein and propionic acid interaction.

The residue Ala a: 86 is predicted as an active site of cytidine that interacted with the conidia protein of *Aspergillus* and distance of 2.78 Å (Figure 3). In addition, the Pi-alkyl interactions are obtained between the cytidine and the residues Pro a: 90 and Ala a: 93 which show a distance of 4.57 Å and 4.95 Å respectively. The binding energy score of the interaction of cytidine with the conidia protein of *Aspergillus* was predicted

as −5.0 kcal/mol, which is the third strongest interaction. Moreover, decanoic acid interaction with the conidia protein of *Aspergillus* shows the conventional hydrogen bond with the residue Leu a: 77 which show a distance of 2.00 and 2.22 Å (Figure 4). The binding energy score of the corresponding interaction is obtained as −4.1 kcal/mol.

As shown in Figure 5, the residue Leu a: 77 with distance of 2.14 Å is predicted as an active site for the interaction of diacetyl with the conidia protein of *Aspergillus,* and the binding energy score is calculated as −3.0 kcal/mol. The interaction of formic acid and conidia protein of *Aspergillus* is shown in Figure 4, which indicates that the active site for the corresponding interaction is Ala a: 86 and Leu a: 77 with distance of 2.07, 2.63 and 2.01 Å. The binding energy score of this interaction is calculated as−2.3 kcal/mol. The active site for the propionic acid interacted with the conidia protein of *Aspergillus* is predicted as Leu a: 77 and Ser a: 76 with distance of 1.96, 3.58 Å respectively (Figure 5). The binding energy score of this interaction is predicted as −2.6 kcal/mol.

Moreover, the valeric acid interacted with the conidia protein of *Aspergillus* is depicted in Figure 6. The residue Leu a: 77 is predicted as an active site for this interaction as well as the binding energy score of the interaction is calculated as −3.3 kcal/mol and the distance is 3.28 and 2.12 Å. The residue Ser a: 74 is predicted as an active site for the interaction between linoleic acid and conidia protein of *Aspergillus* (Figure 7). Ser a: 74 is the second important active site of the targeted protein than the residue Leu a: 77. The binding energy score of the corresponding interaction is predicted as −4.1 kcal/mol. Fatty acids show score better then organic acids. The active site for the malonic acid interacted with conidia protein of *Aspergillus* is indicated as Ser a: 74, 134 with distance of 2.20 and 2.11 Å respectively (Figure 8), and the binding energy score of this interaction is obtained as −4.3 kcal/mol.

Furthermore, Figure 9 shows the interaction of oxalic acid with the conidia protein of *Aspergillus.* The residues including Ser a: 74, 76, 134 are predicted as active sites of the corresponding interaction. This interaction's binding energy score is calculated as −3.0 kcal/mol, which also shows shallow interaction with targeted protein than that of stearic acid and palmitic acid interacted with the targeted protein. The docked pose of oleic acid interacted with the conidia protein of *Aspergillus* is shown in Figure 10. The amino acid residue Ser a: 76, Leu a: 77, Ala a: 93, Pro a: 90, Leu a: 94 and Pro a: 85 are predicted as an active site of this interaction, and the binding energy score is obtained as −4.1 kcal/mol.

The interaction of palmitic acid with the conidia protein of *Aspergillus* is depicted in Figure 11. Significantly, the pi-pi-stacked and pi-pi-t-shaped interactions are obtained in the interaction of palmitic acid with the targeted protein. The pi-pi-stacked interactions are predicted between palmitic acid and the residues Phe a: 136, 145 with distance of 4.63, 3.71 and 4.56 Å. In addition, the pi-pi-t-shaped interactions are obtained between the palmitic acid and the residues Phe a: 136, 145 with distance of 4.63, 3.71 and 4.56 Å. The residue Ser a: 148 is predicted as an active site for the corresponding interaction with distance of 2.56 Å. Interestingly, the corresponding interaction's binding energy score is predicted as −6.0 kcal/mol, which is the second-highest binding energy score in the present study. The stearic acid docked with the conidia protein of *Aspergillus* is depicted in Figure 12. The pi-pi-stacked interaction is obtained between the stearic acid and the targeted protein residue Phe a: 145. Also, the residue Ser a: 134 is accepted as an active site for this interaction by the distance of 2.15 Å. It is important to note that the corresponding interaction's binding energy score is calculated as −6.3 kcal/mol, which is the highest binding energy score compared to that of other compound's interactions with the targeted protein.

Figure 14 describe the interaction between formic acid and VosA protein, the binding energy score is obtained as −2.3 kcal/mol, with the residue Leu a: 86 and 77 as predicted an active site with distance of 2.07 and 2.63, 2.01 Å respectively, this interaction due to conventional hydrogen bond, the same type of interaction was showed by propionic acid in Figure 15 with energy of −2.6 kcal/mol by the residue Leu a: 77 and Ser a: 76, but with carbon hydrogen bond, the distance given by these residue is 1.96 and 3.58 respectively.

Finally, reuterin interacted with the targeted protein conidia protein of *Aspergillus*, which is depicted in Figure 13. The residues Leu a: 77 and Ala a: 86 are predicted as an active site of the targeted protein for the reuterin interaction. The binding energy score for the corresponding interaction is obtained as −2.8 kcal/mol.

The obtained results clearly evidence that the stearic acid metabolite of lactic acid bacteria show better inhibitory activity against the targeted protein of *Aspergillus* than other selected fourteen compounds.

4. DISCUSSION

Fungal growth and mycotoxin production on foods and feed cause poisoning and serious human diseases that may lead to death. The chemical preservatives and fungicides have negative impacts on both health and the environment. In contrast, biopreservatives such as lactic acid bacteria (LAB) are effective, safe, biodegradable and have additional health benefits. The antifungal compounds produced by LAB include organic acids, short-chain fatty acids, hydrogen peroxide, reuterin, diacetyl, bacteriocins, and bacteriocin-like inhibitory substances have been documented previously for *in vitro* and *in vivo* study [9] which support our *in silico* analysis.

Fifty compounds (reported in Table 1) previously were cited as antifungal metabolites of LAB were selected for this study to show how these molecules interact with the conidia VosA protein of *Aspergillus*. Lactic acid which is produced by lactic acid bacteria in 1980 was determined as an antifungal metabolite [10], is considered as the main product by these bacteria, its inhibition is due to the acidification of the environment [11] nevertheless it has a weak inhibition of the fungi which shows a score of binding affinity -3.6 kcal/mol, this record does not make it a good inhibitor, also acetic acid which was described too in 1980 as an antifungal metabolite shows a record of -2.3 kcal/mol. Generally, the activity of organic acids is due to their undissociation form, research shows that the association of several organic acids provides a strong inhibition of the fungi caused by the synergistic activity , it was proven that when the acetic acid is associated with lactic acid it results in a good inhibition [11].

Cytidine and diacetyl are both antifungal metabolite which were detected by Arendt et al. [12] and Garofalo et al. [13] respectively, their action mode have not been studied yet. This study design seems to be advantageous to study the interaction mode to know that each target can be inhibited.

Reuterin was not well documented for its antifungal activity; the first report that shows its ability to inhibit fungi was cited by Dobrogosz and Lindgren [14], this substance can be produced by lactic acid bacteria under aerobic or anaerobic condition in the presence of glycerol in the medium [15]. *In silico* inhibition activity of reuterin is low which shows a score value of binding energy of -2.8 kcal/mol.

The best record was registered by some fatty acids like palmitic and stearic acids with record value of -6 and -6.3 kcal/mol respectively. These substances were detected as antifungal substances by Sangmanee et Hongpattarakere [16].

The antifungal activity of lactic acid bacteria is due to several compounds produced simultaneously, 50% of the activity was due to organic acids [17], the synergistic effect was detected in *in vitro* study by valeric, formic and propionic [18], these molecules have the ability to interact with conidia protein of

Aspergillus which show a value of binding energy -3.3, -2.3 and -2.6 kcal/mol respectively, each substance does not provide a strong activity, but the association of these molecules promotes the inhibition.

This *in silico* study shows that organic acids can be interacted with conidia protein but it interacts better with fatty acids (Table 2), the observed result shows a good agreement with the observation done by other researchers, which shows that the more carbon there is in fatty acids chain the more good the inhibition. [19]. It is supposed that, the action mode creates membrane, vacuole and nucleus alteration, or biomass reduction of fungi and inhibition of blastospore stage formation by some metabolites of LAB like bacteriocins, bacteriocins-like, organic acids or the whole metabolites [20, 21].

In this study we tried to detect the inhibition of conidia protein by some metabolites identified as antifungal compounds produced by lactic acid bacteria and which residue introduced in the inhibition, so, these metabolites that are already considered as metabolites with an antifungal character have the ability to inhibit conidia protein which prevents the hyphae formation, so it's preferable to do this experiment in the laboratory by putting all the substances together in order to have a strong inhibition activity.

5. CONCLUSION

The present study with molecular docking clearly demonstrates the antifungal activity of different metabolites compounds of lactic acid bacteria through conidia germination inhibition. The inhibition of conidia germination is one of the essential protein targets, playing a significant role in the life cycle. So we have found that all the compounds are displaying some good binding affinity values when they are docked with the protein. However, we can determine that palmitic acid and stearic acid are having the lowest binding affinity value.

Authors' Contributions: NL: supervision, conceptualization, project administration, writing original draft, methodology; RP: writing original draft, writing review and editing, data curation; SQ: writing original draft, writing review and editing, resource. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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