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In silico evaluation of the antimicrobial potentials of soluble bioactive compounds derived from *Weissella ciberia* metabolites.

Odion-Owase, Abimbola Onyioza [a] and Adetunji, Oluwaseun Charles [b].
[a] Department of Science Laboratory Technology, School of Applied Sciences and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.
[b] Department of Microbiology, Faculty of Sciences, Edo State University, Uzairue, Edo State, Nigeria.

*Corresponding author: bimbo.owase@gmail.com.

Abstract
Weissella species are a group of lactic acid bacteria gaining rapid popularity as a result of discoveries centered on their biotechnological properties. In this study, an *in silico* approach was imbibed to investigate the antimicrobial potentials of metabolites of *Weissella ciberia*. Soluble compounds of W. *ciberia* were subjected to High Performance Liquid Chromatographic (HPLC) analysis and the inherent metabolites were identified. In order to evaluate their antimicrobial potentials against *Escherichia coli* and *Shigella flexneri*, the identified metabolites of W. *ciberia* were further subjected to geometry optimization of compound structures, ligand/receptor preparation, docking calculations and docking simulations. The HPLC identified metabolites from W. *ciberia* were atropoine, gallic acid, naringinin, caffeine, maleic acid, saponin and glutathione. The results of the *in silico* analysis showed binding affinities of the metabolites against the target microorganisms at a range of -4.6 to 10.7 Kcal/mol. Among metabolites, the highest binding affinity was observed in saponin against E. *coli* and S. *flexneri* at scores of -9.7 Kcal/mol and -10 Kcal/mol respectively. Binding affinities against E. *coli* and S. *flexneri* were also observed in naringinin at binding scores of -7.8 Kcal/mol and -8.5 Kcal/mol respectively. The scores obtained in this study predicts strong antimicrobial potentials that were comparable to those of conventional antibiotics such as ciprofloxacin and meropenem. Hence, the antimicrobial activities of metabolites of W. *ciberia* could be harnessed further for their potential in drug sensitivity against multiple-drug resistant pathogenic microbes.

KEYWORDS: Bioactive, metabolites, fermentate, *in silico* and binding affinity.

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1. INTRODUCTION

Microorganisms have been recognized as a valuable reservoir of bioactive compounds, which have many applications; particularly in food and medical research. The Lactic acid bacteria (LAB) represent a group of microorganisms that have been recognized for their ability to produce bioactive by-products through metabolic processes. Lactic acid bacteria commonly play a pivotal role in the process of fermentation; wherein they metabolize various compounds found in substrates such as carbohydrates for the development of advantageous by-products. These by-products include antimicrobial peptides, such as bacteriocins, as well as ethanol, organic acids, fatty acids, and carbon dioxide, among others. Lactic acid bacteria are commonly regarded as probiotics, with the majority of species being classified as generally recognized as safe (GRAS) and having established health benefits (Marco *et al.*, 2017; Mathur, Beresford, & Cotter, 2020).

A few studies have reported the antimicrobial effects of LAB fermentates on pathogens. In a study (Mathur *et al.*, 2020; Morgan, Galvin, Ross, & Hill, 2001), whey powder-based spray-dried formulations containing the bacteriocin lacticin 3147 were developed from strains of LAB (L. *lactis* DPC3147). The authors assessed the efficacy of its fermentate in the targeting of food-borne pathogens such as *Bacillus cereus* and L. *monocytogenes*. Their study revealed that the fermentate elicited a decrease in L. *monocytogenes* at 99.9% and 85% within 2 hours of incubation in natural yogurt and cottage cheese respectively. Furthermore, they reported that B. *cereus* were decreased by 80% in soup within a 2 hour time-frame in the presence of 1% lacticin 3147 powder.

Weissella species are a group of Lactic acid bacteria that are non-spore forming, gram-positive, catalase-negative coccobacilli. They are often mistaken by traditional phenotypic and biochemical identification methods as Lactobacillus spp. or Lactobacillus-like organisms. In recent times however, Weissella has now constituted a distinct phylogenetic group, separate from all other of genera of lactic acid bacteria, including streptococcus, leuconostoc and lactobacillus (Teixeira *et al*., 2021). To date, nineteen species of Weissella have been identified including *Weissella cibria* (Fusco *et al*., 2015). The strains of *Weissella spp*. strains have been isolated from a

wide variety of habitats such as raw milk, fecal samples, fermented cereals/grains, and vegetables (Kamboj, Vasquez & Balada-Llasat, 2015).

Over the last couple of years, a lot of attention has been on *Weissella spp* as a result of their technological and probiotic properties. A few strains have attracted the attention of the pharmaceutical, medical, and food industries; particularly because of their ability to produce antimicrobial exopolysaccharides. Moreover, Weissella strains are proven to be able to keep foodborne pathogenic microbes in control because of the bacteriocins, hydrogen peroxide, and organic acids they are able to produce. These components all have recognized pathogen antagonistic activities. The Weissella genus has also shown potentials for the treatment atopic dermatitis and some types cancers. W. *cibaria,* W. *confusa*, and W. *paramesenteroides* are particularly of focus as a result of their probiotic functionality and their fermentation of prebiotic fibers as well as their ability to thrive in the gastrointestinal tract. It is important to note that most *Weissella* strains with health-benefiting properties have been demonstrated to be safe, due to the complete absence or the low occurrence of pathogenicity or antibiotic-resistant genes. A vast number of scientific experiments have continued to report on as well as support the employment of *Weissella* strains in the food and pharmaceutical industries (Teixeira *et al.*, 2021).

Food-borne pathogens are organisms capable of causing of causing illnesses when consumed with food (food infection) or when they produce toxins in food before consumption (food intoxication). There are a vast array of food-borne pathogens including *Shigella species* and toxin-producing *Escherichia coli* (Gourama, 2020). The fermentates of lactic acid bacteria are being investigated for their application in food preservation and the control of food-borne pathogens (Mathur *et al*., 2020).

In silico evaluations facilitates computer-aided discoveries of antimicrobial agents. These approaches are attracting increasing attention as they can help reduce the scale, time, and cost issues faced by conventional experimental approaches. *In silico* analysis includes computational identification of potential drug targets, virtual screening of large chemical libraries for effective antimicrobial candidates, further optimization of candidate compounds, and *in silico* assessment of their potential toxicity. After these processes are conducted computationally, candidate compounds can be subjected to *in vitro* and *in vivo* experiments for confirmation (Shaker, Ahmad, Lee, Jung & Na, 2021).

Despite the recent interest in the employment of *Weissella* strains in food and pharmaceutical industries, there is still a dearth of study that initiates *in silico* approach to harness its antimicrobial potential. Hence, this study aimed to identify and quantify the bioactive compounds of metabolites derived from *Weissella ciberia* using high performance liquid chromatography (HPLC) analysis. Further, this study is aimed at evaluating *in silico*, the binding affinities of the isolated compounds in order to validate their potency as antimicrobial agents.

2. MATERIALS AND METHODS

2.1 Materials

Microbiological media were from Hi-Media Laboratories, Mumbai, India. Acetonitrile, methanol, deuterated methanol (CD_3OD), trimethlsilyl propionate sodium salt (TSP), triflouroacetic acid (TFA) and formic acid were HPLC-grades and were purchased from Fisher Scientific, USA. All other reagents were of analytical grade and the other chemicals used in this study were of the highest purity.

2.2 Extraction of crude metabolites from isolates of *Weissella ciberia*

Pure strains of *Weissella ciberia* were sub-cultured in 15 mL of MRS broth, which had a pH of 7.0 and contained glucose and peptone at concentrations of 0.25% w/v and 0.5% w/v respectively. The sub-cultured strains were then incubated under microaerophilic conditions for a period of 48 hours and at a temperature of 28±2 ºC, and subsequently incubated in a sterile MRS broth (1000 mL) for 5 days. The culture media were then centrifuged (10,000 g, 20 min, 4 °C) followed by filtration through a 0.45-µm filter, to obtain cell-free culture filtrate. 30 L of the cell-free culture filtrate were neutralized with concentrated hydrochloric acid and then extracted twice with an equal volume of ethyl acetate. The layers of ethyl acetate were combined and then dried over anhydrous sodium sulphate. The concentrates of metabolites were obtained using a rotary flash evaporator at 30 °C.

2.2 Chromatographic and spectrophotometric characterization of metabolites

Metabolites of *Weissella ciberia* were subjected to High-Performance Liquid Chromatography (HPLC) analysis and their identification and quantification were determined according to the method of Álvarez, Araya, Navarro-Lisboa and Lopez de Dicastillo (2016). The column thermostat was set at 40 °C, with a flow rate of 1 mL/min and an injection volume of 20 µL. The metabolites were identified based on the retention time of internal standards, and quantified from their standard curve calibration.

2.3 *In silico* analysis of antimicrobial potential of compounds of W. *ciberia* metabolites

2.3.1 Geometry optimization

The geometric optimization was conducted by employing Density Functional Theory (DFT) methodologies, which were implemented in the Spartan 14 software programme developed by Wavefunction Inc. (Hehre & Ohlinger, 2014). The chemical structures of the two-dimensional molecules were produced using Chemdraw software version 12.0.2. The DFT/B3LYP/6-31G* refers to the use of the Density Function Theory (DFT) methodology in conjunction with the B3LYP/6-31G* basis set. The technique of vacuum geometry optimisation, as described by Benarous, Cherouana, Aubert, Durand and Dahaoui (2016) was employed in order to mitigate strain and identify the chemically most efficient structure. Ultimately, the optimized structure was exported to an SDF file format to facilitate the generation of descriptors.

2.3.2 Optimization of receptor and ligands for docking

The crystal structures of receptors derived from *Escherichia coli* (PDB ID: 5R1R), and *Shigella flexneri* (PDB ID: 3LX6) were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) website (http://www.rcsb.org/). Following the acquisition of receptor designs, the Discovery Studio Visualizer version 2017 was employed to execute a pretreatment procedure encompassing the elimination of hetero atoms and water molecules. Furthermore, the SDF files holding the optimized molecules, also known as ligands, were transformed into PDB files to facilitate their use in the docking procedure. After being subjected to priming, the receptors were successfully aligned with their corresponding ligands (molecules).

2.3.3 The estimation of molecular docking Using PyRx

The molecular docking was performed using AutoDock Vina (version 4.2) in PyRx 30.8 (Dallakyan & Olson, 2015). The active binding site of the α-amylase with the mini-montbretin A was chosen as the grid centres. The centre grid box dimensions were chosen to include all atoms of the ligand set. The site of the grid box in α-amylase was set at −7.946, 10.438, and −21.863 Å (for x, y and z) by means of a grid of 40, 40, and 40 points (for x, y and z). The structures of metabolites found in W. *ciberia* were retrieved from PubChem database (Kim et al., 2019). The docking scores of the optimized metabolites (ligands) (described in section 2.3.2) were generated and the output of docking scores were defined as affinity binding (Kcal/mol). The docking scoring function was calculated as:

$$\Delta G_{bind} = \Delta G_{gauss} + \Delta G_{repulsion} + \Delta G_{hbond} + \Delta G_{hydrophobic} + \Delta G_{tors}$$

Where; ΔG_{gauss} = gauss attractive term for dispersion (two Gaussian functions); $\Delta G_{repulsion}$ = Square of the distance when closer than a threshold value; ΔG_{hbond} = Ramp function. $\Delta G_{hydrophobic}$ = interactions with metal ions; and ΔG_{tors} = number of rotatable bonds.

The interactions of ligands protein (docked complexes) were created and visualized using the Discovery Studio Visualizer (version v19.1.0.18287) (BIOVIA, San Diego, CA, USA) (Dassault Systèmes BIOVIA, 2017) (Adawara, Shallangwa, Mamza & Ibrahim, 2021).

3. RESULTS AND DISCUSSION

The bioactive compounds of metabolites inherent in isolated pure strains of *Weissella ciberia* were identified and quantified using HPLC, and the presence of atropine, gallic acid, naringinin, caffeine, maleic acid, gluthathione and saponin were verified. Table 1 depicts the chemical composition, quantification as well as retention time of the bioactive compounds in *Weissella ciberia*. Among identified compounds, it was observed that atropine had the highest quantity (10.193 mg/L) at a retention time of 1.764. Atropine has been established in several therapeutic applications for potentials in increasing the heart rate of individuals with abnormally low heart rates. Gallic acid, an important polyphenolic compound known for its antioxidant potential was identified (retention time 2.129) in the metabolites of W. *ciberia*. Although, it was detected at the lowest quantity (0.106 mg/L). The reports of the identified compounds in this study, corroborates with those of Kiran, Kibar, Haliscelik and Zatari (2023) who mentioned that the metabolites of *Weissella* sp. mostly contains antimicrobial, phenolic and flavonoid compounds.

Table 1: HPLC Identification and quantification of bioactive compounds derived from metabolites of *Weissella ciberia.*

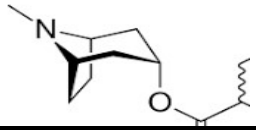
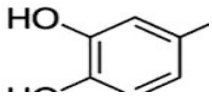
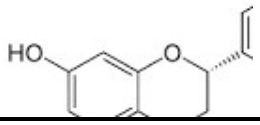
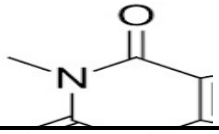
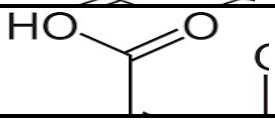
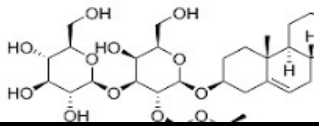
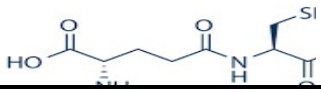
Compound	2D structure	Chemical quantification (mg/L)	Retention time (min)	PUBMED ID/Molecular formular/Molecular weight
Atropine		10.193	1.764	CID:174174 C17H23NO3 289.4 g/mol
Gallic acid		0.106	2.129	CID:370 C7H6O5 170.12 g/mol
Naringinin		0.357	2.322	CID:439246 C15H12O5 272.25 g/mol
Caffeine		0.817	3.208	CID:2519 C8H1ON402 194.19 g/mol
Maleic acid		7.341	3.778	CID:444266 C4H4O4 116.07 g/mol
Saponin		0.461	5.34	CID:198016 C58H94O27 12223.3 g/mol
Glutathione		0.796	6.338	CID:124886 C10H17N3O6S 307.33 g/mol

Figure 1 showed the gradient HPLC chromatograms and peak identification of the identified compounds of metabolites derived from W. *ciberia*. Owing to the fact that the highest quantity (10.193 mg/L) was observed in atropine, it concomitantly showed the highest peak.

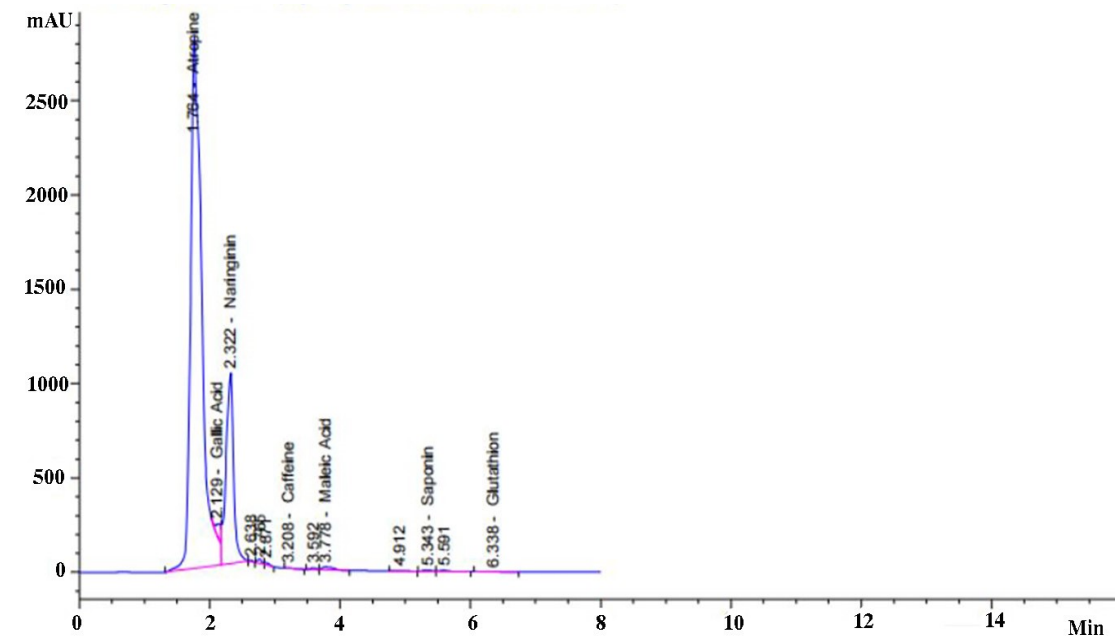


Figure 1: HPLC Chromatogram of identified liquid compounds in metabolites derived from W. *ciberia*.

Basically, *in silico* analysis centres on the evaluation of binding affinities of protein ligands to receptors. As receptors to the ligands (compounds of W. *ciberia* metabolites), the target microrganisms (*Escherichia coli* and *Shigella flexneri*) were prepared and their crystal structures are shown in figure 2.

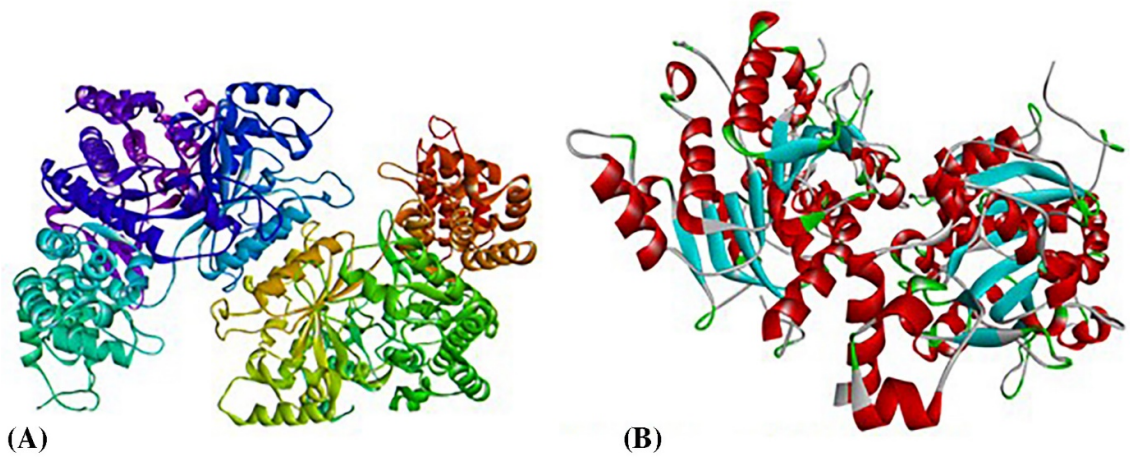


Figure 2: Prepared crystal structure of the receptors **(A)** *Escherichia coli* PDB ID: 5RIR **(B)** *Shigella flexneri* PDB ID: 3LX6.

The binding affinity of the compounds to the target microorganisms are reported in table 2. The binding scores are represented by their binding energies, calculated in kcal/mol. A lower binding energy value represents higher affinity. In this study, the results showed that the binding scores of atropine, gallic acid, naringinin, caffeine, maleic acid, saponin and glutathione against E. *coli* were -6.5 kcal/mol, -6.4 kcal/mol, -7.8 kcal/mol, -6.2 kcal/mol, -5.1 kcal/mol, -9.7 kcal/mol, and -6.4 kcal/mol respectively. Similarly, the binding affinity of atropine, gallic acid, naringinin, caffeine, maleic acid, saponin and glutathione against S. *flexneri* were 7.2 kcal/mol, -6.4 kcal/mol, -8.5 kcal/mol, -6.2 kcal/mol, -4.6 kcal/mol, -10.7 kcal/mol and -6.4 kcal/mol respectively. The results competed favorably with the binding scores of conventional standard antibiotics (meropenem and ciprofloxacin), whose binding affinities were -6.8 kcal/mol and -7.1 kcal/mol against E. *coli* respectively, and 7.0 kcal/mol and -

7.8 kcal/mol against S. *flexneri* respectively. Among all identified compounds, naringinin and saponin were observed to have higher binding scores; hence, higher binding affinities and was comparable to the standard conventional antibiotics tested. Thus, their high binding affinities indicates their potentials as antimicrobial agents.

Table 2: *In silico* binding scores of HPLC identified compounds and standard antimicrobial drugs with selected target microorganisms

Compound	*Shigella flexneri*	*Escherichia coli*
Atropine	-7.2	-6.5
Gallic Acid	-6.4	-6.4
Naringinin	-8.5	-7.8
Caffeine	-6.2	-6.2
Maleic Acid	-4.6	-5.1
Saponin	-10.7	-9.7
Glutathion	-6.4	-6.4
Ciprofloxacin	-7.8	-7.0
Meropenem	-7.1	-6.8

In order to evaluate the interactions between the ligands and target microorganisms (receptors), molecular docking interactions of nariginin and saponin (having the highest binding scores) with E. *coli* were assessed and their level of interactions were compared with standard conventional antibiotics (ciprofloxacin). The 2D molecular docking interactions are shown in figure 3, and the result revealed largely favourable interactions between the compounds and the proteases of the receptors of the microbe. The interaction between naringinin and E. *coli* showed three covalent hydrogen bonds and one alkyl bond. Between saponin and E. *coli*, the interaction of 2D molecular docking showed six covalent hydrogen bonds, three alkyl bonds, one carbon-hydrogen bond and one unfavourable interaction; while the interaction between ciprofloxacin and E. *coli* showed two covalent hydrogen bonds and one Pi bond.

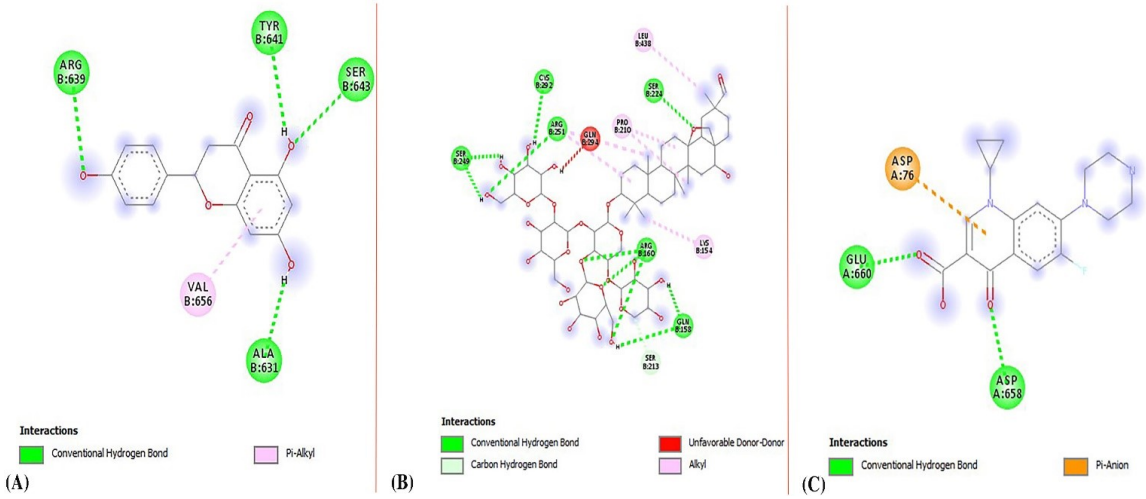


Figure 3: 2D molecular docking interactions of **(A)** Naringinin and E. *coli* **(B)** Saponin and E. *coli* and **(C)** Ciprofloxacin and E. *coli*.

Similarly, the interactions between naringinin, saponin and ciprofloxacin with S. *flexneri* are shown in Figure 4, and the results were largely favourable. The interactions between saponin and S. *flexneri* showed ten covalent hydrogen bonds, five alkyl bonds and one unfavourable bond. The interaction of naringinin with S. *flexneri*

showed three covalent hydrogen bonds and two unfavourable interactions; while that for ciprofloxacin and S. *flexneri* showed three covalent hydrogen bonds, one alkyl bond and one halogen interaction. These largely favourable interactions further established possible antimicrobial potentials of saponin and naringinin which are components of *Weissella ciberia* metabolites.

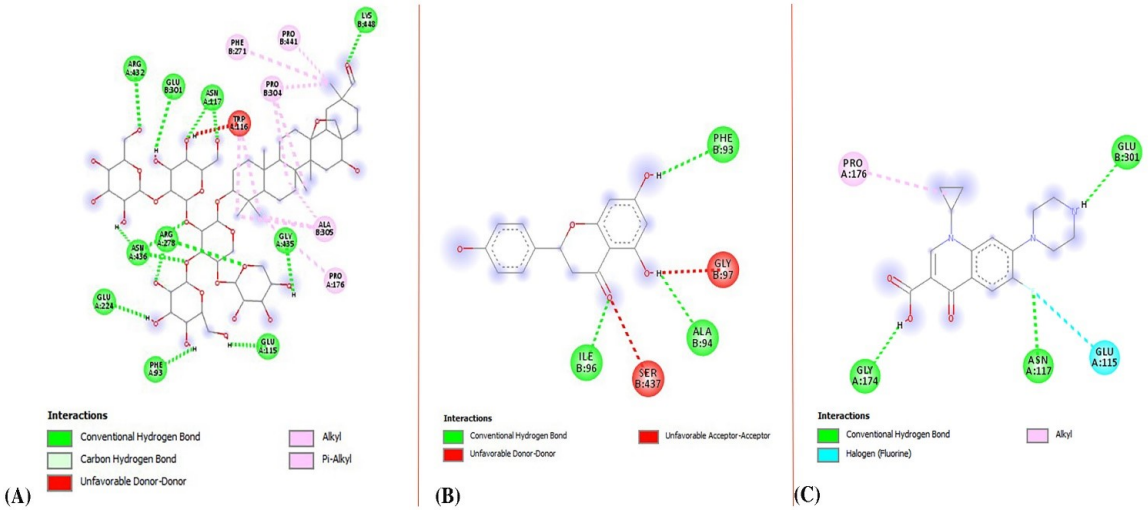


Figure 4: 2D molecular docking interactions of **(A)** Naringinin and S. *flexneri* **(B)** Saponin and S. *flexneri* and **(C)** Ciprofloxacin and S. *flexneri*.

4. CONCLUSION

The *in silico* evaluation has shown that metabolites of *Weissella ciberia* have several compounds with antimicrobial potentials as a result of their binding affinities with the target microorganisms investigated in this study. Significantly high potential qualities as antimicrobial agents were exhibited by nariginin and saponin, and they were favorably competitive with conventional antibiotics (ciprofloxacin and meropenem) commonly used against the target microorganisms. This study demonstrated the efficacy of compounds of *Weissella ciberia* metabolites as antimicrobial agents. The potential antimicrobial activity of the compounds was presumed to have been influenced by the binding affinity of the protein ligands against the receptors.

For further studies, these compounds could be subjected to validation studies involving structural elucidation for possible optimization of its binding affinities for enhanced antimicrobial efficacy. In addition, *in vitro* and *in vivo* studies could be imbibed for confirmation of its potentials and possible application in the food industry (for biopreservation) as well as medical industry.

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