

***In silico* antimicrobial properties of saponins against food pathogens and spoilage microorganism**

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ABSTRACT

Most recent outbreaks of foodborne diseases and illnesses have been linked to microbial contamination of food products, which can most definitely cause food loss due to food spoilage and eventual wastage and hence play a key role in global food security. Prevention of food spoilage is commonly done with the use of chemical preservatives, most of which possess negative impacts on human health such as carcinogenicity, gastrointestinal upset and cardiovascular diseases. This necessitate a healthier and natural alternative preservative. Saponins are natural plants compounds with many health benefits. This current study evaluates the antimicrobial potential of saponins on some food pathogens and spoilage microorganisms such as *Staphylococcus aureus* (Gram positive bacterium), *Escherichia coli* (Gram negative bacterium) and *Aspergillus niger* (fungus). Molecular docking of different saponins were carried out with drug targets peptide deformylase (*Staphylococcus aureus*), DNA gyrase (*E. coli*) and DNA topoisomerase (*Aspergillus niger*) using Autodock Vina. Cucurbitacin and diosgenin exhibited better interaction (-7.9 kcal/mol) with peptide deformylase when compared with actinonin (-7.6 kcal/mol), while momordin 1C showed more binding affinity with DNA gyrase (-8.6 kcal/mol) and DNA topoisomerase (-9.5 kcal/mol) respectively. Glu 155 (peptide deformylase), Asn-46, Glu-50, Gly-77, Thr-165, Val-171 (DNA gyrase) and Glu-382, Arg-390, Asn-517, Thr-526, Asp-553 are important amino acid residues enhancing protein-saponin interaction. Saponins may help to improve global food security and shelf life by preventing food spoilage and wastage due to their excellent antimicrobial activity occasioned by their inhibitory action on key proteins essential for survival of microbes investigated in this study.

Keywords: Phytochemicals, food security, Food spoilage, molecular docking, preservatives.

Introduction

Universally, food spoilage instigated by microbes still extensively affects all kinds of food and leads to food waste and forfeiture, even in industrialized nations. It has been predicted that the annual losses

of global food grasp about 40% owing to numerous reasons comprising spoilage by microorganisms (Gustavsson *et al.*, 2011). The most common types of microbes that cause the spoilage of a substantial

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amount of food and food products are Bacteria, yeast, and molds (Lianou *et al.*, 2016). As soon as these microbes reach food products, they propagate by exploiting the nutrients and thereafter produce metabolites that lead to food spoilage (Parlapani *et al.*, 2017). Another persistent food safety problem triggered by intake of polluted food products, which has been a crucial safety distress to public health is food-borne disease (Kirk *et al.*, 2017). Microorganisms are ubiquitous and they can stay alive under hostile and extreme circumstances employed in the food preservation, some of which include modified atmosphere packaging, low temperature, vacuum packaging, in addition to conventional pasteurization (Provincial *et al.*, 2013; Saraiva *et al.*, 2016; Säde *et al.*, 2017). Therefore, there is a sizeable disquiet amongst consumers concerning the jeopardy of using artificial additives and preservatives for human well-being as the use of these synthetic chemicals in food preservation may lead to deleterious side effects (Gyawali and Ibrahim, 2014; Caleja *et al.*, 2016; Kalem *et al.*, 2017).

The development of food preservation processes has been driven by the need to extend the shelf-life of foods. Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Several food preservation systems such as heating, refrigeration and addition of antimicrobial synthetic chemical compounds can be used to reduce the risk of outbreaks of food poisoning. However, these techniques have been frequently associated with adverse changes in organoleptic characteristics and loss of nutrients in food (Gul and Bakht, 2015; Rawat, 2015).

Consequently, new environmental physiologically pleasant procedures are essential to diminish the growth of disease-causing pathogens and lengthen the shelf-life of food yields, without employing synthetic/inorganic chemical preservatives. In recent times, numerous investigations have confirmed the potential application of some plant extracts as effective natural preservatives (Suppakul *et al.*, 2016;). This is not far-fetched

from the fact that phytochemicals possess the ability to bind with certain proteins (drug targets) such as DNA topoisomerase (Suppakul *et al.*, 2016), peptide deformylase, DNA gyrase (Clarke *et al.*, 2017) present in the microorganism. Medicinal plants comprise numerous phytochemicals including flavonoids, alkaloids, tannins, saponins and terpenoids, which have antimicrobial and antioxidant properties (Talib and Mahasneh, 2010; Khan *et al.*, 2013).

The antimicrobial activities of some plant species have been extensively investigated. The crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other aromatic plant display antimicrobial properties against a wide variety of Gram-positive and Gram-negative bacteria (Alzoreky and Nakahara, 2003; Castro *et al.*, 2008). Furthermore, it has been testified that extracts from Chinese chives and cassia can effectively decrease the growth of *Escherichia coli* and other bacteria for the period of food (meat, juices and milk) storage (Mau *et al.*, 2001).

Proper comprehension of the mechanism of antimicrobial action of phytochemicals is key as it is the first step in the prime application of plant extracts as natural antimicrobial mediators in order to prolong the shelf-life and uphold food quality (Gonelimali *et al.*, 2018). Thus, this work seeks to investigate the interaction of saponins with peptide deformylase as drug target for *Staphylococcus aureus* (Gram positive bacterium), DNA gyrase for *E. coli* (Gram negative bacterium) and DNA topoisomerase for *Aspergillus niger* (a fungus) using molecular docking.

Materials and methods

Retrieval of 3D structure of target enzymes

The 3D crystal structures of peptide deformylase from *Staphylococcus aureus* complexed with actinonin (PDB ID: 1Q1Y) with resolution 1.9 Å and crystal structure of DNA gyrase (1KZN) from *E. coli* in complex with clorobiocin with resolution 2.3 Å were retrieved from the Protein Data Bank

(<http://www.rcsb.org>) while the 3D structure of (GenBank GAQ33639.1) was modeled using SWISSMODEL server.

Ligand selection

Saponins are secondary metabolites synthesized by plants to protect them against microbes. We hypothesize that saponins can be extracted and used in preserving food against microbes. The structures of saponins (arjunolic acid, charantin, cucurbitacin, diosgenin, momordicoside D, momordin C and oleanolic acid) were drawn using Marvin suites after retrieval from Pubmed (<https://www.ncbi.nlm.nih.gov/pubmed/>).

All ligands selected for docking studies were optimized using the MMFF94 force-field.

Docking

The docking analyses of various saponins with peptide deformylase, DNA gyrase and DNA topoisomerase were carried out using AutoDock Vina. This program was run using a searching grid extended over ligand molecules with box spacing and coordinates listed in Table 1. The saponins into various targets and the highest scoring pose was selected for each of the compound. The best docking poses are predicted to be the most stable conformation of each compound.

DNA topoisomerase for *Aspergillus niger*

Results

Table 1 shows the binding affinity, Hydrogen bonding and hydrophobic interaction of saponins (Arjunolic acid, Charantin, Cucurbitacin, Diosgenin, Momordicoside D, Momordin 1C, Oleanolic acid) with peptide deformylase (1Q1Y) of *Staphylococcus aureus*. Both Cucurbitacin and Diosgenin scored highest (-7.9 kcal/mol) in binding affinity with peptide deformylase (1Q1Y) of *Staphylococcus aureus* possessing both hydrogen bonding and hydrophobic interaction with the enzyme.

As depicted by Table 2 and 3 which present the binding affinities, Hydrogen bonding and hydrophobic interactions of Arjunolic acid, Charantin, Cucurbitacin, Diosgenin, Momordicoside D, Momordin 1C and Oleanolic acid, when docked with DNA gyrase (1KZN) of *E. coli*. and DNA topoisomerase of *Aspergillus niger* respectively, Momordin 1C demonstrated the best binding affinity score with both DNA gyrase (1KZN) of *E. coli*. (-8.6 kcal/mol) and DNA topoisomerase of *Aspergillus niger* (-9.5 kcal/mol) - an interaction which was strengthened vehemently by various forms of hydrogen bonding and hydrophobic interactions.

Table 1: Binding affinity, H-bonding and hydrophobic interaction of saponins with peptide deformylase (1Q1Y) of *Staphylococcus aureus*.

Compounds	Binding Energy (Kcal/mol)	Hydrogen bonding	Hydrophobic interaction
1 Arjunolic acid	-6.8	Nil	Ser57, Leu112, Gly110, Gly58, Val151, Leu105, Tyr147, Val 59, Glu109, His154, Glu185
2 Charantin	-7.6	3 (Ser57, Val118, Asn117)	Thr107, Ala119, Arg56, Gly60, Leu112, Gly110, Gly58, Glu155, Tyr147, Leu105, Glu109, Val59, ile150, His154, Val151
3 Cucubitalcin	-7.9	4 (Gly60, Gly110, Tyr147, Glu155)	Leu112, Gly58, His154, Glu109, Leu105, Glu185, His186, Pro78, Val59
4 Diosgenin	-7.9	2 (Val151, Glu155)	Arg56, Ser57, His154, Val59, Tyr147, Gly110, Asn117
5 Momordicoside D	-7.6	1 (Glu155)	Val151, Val159, Glu45, His186, Pro78, Tyr147, Glu185, Ser57, Gly58, Gly111, His 154, leu 112
6 Momordin 1C	-7.8	8 (Gly185, Gly108, Gly110, Gly60, Glu155, Thr107, Ser57, val59)	Asp80, Pro78, Leu105, Glu109, Gly58, His154, Leu112
7 Oleanolic acid	-7.7	1 (Arg56)	Ser57, Glu155, Gly110, His154, Val59, Val151, Tyr147, Leu105, Glu109, Glu185

Table 2. Binding affinity, H-bonding and hydrophobic interaction of saponins with DNA gyrase (1KZN) of *E. coli*.

Compounds	Binding Energy (Kcal/mol)	Hydrogen bonding	Hydrophobic interaction
1 Arjunolic acid	-6.8	1(Ser121)	Glu50, Arg76, Ala53, Asp49, Ile90, His95, Gly119, Val120, Ala96
2 Charantin	-6.6	2 (Thr165, Asn46)	Gly67, Ile78, Arg76, Glu50, Ile90, Ala96, His95, Gly117, Glu42, Asp49, Asp45
3 Cucubitalcin	-8.3	4 (Gly117, Asn46, Val43, Glu50)	Asp73, Thr165, Val167, Gly119, Ala47, Ile78, Ile90, Asp49, Val118
4 Diosgenin	-8.1	Nil	Ile78, Ile90, Asn46, Asp73, Glu50, Pro79
5 Momordicoside D	-7.4	1 (His95)	Ala96, Asn46, Val171, Ile90, Glu50, Gly77, Val43, Ile78, Ala47, Thr165
6 Momordin 1C	-8.6	5 (Gly77, Glu50, Thr165, Val171, Asn46)	Ala86, Pro79, Ile90, Ile78, Val120, Arg76, Ala47, Val43, Asp 73, Val167
7 Oleanolic acid	-7.7	2 (Arg76, Glu50)	Ile78, Ile90, Ala56

Table 3. Binding affinity, H-bonding and hydrophobic interaction of saponins with DNA topoisomerase of *Aspergillus niger*.

Compounds	Binding Energy (Kcal/mol)	Hydrogen bonding	Hydrophobic interaction
1 Arjunolic acid	-7.9	4 (Lys451, Lys400, Arg390, His393)	Thr526, Glu523, Ala524, Asp553, Lys519, Phe387, Glu382, Gly389
2 Charantin	-8.1	2 (Asp269, Ser401)	Asn445, Asp299, Asp302, Ile303, Pro247, Phe244, Pro245, Val403, Gln404, Tyr248, Glu272, Arg402
3 Cucubitalcin	-8.5	3 (Arg300, Lys519, Lys551)	Pre387, His393, Asp553, Ala524, Glu523, Ser531, Gly528, Gly389, Gly551, Arg514, Val527, Asn517, Gly516
4 Diosgenin	-9.2	2 (Glu523, Asn447)	His393, Thr526, Lys519, Asn517, Ala515, Arg514, Gly516
5 Momordicoside D	-8.2	4 (Asp553, Asn517, Lys519, Thr526)	Thr591, Asn595, Lys608, Arg514, His393, Ala524, Glu523, Lys552
6 Momordin 1C	-9.5	5 (Glu382, Arg390, Asp553, Thr526, Asn517)	Lys522, Lys519, Ser554, Val527, Gly516, Gly528, Phe387, Gly389, Lys451, Leu386, Lys400, Met280
7 Oleanolic acid	-7.7	2 (Pro246, Arg402)	Pro247, Glu249, Pro245, Arg339, Gly279, Pro383, Pro276, Pro384

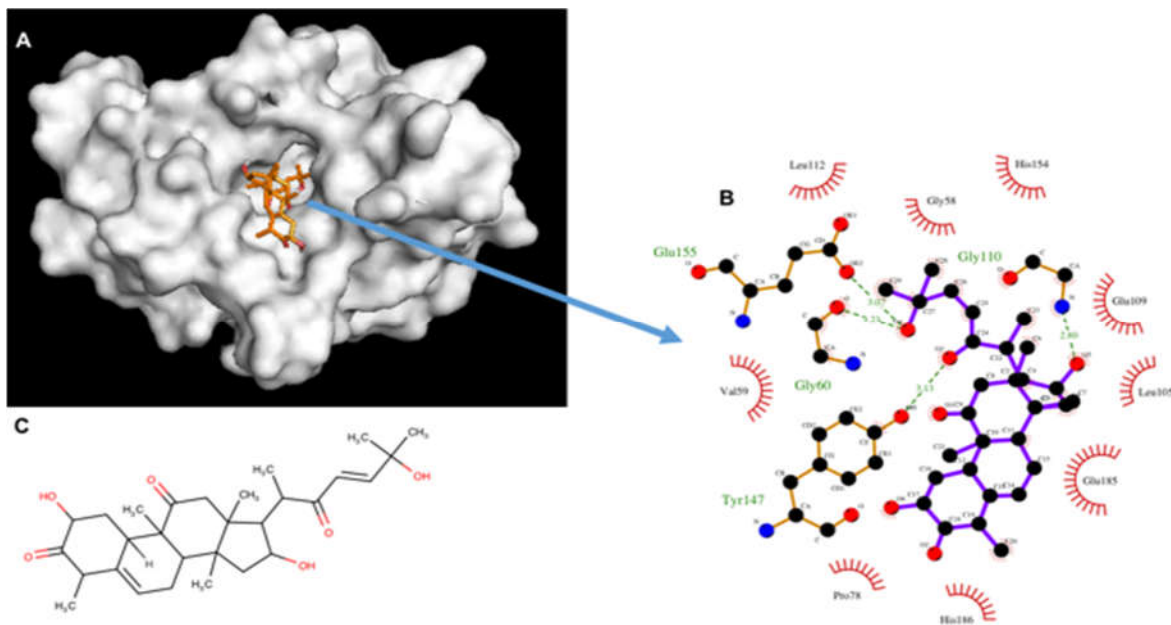


Figure 1: Binding pose of cucubitalcin (-7.9 kcal/mol) with peptide deformylase of *S. aureus* (A), molecular interaction of cucubitalcin with amino acid residues within the active site of peptide deformylase (B), 2D structure of cucubitalcin.

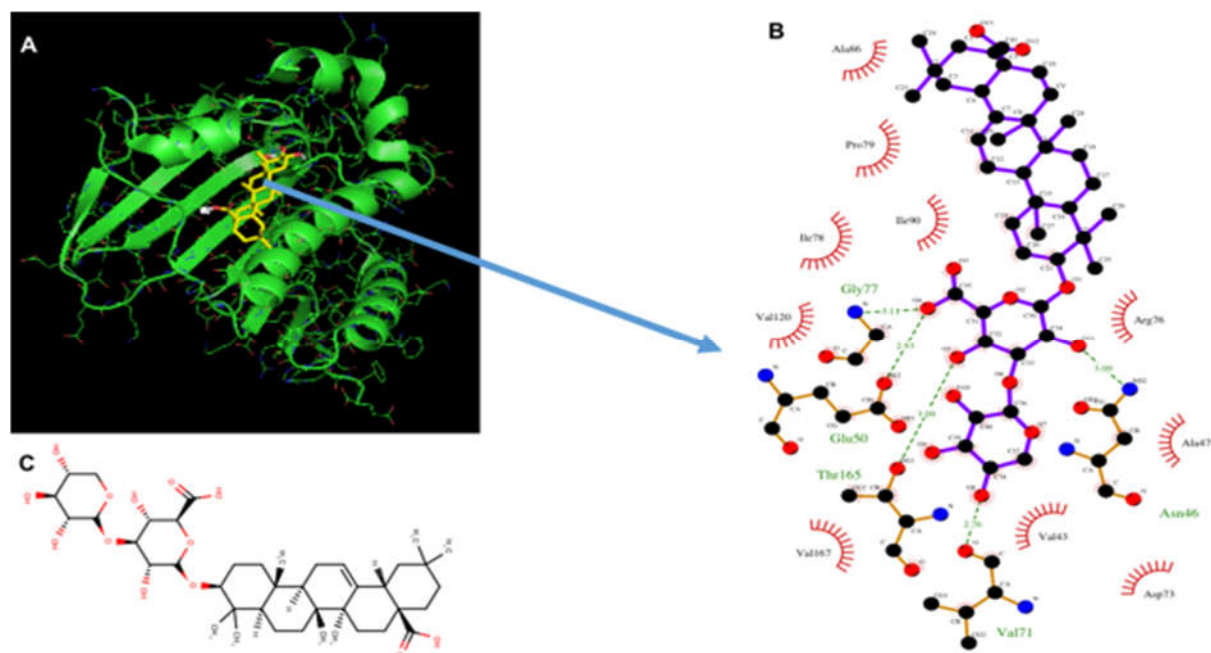


Figure 2: Binding pose of momordin C (-8.6 kcal/mol) with DNA gyrase of *E. coli* (A), molecular interaction of momordin C with amino acid residues within the active site of DNA gyrase (B), 2D structure of momordin C.

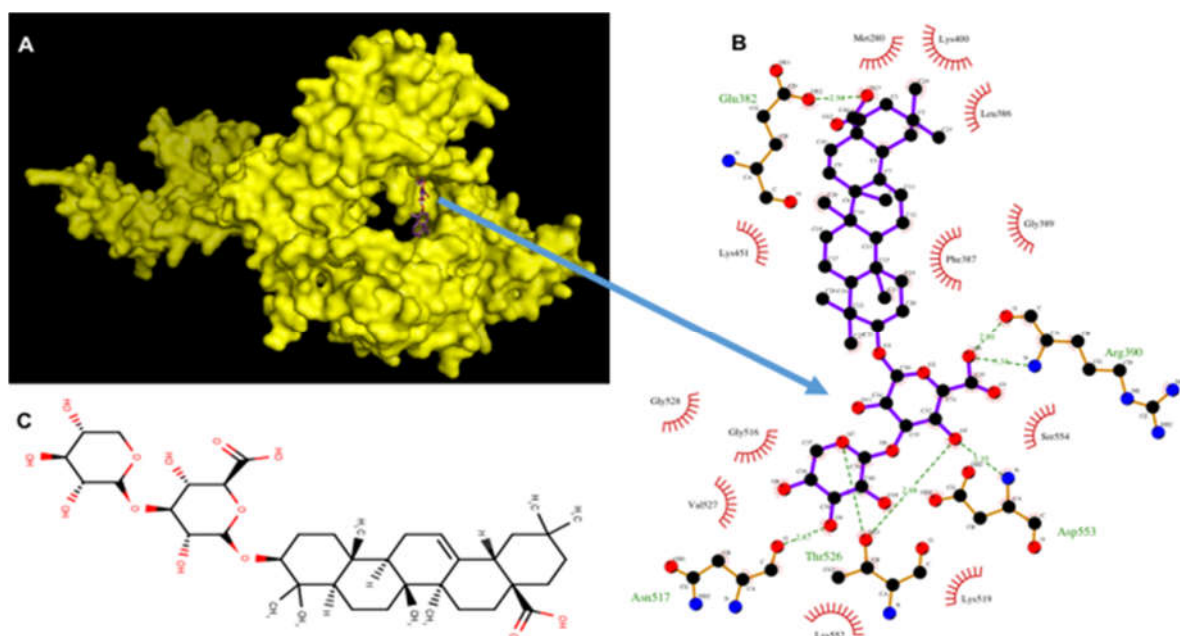


Figure 3: Binding pose of momordin C (-9.5 kcal/mol) with DNA topoisomerase of *A. niger* (A), molecular interaction of momordin C with amino acid residues within the active site of DNA topoisomerase (B), 2D structure of momordin C.

Discussion

Food spoilage is a metabolic process that causes foods to be unattractive or obnoxious for human consumption due to changes in sensory characteristics. The issue of food loss is of great prominence in the efforts to fight hunger, raise income and improve food security in the world's developing countries. Food losses have an influence on food security for poor people, on food quality and safety, on economic development and on the environment (Rawat, 2015). Improving the proficiency of the food supply chain could assist in lowering the cost of food to the purchaser and therefore upsurge food access. Given the degree of food loss, making cost-effective investments in plummeting food loss could be one way of decreasing the cost of food. Chemical reactions that cause offensive sensory changes in foods are mediated by a range of microbes that use food as a carbon and energy source (Burkepile *et al.*, 2006; Rawat, 2015). Because food products are nowadays frequently retailed in areas of the world farther away from their production locations, the need for protracted safe shelf-life for these products cannot be over-emphasized.

Saponins - secondary metabolites manufactured by plants - are amphipathic glycoside grouped phenomenologically by the soap-like foam they give when shaken in aqueous solution, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroids derivative (Gershenson and Kreis, 2018). Over the years, saponins have been revealed to own vast

pharmacological and therapeutic capabilities ranging from antioxidant to immune-therapeutic, anticancer, neuroprotective as well as surfactant (Singh and Goel, 2016; Kang *et al.*, 2016; Wang *et al.*, 2018). The effect of saponins from various plants against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, *Candida parapsilosis*, *Candida pseudotropicalis*, and *Candida stellatoidea* (Abbasolu and Turkoz, 2008) and their effect on multidrug resistant *E. coli* have been documented (Arabski *et al.*, 1995). Saponins selected for this study to investigate its possible mechanism against *Staphylococcus aureus* (Gram positive bacterium), *E. coli* (Gram negative bacterium) and for *Aspergillus niger* (a fungus) include Oleanolic acid, Momordin 1C, Momordicoside D, Diosgenin, Cucurbitacin, Charantin and Arjunolic acid.

Peptide deformylase (PDF) is a metalloenzyme that eliminates the formyl group during eubacterial peptide elongation, which in turn, play a central part in protein maturation. PDF has been shown to be crucial for bacterial growth and highly conserved in bacteria (Lewandowski *et al.*, 2013). This makes PDF an appropriate novel target for antibiotic discovery and development. Thus, a large number of PDF inhibitors have been obtained over the past decade (Chung and Huh, 2015) by researchers who were partly inspired by studies of actinonin, a naturally occurring antibacterial that acts by inhibiting PDF (Peyrusson *et al.*, 2015). Cucurbitacin and Diosgenin ranked highest (-7.9 kcal/mol) in binding affinity with peptide deformylase (1Q1Y) of

Staphylococcus aureus (Table 1) better than that of a standard drug, actinonin (-7.6 kcal/mol). The interactions of these compounds were stabilized by hydrogen bonding and hydrophobic interaction. During the docking simulation of peptide deformylase with the selected saponins, four residues within the active site of peptide deformylase (Gly-60, Gly-110, Tyr-147, Glu-155) were intricate in hydrogen bond formation with cucubitaicin while two residues within the active site of peptide deformylase (Glu-155, Val-151) were saliently involved in hydrogen bond formation with diosgenin. Hydrophobic interactions also contributed to the interaction of cucubitaicin with amino acid residues (Leu 112, Gly 58, His 154, Glu 109, Leu 105, Glu 185, His 186, Pro 78, Val 59) and Diosgenin with amino acid residues (Arg 56, Ser 57, His 154, Val 59, Tyr 147, Gly 110, Asn 117) within the active site of with peptide deformylase of *Staphylococcus aureus* (Table 1). Therefore, inhibition of peptide deformylase is a potent target for alternative food preservation as it prevents the rapid growth and development of bacteria.

DNA gyrase is an important bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase are involved in the control of topological interconversions of DNA molecules and also capable of relaxing negatively supercoiled DNA in the absence of ATP (Sutormin *et al.*, 2018). The unique capability of gyrase to insert negative supercoils into DNA at the expense of ATP hydrolysis is what permits bacterial DNA to possess free negative supercoils and this ability comes into play

during DNA replication and prokaryotic transcription (Caamaño-Antelo *et al.*, 2015; Sutormin *et al.*, 2018). The helical nature of the DNA causes positive supercoils to accumulate ahead of a translocating enzyme, in the case of DNA replication, a DNA polymerase. The ability of gyrase (and topoisomerase IV) to relax positive supercoils allows the release of superhelical tension ahead of the polymerase so that replication can continue (Chan *et al.*, 2017). Gyrase is present in prokaryotes and some eukaryotes, but the enzymes are not completely similar in structure or sequence, and have varying affinities for different molecules. This makes gyrase a decent target for antibiotics (Chan *et al.*, 2017; Sutormin *et al.*, 2018). Momordin 1C exhibited better interaction by showing more binding affinity with DNA gyrase (1KZN) of *E. coli* (-8.6 kcal/mol) (Table 2) and this interaction was stabilized and sustained by hydrophobic interaction and hydrogen bonding. Gly-77, Glu-50, Thr-165, Val-171, Asn-46 are important residues for hydrogen bonding when momordin 1C interacted with DNA gyrase of *E. coli* and Ten residues were responsible for hydrophobic interaction (Ala-86, Pro-79, Ile-90, Ile-78, Val-120, Arg-76, Ala-47, Val-43, Asp-73, Val-167) (Table 2). Hence, the inhibition of DNA gyrase by momordin 1C is implicative of its vast antimicrobial abilities and thus, is probably a potent alternative for synthetic chemical preservatives.

Bacterial topoisomerases are able to catalyze changes in the topology of DNA and are clinically valid targets for antibacterial drug discovery. The complexity of reactions in DNA replication catalyzed by DNA topoisomerase offers multiple opportunities

for therapeutic intervention (Zhang *et al.*, 2016). Momordin 1C, among all other saponins used, showed superior interaction with DNA topoisomerases during the docking simulation by showing more binding affinity with DNA topoisomerase of *Aspergillus niger* (-9.5 kcal/mol) (Table 3). The hydrogen bonding interaction of Momordin 1C with DNA topoisomerase of *Aspergillus niger* was enhanced by five amino acid residues (Glu 382, Arg 390, Asp 553, Thr 526, Asn 517) at the active site and consequentially, twelve residues are responsible for its hydrophobic interaction (Lys522, Lys519, Ser554, Val527, Gly516, Gly528, Phe387, Gly389, Lys451, Leu386, Lys400, Met280) (Table 3). This points to the fact that Momordin 1C possess the ability to inhibit DNA topoisomerase in bacteria and therefore is a probable answer to the long-awaited search for alternative food preservative.

Conclusion

The results of this *in silico* study suggest that cucubitaicin, diosgenin and momordin 1C are potential antimicrobial saponins and their mechanisms of action are probably inhibition of peptide deformylase, DNA gyrase and DNA topoisomerase respectively and these specific target proteins are required by the microorganism for growth and replication. Therefore, they may be used in food industries as an alternative natural food preservative.

Conflict of interest

The authors declare no conflict of interest.

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