ANTIVIRAL EFFECTS OF GARLIC SPICE USED IN ANIMAL PRODUCTS FOR THE DEVELOPMENT OF FUNCTIONAL FOODS

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ABSTRACT
The study evaluates the docking scores, LD50 values, toxicity classes, amino acid affinities, bonds, and charges of garlic compounds with crystal structure of Hepatitis B X-Interacting Protein. An in-silico approach was employed to assess the interactions between compounds present in garlic and molecular targets implicated in hepatitis pathogenesis which was sourced from ethnobotanical databases, were prepared using the Ligprep module for molecular docking. The crystal structure of Hepatitis B X-Interacting Protein with PDB ID 3MSH was employed molecular docking using the Glide ligand docking in Schrödinger Suite 2023. The result obtained shows Garlicin, Gallic Acid, 3,4-Dihydroxybenzoic Acid, and 2,4-Dihydroxybenzoic Acid demonstrate strong binding potential with diverse toxicity profiles. Amino acid analysis reveals common interacting residues like ASP 80, suggesting significant roles in ligand binding. Bonds such as salt bridges and pi-pi stacking interactions contribute to ligand affinity, while charge characteristics influence electrostatic interactions with amino acids. Overall, this study identifies Garlicin, Gallic Acid, and 3,4-Dihydroxybenzoic Acid as leading candidates for therapeutic use, demonstrating strong binding potential against virus such as Hepatitis.
Keywords: Antiviral, Garlic, Spice, Animal Products, Functional Foods

INTRODUCTION

Spices are aromatic plant parts that are mostly used to enhance the taste and flavor of foods. However, their advantages are not limited to culinary purposes alone; they are also used in agriculture. Spices contain several bioactive compounds that have antimicrobial, antioxidant, and anti-inflammatory properties, among others. Spices are commonly used in animal products as flavour enhancers, preservatives, and antimicrobial agents during processing. In beef, pork, and lamb production, spices are used to mask the gaminess of meat during processing and increase its shelf life (Finzi, 2014). Similarly, spices are also used in poultry processing to improve the taste, texture, and appearance of the meat while extending its shelf life (Huque & Haque, 2011)

Moreover, the use of spices in animal products has been known to add nutritional value to the products as well. For instance, ginger and garlic contain antioxidants that can help protect meat from oxidative damage caused by processing (Gupta *et al*., 2014). Similarly, turmeric has been found to contain anti-carcinogenic properties, making it a valuable ingredient for processed meat products (Finzi, 2014).

Garlic has potent antimicrobial properties that make it an effective natural remedy for treating various infections. According to a review by (Naganuma *et al.,*2006), garlic extract possesses antiviral and antibacterial activities against several strains of bacteria such as Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, and Streptococcus pyogenes. The authors also reported the ability of garlic extract to inhibit the growth of influenza A and B viruses, respiratory syncytial virus, and herpes simplex virus type 1 and 2.

Apart from its antimicrobial properties, garlic is also known for its cardioprotective effects. A study by (Ashraf *et al.,* 2018) found that garlic supplementation significantly reduced total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride levels in hyperlipidemic individuals compared to placebo. Additionally, garlic demonstrated antiplatelet effects and improved endothelial function, which are considered crucial factors in preventing the development of cardiovascular diseases.

Functional foods are foods that have been shown to have health benefits beyond their basic nutritional content. These foods contain bioactive compounds that can improve various aspects of health, including cardiovascular and immune function. The concept of functional foods has gained popularity in recent years as consumers become more interested in foods that not only taste good but also offer health benefits (Mozaffarian, 2016).

Hepatitis B virus (HBV) is a major global health concern, causing chronic liver infection and inflammation. It is a double-stranded DNA virus belonging to the Hepadnaviridae family (Kao *et al.,*2018). HBV infection is transmitted through bodily fluids, most commonly through blood-to-blood contact or exposure to infected mucous membranes (8). Chronic HBV infection can lead to serious complications like cirrhosis, liver failure, and even

hepatocellular carcinoma (HCC), a type of liver cancer (Chen *et al.,* 2019). Fortunately, an effective vaccine exists for HBV, and vaccination remains the primary strategy for preventing infection World Health Organization. (2021) Despite the availability of a vaccine, HBV remains a significant public health burden, particularly in resource-limited settings. Globally, an estimated 257 million people were living with chronic HBV infection in 2019 World Health Organization. (2021). However, ongoing efforts towards promoting vaccination and early detection & treatment offer hope for controlling and eventually eliminating HBV as a public health threat.

MATERIALS AND METHODS

Materials: Computer (laptop), Schrödinger Suite 2023 13.5, Web servers (Protox-II) and selected bioactive compound of turmeric which was obtained from Pubchem site (https://pubchem.ncbi.nlm.nih.gov)

Ligand Preparation: The Ligprep module from Schrödinger Suite 2023 13.5 was utilized to prepare a total of 19 phytocompounds extracted from garlic, as identified from ethnobotanical databases in preparation for molecular docking.

Protein Preparation: The crystal structure of Hepatitis B X-Interacting Protein with PDB ID: 3MSH were obtained from the RCSB protein data bank (PDB) method: X-RAY diffraction, with a resolution 1.51 Å, R-Value Free: 0.212, R-Value Work: 0.180 and R-Value Observed: 0.183.

Receptor Grid Generation: Using the receptor grid construction tool in Schrödinger Maestro 13.5, the scoring grid was defined and supported by the co-crystalized ligand GK.

Molecular Docking: Molecular docking of the prepared ligands and proteins were performed using the glide ligand docking in Schrödinger Suite 2023.

RESULTS AND DISCUSSIONS

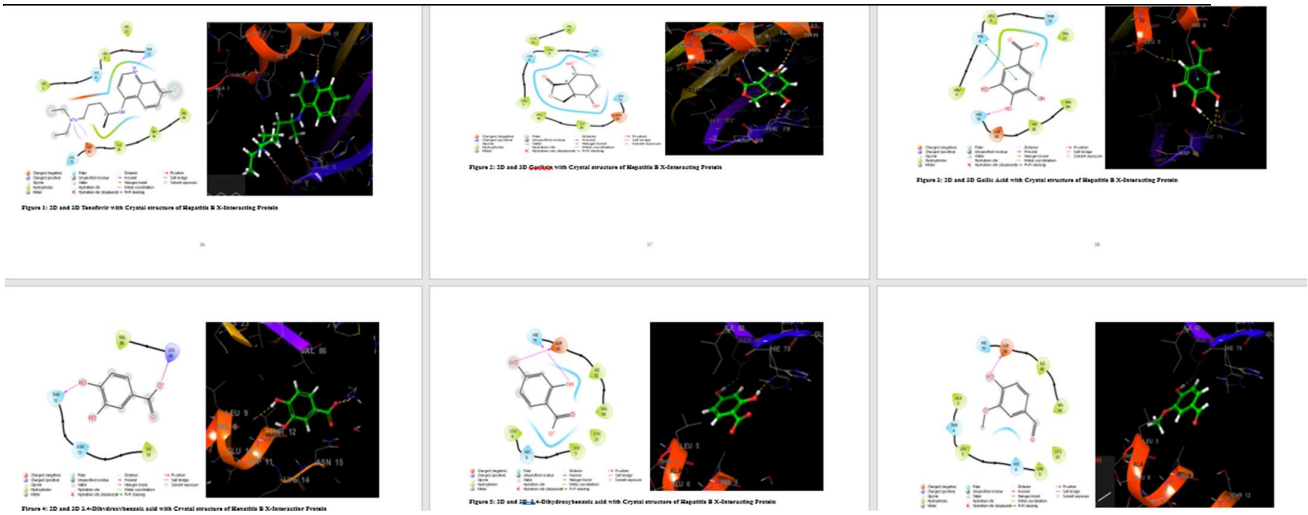
Table1 shows the docking score, LD50 and toxicity class. Tenofovir has a relatively low docking score of -3.69, indicating strong binding potential. The LD50 value is 13 mg/kg, placing it in toxicity class 2, which suggests moderate toxicity. Garlicin has a docking score of -5.24, indicating strong binding affinity. The LD50 value is 100 mg/kg, placing it in toxicity class 3, suggesting moderate toxicity. Gallic Acid has a docking score of -4.95, indicating strong binding potential. The LD50 value is 2000 mg/kg, placing it in toxicity class 4. 3,4-Dihydroxybenzoic acid has a docking score of -4.7, indicating strong binding potential. The LD50 value is 2000 mg/kg, placing it in toxicity class 4. 2,4-Dihydroxybenzoic acid has a docking score of -4.62, indicating strong binding potential.

The LD50 value is 1250 mg/kg, placing it in toxicity class 4, suggesting relatively low toxicity. Vanillin has a docking score of -4.59, indicating strong binding potential. The LD50 value is 1000 mg/kg, placing it in toxicity class 4. Terpinolene has a docking score of -4.54, indicating strong binding potential. The LD50 value is 4390 mg/kg, placing it in toxicity class 5, while Vinyl dithiin has a docking score of -4.32, indicating strong binding potential. The LD50 value is 260 mg/kg, placing it in toxicity class 3. Biochanin A has a docking score of -4.29, indicating strong binding potential. The LD50 value is 2500 mg/kg, placing it in toxicity class 5. 1,3-Dithiane has a docking score of -4.22, indicating strong binding potential. The LD50 value is 2400 mg/kg, placing it in toxicity class 5. Dithiin has a docking score of -4.08, indicating strong binding potential. The LD50 value is 260 mg/kg, placing it in toxicity class 3 while Allixin has a docking score of -4.07, indicating strong binding potential. The LD50 value is 1000 mg/kg, placing it in toxicity class 4.

Table 1: The Docking Score, LD50 and Toxicity Class

Ligand	CID compound	Docking score	LD50(mg/kg)	Toxicity class
Tenofovir	464205	-3.69	13	2
Garlicin	15144051	-5.24	100	3
Gallic Acid	370	-4.95	2000	4
3,4-Dihydroxybenzoic acid	72	-4.7	2000	4
2, 4-Dihydroxybenzoic acid	1491	-4.62	1250	4
Vanillin	1183	-4.59	1000	4
Terpinolene	11463	-4.54	4390	5
Vinyl dithiin	150636	-4.32	260	3
Biochanin A	5280373	-4.29	2500	5
1, 3-Dithiane	10451	-4.22	2400	5
Dithiin	12044565	-4.08	260	3

Allixin	86374	-4.07	1000	4



2D and 3D of Garlic compound with crystal structure of Hepatitis B X-Interacting Protein with PDB ID: 3MSH

DISCUSSION

Tenofovir is a nucleotide reverse transcriptase inhibitor commonly used to treat HIV/AIDS and hepatitis B (Web MD, 2024). It helps to decrease the amount of hepatitis B virus in the body by interfering with virus growth.

Studies have shown that garlicin has potent antibacterial, antifungal, and antiviral activities. It appears to inhibit the growth and proliferation of a wide range of pathogens, including bacteria, viruses, and fungi. The mechanism of action of garlicin is not fully understood, but it is thought to involve disruption of microbial cell membranes, inhibition of enzyme activity, and interference with viral replication. Garlicin has demonstrated a strong binding affinity to various microbial targets, suggesting its potential as a therapeutic agent (Ján *et al*., 2019).).

Gallic acid has been extensively studied for its diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Monika *et al.,* 2022). The strong binding affinity of gallic acid to various molecular targets, combined with its low toxicity profile (Beato *et al*., 2011).

3,4-Dihydroxybenzoic acid, also known as protocatechuic acid (PCA), is a phenolic acid found in various plants. Its pharmacological properties include antioxidant, anti-inflammatory, anti-hyperglycemic, and antiapoptotic activities (Mahfuz *et al.,* 2022). The compound has a wide range of pharmacological activities, such as antioxidant, anti-inflammatory, neuroprotective, antibacterial, and antiviral effects (Jiao *et al.,* 2020).

Vanillin has strong binding affinity and moderate toxicity, suggesting potential therapeutic applications with careful dosage considerations (Bezerra-Filho *et al.,* 2019). It exhibits various bioactive properties, including anticancer, antidiabetic, antioxidant, antimicrobial, anti-inflammatory and antiviral effects (Arya *et al.,* 2021).

Vinyl dithiins are organosulfur compounds formed during the breakdown of allicin from crushed garlic. Vinyl dithiins can also release small amounts of hydrogen sulfide, which has cardioprotective effects, and they can reduce oxidative stress and virus *(Block, 2010).*

Biochanin A has demonstrated antioxidant, anti-inflammatory, anticancer and antiviral properties in various studies (Feng, & Lai, 2023 and Anuranjana *et al.,* 2023).

CONCLUSION

Garlic as one of the major spices used in animal products has many bioactive compounds also called phytochemical. Overall, Garlicin, Gallic Acid, and 3,4-Dihydroxybenzoic Acid demonstrate strong binding potential which shows an antiviral activity against hepatitis.

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