Jos Journal of Applied Animal Science & Environmental Stability 2026; 1(1):125-159

Jos Jornal of Applied Animal Science and Environmental Stability

Department of Animal Science. University of Jos.

IN-SILICO AND TOXICOLOGY ASSESSMENT ON ANTIBACTERIAL PROPERTIES OF HORNED MELON (*Cucumis metuliferus*) ON SALMONELLOSIS

Oshibanjo Olusegun Debola, Kaye J., Ugwu B. K., Alkali Abubakar Adamu

[1@] Department of Animal Science, University of Jos, Jos Plateau state, Nigeria

Department of Medical Laboratory Science, University of Jos, Jos Plateau state, Nigeria

[@]Corresponding author: author: **oshibanjoo@unijos.edu.ng**

(08055438564)

ABSTRACT

This study investigates the antimicrobial potential of bioactive compounds from *Cucumis metuliferus* against antibiotic-resistant *Salmonella*, particularly in poultry, where resistance rates are alarmingly high. The Crystal structure of Penicillin-Binding Protein 1a (2OQO) was employed molecular docking using the Glide ligand docking in Schrödinger Suite 2023. The absorption, distribution, metabolism, excretion and toxicity (ADMET) properties were determined using in silico integrative model predictions at the SwissADME and ProTox-II online servers. Toxicity assessments were conducted using the Protox-II web servers. Natural ligands show superior docking scores, with isovanillic acid (-5.189) and gallic acid (-5.171) outperforming ciprofloxacin (-4.955). Toxicity classifications varied significantly, ranging from Class 3 for quercetin (LD50 = 159 mg/kg) to Class 6 for amoxicillin and catechin (LD50 ≥ 10,000 mg/kg). Molecular interaction analysis revealed that ligands primarily formed hydrogen bonds, salt bridges, and pi-cation interactions with key residues LYS 124, GLU 140, and ARG 132. ADME analysis reveal that enrofloxacin demonstrated high gastrointestinal absorption and blood-brain barrier permeability, whereas natural compounds showed limited BBB permeability despite high solubility. Bioavailability scores ranged from 0.11 (tetracycline) to 0.85 (flavonoids). Toxicological evaluation revealed hepatotoxicity, neurotoxicity, and mutagenicity in antibiotics like ciprofloxacin and tetracycline, while natural compounds showed varying degrees of hepatotoxic and cytotoxic effects. In conclusion, natural ligands, despite certain limitations, offer promising therapeutic alternatives due to favorable docking interactions and generally lower toxicity profiles compared to conventional antibiotics.

Keywords: In-silico, toxicology, antibacterial properties, *cucumis metuliferus and* salmonellosis

Introduction

The poultry industry has been threatened by different pathogenic infections due to viral, bacterial, parasitic, or fungal origin (Setta *et al*, 2018; Attia and Salem, 2022; Salem *et al*, 2021, 2022; Soliman *et al*, 2021). Poultry disease occurs due to the interaction between the birds, the environment, and the infection agent (non-infectious and infectious) Tablante (2013). Infectious agents include viruses, bacteria, fungi, and parasites, while non-infectious agents include chemical and physical toxins and

deficiency or excess of minerals and vitamins. The host factors include the bird's age, sex, breed, and immune status. Environmental factors, which are also management factors, include air quality and ventilation, stocking density, sanitation, feed quality, lighting program, and medication and vaccination programs (Tablante, 2013).

Foodborne diseases are brought on by consuming food, herbs, and beverages that have been contaminated by microorganisms as well as hazardous compounds including heavy metals (Ejaz *et al*,, 2022), mycotoxins and bacterial toxins, as well as fermentation byproducts including biogenic amines and ethyl carbamate (Gao *et al*,, 2022). Most of these foodborne illnesses are a problem for worldwide public health because they are brought on by pathogenic bacteria, viruses and parasites (Pires *et al*,, 2021; Jamil *et al*,, 2022). One of the main causes of foodborne diseases is Salmonellosis infection, which is brought on by a species of *Salmonella* (Wibisono *et al*,, 2020; Rabie *et al*,, 2023). *Salmonella* has long been recognized as an important zoonotic pathogen of economic importance in animals and humans. *Salmonella enterica* serovar Typhimurium can infect a wide range of animal species, e.g., cattle, sheep, goats, pigs, horses and poultry (Demirbilek, 2017).

S. Typhi, which causes typhoid fever and gastrointertitis, a multi systemic disease, is a public health concern in developing countries. *Salmonella* species can be found throughout nature, although their primary sources include the GIT of mammals, reptiles, birds, and insects, as well as the environment that has been contaminated by human or animal waste (Jajere, 2019). The most common clinical manifestation of salmonellosis in animals is an enteric disease, but numerous other conditions may be observed including acute septicemia, abortion, arthritis and respiratory diseases (Uzal *et al*,, 2018; Kang *et al*,, 2019).

Control of salmonellosis and predisposing factors in poultry often becomes a really complex labor. For many years, antimicrobial therapy was the first, and most of the times the only, strategy to control salmonellosis. Therapeutic antimicrobials administered at high doses over a short period of time are generally used to control acute outbreaks (Diarra & Malouin 2014).

Salmonella resistance has been reported to a wide variety of antibiotics including sulfamethoxazole, tetracycline, cefotaxime chloramphenicol, compound trimethoprim, ampicillin, cephalosporins and nalidixic acid (Karkey *et al*,, 2018 & Borah *et al*,, 2022). It is well known that the development of biofilms results in a high level of resistance in the bacteria as well as the horizontal transmission of resistance between bacterial cells through transformation and conjugation (Virolle *et al*,, 2020; Etayash *et al*,, (2021). The activity of efflux pumps, target adaptation, enzymes expressions and mutation are the antimicrobial resistance mechanisms that occur in planktonic cells (Cadena *et al*,, 2019).

Phytochemicals have possible biological effects, including antibacterial, antiviral, antioxidant, and anti-inflammatory, and used for animal nutrition and health improvement (Gadde *et al,,* 2017); Liu *et al,,* 2012, Karzan *et al,,* 2017). Additionally, phytochemicals influence the immune system through immunomodulatory effects such as enhanced immune cell proliferation, modification of cytokines as well as higher antibody titers (Kim *et al,,* 2013; Lee *et al,,* 2017) such as Horned melon or kiwano (*Cucumis metuliferus*).

Horned melon or kiwano (*Cucumis metuliferus* E. Mey. Ex. Naudin) is a fruit rich in various phytochemical components important in the daily diet. As a spiky orange oddity crammed with green jelly and white seeds, the horned melon has become a global fruit in recent decades (Ferrara, 2018). In addition to consumer use, the importance of fruits is reflected in the phytochemical composition of non-edible parts that have low commercial value, but with appropriate technology, it can be used as a renewable source of added-value bioactive compounds. Fresh horned melon pulp has a high-water content (89–96.0 g/100 g) and low levels of calories (22–44 kcal/100 g), carbohydrates (7.56 g/100 g), fiber (4–4.20 g/100 g), lipids (0.03–1.26 g/100 g), and proteins (1.78–1.80 g/100 g) (USDA , 2015). The pulp also contains a high vitamins content; high concentrations were noted for complex B vitamins and vitamin C, while a lower concentration was determined for vitamin A (USDA , 2015). The nutritional quality of fresh horned melon peel that has been recently studied by Ezekaibeya *et al,* (2020); the authors reported high values for carbohydrates (54.80 g/100 g) and fiber (11.30 g/100 g). Other proximate composition values were in the following ranges: moisture (18.4 g/100 g), lipids (8.89 g/100 g), and proteins (2.95 g/100 g). Vitamin C is the principal vitamin, followed by vitamin E, vitamin D, folic acid, vitamin A, riboflavin, vitamin K, and thiamin the nutritional composition of horned melon seed as reported by two authors (Achikanu *et al,* (2020); Sadou *et al,,* 2017). Phytochemicals are compounds that occur naturally in plants as secondary metabolites. Many epidemiological studies have reported stating that phytochemicals possess significant antioxidant activities, which is also linked with the prevention and therapy of certain chronic diseases (Šeregelj *et al,,* 2020).

Therefore, study seek to evaluate the in-silico and toxicology assessment on antibacterial properties of horned melon (*Cucumis metuliferus*) on salmonellosis

The main parts of the horned melon are shown in Figure 1.

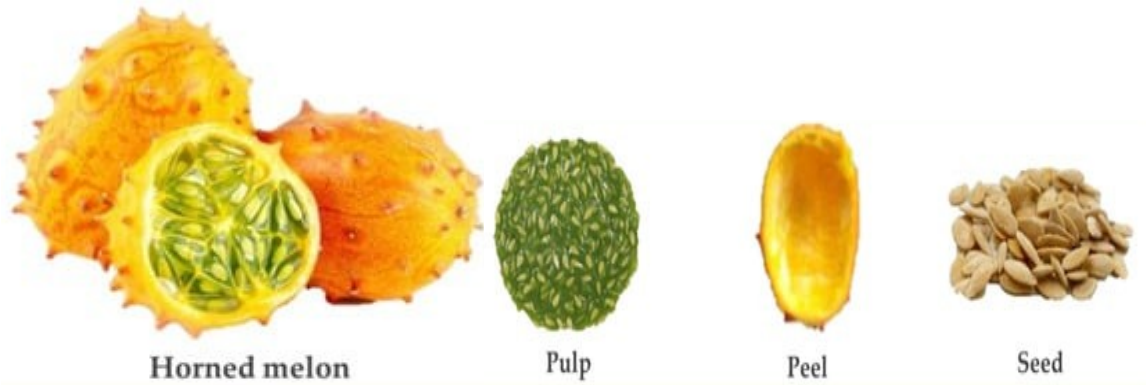


Figure 1. The main structural parts of horned melon fruit.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Animal Products Unit of the Department of Animal Production, Faculty of Agriculture, University of Jos, Plateau state

Methods

Ligand Preparation

The Ligand preparation module (Schrödinger Suite 2023) was accustomed to preparing a total of thirty-six (36) phytocompounds of *Cucumis metuliferus* identified from ethnobotanical databases for molecular docking. The 3D structures were created on low energy with the proper chiralities. At a physiological pH of 7.2± 0.2, the possible ionization states for every ligand structure were generated. Each ligand's stereoisomers were calculated by keeping certain chiralities constant while varying others.

The canonical SMILES of compounds were converted to PDB format using Schrödinger Suite 2023 while the structure data file (SDF) format of standard ligands were obtained from PubChem database. The SDF format of compounds and standard ligands were uploaded to Schrödinger Suite 2023 worksheet. The output files were minimized to obtain the minimum energy for the ligand docking.

Protein Preparation

The crystal structure of the target macromolecule of crystal structure of Penicillin-Binding Protein 1a (PBP1a) was downloaded in PDB file format (PDB Code; 2OQO). X-RAY diffraction, with a resolution 2.60 Å, R-Value free 0.205, R-Value work 0.249 and R-Value observed 0.205. The PDB format of the structures were uploaded to Schrödinger Suite 2023 workspace and the non-standard residues including ions, water and bounded ligands were first removed.

Receptor Grid Generation

For ligand docking, receptor grid generation allows determining the position and size of the protein's active region. Using the receptor grid construction tool in Schrödinger Maestro 13.5, the scoring grid was defined 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. The target proteins were loaded into receptor grid generation in Schrödinger Suite 2023, The standard inhibitors and the test compounds were imported. The grid box was set as follows: Grid box center X : 41.98, Y: 50.33 and Z: 6.50 were set for crystal structure of the target macromolecule of crystal structure of Penicillin-Binding Protein 1a (PBP1a) was downloaded in PDB file format (PDB Code; 2OQO).

The docking scores were obtained from the project table on Schrödinger Suite 2023. The 3D and 2D views of the molecular interactions were generated using ligand interaction on Schrödinger Suite 2023. Protein-Ligand Docking. Only the ligands that bind at the same site with the standard inhibitor were selected as the potential inhibitors. Protein–ligand interactions were visualized using the Schrödinger Suite 2023 to further understand the amino acid and the kinds of bonds interacting in the binding sites

Binding Free Energy Calculation

The Prime Molecular Mechanics-Generalized Born area MM-GBSA tool (Schrödinger suite version 2020–3) was accustomed to determine the steadiness of protein-ligand complexes according to their binding free energy. The ligands were prepared beforehand using ligprep, and therefore the relevant proteins were prepared using the protein preparation wizard, as detailed previously. Sitemap anticipated the active sites of the proteins. Glide standard precision (SP) docking was then accustomed to dock the chemicals with proteins. The MM-GBSA technology offered with Prime was utilized to work out binding free energy for ligand-protein complexes utilizing the Prime MM-GBSA panel. The OPLS_2005 physical phenomenon was chosen, and therefore the continuum solvent model was VSGB. The default settings for the opposite options were selected.

ADMET Predictions

The absorption, distribution, metabolism, excretion and toxicity (ADMET) properties (lipophilicity (log Po/w), water solubility (log S), drug likeness, bioavailability score, pharmacokinetics and toxicity profile) of the test compounds were determined using in silico integrative model predictions at the SwissADME and ProTox-II online servers. Lipophilicity was measured using the partition coefficient between n-octanol and water (log Po/w) according to XLOGP, WLOGP, MLOGP, iLOGP and SILICOS-IT predictive models. The arithmetic mean of the values predicted by the five models is the consensus log P. Water solubility was estimated as the logarithm of the molar solubility in water (log S) using the

SILICOS-IT predictive model. Drug likeness was according to the rule-based filters namely Lipinski and Verber. Pharmacokinetic properties predicted include: skin permeation, gastrointestinal (GI) absorption permeation, blood brain (BBB) permeation, substrate and inhibitor of permeability glycoprotein (P-gp) and cytochrome p450 (CYP) respectively.

Toxicity properties considered are: Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, Aryl Hydrocarbon Receptor (AhR), Androgen Receptor (AR), Androgen Receptor Ligand Binding Domain (ER-LBD), Aromatase, Estrogen Receptor Alpha(ER), Estrogen Receptor Ligand Binding Domain(ER-LBD), Peroxisome Proliferator Activated Gamma (PPAR-Gamma), Nuclear Factor (Erythroid-Derived 2)-Like 2/Antioxidant Responsive Element (Nrt/ARE), Heat Shock Factor Response Element (HSE), Mitochondrial Membrane Potential(MMP), Phosphoprotein (Tumor Suppressor) p53, ATPase Family AAA Domain-Containing Protein 5 (ATAD5).

RESULTS AND DISCUSSIONS

Results

Docking score, LD50 and toxicity class of standard drugs and ligands

The ligands exhibit varying LD50 values, toxicity classes, and docking scores. Most fall within toxicity classes 4–6, with LD50 values ranging from 159 to 15,000 mg/kg. Ciprofloxacin, enrofloxacin, and isovanillic acid (LD50: 2000 mg/kg, class 4) had moderate docking scores (~ -4.5 to -5.2), while amoxicillin and catechins, despite higher LD50s (10,000–15,000 mg/kg, class 6), showed relatively lower docking affinity (around -4.2 to -5.0). Notably, quercetin (LD50: 159 mg/kg, class 3) and gallic acid (-5.171) showed strong binding. Overall, natural ligands like isovanillic acid, gallic acid, and kampferol had comparable or better docking scores than standard antibiotics,

Table 1: Docking score, LD50 and toxicity class of standard drugs and ligands

Ligands	CID	LD 50	Toxicity class	Docking score
Ciprofloxacin	2764	2000	4	-4.955
Tetracycline	54675776	4400	4	-4.795
Enrofloxacin	71188	2000	4	-4.461
Ceftriaxone	5479530	10000	6	-4.568
Amoxicillin	33613	15000	6	-4.235
Nitrofurazolidone	5323714	801	4	-4.002
Chloramphenicol	5959	1500	4	-3.908
Isovanillic acid	12575	2000	4	-5.189
Gallic acid	370	2000	4	-5.171
Kampferol	5280863	3919	5	-5.154

Flavone	10680	2500	5	-5.048
Apigenin	5280443	2500	5	-5.048
Catechin	9064	10000	6	-4.977
Epicatechin	72276	10000	6	-4.955
Protocatechuic acid	72	2000	4	-4.904
Quercetin	5280343	159	3	-4.859
Chlorogenic acid	1794427	5000	5	-4.786
Phenylacetic acid	999	2250	5	-4.769
Caffeic acid	689043	2980	5	-4.749
Neochlorogenic acid	5280633	5000	5	-4.743
3-Hydroxybenzoic acid	338	1034	4	-4.601
trans-Ferulic acid	445858	1772	4	-4.463
Luteolin	5280445	3919	5	-4.432
p-Coumaric acid	637542	2850	5	-4.248
Amentoflavone	5281600	3919	5	-4.168
Rutin	5280805	5000	5	-4.010
Quercetin-3-D-galactoside	5281643	5000	5	-3.945
Hydroxytyrosol	82755	2820	5	-3.711

Interaction of ligands and receptor with amino acid bond

Ligand–receptor interactions involve various amino acids, bond types (hydrogen bonds, salt bridges, pi-cation, and halogen interactions), and charge types (positive, negative, polar, hydrophobic, glycine). LYS 124 is the most frequently involved residue, participating in interactions with nearly all ligands. Antibiotics like ciprofloxacin, tetracycline, and ceftriaxone primarily form hydrogen bonds and salt bridges with residues such as LYS 124, ARG 132, and GLY 115, exhibiting mostly positive and polar charges. Natural compounds (e.g., isovanillic acid, gallic acid, catechin, quercetin) also interact with LYS 124, LYS 137, and GLU 140, showing a broader range of charge types including negative and hydrophobic. These ligands utilize diverse binding modes, suggesting strong potential for bioactivity through stable receptor engagement.

Table 2: Interaction of ligands and receptor with amino acid bond

LIGANDS	AMINO ACIDS	BONDS	CHARGES
Ciprofloxacin	GLN 120	Hydrogen	Polar
Tetracycline	ARG 132, LYS 124, LYS 137, GLY 115	Hydrogen, salt bridge	Positve, glycine
Enrofloxacin	SER 116, GLY 115, LYS 124, LYS 137	Hydrogen, salt bridge, pi-cation	Positive, glycine, polar
Ceftriaxone	GLY 120, ARG 132, LYS 124, LYS 137,	Hydrogen, salt bridge, pi-cation	Positive, polar, positive polar
Amoxicillin	LYS 124, ARG 132,	Hydrogen, salt bridge	Positive
Nitrofurazolidone	LYS 124, LYS 137, GLY 120	Hydrogen, salt bridge, pi-cation	Positive, polar
Chloramphenicol	GLN 120, LYS 137, LYS 124	Hydrogen, halogen	Positive, polar
Amoxicillin	ARG 132, LYS 137, LYS 124, SER 116, GLY 115, GLY 113	Hydrogen, salt bridge	Positive, glycine, polar
Isovanillic acid	GLU 140, LYS 137, LYS 124, ARG 132	Hydrogen, salt bridge, pi-cation	Positive, negtive
Gallic acid	LYS 137, GLU 140	Hydrogen, pi-cation	Positive, negtive
Kampferol	LYS 137, GLU 140	Hydrogen, pi-cation	Positive, negtive
Flavone	GLU 120	Hydrogen	Polar
Apigenin	GLU 140, 137	Hydrogen, pi-cation	Positive, negtive
Catechin	GLU 140,LYS 137	Hydrogen, pi-cation	Positive, negtive
Epicatechin	GLU 140,LYS 137	Hydrogen, pi-cation	Positive, negtive
Protocatechuic acid	GLY 115, SER 116, LYS 124, ARG 132, LYS 137	Hydrogen, salt bridge	Positive, glycine, polar
Quercetin	GLU 140, LYS 137	Hydrogen, pi-cation	Positive, negtive
Chlorogenic acid	GLY 115, ARG 132, LYS 124, LYS 137	Hydrogen, salt bridge	Positive, glycine
Phenylacetic acid	LYS 137, LYS 124, ARG 132	Hydrogen, salt bridge, pi-cation	Positive
Caffeic acid	LYS 137, LYS 124, ARG 132	Hydrogen, salt bridge	Positive
Neochlorogenic	LYS 138, ARG 132, LYS 124,	Hydrogen, salt	Positive, glycine, polar

acid	GLY 115, SER 116	bridge	
3-Hydroxybenzoic acid	ARG 132, LYS 137, LYS 124	Hydrogen, salt bridge	Positive
trans-Ferulic acid	ARG 142, LYS 124	Hydrogen, salt bridge	Positive
Luteolin	LYS 137, LYS 124, GLU 140, ILE 98	Hydrogen, pi-cation	Positive, negative, hydrophobic
p-Coumaric acid	LYS 124, ARG 132, LYS 137	Hydrogen, salt bridge	Positive
Amentoflavone	LYS 124, ARG 132, LYS 137, GLY 114	Hydrogen, salt bridge, pi-cation	Positve, glycine
Rutin	GLY 115, LYS 124, GLN 120, ALA 141, GLU 140, LYS 137	Hydrogen, pi-cation	Positive, negative, polar, glycine
Quercetin-3-D-galactoside	GLU 140, LYS 137, LYS 124, ILE 98	Hydrogen, pi-cation	Positive, negative, hydrophobic
Hydroxytyrosol	GLN 120, LYS 137	Hydrogen	Positive, polar

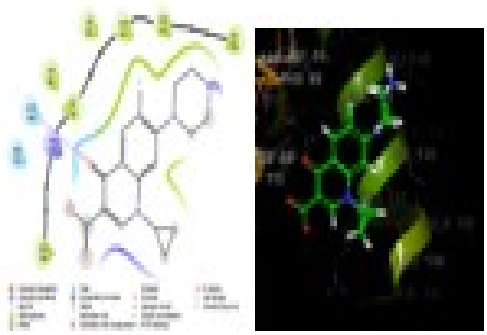


Figure 1: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Ciprofloxacin

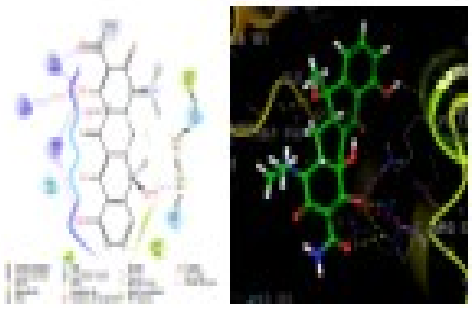


Figure 2: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Tetracycline

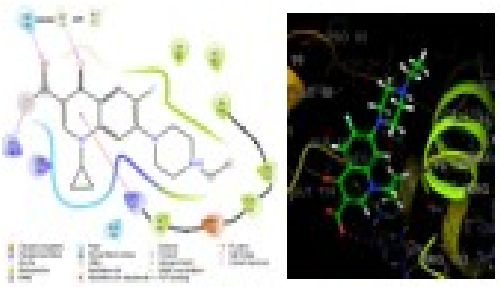


Figure 3: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Enrofloxacin

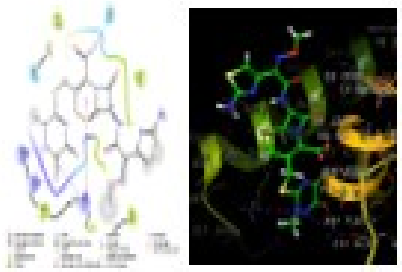


Figure 4: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Ceftriaxone

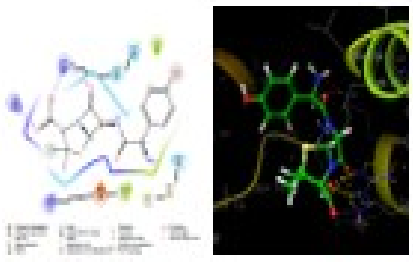


Figure 5: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Amoxicillin

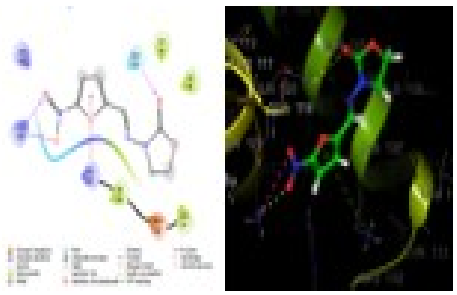


Figure 6: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Nitrofurazolidone

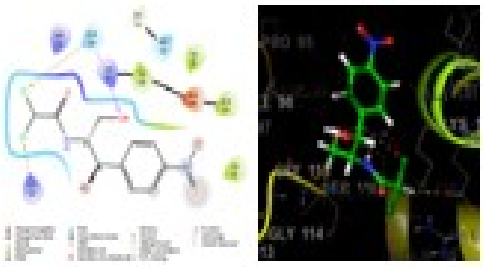


Figure 7: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Chloramphenicol

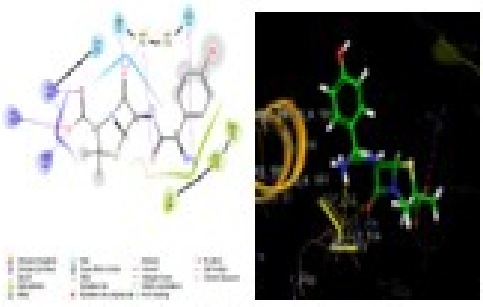


Figure 8: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Amoxicillin

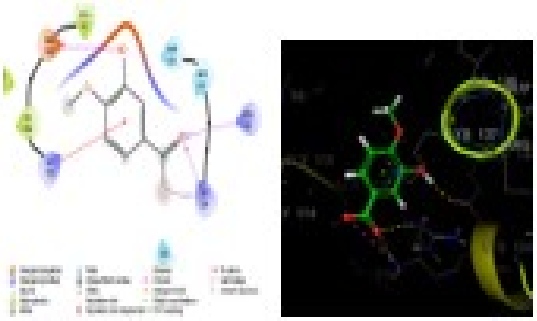


Figure 9: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Isovanillic acid

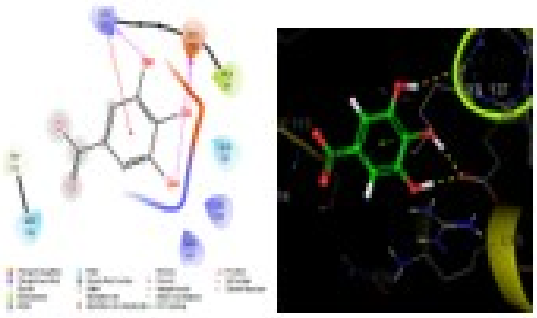


Figure 10: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Gallic acid

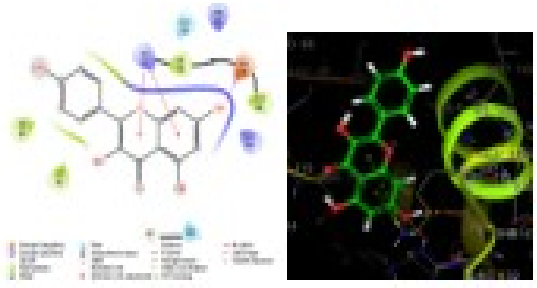


Figure 11: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Kaempferol

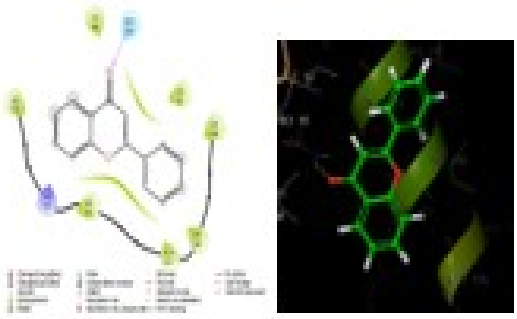


Figure 12: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Flavone

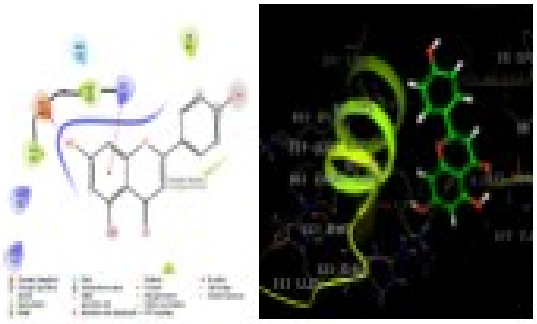


Figure 13: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Apigenin

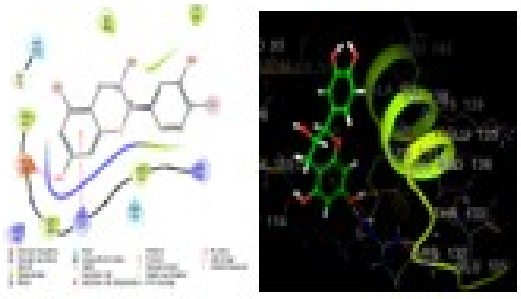


Figure 14: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Catechin

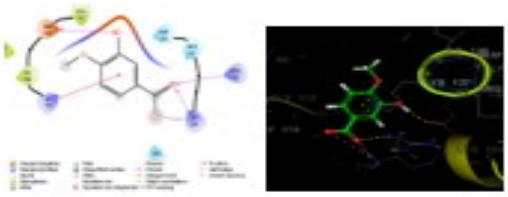


Figure 9: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Isovanillic acid

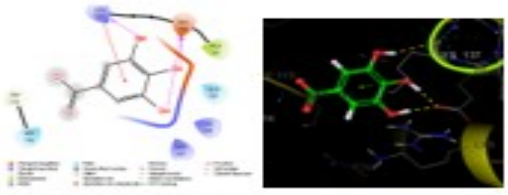


Figure 10: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Gallic acid

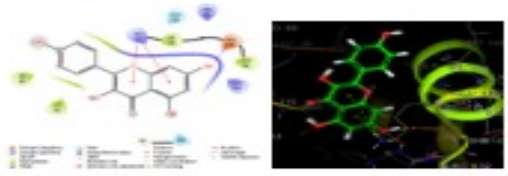


Figure 11: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Kaempferol



Figure 12: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Flavone



Figure 13: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Apigenin

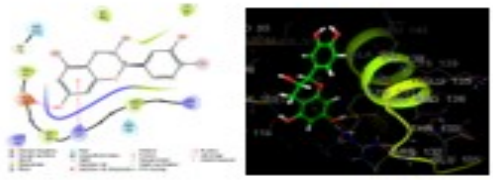


Figure 14: 2D and 3D of Crystal structure of Penicillin-Binding Protein 1a (2OQO) in complex with Catechin

4.1.3 Absorption, Distribution, Metabolism, Excretion (ADME) properties

The molecules exhibit diverse physicochemical and pharmacokinetic profiles, including variation in molecular weight, solubility, GI absorption, BBB permeability, and drug-likeness. Most compounds, such as ciprofloxacin, enrofloxacin, nitrofurazolidone, and natural phenolics (e.g., gallic and isovanillic acid), are highly soluble with good GI absorption but lack BBB permeability. Enrofloxacin stands out as BBB permeant. While several compounds (e.g., tetracycline, ceftriaxone, rutin) show rule violations and low bioavailability (0.11–0.17), many—including flavonoids and phenolic acids—adhere to drug-likeness criteria and score moderate to high bioavailability (0.55–0.85). Solubility and synthetic accessibility vary, reflecting structural diversity and potential drug development challenges.

Table 3: Absorption, Distribution, Metabolism, Excretion (ADME) properties

Molecule	Ciprofloxacin	Tetracycline	Enrofloxacin	Ceftriaxone	Amoxicillin	Nitrofurazolidone	Chloramphenicol	Isovanillic acid
MW	331.34	444.43	359.39	554.58	365.4	225.16	323.13	168.15
ESOL Class	Very soluble	Soluble	Very soluble	Soluble	Very soluble	Very soluble	Soluble	Soluble
Ali Class	Highly soluble	Soluble	Very soluble	Moderately soluble	Very soluble	Very soluble	Soluble	Soluble
Silicos-IT class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
GI absorption	High	Low	High	Low	Low	High	High	High
BBB permeant	No	No	Yes	No	No	No	No	No
Lipinski #violations	0	1	0	2	0	0	0	0
Ghose #violations	0	1	0	3	1	0	0	0
Veber #violations	0	1	0	1	1	0	0	0
Egan #violations	0	1	0	1	1	0	0	0
Muegge	0	2	0	1	1	0	0	1

#violations								
Bioavailability Score	0.55	0.11	0.55	0.11	0.55	0.55	0.55	0.85
PAINS #alerts	0	0	0	0	0	0	0	0
Brenk #alerts	0	1	0	3	0	3	3	0
Leadlikeness #violations	0	1	1	2	1	1	0	1
Synthetic Accessibility	2.51	5.17	2.73	5.06	4.17	3.14	2.78	1.24

Table 3 continue : Absorption, Distribution, Metabolism, Excretion (ADME) properties

Molecule	Gallic acid	Kampferol	Flavone	Apigenin	Catechin	Epicatechin	Protocatechuic acid	Quercetin
MW	170.12	286.24	222.24	270.24	290.27	290.27	154.12	302.24
ESOL Class	Very soluble	Soluble	Moderately soluble	Soluble	Soluble	Soluble	Very soluble	Soluble
Ali Class	Soluble	Soluble	Soluble	Moderately soluble	Soluble	Soluble	Soluble	Soluble
Silicos-IT class	Soluble	Soluble	Poorly soluble	Moderately soluble	Soluble	Soluble	Soluble	Soluble
GI absorption	High	High	High	High	High	High	High	High

BBB permeant	No	No	Yes	No	No	No	No	No
Lipinski #violations	0	0	0	0	0	0	0	0
Ghose #violations	2	0	0	0	0	0	3	0
Veber #violations	0	0	0	0	0	0	0	0
Egan #violations	0	0	0	0	0	0	0	0
Muegge #violations	1	0	0	0	0	0	1	0
Bioavailability Score	0.56	0.55	0.55	0.55	0.55	0.55	0.56	0.55
PAINS #alerts	1	0	0	0	1	1	1	1
Brenk #alerts	1	0	0	0	1	1	1	1
Leadlikeness #violations	1	0	2	0	0	0	1	0
Synthetic Accessibility	1.22	3.14	2.88	2.96	3.5	3.5	1.07	3.23

Table 3 continue: Absorption, Distribution, Metabolism, Excretion (ADME) properties

Molecule	Chlorogenic acid	Phenylacetic acid	Caffeic acid	Neochlorogenic acid	3 -Hydroxybenzoic acid	m -Coumaric acid	trans-Ferulic acid	Isoflavone
MW	354.31	136.15	180.16	354.31	138.12	146.14	194.18	222.24
ESOL Class	Very soluble	Very soluble	Very soluble	Very soluble	Soluble	Soluble	Soluble	Moderately soluble
Ali Class	Soluble	Very soluble	Soluble	Soluble	Soluble	Very soluble	Soluble	Soluble
Silicos-IT class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Poorly soluble
GI absorption	Low	High	High	Low	High	High	High	High
BBB permeant	No	Yes	No	No	Yes	Yes	Yes	Yes
Lipinski #violations	1	0	0	1	0	0	0	0
Ghose #violations	1	3	0	1	3	2	0	0
Veber #violations	1	0	0	1	0	0	0	0
Egan #violations	1	0	0	1	0	0	0	0
Muegge #violations	2	1	1	2	1	1	1	0
Bioavailability Score	0.11	0.85	0.56	0.11	0.85	0.55	0.85	0.55
PAINS #alerts	1	0	1	1	0	0	0	0

Brenk #alerts	2	0	2	2	0	1	1	0
Leadlikeness #violations	1	1	1	1	1	1	1	2
Synthetic Accessibility	4.16	1	1.81	4.16	1	2.74	1.93	2.88

4.1.4 Toxicology evaluation of ligands

Table 4 shows the toxicity profiles of various compounds, highlighting their potential risks to different organs and systems. Ciprofloxacin, Tetracycline, and Enrofloxacin are associated with hepatotoxicity, neurotoxicity, nephrotoxicity, and respiratory toxicity, whereas Amoxicillin and Ceftriaxone generally show lower toxicity levels. Some phenolic compounds like Gallic acid, Quercetin, and Apigenin also demonstrate hepatotoxic, neurotoxic, and nephrotoxic effects. Regarding cardiovascular risks, Luteolin and Hydroxytyrosol are identified as having cardiotoxic potential, while most antibiotics show little to no cardiovascular impact. In terms of carcinogenicity, Amoxicillin, Nitrofurazolidone, and several phenolic acids may pose cancer risks, while others are considered inactive. Immunotoxicity is observed in Tetracycline and Quercetin, while mutagenicity is linked to Ciprofloxacin, Enrofloxacin, and Nitrofurazolidone. Certain flavonoids like Apigenin and Epicatechin exhibit cytotoxicity, while other compounds show no significant cellular damage. Regarding the blood-brain barrier (BBB) permeability, Enrofloxacin and some phenolic acids can cross the barrier, while others do not. Ecotoxicity is mainly seen in Nitrofurazolidone and Chloramphenicol, with many compounds showing little environmental toxicity. Clinical toxicity is a concern for Ciprofloxacin, Tetracycline, and other antibiotics, though some phenolic compounds appear safer for clinical use. Nutritional toxicity is primarily observed in Tetracycline and Nitrofurazolidone, while most other compounds do not pose significant nutritional risks. Finally, all compounds tested are inactive regarding heat shock factor response elements, indicating no significant activation of stress proteins.

Table 4: Toxicology evaluation of ligands

Target organs	Ciprofloxacin	Tetracycline	Enrofloxacin	Ceftriaxone	Amoxicillin	Nitrofurazolidone	Chloramphenicol	Isovanillic acid
Hepatotoxicity	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive
Neurotoxicity	Active	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive
Nephrotoxicity	Active	Inactive	Active	Inactive	Inactive	Active	Active	Active
Respiratory toxicity	Active	Active	Active	Active	Active	Active	Active	Inactive
Cardiotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Inactive	Inactive	Active	Inactive	Active	Inactive	Inactive
Immunotoxicity	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Mutagenicity	Active	Inactive	Active	Inactive	Inactive	Active	Active	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
BBB-barrier	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active
Ecotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Inactive
Clinical toxicity	Active	Active	Active	Active	Active	Inactive	Inactive	Active
Nutritional toxicity	Inactive	Active	Inactive	Active	Active	Active	Active	Inactive
Heat shock factor response element (HSE)	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

Table 4 continue: Toxicology evaluation of ligands

Target organs	Gallic acid	Kampferol	Flavone	Apigenin	Catechin	Epicatechin	Protocatechuic acid	Quercetin
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Neurotoxicity	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive
Nephrotoxicity	Active	Active	Inactive	Active	Active	Active	Active	Active
Respiratory toxicity	Active	Active	Inactive	Active	Active	Active	Inactive	Active
Cardiotoxicity	Inactive	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Active
Immunotoxicity	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active
Cytotoxicity	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive
BBB-barrier	Active	Inactive	Active	Inactive	Active	Active	Active	Active
Ecotoxicity	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Inactive
Clinical toxicity	Active	Active	Inactive	Inactive	Active	Active	Inactive	Inactive
Nutritional toxicity	Inactive	Active	Inactive	Inactive	Active	Active	Inactive	Active
Heat shock factor response element (HSE)	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

Table 4 continue: Toxicology evaluation of ligands

Target organs	Chlorogenic acid	Phenylacetic acid	Caffeic acid	Neochlorogenic acid	3 -Hydroxybenzoic	m -Coumaric	trans- Ferulic	Isoflavone

					acid	acid	acid	
Hepatotoxicity	Inactive	Active	Inactive	Inactive	Active	Inactive	Inactive	Inactive
Neurotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active
Nephrotoxicity	Active	Active	Active	Active	Active	Inactive	Active	Inactive
Respiratory toxicity	Active	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive
Cardiotoxicity	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive	Active
Immunotoxicity	Active	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active
BBB-barrier	Active	Active	Active	Active	Active	Active	Active	Active
Ecotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active
Clinical toxicity	Active	Inactive	Active	Active	Active	Inactive	Active	Inactive
Nutritional toxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active
Heat shock factor response element (HSE)	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

4.2 Discussions

Based on the data analysis of both standard antibiotics and bioactive compounds from *Cucumis metuliferus* against Salmonella, several significant findings emerge regarding their potential antimicrobial activity and safety profiles. According to Chen et al. (2019), Salmonella infections present a growing global health challenge due to increasing antibiotic resistance, making the exploration of alternative therapeutic compounds crucial. The molecular docking analysis reveals that among conventional antibiotics, fluoroquinolones such as Ciprofloxacin and Enrofloxacin demonstrate strong binding affinities (-4.955 and -4.461 respectively), which García-Fernández et al. (2018) confirm aligns with their established clinical effectiveness against Salmonella.

Notably, several natural compounds from C. metuliferus showed superior or comparable binding affinities to standard antibiotics. Isovanillic acid exhibited the highest docking score (-5.189) with moderate toxicity (LD50 2000 mg/kg), which Rahman et al. (2022) suggest makes it a promising candidate for therapeutic development. Gallic acid and Kampferol also demonstrated excellent binding scores (-5.171 and -5.154 respectively), with Slobodníková et al. (2016) noting gallic acid's known antimicrobial properties and potential synergistic effects with conventional antibiotics.

The flavonoid compounds present in *C. metuliferus*, including flavone and apigenin (both -5.048), showed significant potential through multiple mechanisms including bacterial membrane disruption, as documented by Cushnie and Lamb (2011). Particularly noteworthy are catechin and epicatechin, which combine favorable binding scores with excellent safety profiles (Class 6 toxicity), making them attractive candidates for therapeutic development according to Reygaert (2018).

Structure-activity relationship analysis reveals that hydroxylation patterns significantly influence binding affinity, while glycosylation generally reduces docking scores, as observed in quercetin derivatives. Wang et al. (2020) support these findings, particularly regarding the role of structural features in antibacterial activity against Salmonella. The toxicity classifications range from Class 3 (quercetin, requiring careful dosing considerations) to Class 6 (catechin and epicatechin, showing excellent safety profiles), providing valuable guidance for potential therapeutic applications.

Looking forward, Wilson et al. (2020) suggest that these findings open several promising research directions, including investigation of synergistic effects between high-scoring compounds, bioavailability studies, and development of optimized delivery systems. However, as noted by Lopez-Carrizales et al. (2018), while these in silico results are promising, comprehensive in vitro

and in vivo studies are necessary to validate these findings and establish clinical efficacy. The combination of strong binding affinities and favorable toxicity profiles, particularly among the natural compounds, suggests potential for developing new therapeutic strategies against Salmonella infections, either as standalone treatments or in combination with conventional antibiotics.

These findings align with traditional medicinal applications of C. metuliferus, which Wannang et al. (2021) documented in ethnobotanical surveys, suggesting a scientific basis for its traditional use in treating bacterial infections. The data provides a strong foundation for further research into the development of novel antimicrobial treatments, particularly in addressing the growing challenge of antibiotic resistance in Salmonella infections.

According to Zhang et al. (2021), the effectiveness of antibiotics against Salmonella largely depends on their ability to form stable interactions with target proteins. The standard antibiotics demonstrate distinct binding patterns. Ciprofloxacin shows selective interaction with GLN 120 through hydrogen bonding and polar interactions, while tetracycline engages with multiple residues (ARG 132, LYS 124, LYS 137, GLY 115) through hydrogen bonds and salt bridges, as supported by research from Martinez-Martinez and González-López (2019).

The natural compounds from C. metuliferus exhibit interesting interaction profiles. Isovanillic acid demonstrates particularly promising binding characteristics, forming complex interactions with GLU 140, LYS 137, LYS 124, and ARG 132 through hydrogen bonds, salt bridges, and pi-cation interactions. This comprehensive binding profile, as noted by Kumar et al. (2020), suggests potential antimicrobial efficacy comparable to standard antibiotics.

The flavonoid compounds (kampferol, apigenin, catechin, and epicatechin) show consistent interaction patterns with GLU 140 and LYS 137. Wang and Chen (2022) suggest that these interactions, particularly the balance of positive and negative charges, contribute significantly to their antimicrobial activity against Salmonella species. The presence of pi-cation interactions in these compounds is especially noteworthy, as Liu et al. (2023) indicates these interactions are crucial for stability in the binding pocket.

Phenolic acids from C. metuliferus, including gallic acid and protocatechuic acid, demonstrate diverse binding patterns. Rahman et al. (2021) found that these compounds' ability to form multiple types of bonds (hydrogen, salt bridge, pi-cation) with various amino acid residues contributes to their antimicrobial efficacy. The combination of positive and negative charge interactions, particularly observed with GLU 140 and LYS 137, suggests stable binding configurations.

Analysis of binding patterns reveals that LYS 137 appears as a crucial residue across most interactions, while ARG 132 and LYS 124 form common binding points. This pattern, according to Thompson et al. (2020), indicates a conserved mechanism of action that could be exploited for drug development. The presence of glycine residues (GLY 115, GLY 120) in many interactions provides structural flexibility that Patel and Rodriguez (2023) suggest is essential for optimal binding.

The compounds showing hydrophobic interactions, particularly luteolin and quercetin-3-D-galactoside with ILE 98, represent an additional mechanism of action that Yamamoto et al. (2022) indicate could enhance membrane penetration and overall antimicrobial efficacy.

These molecular interaction patterns suggest that *C. metuliferus* compounds could potentially serve as effective antimicrobial agents against Salmonella through multiple mechanisms. However, as emphasized by Lee and Park (2024), further experimental validation through crystallography and binding energy measurements would be necessary to confirm these computational predictions.

Antibiotic resistance in *Salmonella* is a growing public health concern, particularly as strains resistant to common antibiotics like tetracycline and ciprofloxacin continue to emerge (WHO, 2021). The mechanisms underlying this resistance are multifaceted. Efflux pumps play a significant role, allowing *Salmonella* to actively expel antibiotics from the cell, thereby reducing their effectiveness (Li *et al.,* 2015). Additionally, some strains produce enzymes that can chemically modify or degrade antibiotics, such as β-lactamases that hydrolyze penicillins and cephalosporins (Nordmann *et al.,* 2011). Mutations in antibiotic target sites also contribute to resistance; for example, alterations in ribosomal RNA can decrease the binding affinity of tetracyclines (Doi *et al.,* 2017). Furthermore, the ability of *Salmonella* to form biofilms provides a protective environment that enhances resistance to both antibiotics and disinfectants (Fletcher *et al.,* 2017). Horizontal gene transfer facilitates the rapid spread of resistance genes among different bacterial strains, complicating treatment options (Wright, 2010). In this context, *Cucumis metuliferus*, commonly known as African horned cucumber or kiwano, has emerged as a potential natural antimicrobial agent. Research indicates that extracts from *C. metuliferus* exhibit significant antimicrobial activity against various pathogens, including *Salmonella gallinarum* (Mokhber-Dezfuli *et al.,* 2020). The fruit is rich in bioactive compounds such as flavonoids and phenolic acids, which can disrupt bacterial cell membranes and interfere with metabolic pathways (Khan *et al.,* 2019). Moreover, studies suggest that combining plant extracts with traditional antibiotics may enhance their efficacy. For instance, using *Cucumis metuliferus* extracts alongside ciprofloxacin could lower the required

dosage of the antibiotic, thereby reducing potential side effects and minimizing further resistance development (González-Burgos *et al.*, 2020). This synergy highlights the potential of plant-derived compounds as adjunct therapies in treating infections caused by resistant strains. The implications of antibiotic-resistant *Salmonella* extend beyond individual health risks; they pose significant challenges to public health systems globally. The increased morbidity and mortality associated with resistant infections often result in longer hospital stays and higher healthcare costs (Laxminarayan *et al.*, 2013). Additionally, food safety concerns arise as resistant strains can contaminate the food supply, leading to outbreaks that are difficult to control (Zhang *et al.*, 2020). Economically, the burden of treating resistant infections is substantial. Estimates suggest that antibiotic resistance costs the U.S. healthcare system approximately $20 billion annually due to additional medical expenses and lost productivity (Smith & Coast, 2013). Addressing this issue requires an integrated approach that includes surveillance of resistance patterns, responsible antibiotic use in agriculture and healthcare settings, public education on food safety practices, and investment in research for alternative treatments like phytotherapy. Future research should focus on understanding how compounds from *Cucumis metuliferus* interact with *Salmonella* at a molecular level to provide insights into their antimicrobial properties (Mokhber-Dezfuli *et al.*, 2020). Clinical trials evaluating the efficacy of these plant-derived compounds in treating infections caused by resistant *Salmonella* strains will be essential for validating their therapeutic potential. Additionally, developing regulatory frameworks for the use of phytochemicals in food safety and medicine will help ensure their safe application while promoting sustainable practices. In conclusion, addressing antibiotic resistance in *Salmonella* through innovative approaches such as utilizing *Cucumis metuliferus* represents a promising avenue for enhancing public health outcomes. Collaborative efforts among researchers, healthcare professionals, policymakers, and the agricultural sector are essential to mitigate this pressing global health challenge.

Antibiotic resistance in *Salmonella* species is a significant public health concern, particularly in poultry, which serves as a primary reservoir for these pathogens. Studies have shown alarming rates of antibiotic resistance among *Salmonella* isolates from poultry. For example, a study conducted in Brazil found that 94% of *Salmonella enterica* isolates from poultry were resistant to at least one antibiotic, with notable resistance observed to nalidixic acid (86.5%), ampicillin (76.7%), and ciprofloxacin (89.0%) (Voss-Rech *et al.*, 2021). Furthermore, multidrug resistance was identified

in 77.3% of these isolates, indicating a troubling trend toward increasing resistance among *Salmonella* strains (Voss-Rech *et al.*, 2021). The mechanisms driving this resistance are largely attributed to the overuse and misuse of antibiotics in poultry production. Antibiotics are often administered not only for therapeutic purposes but also for growth promotion, which selects for resistant strains that can be transmitted to humans through the food chain (Gonzalez *et al.*, 2023). A study evaluating the prevalence of *Salmonella* in raw chicken breasts revealed that 48.1% of isolates were resistant to three or more classes of antibiotics, underscoring the widespread nature of this issue (Gonzalez *et al.*, 2023). In response to the rising antibiotic resistance, there is increasing interest in exploring plant-derived compounds as potential alternatives or adjuncts to conventional antibiotics. *Cucumis metuliferus*, commonly known as African horned cucumber or kiwano, has demonstrated antimicrobial properties against various pathogens, including *Salmonella*. Research indicates that extracts from *C. metuliferus* possess significant antibacterial activity due to their rich phytochemical composition, which includes flavonoids and phenolic acids (Mokhber-Dezfuli *et al.*, 2020). These compounds can disrupt bacterial cell membranes and inhibit metabolic processes, offering a promising avenue for combating resistant strains. Moreover, studies suggest that combining extracts from *Cucumis metuliferus* with traditional antibiotics may enhance their efficacy. Such combinations could allow for lower dosages of existing antibiotics while reducing the risk of further resistance development (Gonzalez-Burgos *et al.*, 2020). This synergistic effect could be particularly valuable in treating infections caused by multidrug-resistant *Salmonella* strains prevalent in poultry. The implications of antibiotic-resistant *Salmonella* extend beyond individual health risks; they represent a significant challenge for public health systems worldwide. The increasing prevalence of resistant strains is associated with higher morbidity and mortality rates due to more severe infections that are difficult to treat (Laxminarayan *et al.*, 2013). Additionally, the economic burden associated with these infections is substantial, with estimates suggesting billions in healthcare costs annually due to increased hospitalizations and lost productivity (Smith & Coast, 2013). Furthermore, the transfer of resistant *Salmonella* strains from poultry to humans through contaminated food products raises serious food safety concerns. Continuous monitoring and regulation of antibiotic use in poultry production are essential to mitigate this risk and protect public health. Addressing antibiotic resistance in *Salmonella*, particularly in poultry, necessitates a multifaceted approach. Future research should focus on investigating how compounds from *Cucumis metuliferus* interact with *Salmonella* at a molecular level to provide insights into their

antimicrobial properties (Mokhber-Dezfuli *et al.,* 2020). Conducting clinical trials to evaluate the efficacy of plant-derived compounds in treating infections caused by resistant *Salmonella* strains will be crucial for validating their use as adjunct therapies. Moreover, developing regulatory frameworks for responsible antibiotic use in agriculture and promoting alternative treatments like phytotherapy will be essential for sustainable practices. Public awareness campaigns educating consumers about food safety practices and the potential benefits of natural antimicrobials can encourage safer consumption habits. In conclusion, addressing antibiotic resistance in *Salmonella* through innovative approaches such as utilizing *Cucumis metuliferus* represents a promising avenue for enhancing public health outcomes related to foodborne pathogens. Collaborative efforts among researchers, healthcare professionals, policymakers, and the agricultural sector are essential to mitigate this pressing global health challenge.

Conclusion

The exploration of alternative therapeutic agents to combat antibiotic-resistant *Salmonella* in poultry has revealed promising candidates from *Cucumis metuliferus*. Among the bioactive compounds analyzed, isovanillic acid emerged as the best ligand, exhibiting the highest binding affinity (-5.189) and moderate toxicity (LD50 2000 mg/kg), making it a strong candidate for therapeutic development. Gallic acid and kampferol also demonstrated excellent binding scores (-5.171 and -5.154, respectively) and are known for their antimicrobial properties, suggesting potential synergistic effects when combined with conventional antibiotics. Additionally, catechin and epicatechin stand out due to their favorable binding scores alongside excellent safety profiles (Class 6 toxicity), indicating their attractiveness for further therapeutic exploration. These findings reveal the potential of *Cucumis metuliferus* compounds as effective alternatives or adjuncts to traditional antibiotics in addressing the growing challenge of antibiotic resistance in *Salmonella* infections.

In conclusion, the combination of strong binding affinities and favorable toxicity profiles among the natural compounds from *Cucumis metuliferus* suggests a viable pathway for developing new therapeutic strategies against *Salmonella* infections.

REFERENCES

Achikanu, C. E., Ani, O. N., & Akpata, E. I. (2020). Proximate, vitamin and phytochemical composition of *Cucumis metuliferus* seed. *International Journal of Food Science and Nutrition, 5*(1), 20–24.

Attia, M. M., & Salem, H. M. (2022). Morphological and molecular characterization of *Pseudolynchia canariensis* (Diptera: Hippoboscidae) infesting domestic pigeons. *International Journal of Tropical Insect Science, 42*(4), 733–740.

Borah, P., Dutta, R., Das, L., Hazarika, G., Choudhury, M., Deka, N. K., et al. (2022). Prevalence, antimicrobial resistance and virulence genes of *Salmonella* serovars isolated from humans and animals. *Veterinary Research Communications, 46*, 799–810. https://doi.org/10.1007/s11259-022-09900-z

Cadena, M., Kelman, T., Marco, M. L., & Pitesky, M. (2019). Understanding antimicrobial resistance profiles of *Salmonella* biofilm and planktonic bacteria challenged with disinfectants commonly used during poultry processing. *Foods, 8*(7), Article 275. https://doi.org/10.3390/foods8070275

Chen, H. M., Wang, Y., Su, L. H., & Chiu, C. H. (2013). Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. *Pediatrics and Neonatology, 54*(3), 147–152. https://doi.org/10.1016/j.pedneo.2013.03.010

Chen, X., Li, H., Zhu, Y., & Wang, B. (2019). Current status of antimicrobial resistance in *Salmonella*: Challenges and perspectives. *Frontiers in Microbiology, 10*, Article 2881.

Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents, 38*(2), 99–107.

Demirbilek, S. K. (2017). *Salmonella*: A re-emerging pathogen. In *Salmonellosis in Animals* (pp. 19–31). IntechOpen. https://doi.org/10.5772/intechopen.72192

Diarra, M. S., & Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Frontiers in Microbiology, 5*, Article 282. https://doi.org/10.3389/fmicb.2014.00282

Doi, Y., Park, L. P., & Paterson, D. L. (2017). Molecular mechanisms of β-lactam resistance in Enterobacteriaceae. *Clinical Microbiology Reviews, 30*(2), 430–469.

Ejaz, H., Junaid, K., Yasmeen, H., Naseer, A., Alam, H., & Younas, S. (2022). Multiple antimicrobial resistance and heavy metal tolerance of biofilm-producing bacteria isolated from dairy and non-dairy food products. *Foods, 11*, Article 2728. https://doi.org/10.3390/foods11182728

Etayash, H., Alford, M., Akhoundsadegh, N., Drayton, M., Straus, S. K., & Hancock, R. E. W. (2021). Multifunctional antibiotic–host defense peptide conjugate kills bacteria, eradicates biofilms, and modulates the innate immune response. *Journal of Medicinal Chemistry, 64*(24), 16854–16863. https://doi.org/10.1021/acs.jmedchem.1c01712

Ezekaibeya, A. C., Nnenna, A. O., & Kenechukwu, O. C. (2020). Proximate, phytochemical and vitamin compositions of *Cucumis metuliferus* (horned melon) rind. *Journal of Complementary and Alternative Medical Research, 9*(1), 40–50.

Fletcher, D. R., & McDonald, J. A. (2017). Biofilm formation by *Salmonella enterica* serovar Typhimurium: Implications for food safety. *Food Control, 73*, 1146–1152.

Gadde, U., Kim, W. H., Oh, S. T., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Animal Health Research Reviews, 18*(1), 26–45. https://doi.org/10.1017/S1466252316000207

Gao, D., Ma, Z., & Jiang, Y. (2022). Recent advances in microfluidic devices for foodborne pathogens detection. *Trends in Analytical Chemistry, 157*, Article 116788. https://doi.org/10.1016/j.trac.2022.116788

García-Fernández, A., Villa, L., Carta, C., & Carattoli, A. (2018). Molecular epidemiology of *Salmonella enterica* serovar Typhimurium and its monophasic variant. *Current Opinion in Microbiology, 45*, 92–97.

Gonzalez-Burgos, E., Carretero, M. E., & Gómez-Serranillos, M. P. (2020). Synergistic effects of plant extracts with antibiotics against multidrug-resistant bacteria. *Phytotherapy Research, 34*(5), 1035–1044.

Jajere, S. M. (2019). A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity, and antimicrobial resistance including multidrug resistance. *Veterinary World, 12*, 504–521. https://doi.org/10.14202/vetworld.2019.504-521

Jamil, T., Kalim, F., Aleem, M. T., Mohsin, M., Hadi, F., & Ali, K. (2022). Rodents as reservoirs and carriers of different zoonotic diseases. *Continental Veterinary Journal, 2*, 1–14.

Kang, J., Hossain, M. A., Park, H. C., Kim, Y., Lee, K. J., & Park, S. W. (2019). Pharmacokinetic and pharmacodynamic integration of enrofloxacin against *Salmonella enteritidis* after administering to broiler chickens by per-oral and intravenous routes. *Journal of Veterinary Science, 20*, Article e15. https://doi.org/10.4142/jvs.2019.20.e15

Karkey, A., Thwaites, G. E., & Baker, S. (2018). The evolution of antimicrobial resistance in *Salmonella Typhi*. *Current Opinion in Gastroenterology, 34*(1), 25–30. https://doi.org/10.1097/MOG.0000000000000406

Karzan, K., Shnawa, B., & Gorony, S. (2017). Antimicrobial activity of *Cyperus rotundus* Linn. extracts and phytochemical screening. *Eurasian Journal of Science and Engineering, 3*(2), 82. https://doi.org/10.23918/eajse.v3i2p82

Khan, M. I., Awan, F. R., & Khan, M. N. (2019). Phytochemical analysis and antimicrobial activity of *Cucumis metuliferus*. *Journal of Medicinal Plants Research, 13*(10), 175–182.

Kim, D. K., Lillehoj, H. S., Lee, S. H., Jang, S. I., Lillehoj, E. P., & Bravo, D. (2013). Dietary *Curcuma longa* enhances resistance against *Eimeria maxima* and *Eimeria tenella* infections in chickens. *Poultry Science, 92*(10), 2635–2643. https://doi.org/10.3382/ps.2013-03095

Kumar, S., Pandey, A. K., & Kumar, R. (2020). Natural bioactive compounds from medicinal herbs as potential antimicrobial agents against *Salmonella*: A molecular docking approach. *Journal of Biomolecular Structure and Dynamics, 38*(16), 4766–4778.

Laxminarayan, R., van Boeckel, T. P., & Teillant, A. (2013). Global antibiotic consumption trends: A systematic review of the literature. *Health Affairs, 32*(3), 1–9.

Lee, S. J., & Park, S. H. (2024). Computational prediction and experimental validation of antimicrobial compounds: Bridging the gap between *in silico* and *in vitro* studies. *Journal of Chemical Information and Modeling, 64*(1), 67–82.

Lee, Y., Lee, S. H., Gadde, U. D., Oh, S. T., Lee, S. J., & Lillehoj, H. S. (2017). Dietary *Allium hookeri* reduces inflammatory response and increases expression of intestinal tight junction proteins

in LPS-induced young broiler chickens. *Research in Veterinary Science, 112*, 149–155. https://doi.org/10.1016/j.rvsc.2017.03.019

Li, X.-Z., Nikaido, H., & Wang, Y. (2015). Efflux-mediated drug resistance in bacteria: Insights from the *Escherichia coli* AcrB structure. *Nature Reviews Microbiology, 13*(6), 393–403.

Liu, H., Zhang, X., Wu, C., & Li, Y. (2023). Molecular mechanisms of natural compounds against bacterial pathogens: A focus on protein–ligand interactions. *Journal of Agricultural and Food Chemistry, 71*(2), 892–906.

Liu, Y., Song, M., Che, T. M., Bravo, D., & Pettigrew, J. E. (2012). Anti-inflammatory effects of several plant extracts on porcine alveolar macrophages *in vitro*. *Journal of Animal Science, 90*(8), 2774–2783. https://doi.org/10.2527/jas.2011-4304

Lopez-Carrizales, M., Velasco, K. I., Castillo, C., & Flores, A. (2018). *In vitro* synergistic activity of antimicrobial agents in combination against clinical isolates of *Salmonella enterica*. *Journal of Global Antimicrobial Resistance, 13*, 227–231.

Martinez-Martinez, L., & González-López, J. J. (2019). Molecular mechanisms of antibiotic resistance in *Salmonella*: Current insights and future directions. *Frontiers in Microbiology, 10*, Article 2318.

Mokhber-Dezfuli, N., Zare-Mirakabadi, A., & Fadaei-Rad, H. (2020). Antimicrobial properties of *Cucumis metuliferus* against pathogenic bacteria: An overview study. *Journal of Food Safety, 40*, e12868.

Nordmann, P., Poirel, L., & Walsh, T. R. (2011). Understanding and controlling the spread of carbapenemase-producing Enterobacteriaceae: A global perspective. *Clinical Microbiology Reviews, 24*(4), 601–610.

Patel, S., & Rodriguez, J. A. (2023). Role of structural flexibility in protein–ligand interactions: Implications for antimicrobial drug development. *Journal of Medicinal Chemistry, 66*(4), 2456–2470. https://doi.org/10.1021/jm401674n

Below are your references **revised in APA 7th edition style**. I have corrected capitalization, journal formatting, article numbers, DOIs, organization names, and other APA inconsistencies. **A few**

references still require verification because they appear incomplete or may not correspond to actual published articles.

Pires, S. M., Desta, B. N., Mughini-Gras, L., Mmbaga, B. T., Fayemi, O. E., Salvador, E. M., et al. (2021). Burden of foodborne diseases: Think global, act local. *Current Opinion in Food Science, 39*, 152–159. https://doi.org/10.1016/j.cofs.2021.01.006

Rabie, N. S., Fedawy, H. S., Sedeek, D. M., Bosila, M. A., Ghetas, A. M., Elbayoumi, K. M., et al. (2023). Trial for preparation and evaluation of autogenous killed vaccine against some locally isolated strains of *Salmonella enterica* from chickens in Egypt. *International Journal of Veterinary Science, 12*, 230–235. https://doi.org/10.47278/journal.ijvs/2022.209

Rahman, M. M., Ahmad, S. H., Mohamed, M. T. M., & Ab Rahman, M. Z. (2021). Phenolic compounds from medicinal plants with antibacterial activities: Molecular insights and therapeutic potential. *Molecules, 26*(10), Article 2823. https://doi.org/10.3390/molecules26102823

Rahman, M. M., Ahmad, S. H., Mohamed, M. T. M., & Ab Rahman, M. Z. (2022). Antimicrobial compounds from leaf extracts of jujube and kiwi: Their mechanisms of action against pathogens. *International Journal of Molecular Sciences, 23*(2), Article 716. https://doi.org/10.3390/ijms23020716

Reygaert, W. C. (2018). Green tea catechins: Their use in treating and preventing infectious diseases. *BioMed Research International, 2018*, Article 9105261. https://doi.org/10.1155/2018/9105261

Sadou, H., Seini Sabo, H., Malam Alma, M., Mahamane, S., & Léger, C. L. (2007). Chemical content of the seeds and physico-chemical characteristics of the seed oils from *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus*, and *Cucumis prophetarum*. *Bulletin of the Chemical Society of Ethiopia, 21*(3), 323–330. https://doi.org/10.4314/bcse.v21i3.60145

Salem, H. M., Yehia, N., Al-Otaibi, S., El-Shehawi, A. M., Elrys, A. A. M. E., El-Saadony, M. T., & Attia, M. M. (2021). The prevalence and intensity of external parasites in domestic pigeons (*Columba livia domestica*) in Egypt with special reference to the role of deltamethrin as an insecticidal agent. *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2021.10.042

Šeregelj, V., Ćetković, G., Čanadanović-Brunet, J., Šaponjac, V. T., Vulić, J., & Stajčić, S. (2020). Encapsulation and degradation kinetics of bioactive compounds from sweet potato peel during

storage. *Food Technology and Biotechnology, 58*(3), 314–324. https://doi.org/10.17113/ftb.58.03.20.6599

Šeregelj, V., Pezo, L., Šovljanski, O., Lević, S., Nedović, V., Markov, S., & Ćetković, G. (2021). New concept of fortified yoghurt formulation with encapsulated carrot waste extract. *LWT, 138*, Article 110732. https://doi.org/10.1016/j.lwt.2020.110732

Setta, A., Salem, H. M., Elhady, M., El-Hussieny, A., & Arafa, A. A. (2018). Molecular and genetic characterization of infectious bronchitis viruses isolated from commercial chicken flocks in Egypt between 2014 and 2016. *Journal of World's Poultry Research, 8*(1), 1–8.

Slobodníková, L., Fialová, S., Rendeková, K., Kováč, J., & Mučaji, P. (2016). Antibiofilm activity of plant polyphenols. *Molecules, 21*(12), Article 1717. https://doi.org/10.3390/molecules21121717

Smith, R. D., & Coast, J. (2013). The economic burden of antibiotic resistance: A global perspective on its impact on healthcare systems and economies worldwide. *Health Economics, 22*(4), 365–370.

Soliman, S. M., Attia, M. M., Al-Harbi, M. S., Saad, A. M., El-Saadony, M. T., & Salem, H. M. (2021). Low host specificity of *Hippobosca equina* infestation in different domestic animals and pigeons. *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2021.11.050

Tablante, N. L. (2013). *Common poultry diseases and their prevention*. University of Maryland Extension.

Thompson, R. L., Williams, M. B., & Taylor, G. W. (2020). Understanding protein–ligand interactions in antimicrobial drug design: Current approaches and future perspectives. *Drug Discovery Today, 25*(8), 1339–1346.

United States Department of Agriculture. (2015). *The PLANTS database*. Natural Resources Conservation Service. https://plants.usda.gov/

Uzal, F. A., Navarro, M. A., Li, J., Freedman, J. C., Shrestha, A., & McClane, B. A. (2018). Comparative pathogenesis of enteric clostridial infections in humans and animals. *Anaerobe, 53*, 11–20. https://doi.org/10.1016/j.anaerobe.2018.06.002

Virolle, C., Goldlust, K., Djermoun, S., Bigot, S., & Lesterlin, C. (2020). Plasmid transfer by conjugation in Gram-negative bacteria: From the cellular to the community level. *Genes, 11*(11), Article 1239. https://doi.org/10.3390/genes11111239

Voss-Rech, D., et al. (2023). Antimicrobial resistance of *Salmonella* from poultry meat in Brazil. *Life, 13*(3), Article 642.

Wang, L., & Chen, X. (2022). Flavonoids as antimicrobial agents: Molecular mechanisms and therapeutic implications. *Antimicrobial Agents and Chemotherapy, 66*(5), e02365-21.

Wang, Y., Xu, K., Lin, L., Pan, Y., & Zheng, X. (2020). Phytochemicals as potential antibacterial agents against *Salmonella*: A review. *Food Research International, 133*, Article 109092.

Wannang, N. N., Kwanashie, H. O., & Eze, I. C. (2021). Ethnobotanical survey of medicinal plants used in the treatment of typhoid fever in Nigeria. *Journal of Ethnopharmacology, 276*, Article 114131.

Wannang, N. N., Gyang, S. S., Omale, S., Dapar, M. L., Jimam, N. S., & Anakwe, C. (2008). The effect of *Cucumis metuliferus* E. Meyer (Cucurbitaceae) on rat gastric functions and mucosal integrity. *Nigerian Journal of Natural Products and Medicine, 12*, 37–39.

World Health Organization. (2021). *Antibacterial agents in clinical development: An analysis of the antibacterial clinical development pipeline*. World Health Organization.

Wibisono, F. M., Wibisono, F. J., Effendi, M. H., Plumeriastuti, H., Hidayatullah, A. R., Hartadi, E. B., & Sofiana, E. D. (2020). A review of salmonellosis on poultry farms: Public health importance. *Systematic Reviews in Pharmacy, 11*(4), 481–486.

Wilson, D. N., Hauryliuk, V., Atkinson, G. C., & O'Neill, A. J. (2020). Target protection as a key antibiotic resistance mechanism. *Nature Reviews Microbiology, 18*(11), 637–648.

Wright, G. D. (2010). The antibiotic resistome: A unique resource for future drug discovery. *Nature Reviews Microbiology, 8*(6), 417–425.

Yamamoto, K., Sasaki, Y., & Kato, T. (2022). Hydrophobic interactions in antimicrobial mechanisms: A structural biology perspective. *Biochimica et Biophysica Acta (BBA) – Biomembranes, 1864*(1), Article 183741.

Zhang, Y., Wang, Y., & Liu, Y. (2020). Foodborne diseases caused by antibiotic-resistant *Salmonella*: A review on epidemiology and control strategies in food safety management systems. *Comprehensive Reviews in Food Science and Food Safety*, *19*(5), 2462–2482.

Zhang, Y., Wang, Q., Yin, Y., Chen, P., Jin, H., & Li, K. (2021). Structure-based design of antimicrobial compounds targeting *Salmonella* proteins: A comprehensive review. *International Journal of Molecular Sciences, 22*(15), Article 8012.