ASEC 2025 Conference

The 6th International Electronic Conference on Applied Sciences

09–11 December 2025 | Online

Chaired by Prof. Dr. Nunzio Cennamo and Dr. Stefano Toldo

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Green tea polyphenols inhibit *Helicobacter pylori* virulence factors CagA and VacA: A Computational Study using Molecular Docking

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Università degli Studi della Campania Luigi Vanvitelli — Dipartimento di Ingegneria

Abstract

- Helicobacter pylori (H. pylori) is a Gram-negative bacterium that infects the gastric mucosa and is a major contributor to peptic ulcers, chronic gastritis, and gastric cancer.
- With a large proportion of the global population affected, there is a growing need for improved diagnostic and therapeutic strategies.
- Green tea flavonoids (GTFs) have recently emerged as promising natural agents with anti-H. pylori potential.

Among these, Epigallocatechin Gallate (EGCG)—the principal catechin in green tea—shows strong antibacterial effects through:

1.Disruption of bacterial membranes
2.Inhibition of key bacterial enzymes
3.Modulation of oxidative stress

- The antioxidant and anti-inflammatory activities of GTFs may additionally help reduce H. pylori-induced gastric inflammation.
- In this computational investigation, molecular docking (AutoDock Vina) was used to study the interaction of major GTFs with the virulence factors CagA and VacA.

Results showed strong binding affinities:
EGCG: −198.72 kcal/mol (CagA)
Theaflavin-3-gallate: −177.19 kcal/mol (VacA)

Introduction

- Helicobacter pylori infects nearly 50% of the global population and causes chronic stomach inflammation.
- Long-term infection can lead to peptic ulcers, gastric cancer, and MALT lymphoma.

Two major virulence factors drive its pathogenicity:
1.CagA – its N-terminal region interacts with host proteins like ASPP2, disrupting normal apoptosis.
2.VacA – its p33 and p55 domains form ion channels and help bacterial adhesion.

- Antibiotic resistance in H. pylori is rising and is recognized by the WHO as a top global health concern.
- This has shifted research focus toward targeting virulence factors rather than essential bacterial structures, to reduce infection severity and resistance development.

Green tea polyphenols (GTPs) show strong anti-H. pylori activity:
1.Akai et al. (2007) – reduced epithelial cell proliferation and apoptosis.
2.Mabe et al. – identified catechins as potent inhibitors.
3.Recent studies (Wang 2023, Kamankesh 2023, Bansal 2012) support their therapeutic potential.

- Due to their antibacterial, antioxidant, and bioactive properties, flavonoids are increasingly important in drug discovery.
- Molecular docking studies help understand how these compounds interact with virulence proteins like CagA and VacA.

In this study:
1.GTPs showed strong binding to H. pylori virulence factors.
2.Top two hits underwent in-silico ADME and toxicity screening.
3.The most promising complex, EGCG–CagA, was evaluated using a 20-ns molecular dynamics simulation, confirming stable interactions.

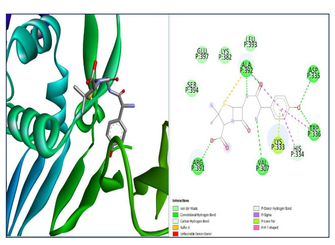

Figure 2-2D and 3D-interaction profiles for docked *Amoxicillin* with **4DVY.**

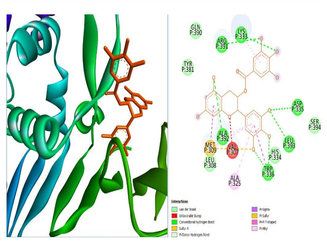

Figure 3- 2D and 3D-interaction profiles for best docked *Epigallocatechin Gallate* with **4DVY.**.

Methods and Materials

2.1. Molecular Docking Simulations
- 3D structures of CagA and VacA were retrieved from the RCSB Protein Data Bank.
- CagA:

Used N-terminal region (residues 24–824) from Hayashi et al. (PDB ID: 4DVY).
Focused on ASPP2-interacting region (residues 24–221) based on Nešić et al.
- VacA:

Used p55 domain (PDB ID: 2QV3) from Gangwer et al.
Binding pocket identified using CASTp server.
- Docking simulations were performed using iGemDock with a 15 Å radius around the binding site.
- Docking results were visualized using Discovery Studio 2020 Visualizer.

2.2. In-Silico Drug-Likeness and ADMET Analysis
- Top three green tea polyphenols (GTPs) with highest docking scores were selected for ADME evaluation.
- Drug-likeness and pharmacokinetic properties assessed using SWISSADME.
- Toxicity predictions conducted using admetSAR (accessed 20-12-2023).

2.3. BOILED-Egg Model Analysis
- BOILED-Egg model on SwissADME used to predict:

1.Gastrointestinal absorption
2.Blood-brain barrier (BBB) permeability
- Provided visual assessment of the absorption/permeation potential of selected molecules

2.4. Molecular Dynamics (MD) Studies
- MD simulations conducted to evaluate protein–ligand stability and account for protein flexibility.
- Focused on the EGCG–4DVY complex.
- Performed 20-ns simulation using Desmond 2022 software.
- System solvated in water and run under standard NPT conditions.
- RMSD values remained below 3 Å, indicating stable interactions throughout the simulation.

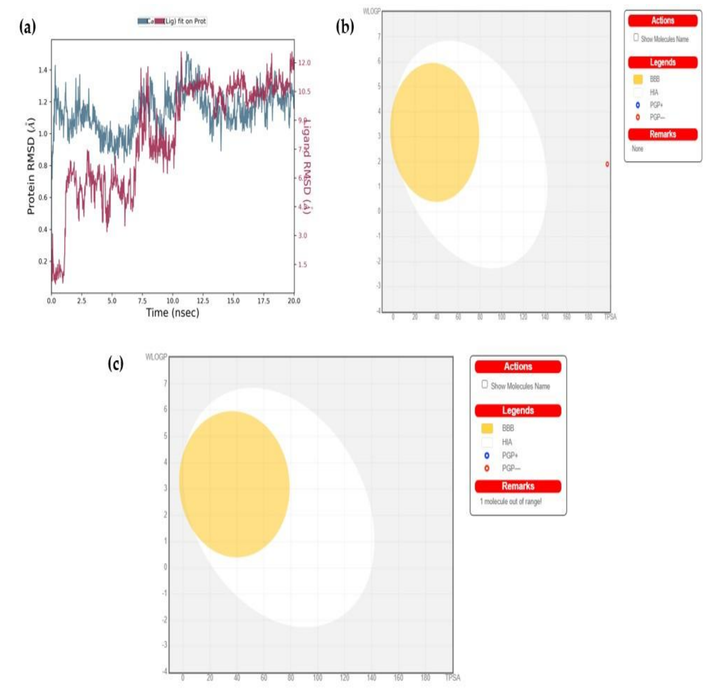

Fig.1-a) The Root Mean Square Deviations (RMSD) of backbone atoms relative to the starting complexes during 20 ns MD; (b) Boiled-egg model analysis for Epigallocatechin Gallate and (c) Boiled-egg model analysis for Theaflavin-3-Gallate.

Results

1. Molecular Docking Findings
- EGCG showed the highest binding affinity towards CagA (4DVY) with a docking score of −198.72 kcal/mol, outperforming all other GTPs.
- Theaflavin-3-gallate demonstrated the best binding towards VacA (2QV3) with a docking score of −177.19 kcal/mol.
- Standard drug Amoxicillin showed comparatively lower affinity toward both targets

CagA: −103.11 kcal/mol
VacA: −119.03 kcal/mol

2. Key Protein–Ligand Interactions
- EGCG–4DVY complex involved key interactions with:
  Arg391, Lys333, Asp335, Leu393, His334, Trp336, Ala392 (H-bonding) and Met309 (hydrophobic).
- Theaflavin-3-Gallate–2QV3 interacted with:
  His531, Tyr529, Ser504 (H-bonding), Phe483, Thr505 (π–π/hydrophobic), Asn463.

3. Molecular Dynamics (MD) Simulation
- The EGCG–4DVY complex remained highly stable during the 20-ns MD simulation.
- RMSD values stayed below 1.4 Å, confirming strong and stable binding.
- RMSF analysis (CABSflex2) also showed minimal fluctuations, supporting structural stability.

4. ADMET & Pharmacokinetic Results
- EGCG and Theaflavin-3-gallate showed:

1.Non-substrate behavior for CYP2C9, CYP2D6, CYP3A4
2.Weak hERG inhibition
3.No AMES toxicity
4.No carcinogenicity predicted
5.Non-inhibitors of P-gp
- BOILED-Egg model: Both compounds fall outside the absorption zone, indicating poor intestinal absorption, consistent with known low bioavailability of GTPs.

5. Overall Outcomes
- EGCG identified as the most promising lead compound against H. pylori virulence factors.
- Both ligands show superior binding compared to standard antibiotic amoxicillin.
- Results support further development of green tea polyphenol-based anti-H. pylori leads.

Table 1. Label in 24pt Calibri.

Molecule	Interaction	Molecule	Interaction
Theaflavin-3-gallate	−197.06	Theobromine	-111.7269
Epigallocatechin Gallate	-198.72	Phenylethanol	−111.468
Epigallocatechin	−177.34	Amoxicillin*	−103.11
Quercetin	−112.834	Epicatechin-3,5-Di-O-Gallate	−105.495
Catechin	−111.992	Coumaroyl Quinic Acid	−94.7189
Succinic Acid	−112.5696	Epicatechin	−98.6033

Discussion

- The molecular docking analysis revealed that Epigallocatechin Gallate (EGCG) and Theaflavin-3-gallate (TG) exhibit strong binding affinities toward the two major H. pylori virulence proteins, CagA (4DVY) and VacA (2QV3), respectively.
- EGCG demonstrated significantly better binding performance than amoxicillin, indicating that natural polyphenols may provide stronger inhibitory interactions than conventional antibiotics in silico.
- The interaction profiles showed multiple stabilizing hydrogen bonds and hydrophobic contacts, supporting the enhanced binding energies of EGCG and TG and suggesting that these ligands may interfere with the functional domains of CagA and VacA.
- Molecular dynamics simulations confirmed the high stability of the EGCG–CagA complex, with low RMSD values indicating minimal structural deviation. This supports the hypothesis that EGCG can maintain a stable and effective interaction with its target under physiological conditions.
- ADMET and toxicity predictions further reinforced the suitability of GTPs as drug candidates, showing no AMES toxicity, weak hERG inhibition, no carcinogenicity, and acceptable pharmacokinetic profiles, despite low intestinal absorption.
- Collectively, these findings highlight the therapeutic promise of green tea polyphenols, especially EGCG, in targeting H. pylori virulence mechanisms. Their strong binding affinity, structural stability, and favorable safety characteristics suggest that they may serve as viable leads for developing novel anti–H. pylori agents.

Conclusions

- EGCG showed strong binding to 4DVY, and Theaflavin-3-gallate (TG) showed strong binding to 2QV3.
- EGCG displayed higher binding affinity than amoxicillin (−103.11 kcal/mol).
- ADMET analysis indicated favorable safety, including low hERG inhibition, no AMES toxicity, and no carcinogenic predictions.
- The known antimicrobial activity of green tea polyphenols supports their potential against H. pylori.
- Overall findings suggest that GTPs, especially EGCG, may serve as promising leads for developing improved anti-H. pylori therapies.

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