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In silico elucidation of the binding affinity of puerarin and adenine with tight junctional proteins (ZO-1, Occludin, and claudin 4) to restore gut barrier integrity

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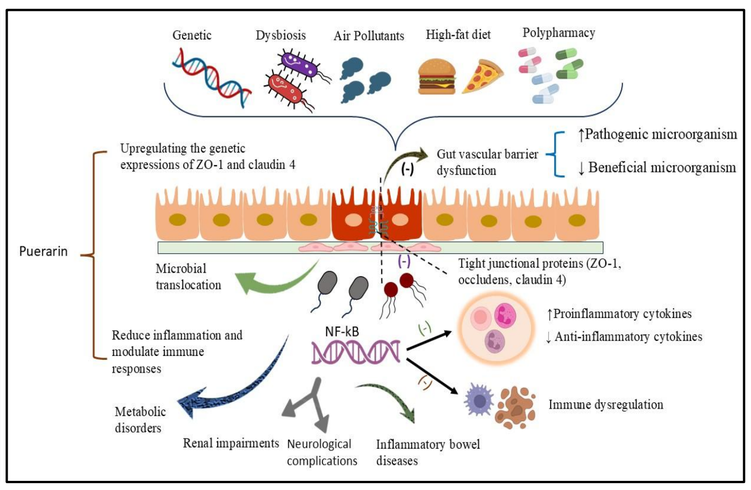
INTRODUCTION & AIM

- Intestinal transmembrane proteins (ZO-1, occludin and claudin 4) can preserve the integrity of the gut and protect it from foreign materials (1).
- Altered expression of these proteins increases intestinal permeability, allowing gut-derived toxicants to enter systemic circulation.
- Gut dysbiosis can result in multiple adverse health effects, including intestinal disorders, metabolic dysfunctions, neurological issues and renal impairments.
- Puerarin is a bioactive isoflavone with anti-inflammatory and antioxidant properties (2).
- It helps restore gut barrier integrity, modulates gut dysbiosis, and supports neurological health by protecting neurons and improving cognitive functions.



METHODS

3D Structures of Zona occludens, claudin 4 and Occludin were downloaded from RSC IN PDB format

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3D Chemical Structures of Puerarin and Adenine was downloaded from the PubChem database in MOLSDF format

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Preparation of target protein
- Remove water molecules
- Polar hydrogens were added
- Adding Kollman charges
- Finally, protein was saved in PDBQT format

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Identify the active binding site through PyMOL software

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Ligand protein docking between ligand (adenine and puerarin) and tight junctional proteins (ZO-1, occludens, and claudin 4) was performed using AutoDock Vina by assigning grid points to the active site and evaluating binding energy. The binding pocket, interacting residues, and ligand poses were analysed using PyMOL.

RESULTS & DISCUSSION

Table 1. Binding energy values, hydrogen bonding, hydrophilic, and polar interactions obtained from molecular docking studies between the ligands (Adenine and Puerarin) and transmembrane proteins (ZO-1, Occludin, and Claudin-4).

Protein	Ligand	Docking Score	H- bond	Hydrophobic interactions	Polar interactions
ZO-1 (4OEO)	Adenine	-3.076	Pro45	Pro45, Phe47, Val81	His46, Gln48, Gln93, Gln94
ZO-1 (4OEO)	Puerarin	-7.187	Phe47, Gln48, Gln94	Pro45, Phe47, Gly50, Val81, Phe90	Gln48, Thr52, Asn79, Gln94
Occludin (1WPA)	Adenine	-2.737	Glu449, Glu452, Asp492	Leu450, Ile453, Val487, Tyr493	-
Occludin (1WPA)	Puerarin	-2.648	Glu449, Glu452, Glu480, Asp492	Leu450, Ile453, Leu484, Val487, Tyr493	-
Claudin 4 (5B2G)	Adenine	-5.409	Leu70, Gln1170, Val1263	Leu70, Leu71, Leu73, Val1171, Ile1174, Val1262, Val1263, Met1281	Ser69, Gln78, Gln1170, Ser1261
Claudin 4 (5B2G)	Puerarin	-9.193	Ser996, Gly998, Gly1265	Leu70, Leu71, Leu73, Val1263, Ala1276	Thr206, Ser996, Ser997, Ser999, Ser1000, Ser1275

Zona occludens- 1 (ZO-1) PDBID: 4OE0

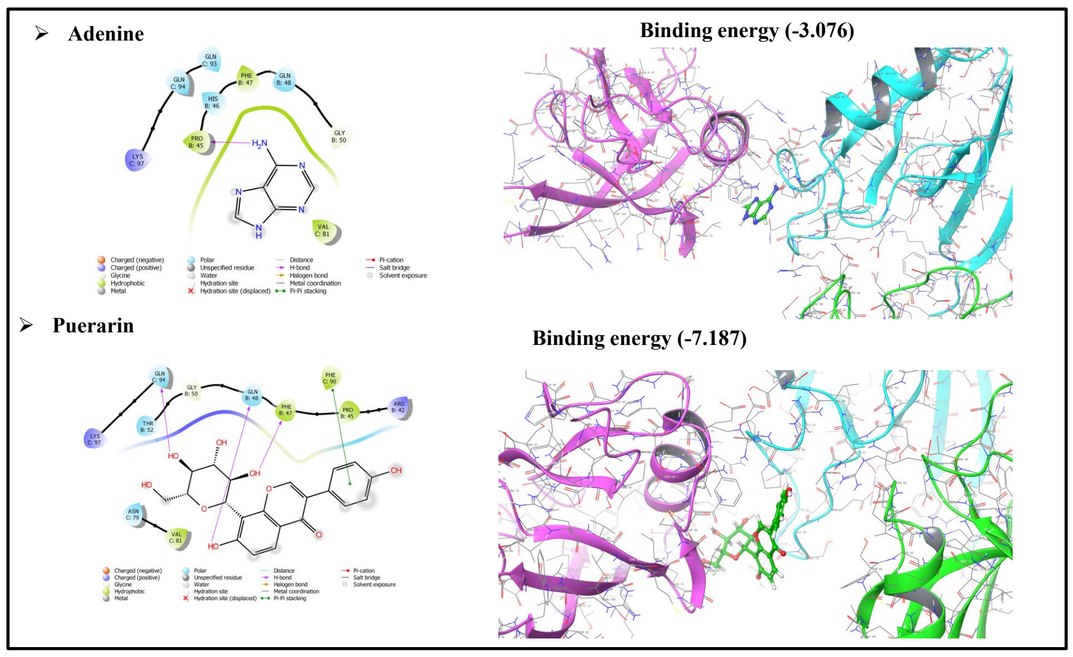

Figure 1: Molecular docking of lignads (Adenine and Puerarin) with Zona occludenes-1 (ZO-1) shows their interactions with key amino acids, where the 2D structure determines the hydrogen bonding, hydrophilic, and hydrophobic contacts. While 3D model shows the binding confirmations within the protein pocket (3D). Adenine exhibits a binding energy of **–3.076 kcal/mol**, while Puerarin shows a stronger affinity with **–7.187 kcal/mol**, **indicating more stable protein–ligand interactions and may possess greater biological activity.**

Occludin PDBID: 1WPA

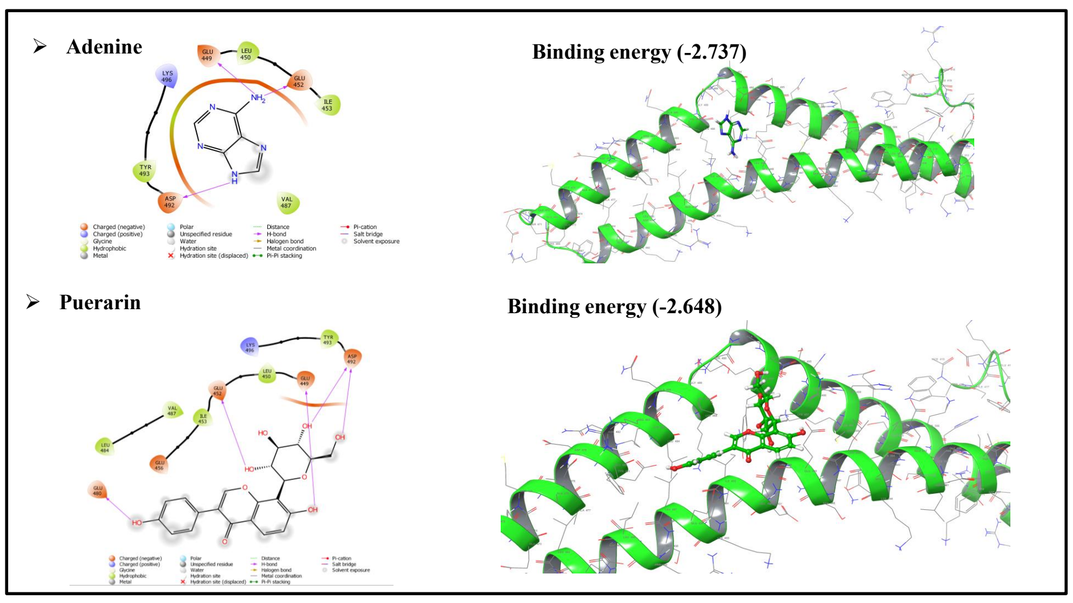

Figure 2: Computational docking of lignads (Adenine and Puerarin) with occludin shows their interactions with key amino acids, where the 2D structure determines the hydrogen bonding, hydrophilic, and hydrophobic contacts. While 3D model shows the binding confirmations within the protein pocket (3D). Adenine demonstrates a binding energy of **–2.737 kcal/mol**, whereas no significant binding interactions were observed for Puerarin. **Hence, Puerarin exhibits lesser biological activity with occludin.**

Claudin-4 PDBID: 5B2G

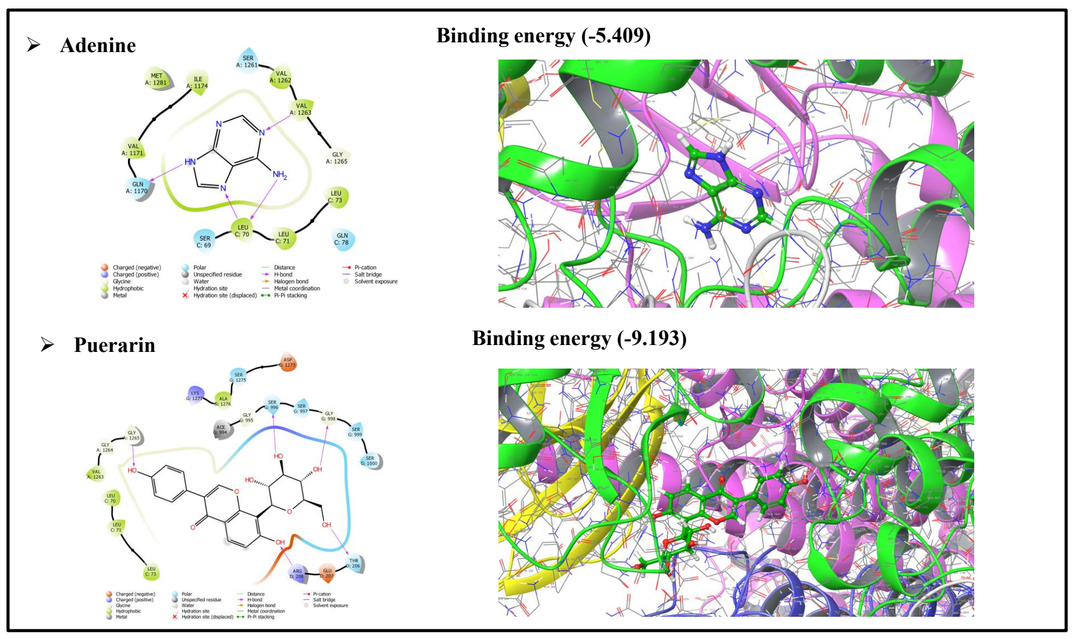

Figure 3: Molecular interaction assessment of lignads (Adenine and Puerarin) with claudin 4 shows their interactions with key amino acids, where the 2D structure determines the hydrogen bonding, hydrophilic, and hydrophobic contacts. While 3D model shows the binding confirmations within the protein pocket (3D). Adenine shows a binding energy of **–5.409 kcal/mol**, whereas Puerarin exhibits a stronger affinity of **–9.193 kcal/mol**, **indicating more stable protein–ligand interactions with claudin 4, which may indicate better therapeutic efficacy.**

CONCLUSION

- Puerarin demonstrated efficient binding interaction with the intestinal epithelial transmembrane proteins, displayed lesser binding energy with ZO-1 (-7.187) and claudin 4 (-9.193), while no significant results were observed with occludin.
- Notably, puerarin exhibits a better docking score compared to Adenine, the positive control, suggesting its potential as a promising phytoconstituent for regulating intestinal barrier functions.
- Further in vitro and in vivo studies are essential to validate its therapeutic efficacy in gut dysbiosis and intestinal-related disorders.

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