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Research Article

An *In-silico* Analysis on Anti-ulcer Activity of Bioactive Compounds Found in Honey

Musab Umar Abubakar and Ankita Mathur*

Department of Life Science, Mewar University, Rajasthan, India

***Corresponding Author:** Ankita Mathur, Department of Life Science, Mewar University, Rajasthan, India.

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Abstract

Ulcer disease has become a disease mostly affecting the older people, with the highest incidence occurring between 55 and 65 years of age. The study was carried out to determine an *in-silico* analysis on anti-ulcer activity of bioactive compounds found in Honey. The crystal structure for 2PVP and 4INP proteins of *Helicobacter pylori* were used for molecular docking in which twenty four ligands compounds of honey were tested, they yielded a good result, Among the docking result of the selected hits obtained, the binding affinities range from -3.3 to -6.3 (kcal/mol) and -2.9 to -6.5 for 2PVP and 4INP protein respectively for which some were higher than the control used. In this study, 11 compounds shows good binding affinity, higher than the control in 2PVP protein while 22 compounds shows good binding affinity, higher than the control in 4INP. 2H-Benzimidazol-2-one with docking score of -6.3kcal/mol while Acetamide has the least docking scores of -3.3 kcal/mol for 2PVP, and Benzophenone was with highest binding affinity in 4INP with docking score -6.5 Kcal/mol while Acetamide has the least docking scores of -2.9 kcal/mol among the selected compounds. The active site of the targeted proteins showed hydrophobic interactions with the help of CEU B: 279, LEU A: 149, TYR A: 131 etc. amino acids. Important Admet properties were examined for the prediction of the physical descriptor and pharmacologically important properties. All the selected compounds displayed important values for the various criteria tested and showed strong drug-like properties based on the Lipinski's rule of five.

Keywords: Ulcer; Phytochemicals; Docking; Virtual Screening; Bacteria; Bioactive Compounds

Introduction

Ulcer disease represents a serious medical problem. Approximately 500,000 new cases are reported each year, with 5 million people affected in the United States. People with the highest risk of contracting peptic ulcer disease are those born around the middle of the 20th century. Ulcer disease has become a disease that usually affect the older people, with the peak incidence occurring between 55 and 65 years of age. In men, duodenal ulcers occurrences were more common than gastric ulcers; in women, the converse was found to be the case. Thirty-five percent (35%) of patients detected with gastric ulcers suffers serious difficulties. Although death rates from peptic ulcer disease are low, the high prevalence and the resulting suffering, and expense are very costly. Ulcers can develop in different body parts, such as esophagus, stomach or duodenum, at the margin of a gastro enterostomy, in the jejunum, in Zollinger-Ellison syndrome, and in association with a Meckel's diverticulum containing ectopic gastric mucosa.

Helicobacter pylori virulence factors are related to genomic regions with high flexibility and diversity, being present, absent, up-regulated or differentially uttered during bacteria growth and gastric settlement tenacity [1]. The flexible *H. pylori* genome present strong phylogeographic structure reached during co-evolution process in human [2], resulting in different bacteria

strains with precise virulence factors and disease outcome according to geographic and population dispersal [3].

The urease molecular composite and *vacA* gene are abundant in *H. pylori* strains corresponding to bacterial crucial virulence factors. Ammonia and bicarbonate produced by *H. pylori* urease enzyme complex can directly or indirectly cause damage to gastric mucosa or toxic compounds derived from ammonia chemical processing respectively. Also, the urease enzyme is a strong antigen, produced in high concentration, which results in proliferations tissue injury by local inflammatory response [4].

When scientists identified *Helicobacter pylori* as an infectious agent responsible for peptic ulcer disease, it completely transformed our understanding of the microbiology and pathology of the human stomach. The widespread existence of unhealed wounds, ulcers, and burns has a great influence on public health and economy. Many interventions, including new medications and technologies, are being used to help achieve substantial wound healing and to eradicate infections. Therefore, finding an intervention that has both therapeutic effect on the healing process and the ability to kill microbes is of great value.

Honey is a natural product that has been recently introduced in recent medical practice. Honey's antibacterial properties and its effects on wound healing have been thoroughly investigated.

As a wound dressing, honey provides a moist environment with antimicrobial properties, has anti-inflammatory effects, reduces oedema and exudates, promotes angiogenesis and granulation tissue formation, induces wound contraction and speeds wound epithelialization [5]. Honey efficacy in the curing of skin ulcers of different etiologies has been acknowledged in numerous studies [6]. Antibacterial action of honey has been attributed to its hyper osmolarity, acidity or other properties that have not been fully elucidated [7].

Material and Methods

Receptor preparation

The structural information of the macromolecules determined by x-ray crystallographic and NMR methods are available in the PDB. The 3D structure of D-alanine-D-alanine ligase (PDB code 2PVP (D-Alanine-D-Alanine), CeuE (PDB code 4INP (*H. pylori* Ceue) were taken from Protein Data Bank (http://www.rcsb.org/pdb/). To validate the capacity of the model in reproducing experimental observation with new ligand, the above proteins were tested for which the binding sites were determined. The proteins were tested with the Following Ligands: 2-Heptanone, Isoborneol, Nonanol, 2H-Benzimidazol-2-one, 4-Hexen-3-ol, Benzophenone, 1-Penten-3-ol, Thymol, 2(3H)-Benzothiazolone, 1,3-Benzenediamine, 2-hydroxymethylbenzimidazole, Acetamide, Guanidine, 2-Furancarboxaldehyde, alpha.-Furfuryl alcohol, 2,4-Dimethyl-1-pentanol, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 5-Hydroxymethylfurfural, 2H-Imidazol-2-one, 1,3-dihydro-4-methyl, 2-Butoxyethyl acetate, n-Hexadecanoic acid, 11-Octadecenoic acid, methyl ester, Oleic Acid.

Bioactive compounds (Ligands) preparation

Most of the 3D structures of drug molecules were downloaded from PubChem Compound section of National Center for Biotechnology Information (NCBI) and the others were drawn by Gauss- View 5 [8]. Ligands during this procedure were also being checked for twisting count to detect currently active bonds with default settings. Importantly, amide bonds were checked and treated as non-rotatable. Ligands also utilized to merge non-polar hydrogens.

S/N	Name of compound	Molecular formular	Molecular weight (g/mol)	PUBCHEM ID
1	2-Heptanone	$C_7H_{14}O$	114.19	8051
2	Isoborneol	$C_{10}H_{18}O$	154.25	64685
3	Nonanol	$C_9H_{20}O$	144.25	8914
4	2H-Benzimidazol-2-one	$C_7H_4N_2O$	132.12	162542
5	4-Hexen-3-ol	$C_6H_{12}O$	100.16	5366234
6	Benzophenone	$C_{13}H_{10}O$	182.22	3102
7	1-Penten-3-ol	$C_5H_{10}O$	86.13	12020

8	Thymol	$C_{10}H_{14}O$	150.22	6989
9	2(3H)-Benzothiazolone	C_7H_5NOS	151.19	13625
10	1,3-Benzenediamine	$C_6H_8N_2$	108.14	7935
11	2-hydroxymethylbenzimidazole	$C_8H_8N_2O$	148.16	78569
12	Acetamide	C_2H_5NO	59.07	178
13	Guanidine	CH_5N_3	59.07	3520
14	2-Furancarboxaldehyde	$C_5H_4O_2$	96.08	7362
15	alpha.-Furfuryl alcohol	$C_5H_6O_2$	98.1	7361
16	2,4-Dimethyl-1-pentanol	$C_7H_{16}O$	116.2	22749
17	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128.13	19309
18	Methyl 1-methylcyclopropyl ketone	$C_6H_{10}O$	98.14	74067
19	2-Furancarboxaldehyde, 5-hydroxymethyl	$C_6H_6O_3$	126.11	237332
20	2H-Imidazol-2-one, 1,3-dihydro-4-methyl	$C_8H_{10}N_2O_3$	182.18	12699181
21	2-Butoxyethyl acetate	$C_8H_{16}O_3$	160.21	8160
22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	985
23	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	5364432
24	Oleic Acid	$C_{18}H_{34}O_2$	282.5	445639

Table 1: GC-MS DATA: Molecular Formula, Molecular Weight and PubChem ID number of Bioactive Compounds of Honey from Ethanol extracts.

Pharmacophore-based virtual screening

In silico pharmacophore-based virtual screening was performed with "Asinex focused protein–protein interaction (PPI)" library having 11,870 small-molecules contrary to the generated pharmacophore model by means of Lignad Scout software. The library contains non-macrocyclic compounds with a diversity of more than 500 scaffolds. The library obtained from (https://www.asinex.com/ppi/) in sdf format and converted into Idb using Ligand Scout library generation tool. The compounds that meets all pharmacophore features considered as hit compounds and rank based on their pharmacophore-fit scores which reflect to which degree the molecules fitting the pharmacophore features.

Docking-based virtual screening

The retrieved hit compounds from prior screening were subjected to docking-based virtual screening against the 3D structure of diverse target proteins (PDB ID) using AutodockVina in PyRx 0.8 program [9]. Before docking, hit compounds energy lessened and transformed from sdf files into pdbqt files using Open Babel tool in PyRx 0.8 program [10]. The grid box cantered which covers' the amino acid residues involved in the topology of the primary pocket of diverse target proteins. Earlier screening, standard inhibitor added to the database as a control. Compounds that bind to target protein with high binding affinities in contrast to standard inhibitor were considered for further analysis.

In silico ADME-T analysis

pkCSM server used to evaluate the absorption, distribution, metabolism and excretion- toxicity (ADME-T) parameters for the recognized hit compounds (Pires., *et al.* 2015). For the compound to be selected as a hit, it must be non-hepatotoxic and non-carcinogenic. Swiss ADME used for evaluating other physiochemical properties of these hit compounds [11].

Molecular docking study

Hit compounds satisfying the pervious filters were docked against the 3D structure of diverse target proteins using Auto docking program [12]. The protein structure (PDB: ID) acquired from RCSB protein data bank [13]. Discovery studio 4.5 (Accelrys, San Diego, CA, USA) used to eradicate the unwanted water molecules and ligands as well as to produce the pdb files for the protein in monomer form. Autodock tools program used to

generate the pdbqt files and prepared the gridbox for the docking configuration files (Sanner, 1999). The grid box centered which covers' the primary pocket with the following parameters; the box size of x = 14 y = 14 z = 22 and the box center: x = 1.605 y = 11.139 z = 23.381. Discovery Studio 4.5 and PyMOL Molecular Graphics System 1.3 used to visualize and examined the docking results.

Pharmacophore analysis

This part of process carried out by using the pharmacophore tool involved in Ligand Scout [14]. The program shows the 2D and 3D structure with the position and collaboration of ligand in the binding pocket of the receptor. From these 2D figures, some types of bond identified by color and symbol. Four features namely hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), negative ionizable area (NIA), hydrophobic interaction were labeled as red arrow, green arrow, red star and orange bubble (supporting information) respectively.

Visualization of binding interaction

The PDB structures of anti-ulcer compounds, Enzymes and the Enzyme-ligand interaction were visualized using the PyMOL.

Result and Discussion

Virtual screening

The crystal structures of the 2PVP (D-Alanine-D-Alanine) and 4INP (*H. pylori* Ceue) proteins of *Helicobacter pylori* were used for molecular docking study, and analysis was performed using PyRx program v 0.8 to determine the binding energy of the hits and visualization was done using Discovery studio 2020. The results obtained shows the binding energy of all the twenty four ligands compounds that are related to the Ulcer as shown in table 1. Among the docking result of the selected hits obtained, the binding affinities ranges from -3.3 to -6.3 (kcal/mol) and -2.9 to -6.5 for 2PVP (D-Alanine-D-Alanine) and 4INP (*H. pylori* Ceue) protein respectively, for which some were higher than the control used. In this study, 11 compounds shows good binding affinity, higher than the control in 2PVP (D-Alanine-D-Alanine) protein, while 22 compounds shows good binding affinity, higher than the control in 4INP (*H. pylori* Ceue). Table 2 showed the binding affinity of the selected compounds. All the selected compounds shows good binding affinity, but the highest binding affinity was obtained from 2H-Benzimidazol-2-one with docking score of -6.3 kcal/mol while Acetamide has the least docking scores of -3.3 kcal/mol for 2PVP (D-Alanine-D-Alanine), and Benzophenone was with highest binding affinity in 4INP (*H. pylori* Ceue) with docking score of -6.5 Kcal/mol while Acetamide has the least docking scores of -2.9 kcal/mol among the selected compounds.

Protein-Ligand interaction of the selected compounds with the target protein

Hydrophobic interaction and hydrogen interaction are playing an integral role in predicting the binding affinity of the selected compounds (or ligands) with the target protein, and this is called Protein-Ligand interaction. All the selected 24 compounds used in this studies were docked in to the active site of the two targeted protein receptors of *Helicobacter pylori*. Figure 5 and 6 represent binding conformations of the selected compounds in the binding pocket of the target protein. The active site of the target protein showed hydrophobic interactions with the help of CEU B: 279, LEU A: 149, TYR A: 131, SER B: 277, VAL A: 125 etc. amino acids.

The amino acids presents in the active site of the proteins like LEU A: 149, CEU B: 279, ALA A: 149, PRO B: 98 etc. are most commonly found to generate hydrophobic interaction via Alkyl and Phi-Alkyl hydrophobic interactions with the top selected ligands. In addition to hydrophobic interaction, hydrogen bonds present in the active site of the target protein play a vital role. The target protein shows hydrogen bond with the help of GLU B: 122, ASN A: 129, SER A: 173, VAL A: 86 etc. amino acids (Table 3 and 4).

Also figure 5 and table 3 and 4 shows protein-ligands interactions of the targets proteins with the selected compound showing the hydrophobic interactions and hydrogen bonds formed on each individual ligand and the target protein while figure 5-8 shows Ribbon presentation for 2PVP (D-Alanine-D-Alanine) and 4INP (*H. pylori* Ceue) with selected compounds.

ADME analysis

Admet properties of the compounds were evaluated using SWISSADME online tool (Table 5). This website allows for the compute of physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, drug-like nature and medicinal chemistry to support drug discovery (SwissADME, n.d.). With the assistance of this online tool, important Admet properties were examined for the prediction of the physical descriptor and pharmacologically significant properties for Admet prediction (Figure 8 and Table 5). All the selected compounds displayed important values for the various criteria tested and showed strong

drug-like properties based on the Lipinski's rule of five. The data obtained were within the range of values for all natural compounds.

Swiss ADME boiled egg (Figure 7) allows for assessment of Human Intestinal Absorption (HIA) as a function of the position of the twenty four molecules in the WLOGP- versus TPSA referential. The significance of polar surface area (PSA) suggested good oral bioavailability of the compounds under study (A. K. Verma., *et al*. 2020). Furthermore, polar surface area, oral bioavailability, H-bond donor, H-bond acceptor are being important criteria for the development of effective therapeutic agent.

All molecules within the white zone of the egg are predicated as absorbed by gastro intestines but are not brain penetrant (York), while molecules that are excluded out of the egg are neither absorbed by human gastro intestines nor brain penetrant (Figure7). All of the molecules evaluated are PGP- not to subject to active efflux (Red dot) none of the molecule was found to have PGP+ (Blue dot). The depiction of figure 8, shows that all the twenty four compounds showed in colored zones are the suitable physiochemical space for oral bioavailability and shows the LIPO(Lipophilicity), SIZE(Molecular Weight), POLAR(Polarity), INSATU(Instauration), and FLIX(Rotable bond flexibility) parameters.

All these models unscrew the qualitative prediction and ranking of absorption, formulation effects on drug permeability, determining the mechanism(s) of permeability, and the potential for transporter-mediated drug-drug interactions similar to the work done by Danyaya (Danyaya., *et al*. 2020). All the good scoring ligands have drug-likeness properties by Lipinski's rule. It may also be predictable that the combination of these drugs may be a good idea for anti-ulcer's drug [15,16].

S/N	Name of compound	PUBCHEM ID	Docking scores for 2PVP (Kcal/mol)	Docking scores for 4INP (Kcal/mol)
1	2-Heptanone	8051	-4.1	-4.3
2	Isoborneol	64685	-5.1	-5.1
3	Nonanol	8914	-4.5	-4.9
4	2H-Benzimidazol-2-one	162542	-6.3	-5.9
5	4-Hexen-3-ol	5366234	-4.6	-4.1
6	Benzophenone	3102	-6.2	-6.5
7	1-Penten-3-ol	12020	-4.0	-3.5
8	Thymol	6989	-5.7	-5.4
9	2(3H)-Benzothiazolone	13625	-5.1	-4.8
10	1,3-Benzenediamine	7935	-5.2	-4.9
11	2-hydroxymethylbenzimidazole	78569	-5.1	-5.2
12	Acetamide	178	-3.3	-2.9
13	Guanidine	3520	-3.5	-3.6
14	2-Furancarboxaldehyde	7362	-4.1	-3.9
15	alpha.-Furfuryl alcohol	7361	-4.3	-4.0
16	2,4-Dimethyl-1-pentanol	22749	-4.2	-4.4
17	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	19309	-5.4	-4.6
18	Methyl 1-methylcyclopropyl ketone	74067	-4.5	-4.0
19	5-Hydroxymethylfurfural	237332	-5.1	-4.2
20	2H-Imidazol-2-one, 1,3-dihydro-4-methyl	12699181	-5.4	-5.0
21	2-Butoxyethyl acetate	8160	-4.1	-4.3
22	n-Hexadecanoic acid	985	-5.5	
23	11-Octadecenoic acid, methyl ester	5364432	-5.6	-6.5
24	Oleic Acid	445639	-4.9	-5.9

Table 2: Docking score (Kcal/mol) of the (2PVP and 4INP) with selected compounds of *Honey* detected by molecular docking.

S/N	Name of compound	PUBCHEM ID	H-Bonding	Hydrophobic interaction
1	2H-Benzimidazol-2-one	162542	Glu203(B), Asn129(B), Ser277(B).	Leu279(A).
2	Benzophenone	3102	Ser277(B), Tyr136(B).	Ceu279(B), Leu149(A).
3	Thymol	6989	Glu122(B), Asn129(A).	Leu279(B), Tyr131(A), Tyr136(B).
4	n-Hexadecanoic acid	985	Ser173(B), Glu203(B), Lys202(B).	Leu279(A), Arg120(A), Tyr128(B), Ile121(A), Val125(B).
5	11-Octadecenoic acid, methyl ester	5364432	Asp278(B), Leu279(B), Ser277(B).	Ile121(B).

Table 3: Protein-ligand interaction on 2pvp protein.

S/N	Name of compound	PUBCHEM ID	H-Bonding	Hydrophobic interaction
1	2H-Benzimidazol-2-one	162542	Glu153(A), Ni402 (A).	Ala149(A), Met152(A), Leu170 (A).
2	Benzophenone	3102	……..	Pro98 (B), Ala149 (A).
3	Thymol	6989	……..	Pro98 (B), Phe81 (B).
4	n-Hexadecanoic acid	985	Val86(B).	Ala149(A), Met152(A), Pro98(B), Phe81(B), Pro93(B).
5	11-Octadecenoic acid, methyl ester	5364432	Glu94(B).	Lys87(B), Val86(B), Leu90(B), Phe81(B), Pro93(B).

Table 4: Protein-ligand interaction on 4INP protein

S/N	PubChem ID of molecules	MW(g/mol)	HBA	HBD	Molar Refractivity	LogP	Lipinski violation
		≤500	≤10	≤5	40-130	≤5	
1	7935	108.14	0	0	35.25	0.52	1
2	12020	86.13	1	1	26.84	1.11	1
3	13625	151.19	1	1	42.45	1.88	0
4	22749	116.20	1	1	36.92	1.81	1
5	19309	128.13	3	1	31.22	0.56	1
6	8160	160.21	3	0	42.94	1.58	0
7	7362	96.08	2	0	24.10	0.69	1
8	237332	126.11	3	1	30.22	0.19	1
9	162542	132.12	3	0	40.80	0.84	0
10	8051	114.19	1	0	35.96	1.97	1
11	12699181	182.18	3	0	46.64	0.27	0
12	78569	148.16	2	2	42.22	1.02	0
13	5366234	100.16	1	1	31.64	1.40	1
14	5364432	296.49	2	0	94.26	5.95	1
15	178	59.07	1	1	14.64	-0.48	1
16	7361	98.10	2	1	24.84	0.62	1

17	3102	182.22	1	0	56.32	2.94	0
18	3520	59.07	1	3	15.93	-1.01	1
19	64685	154.25	1	1	46.60	2.39	0
20	74067	98.14	1	0	28.78	1.30	1
21	985	256.42	2	1	80.80	5.20	1
22	8914	144.25	1	1	46.54	2.87	0
23	445639	282.46	2	1	89.94	5.71	1
24	6989	150.221	1	1	48.91	2.8o	0

Table 5: Prediction of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of the compounds.

a) Molecular Weight (MW) (acceptable range: <500)

b) Hydrogen bond (HB) donor (acceptable range: ≤5)

c) Hydrogen bond (HB) acceptor (acceptable range: ≤10)

d) High lipophilicity (expression as LogP, acceptable range: <5)

e) Molar refractivity should be 40-130.

Figure 1: Showing the Duodenum ulcer.

Figure 3: Ribbon representation *H. pylori* protein (PDB: 2PVP) with promising compounds of Honey.

Figure 2: Shows the photo for Honey.

Figure 4: Ribbon representation *H. pylori* protein (PDB: 4INP) with promising compounds of Honey.

Figure 5: 2D representation of the interaction between the best pose found for Honey compounds in 2PVP protein.

Figure 6: 2D representation of the interaction between the best pose found for Honey compounds in 4INP protein.

Figure 7: Boiled Egg diagram and bioavailability radar map of selected compounds for the treatment of Ulcer disease.

Figure 8: All the twenty four compounds shown in colored zones are the suitable physiochemical space for oral bioavailability and shows the LIPO (Lipophilicity), SIZE (Molecular Weight), POLAR (Polarity), INSATU (Instauration), and FLIX (Rotable bond flexibility) parameters.

Conclusion

Ulcer disease signifies a serious medical problem worldwide; thirty-five percent of patients analyzed with gastric ulcers were suffering with serious difficulties. One of the best ways for developing Ulcer's drugs is through using herbal medicines which will have no side effects. Docking studies of bioactive compounds in Honey against two targeted proteins of *Helicobacter pylori* (causative agent for an ulcer) yielded a good results with prominent inhibitory activity were observed with highest binding affinity were obtained from 2H-Benzimidazol-2-one and Benzophenone on 2PVP (D-Alanine-D-Alanine) and 4INP (*H. pylori* Ceue) respectively. The aforementioned phytochemicals (drugs) can be used for the developing a potent drugs against ulcer.

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