Journal of Advanced Pharmacy Research

Section B: Pharmaceutical Analytical & Organic Chemistry, Medicinal & Biochemistry

Evaluation of *Pomegranate granatum* Compounds' as Potential Inhibitors of *H. pylori* RdxA: A Computational Approach

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Submitted on: 01-15-2024; Revised on: 27-03-2024; Accepted on: 06-04-2024

To cite this article: Elnasri, H. A.; Al-Bykre, A. M. Evaluation of Pomegranate granatum compounds' as Potential Inhibitors of H. pylori RdxA: A Computational Approach. *J. Adv. Pharm. Res.* **2024**, *8* (2), 49-62. DOI: 10.21608/aprh.2024.263114.1248

ABSTRACT

Background and Objective. *Helicobacter pylori (H.pylori*) is among the major risk factors for gastritis. *H pylori* is becoming resistance to commonly used antibiotic and thus it is necessary to search for an alternative treatment. *Pomegranate granatum* (PG) is a plant commonly used for the treatment of variety of diseases as it shown to possess antibacterial and antiviral effect. This study aimed to search for new compounds of *Pomegranate granatum* that can be considered as potential hits against *H. pylori*. **Methods:** Natural compounds of PG were obtained from Dr Dukes ethno botanical database. RdxA enzyme was selected since it is an important bacterial enzyme in the reactions with the commonly used antibiotic Metronidazole. MOE software was used for preparation and docking. Filtration of compounds was done using the Lipinski rule, PAINS, ADME and PASS parameters. **Results:** A total of 76 compounds were obtained. Following the filtration using Lipinski rule and PAINS only 20 compounds passed with zero violations. After studying the biological activity using PASS software, four compounds namely cinnamic acid, ferulic acid, fumaric acid and flavanols showed good affect against *H pylori according to their Pa*. Their ADME properties also showed good pharmacokinetic properties. **Conclusion**: Compounds selected from PG can be useful for treatment of *H. pylori* infection and the obtained compounds can be further investigated using in vivo studies.

Keywords: *Pomegranate granatum; H pylori. Computational study; RdxA enzyme.*

INTRODUCTION

Gastric cancer is a serious health problem and there were more than 1000,000 cases of gastric cancer in 2020.[1] *Helicobacter pylori (H. pylori)* bacteria is the main risk factor for gastric cancer in addition to other behaviors such as alcohol consumption and smoking [2].

H. Pylori is a Gram-negative, microaerobic spiral bacterium, causing several health problems –in addition to the gastric ulcer, such as chronic active gastritis, peptic ulcer, and gastric mucosa -associated lymphoma[3]. *H. pylori* can weaken the protective mucus coating of the stomach and the upper part of the small intestine, making them more vulnerable to acidic gastric juice and thus leading to gastritis and ulcers.[4]

H pylori possess the enzyme RdxA which belongs to the nitroreductase group and is responsible for reduction of many nitro groups of nitrotoluenes, nitrofurans. and nitroimidazoles, an example is the oxygen-insensitive class, represented by the homodimeric NAD(P)H/FMN nitroreductases which is commonly found in bacteria. They catalyze a two-step, 4e´ reduction of the nitro group, producing DNA damaging and mutagenic hydroxylamine adducts[5-8]. The RdxA NTR of *H. pylori* efficiently reduces the commonly used drug Metronidazole, and it is the major activating enzyme in this species [5,9].

Plant species contain a variety of active ingredients such as alkaloids, phenols, tannins, cryogenics, glycocides, terpeniods [10]. They are commonly used as sweeteners in addition to having anti-infections and anti-bacterial properties [11,10].

Pomegranate granatum (PG) commonly referred to as Pomegranate belongs to the genus *Punica, and* family Lythraceae [12] and it is widely consumed in different forms such as fruits, juice or dried peel [13]. It is known to have different health and nutritional benefits [14-15]. It is used for treatment of variety of diseases such as diarrhea, fever, malaria in addition to having an antioxidant, anti-inflammatory anti-obesity, anti-diabetic, anti-ulcer, anti-depressant properties [15-17] and even can be used for treatment of gastric inflammation caused by *H. Pylori* [4]. It also has the ability to inhibit ribonucleic acid (RNA) replication [18]. In Sudan, PG is commonly used by the local people for treatment of *H pylori* infection mainly as dried peel powder.

Recently, the use of computational tools and /or molecular docking methods is becoming widely used. As seen with the COVID19 outbreak, many studies were carried out using insilico approaches to find treatment from different plant sources[19-23]. Molecular docking aims to find the best fit / orientation of some selected compounds (called ligands) and a receptor (usually a protein) as a step to predict new compounds that can be used as drugs. It has many benefits such as reliability, cost effectiveness and time-saving especially in the area of drug design by discovering new promising therapeutic compounds in a short period of time [24].

MATERIAL AND METHODS

Methodology

In the current study MOE (Molecular Operating Environment) software was used [25]. It was used for preparation of ligands, protein and for docking studies. It has a user-friendly graphical interface and provides a good graphical view to show ligand and receptor binding residues with their positions and interactions.[26-27]. The scheme of study is shown in **Figure.1**.

Identification of PG natural compounds

The natural components of *PG* fruit were obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phyto chem.nal.usda.gov/).

Selection and Preparation of target protein (enzyme):

The 3D structure of RdxA enzyme was downloaded from Protein Data Bank (www.rcsb.org) under PDB ID 3QDl. The enzyme was prepared using MOE software before docking.

Identification of Active site

The preferred region of the receptor that interacts with a ligand is known as the active site. It was identified using MOE. The largest site including the naturally isolated ligand FMN was selected.

Filtration and Preparation of ligands

The obtained compounds of *PG* were filtered according to Lipinski's rule of 5 [28] to predict their drug likeliness using Swiss ADME web tool (http://www.swissadme.ch/index.php). The input was the SMILE format obtained from Drug bank. The rule of five implies that a molecule to have a good drug property should have the following characteristics: molecular weight ≤ 500 Da, number of acceptable hydrogen ≤ 10, number of Hydrogen donors ≤ 5 and LOGp ≤ 5.

In addition, compounds were also filtered according to PAINS (Pan Assay Interference Compounds) criteria which identify if a molecule contains any problematic fragments. Only compounds showing zero violation in both tools were selected for further studies. The selected compounds were then prepared using MOE before docking.

Reference Drug

For comparison, the drug Metronidazole (MNZ) was selected. It is used as a first-line antibiotic for *H.pylori* eradication therapy; but still the increasing resistance to metronidazole impairs the efficacy of *H.pylori* eradication, and increasing the dose of metronidazole was recommended to overcome low-level resistance [29].

Docking

Docking of prepared ligands against RdxA protein was done using MOE. The receptor–ligand binding affinities with various binding geometries are prioritized on the basis of a numerical value called s-score.[26]. Docking was done for 30 poses / molecule and the best five poses were chosen. After docking molecules with best s- score and Root mean standard deviation (rmsd) >1.3 were selected.

Prediction of Biological Activity (PASS).

The chosen molecules were further investigated for their biological activity using PASS tool (Prediction of Activity Spectra for Substances prediction) http://www.way2drug.com/passonline/. PASS tool allows the search for possible biological properties of compounds, based on their chemical formula. It uses 2D molecular fragments known as multilevel neighbors of atoms (MNA) descriptors which suggest that the biological activity of a chemical compound is the function of its molecular structure. It gives the prediction score for biological properties based on the ratio of 'probability to be active (Pa)' and 'probability to be inactive (Pi)'.A higher Pa means the biological property is having more probability. The input was the SMILES format for the molecule and the result will be a description of the function with various Pa and Pi. Only activities related to *H. pylori* effect or gastrointestinal complications were selected.

RESULTS

Retrieval of PG Compounds

A total of 76 compounds were obtained from the database (**Table 1**). It included phenolic compounds. organic acids, amino acids and sugars,

Filtration of compounds

The obtained compounds were then filtered according to both Lipinski's rule of 5 and PAINS. Metronidazole was also tested. As shown in (Table 2), only 20 compounds passed both tests with zero violations and zero alert in PAINS.

Identification of RdxA enzyme and Determination of Active Site

The 3D structure of RdxA was downloaded from the PDB data bank **(Figure 2**) .The active site of the enzyme as depicted by MOE composed of the amino acid residues :Ser43- Pro44- Ser45- Ser46- Tyr47- Asn48- Gln50- Trp52- Tyr135- Glu138- Gln139- Ile142- Arg16 -His17- Ser18- Lys20- Phe72- Asn73- Met76 - Tyr141- Cys159- Ile160- Ile161- Gly162- Gly163- Phe164 -Ala183 Cys184 -Ser196- Gln197- Lys198 and, Arg200 (**Figure (3a)).**The naturally isolated ligand FMN was located within this region and it binds with amino acids Asn73-Lys 20- Lys 198- Gly 162- Gly 163- Ser 45- Ser 18- Arg 16 - Arg200 and, Pro 44 (**Figure.(3b)**) FMN is needed as a cofactor in the reduction reactions catalyzed by the enzyme.

Docking Results

After docking, only 14 compounds with s -score lower than Metronidazole were selected (Table 3) in addition to having an rmsd value less than 1.3.Usually for an efficient binding of a ligand and a receptors specific amino acids involved. In the current study, the amino acids Ser18, Arg200, Lys 20, and Lys 198 were found to be the common ones for binding of ligands and RdxA along with the FMN molecule. On the contrary, Metronidazole attached only to two amino acids (Gly 162 and Ser 46) (**Table 3**).

Evaluation of Biological activity

In the current study, many of the compounds had an anti H. pylori activity (**Table 4**). Based on the Pa cinaminc acid, feurlic acid, fumaric acid and Flavanols showed the highest Pa compared to Pi while the other compounds also showed an anti H. pylori activity but with lower probability (**Table 4**). These compounds were further investigated for their ADME properties (**Table 5**).

When studying the docking and binding features of the four compounds, all compounds were found to bind with the important amino acids Ser 18, Arg200, Lys 20, and Lys 198 and in close proximity with FMN either through hydrogen or ionic bonds (**Table 6)**

DISCUSSION

Worldwide, various plant species are used for the treatment of different diseases. The revert to nature for finding new drugs is becoming an important era of research, especially with the increased risk of the side effects of the synthetic drugs and the antimicrobial resistance phenomena.

This study aimed to identify the important compounds from PG that can be used for treatment of .*H pylori* infection. The natural compounds retrieved from the database included phenolic compounds, simple organic acids such as ascorbic acid, citric acid, fumaric acid and some amino acids and sugars. Pomegranate juice is a good source of different sugars like fructose, sucrose, and glucose while the PG juice and peel are rich in polyphenols [30]. As previously reported, PG is a polyphenol-rich fruit that contains tannins from its seeds to its peels [31] and flavonoids[32].Flavonoids were identified in different parts of PG such as peels [33], seeds, leaves, juice[34], flowers, pericarps, and barks. A previous study reported that PG contains 17 types of amino acids, minerals, vitamins C, calcium, iron, phosphorus, retinol, riboflavin, and ferulic acid[12]. Also other bioactive compounds have been identified in PG including alkaloids, phenolics, proanthocyanidins, sterols, terpenes, xanthonoids, fatty acids, organic acids, lignans, saccharides, and vitamin C[35]. The different pharmacological properties of PG such as anti-microbial , anti-viral, antioxidants, anti-atherosclerosis, anti-inflammatory, and anti-cancerous are attributed to the presence and richness of flavonoids and tannins [36-37- 20-21].

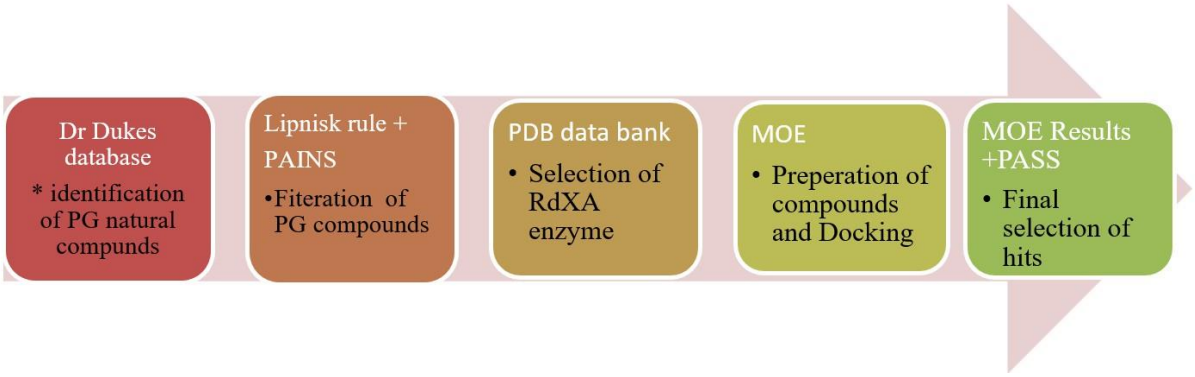

Figure 1. Overall steps followed for prediction of the activity of PG compounds

Table 1. Chemical composition of the PG whole fruit

1	(-)-Catechin	39	Magnesium
2	Anthocyanins	40	Malic-Acid
3	Ascorbic-Acid	41	Maltose
4	Ash	42	Malvidin
5	Beta-Sitosterol	43	Methionine
6	Boric-Acid	44	Myricetin
7	Calcium	45	Neo-Chlorogenic-Acid
8	Carbohydrates	46	Niacin
9	Carotene	47	O-Coumarinic-Acid
10	Catechin-(4,8)-Gallocatechin	48	Oxalic-Acid
11	Catechins	49	P-Coumarinic-Acid
12	Chlorine	50	Pantothenic-Acid
13	Chlorogenic-Acid	51	Pectin
14	Cinnamic-Acid	52	Phosphorus
15	Copper	53	Phytosterols
16	Cyanidin	54	Polyphenols
17	Cyanidin-3,5-Diglucoside	55	Potassium
18	Cyanidin-3-Glucoside	56	Procyanidin-B-1
19	Cyanidin-3-Rutinoside	57	Procyanidin-B-2
20	Delphinidin	58	Proline
21	Delphinidin-3-Glucoside	59	Protein
22	Ellagic-Acid	60	Protocatechuic-Acid
23	Fat	61	Quercetin
24	Ferulic-Acid	62	Quercetin-3,4'-Dimethylether-7-O-Alpha-rabinofuranosyl(1,6) Beta-D-Glucoside
25	Fiber	63	Quercetin-3-O-Rutinoside
26	Flavan-3-Ol	64	Quercimeritrin
27	Flavones	65	Quinic-Acid
28	Flavonoids	66	Riboflavin
29	Flavonols	67	Salicylates
30	Fructose	68	Sodium
31	Fumaric-Acid	69	Succinic-Acid
32	Gallocatechin	70	Sucrose
33	Glucose	71	Sulfur
34	Invert-Sugars	72	Thiamin
35	Iron	73	Ursolic-Acid
36	Kaempferol	74	Valine
37	L-Malic-Acid	75	Vit-B-6
38	Luteolin	76	WATER

Table 2. Lipinski's and PAINS results

No	Compound	Lipinski results	PAINS Alert
1.	Anthocyanins	Mwt 207.23 #H-bond acceptors 1 #H-bond donors 0 LOGp 2.64	0
2.	Ascorbic acid	Mwt 176.12 #H-bond acceptors 6 #H-bond donors 4 LOGp -1.42	0
3.	Cinnamic-Acid	Mwt 148.16 #H-bond acceptors 2 #H-bond donors 2 LOGp1.79	0
4.	Ferulic-Acid	Mwt 194.18 #H-bond acceptors 4 #H-bond donors 2 LOGp 1.36	0
5.	Flavan-3-OL	Mwt 226.27 #H-bond acceptors 2 #H-bond donors 1 LOGP 2.54	0
6.	Flavonols	Mwt 238.24 #H-bond acceptors 3 #H-bond donors 1 LOGp 2.84	0
7.	Fructose	Mwt 180.16 #H-bond acceptors 6 #H-bond donors 5 LOGp -2.02	0
8.	Fumaric-Acid	Mwt 116.07 #H-bond acceptors 4 #H-bond donors 2 LOGP -0.35	0
9.	Glucose	Mwt 180.16 #H-bond acceptors 6 #H-bond donors 5 LOGp -2.23	0
10.	L-malic-acid	Mwt 196.07 #H-bond acceptors 6 #H-bond donors 2 LOGP -5.84	0
11.	Malvidin	Mwt 331.3 #H-bond acceptors 7 #H-bond donors 4 LOGP 0.92	0
12.	Niacin	Mwt 123.11 #H-bond acceptors 3 #H-bond donors 1 LOGp 0.32	0
13.	Pantothenic-Acid	Mwt 219.23 #H-bond acceptors 7 #H-bond donors 5 LOGp-0.48	0
14.	Pectin	Mwt 194.14 #H-bond acceptors 7 #H-bond donors 5 Log p -2.41	0
15.	O-Coumarinic-Acid	Mwt 146.14	0

		#H-bond acceptors 2 #H-bond donors 0 Log P 1.82	
16.	P-Coumarinic-Acid	Mwt 146.16 #H-bond acceptors 3 #H-bond donors 2 LOGp 1.26	0
17.	Quinic-Acid	Mwt 192.17 #H-bond acceptors 6 #H-bond donors 5 LOGP- 1.69	0
18.	Riboflavin	Mwt 376.36 #H-bond acceptors 8 #H-bond donors 5 LOGp-0.19	0
19.	Salicalyates	Mwt 137.11 #H-bond acceptors 3 #H-bond donors 1 LOGp 1.33	0
20.	Succinic acid	Mwt 118.09 #H-bond acceptors 4 #H-bond donors 2 LOGP- 0.3	0
	Metronidazole	Mwt 171.15 #H-bond acceptors 4 #H-bond donors 1 LOGp- 0.23	0

Table 3. Docking results

No	Compound	Amino Acid	S score	Rsmd
1	Metronidazole	Gly 162 Ser46	-5.4964	1.174
2	Anthocyanins	Ser 134	-5.7449	1.1850
3	Cinaminc acid	Ser 18 - Ser 19- Arg 16- Arg 200 - Lys 198	-5.8654	0.9306
4	Feurlic acid	Ser 18- Ser 19- Arg 16- Arg20 – lys 198	-6.0773	1.2962
5	avanols	Cys 159 - Lys 20 - Lys 20-Ser 64	-6.007	1.4195
6	Fumaric-Acid	Ser 18- Ser 196 -Arg 16- Arg 200 Lys 198	-5.6784	0.782
7	L-malic-acid	Ser 45- Ser 18-Ser 196- Arg 16- Arg 200 - Lys 198	-6.078	1.5627
8	Malvidin	Ala 183	-6.4785	1.742
9	Niacin	Ser 18- Ser196- Arg 16- Arg 200 Lys198	-5.8238	1.0484
10	p-coumarinic acid	Ser 196- Arg 16- Arg 200 - Lys 198	-5.837	0.551
11	Pathathoenic acid	Arg 16 - Arg 200- Lys 198- Ser 18 Ser196	-6.7347	1.2307
12	Pectin	Ser45- Ser 18, Arg 16-Arg 200- Lys198	-5.9328	0.9959
13	Quinic-Acid	Ser 45- Ser 18- Ser 196- Arg 16 200 - Lys 198	-6.2604	1.0259
14	Riboflavin	Arg 16- Arg 200 - Lys 20- Asn 48	-7.7315	1.2625
15	Salicayltes	Ser 18- Ser- 196 - Arg 16- Arg 200 - Lys 198 .	-6.0389	0.8554

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6	Fumaric-Acid	Ser 18- Ser 196 -Arg 16- Arg 200 Lys 198	-5.6784	0.782
7	L-malic-acid	Ser 45- Ser 18-Ser 196- Arg 16- Arg 200 - Lys 198	-6.078	1.5627
8	Malvidin	Ala 183	-6.4785	1.742
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11	Pathathoenic acid	Arg 16 - Arg 200- Lys 198- Ser 18 Ser196	-6.7347	1.2307
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13	Quinic-Acid	Ser 45- Ser 18- Ser 196- Arg 16 200 - Lys 198	-6.2604	1.0259
14	Riboflavin	Arg 16- Arg 200 - Lys 20- Asn 48	-7.7315	1.2625
15	Salicayltes	Ser 18- Ser- 196 - Arg 16- Arg 200 - Lys 198 .	-6.0389	0.8554

Table 4: PASS Results

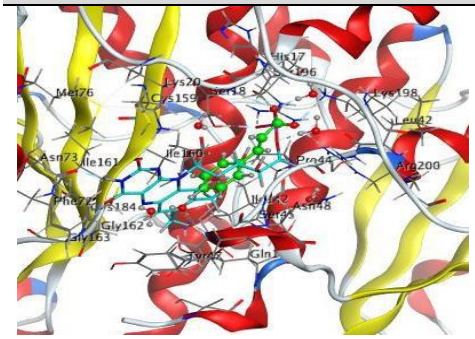
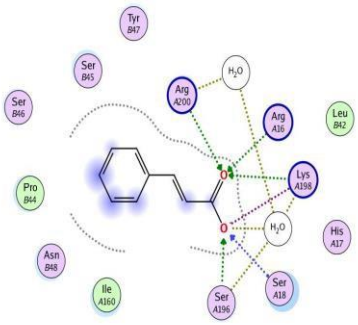
No	Compound	Pa < Pi	PASS activity
1.	Metronidazole	0.679< 0.003	**Anti-Helicobacter pylori**
		0.425 < 0.025	Antibacterial
2.	Cinaminc acid	0,658 < 0,003	Anti inflammatory , intestinal
		0.56< 0.022	Anti inflammatory
		0.358 < 0.014	**Anti-Helicobacter pylori**
3.	Flavan -3-OL	0.889 < 0.002	Antihemorrhagic
		0.556 < 0.042	Anti-inflammatory
		0.308 < 0.064	Anti-inflammatory , intestinal
		0.296 < 0.062	Antibacterial
		0.214 < 0.042	Gastritis treatment
		0.216 < 0.102	**Anti-Helicobacter pylori**
4.	Feurlic acid	0.661< 0,003	Anti inflammatory , intestinal
		0.604< 0.031	Anti inflammatory
		0.433< 0.008	**Anti-Helicobacter pylori**
		0.384< 0.004	Gastritis treatment
		0.333 < 0.048	Antibacterial
		0,168 < 0,036	Antihemorrhagic
5.	Flavanols	0.764 0.002	Antihemorrhagic
		0.603 0,031	Anti inflammatory
		0.365 0.034	Anti inflammatory , intestinal
		0,331 0.049	Antibacterial
		0.306 0.026	**Anti-Helicobacter pylori**
		0.280 0.013	Gastritis treatment
6.	Fumaric-Acid	0.602 < 0.031	Anti inflammatory
		0,545 < 0,005	Anti inflammatory , intestinal
		0.358 < 0.014	**Anti-Helicobacter pylori**
7.	Malvidin	0.499 < 0.057	Anti inflammatory
		0.499 < 0.057	Antibacterial
		0.255< 0.113	Anti inflammatory intestinal
8.	Niacin	0.459<0,070	Anti inflammatory intestinal
		0.291 < 0,004	Anti-inflammatory
		0,457<0,012	Antianemic
		0,309< 0,057	Antibacterial
		0.280< 0,039	**Anti-Helicobacter pylori**
9.	p-coumarinic-acid	0.684< 0.003	Anti inflammatory, intestinal
		0.641 < 0.024	Anti inflammatory

		0.581 < 0.013	Antiulcerative
		0.344< 0,005	Gastritis treatment
10.	Pathathoenic acid	0.291 < 0,063	Antibacterial
		0.247< 0,004	**Anti-Helicobacter pylori**
		0.270< 0.097	Anti inflammatory intestinal
11.	Pectin	0,730 < 0,012	Anti inflammatory
		0.569 < 0.011	Antibacterial
		0,424 < 0,003	Gastritis treatment
12.	Quinic-Acid	0.75 < 0.015	Anti inflammatory
		0.478 < 0.019	Antibacteria
13.	Salicayltes	0.812 < 0,002	Anti inflammatory intestinal
		0.713 <0,014	Anti inflammatory
		0.404 < 0.029	Antibacterial
		0.374 < 0.004	Gastritis treatment
		0.283 < 0.037	**Anti-Helicobacter pylori**

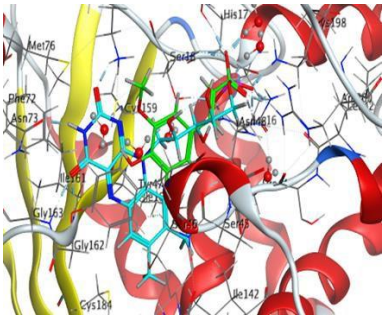
Table 5. ADME characteristics of the selected compounds

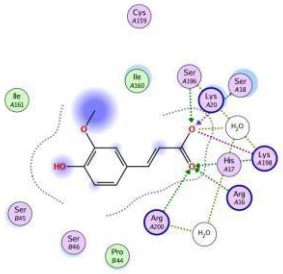
Compound (chemical classification)	Solubility	GI Absorption	Pgp substrate	BBB Permeation	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP34A inhibitor
Cinaminic acid Phenolic	Soluble	High	No	Yes	No	No	No	No	no
Ferulic acid Tannins	Soluble	High	No	Yes	No	No	No	No	no
Fumaric acid Organic acid	very soluble	High	No	No	No	No	No	No	no
Flavanols (flavonoids with a ketone group)	Moderate	High	No	Yes	Yes	Yes	No	Yes	yes

Table 6. Binding of selected compounds with the RDxA*

Binding of compound (ligand) and FMN to Protein	Ligand and Protein Interaction	Amino Acid	Interaction -distance –E (kcal\mol)
		Ser 18	H-acceptor 3.02 -3.6
		Ser198	H-acceptor 2.66 -3.8
		Arg 16	H-acceptor 2.86 -5.4
		Lys 198	H-acceptor 2.92 -17.8
		Arg 200	H-acceptor 2.89 -7.6
		Lys 198	ionic 3.70 -1.2
		Arg 16	ionic 2.86 -5.5
		Arg 16	ionic 3.62 -1.5
		Lys 198	ionic 2.92 -5.0
		Arg200	ionic 3.92 -0.7
		Arg200	ionic 2.89 -5.3

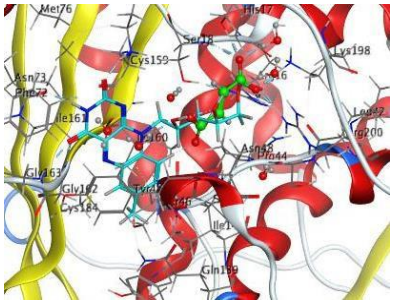
a) Cinamininc acid

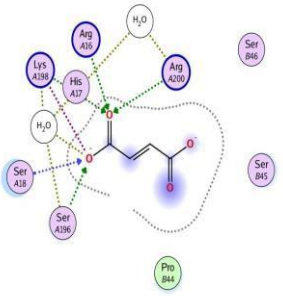




Ser 18	H-acceptor	3.16	-2.5
Ser 196	H-acceptor	2.78	-4.1
Arg 16	H-acceptor	2.88	-6.2
Lys 198	H-acceptor	3.32	-8.7
Arg 200	H-acceptor	2.83	-6.7
Lys 198	ionic	3.74	-1.1
Arg 16	ionic	2.88	-5.3
Arg 16	ionic	3.57	-1.6
Lys 198	ionic	3.32	-2.7
Arg200	ionic	3.92	-0.7
Arg 200	ionic	2.83	-5.7

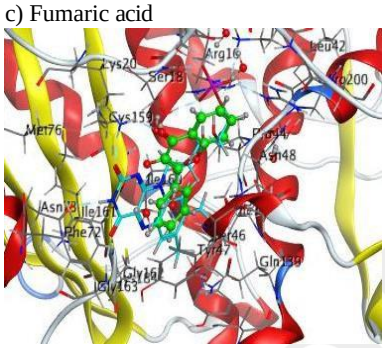
b) Ferulic acid

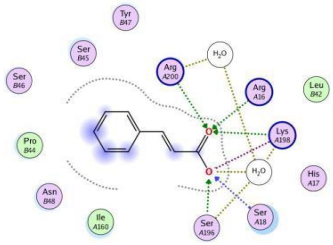




Ser18	H-acceptor	3.04	-3.2
Ser196	H-acceptor	2.68	-3.9
Arg16	H-acceptor	2.85	-5.8
Lys198	H-acceptor	3.07	-14.8
Arg200	H-acceptor	2.89	-7.1
Lys198	ionic	3.75	-1.1
Arg16	ionic	2.85	-5.5
Arg16	ionic	3.57	-1.7
Lys198	ionic	3.07	-4.0
Arg200	ionic	3.95	-0.6
Arg200	ionic	2.89	-5.2

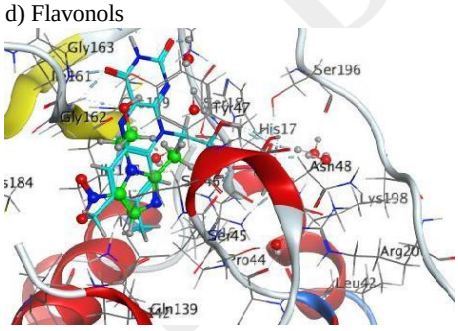
c) Fumaric acid

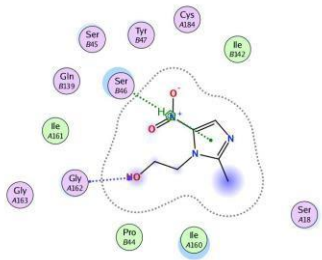




Ser18	H-acceptor	3.02	-3.6
Ser196	H-acceptor	2.66	-3.8
Arg16	H-acceptor	2.86	-5.4
Lys198	H-acceptor	2.92	-17.8
Arg200	H-acceptor	2.89	-7.6
Lys198	ionic	3.70	-1.2
Arg16	ionic	2.86	-5.5
Arg16	ionic	3.62	-1.5
Lys198	ionic	2.92	-5.0
Arg200	ionic	3.92	-0.7
Arg200	ionic	2.89	-5.3

d) Flavonols





Gly62	H-acceptor	2.96	-0.9
Ser 46	pi-H	3.81	-1.2

e) Metronidazole

**Binding of the selected compounds to receptor (shown in green colour), FMN in Turquoise, protein part is shown in red yellow and grey*

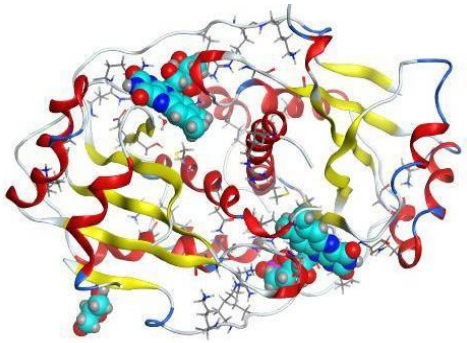


Figure 2: Structure of the Rdx A Enzyme. (Yellow and red colored chains are protein chains while the 2 FMN molecules are shown in turquoise.

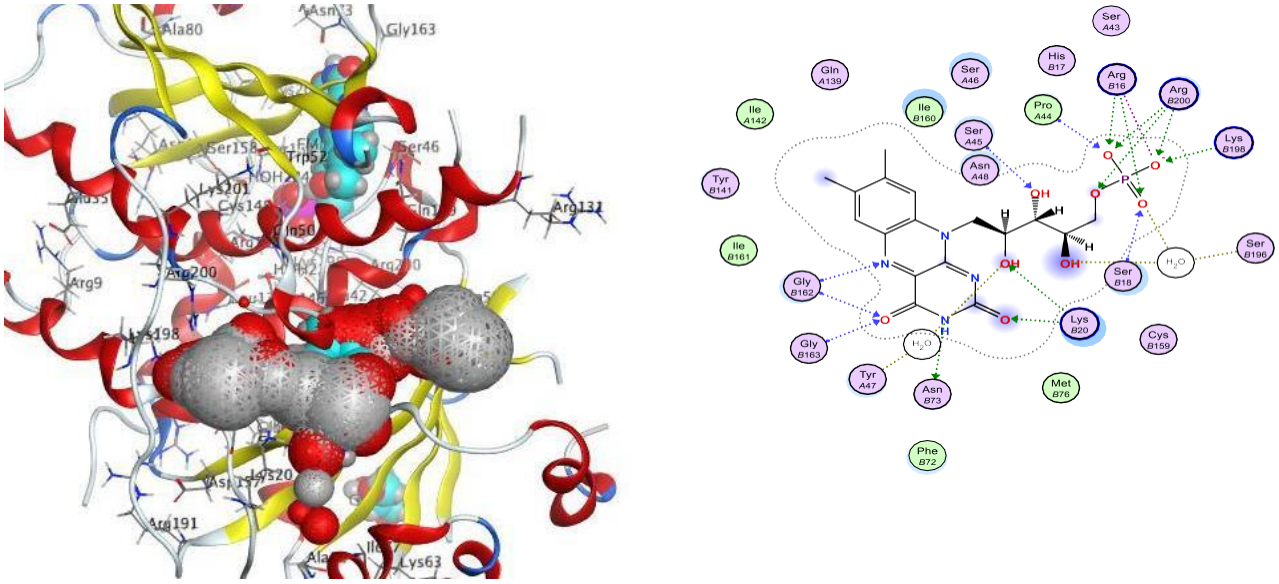


Figure 3. (a) Predicted active site(Dummy shape). (3b). Binding of FMN with the protein. One FMN is loacted within it coloured in Turquoise).

The 76 compounds retrieved were then filtered according to Lipinski's rule of 5 and PANS, only 20 compounds passed with zero violation (**Table 2**). Lipinski's rule generally shows the physiochemical properties of a molecule. The poor absorption or permeation of a molecule is more likely to occur if the molecular weight (MWT) is greater than 500 Da, there are more than 5 H-bond donors, 10 H-bond acceptors, and the calculated LOGp (octanol–water partition coefficient) is greater than 5.[28] Another criterion was the PAINS which shows if the molecule contains any problematic parts and thus may be excluded.

RdxA is an important bacterial enzyme that plays a role in reactions with drugs. It is dimer protein; each monomer consists of 210 amino acids folded in an a+ b motif. In addition to the protein chains, there are two Flavin adenine dinucleotide (FMN) molecules inserted in the interface of the dimer [38]. The FMN ribityl group forms hydrogen bonds with several amino acids such as Ser18 of the same monomer, Ser45 of the other monomer and with two water molecules. Also, its phosphoryl group is tightly bound to the structure through contact with the side chains of amino acids of the same monomer such as Arg16, Ser18 Lys198 and Arg200, plus hydrogen bonds with the N atom of Ser18 and a water molecule [38].

These FMN also bound to the active site predicted by MOE Docking studies are usually carried out to show how ligands and receptors interact, During the in silico studies an important determinant for the selection of hits is the s- score (ie binding energy) [26-22] and the rmsd which shows the distance of bond formation between interested molecules and the receptor. In the current study, 14 compounds had a sore lower than metronidazole. Usually, inhibitors with low *s*-score ie. with high binding energy tend to establish strong

interaction with protein's active sites[26] and thus can be considered good hits during drug discovery.

To predict the biological activity of the selected various compounds against H pylori , PASS software predicted four compounds had a high Pa score namely ferulic acid, fumaric acid, cinamininc acid and flavanols . Usually a higher Pa value compared to the Pi, suggests the likelihood of the compound to have an inhibitory effect towards enzymes [39] and can thus be subjected for further experimental evaluation.

Ferulic acid belongs to the tannin group and some of the antimicrobial and anitviral properties of PG are due to the impacts of tannins bioactive compounds[35]. Fumaric acid and essential oils including fatty acids and organic acids have been documented to possess various pharmacological effects including anti-microbial, anti-oxidant, insecticide, anti-helminthic, and ant nociceptive activity [40].

Flavonoids are found in various plant parts where they serve important functions in growth and defense and are also responsible for the attractive color, odor, and flavor of plants to pollinators [41]. Flavonoid can be classified into several groups, such as flavones, flavonols, catechins, and chalcones, with various subgroups [42]. Flavonoids are known for their antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, antifungal, antiviral and antibacterial properties and have been identified as potent inhibitors of many enzymes, making them an important area in pharmaceutical industry [41] .

Phenolic substances or polyphenols include a variety of compounds, simple flavonoids phenolic acid and, colored anthocyanins[43]. As reported, the potent antibacterial activity of the pomegranate extracts might be due to the potential synergistic action of ellagitannins, anthocyanins and flavonols [44].The mechanisms of action of simple phenols are probably proceed through interaction with sulfhydryl groups in microbial enzymes, leading to inhibition of those enzymes or due to nonspecific proteins interaction [45].

The ADME properties of molecules are important for selection of good hits [23-27]. These pharmacokinetics properties are important for determination of various drug properties such as the movement of a drug in the body and its ability to reach its site of action [46-47]. The first criterion is the absorption phenomena which is usually affected by solubility and lipophilicity of a molecule. Water soluble compounds facilitate drug development due to ease of handling and formulation and they highly influence oral absorption. In addition, a high gastrointestinal absorption (GI) means the administered drugs can easily be absorbed in the intestines [48]. All 4 selected compounds were found to be water soluble and have a high GI absorption rate (**Table 5**).

The second property is distribution which shows how a drug passes throughout different body parts and is identified mainly by Blood Brain Barrier (BBB) Permeability and Glycoprotein (P-gp) Substrate. Regarding permeability, three of the four selected molecules had the ability to cross the brain blood barrier (Table 5) and the drugs which can cross the brain-barrier can reach the brain and bind to specific receptors to elicit specific reactions [49]. BBB protects the central nervous system (CNS) by separating the brain tissues from the bloodstream. It is responsible for preventing larger molecules (100%) and small molecules (98%) from entering into the CNS and it allows transport of only water- and lipid-soluble molecules and the selective transport of molecules [50].

P-gp are proteins that act as drug extracting pump and molecules or drugs that are inhibitors of P-gp will possibly have an increased bioavailability at the site of activity [51]. All compounds were inhibitors of P-gp (**Table 5**).

The third criteria is metabolism, which is a series of reactions involving many enzymes that can modify drugs. The main enzymes involved are the Cytochrome P450 system and its isoform CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP34A. About 50 – 90% of therapeutic molecules are substrates for them [52-53].They also play an important role in the detoxification of drugs and cellular metabolism and approximately , 50% of the overall elimination of common clinical drugs may be attributed to the CYPs [54].The activity of the four molecules towards the different Cytochromes are shown in Table 5 , with the exception of flavanols the others were non inhibitors for the Cytochrmoe isozymes. It has been suggested that both Cytochromes and P-gp can process small molecules synergistically and thus improve protection of tissues and organisms [55]. Inhibition of these isoenzymes is one major cause of pharmacokinetics-related drug-drug interactions [56 -57] leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites [58].

It is necessary that potential novel inhibitors should bind at the active site of the target protein [27] so as a chemical reaction will take place. The four selected molecules were found to bind to different amino acids in the predicted active site with different types of bonds/interaction (**Table 6**). Hydrophobic and hydrogen-bonding interactions are the most frequent type of interactions[59]. The hydrogen-bonding interactions are mainly governed by, by two requirements ie: the distance and the geometry. The distance shows ow the two closest atoms in the ligand and the receptor bind and should be within the optimal range of 2.8-3.2 Å (or at least within the *acceptable* range of 2.5-3.5 Å) for a hydrogen-bond interaction to be effective.[59]

CONCLUSION

The current study revealed that cinaminic acid, fumaric acid, ferulic acid and flavanols compounds of the PG can be considered promising molecules for the treatment of *H pylori* infection. These four compounds gave good results using the different criteria in the in silico study and may be selected further invivo investigation.

Funding Acknowledgment

Non.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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