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Molecular modeling of *Ruellia tuberosa L* compounds as α-amylase inhibitor: an *in silico* comparation between human and rat enzyme model

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Abstract:
Inhibition of α-amylase is an important strategy to control post-prandial hyperglycemia. The present study on *Ruellia tuberosa,* known as traditional anti-diabetic agent, is being provided in silico study to identify compounds inhibiting α-amylase in rat and human. Compounds were explored from PubChem database. Molecular docking was studied using the autodock4. The interactions were further visualized and analyzed using the Accelrys Discovery Studio version 3.5. Binding energy of compounds to α-amylase was varying between -1.92 to -6.66 kcal/mol in rat pancreatic alpha amylase and -3.06 to -8.42kcal/mol in human pancreatic alpha amylase, and inhibition konstanta (ki) was varying between 13.12- 39460μM in rat and 0.67-5600μM in human. The docking results verify that betulin is the most potential inhibitor of all towards rat model alpha amylase and human alpha amylase. Further analysis reveals that betulin could be a potential inhibitor with non-competitive pattern like betulinic acid. In comparison, betulin has smaller Ki (0.67μM) than acarbose (2.6 μM), which suggesting that betulin is more potential as inhibitor than acarbose, but this assumption must be verified in vitro.

Keywords: alpha amylase inhibitor, betulin, docking, *Ruellia tuberosa L.*

Background:
Diabetes mellitus is a metabolic diseases characterized by hyperglycemia. *Ruellia tuberosa* L., is widely disseminated in South East Asian including in Indonesia, and is used as an anti-diabetic agent traditionally.

R. tuberosa possesses significant blood glucose lowering effect in aloxan induced diabetic rat and rabbit **[1, 2].** The active compound from *R. Tuberosa* that has hypoglycaemic effect has not been studied yet. It was reported five flavanoids: cirsimaritin, cirsimarin, cirsiliol 4-glucoside, sorbifolin, and pedalitin along with betulin, vanillic acid, and indole-3-carboxaldehyde were isolated from the ethyl acetate fraction of methanolic extracts of *R. tuberosa* **[3].** Apigenin, luteolin, 3,5-diglucoside, apigenin-7-O-glucoronide, apigenin glucoside, apigenin rutinoside, luteolin glucoside, flavone glycoside were also reported in *R. tuberosa* **[4, 5].**

Inhibitor of carbohydrate digesting enzyme as alpha amylase is now actively searched for the medicine against diabetes, since it could control postprandial increase of blood glucose **[6].** Alpha-amylase, multidomain protein, has a catalitic $(\beta/\alpha)_8$-barrel with catalitic triads of Asp197, Glu233, and Asp300. The interactions between ligand and catalytic domain can inhibit the enzyme activities.

This research points out the modeling on an interaction between Alpha-amylase and compounds of *R. tuberosa* that has

an anti-diabetic activity. The molecular modeling will show an energy binding afinity (Ea), and an inhibition constant (Ki) of the compound.

Alpha amylase inhibitor becomes a part of drug used for diabetes. Although the final target of inhibitor is human pancreatic alpha amylase, it is still common use in vitro or in vivo studies on rat. It would be interesting to see the interaction between inhibitor of rat pancreatic alpha amylase (RPA) and human pancreatic alpha amylase (HPA) using a molecular docking.

Methodology:

Sequences alignments

Sequences of pancreatic alpha amyle *Rattus nover*gicus (Gen Bank ID AAA40725.2/GI: 11528629) and human pancreatic alpha amylase structure (PDB ID: 3OLD) **[7]** were downloaded from NCBI (http: // www.ncbi.nlm.nih.gov/ structure). blastp from NCBI tools online were used to perform alignments alpha amylase from human and rattus (http://blast.ncbi. nlm.nih. .). This tool reports residu that are identical (percentage of identity), and conserve (percentage of similarity/positive).

Model generation

The Swiss model program was used in order to make a RPA model. SWISS-MODEL workspace (http://swissmodel.expasy. org/) is a web-based integrated service dedicated to protein structure homology modeling **[8].** To make a three dimensional protein model, the program uses the protein sequence (model), and a three dimensional structure (template) that has a high enough similarity to the sequence. In this case, porcine pancreatic alpha amylase (PDB ID: 1BVN) with 1.97 Å was used as a template.

Ligand preparation

Three dimensional ligand structure was downloaded from PubChem Compound (http://pubchem.ncbi.nlm.nih.gov). The ID of Betulin [CID 72326], Vanilic acid [CID 8468], Indole-3-carboxaldehyde [CID 10256], Cirsimarin [CID 188323], Sorbifolin [CID 3084390], Pedalitin [CID 31161], Apigenin [CID 5280443], Luteolin [CID 5280445], Flavone [CID 10680], and Cirsiliol 4'-glucoside were created with the HyperChem version 7. Their energy forms were minimized, geometrical structure were optimized semi empirically AM1 with conjugate direction algoritm using the HyperChem and were converted to PDB format by the Open Babel 2.3.1. All ligands were prepared to pdbqt format using the AutoDock Tools 1.5.6.

Docking ligand-receptor

All receptors (alpha amylase model and 3OLD.pdb) were prepared with the AudoDock Tools 1.5.6 for docking. Docking (rigid docking with genetic algorithm parameter) was performed with the autodock version 4.2.5.1 **[9].** Additional molecules to alpha amylase, except cofactor (Ca^{2+}, Cl^{-}) and solvent were deleted prior to the docking using the Accelrys Discovery Studio version 3.5. The bonds in the ligands were set to be rotatable to maximize the flexibility of the ligand. The Autodock Tools is the graphical interface to assign gasteiger charge to reseptor and ligand molecule. The docking box was positioned at x = 8.458, y = -5.795, z = 15.737 with a size of 62x76x66 for 3OLD.pdbqt and x = 37.309, y = 31.28, z = 44.36 with a size of 60x72x74 for RPA model. To validate the docking method that was used, we calculate RMSD between actual pose of the co-crystallized ligand and the redocking co-crystallized ligand (pseudo-pentasaccharide of trestatin family) into their respective binding sites in HPA (ib2y.pdb).

Further interaction analysis was done using the autodock tools and was visualized using the Accelrys Discovery Studio version 3.5. The predicted binding energy (kcal/mol), which indicates how strongly a ligand binds to the receptor, was calculated based on the scoring function used in the AutoDock. A more negative binding affinity indicates stronger binding.

Discussion:

Supplementary Figure 1 shows the result of multiple alignment between RPA and HPA sequences. It reveals that rat and human have a high identity (84%) and a similarity (92%). It means that the homology between the two species is very high. However, the rat sequences are shorter than the human sequences. There is a gap in the rat sequence at the position of amino acids 142-144 in HPA.

Since there is no crystal structure of rat enzymes, computer generated model was used in this study. Quality assesment of generated model indicated to be reliable. Identity more than 30% between template and target is sufficient to obtain a reliable model **[10].** Rat pancreatic alpha amylase model has a high sequence identity 84,677%. Futhermore the good model show Z-score Q MEAN -0.723 and QMEAN 0.715 with a residual error < 1 Å. The resulting QMEAN z-score provides an estimate of the 'degree of nativeness' of the structural features observed in a model and indicates whether the model is of comparable quality to experimental structures **[11].** QMEAN is a scoring fuction consisting of a linear combination of structural descriptor: two distance-dependent interaction potentials of mean force based on C-β atoms and on all atom types are used to assess long-range interactions both are secondary structure dependent; a torsion angle potential; finally, the agreement of predicted and calculated secondary structure and solvent accessibility is included in the form of two agreement terms **[12].** QMEAN and agreement terms range from 0 to 1 with higher values for more reliable candidates. Ramachandran plot of RPA model indicates that 96.5% of its residues are situated in the favoured and 3.15% in allowed region. According to this quality assessment results, we believe that this model could be considered to have enough accuracy and biological posibility for further ligand binding studies.

An RMSD of 0.0011 Å was obtained between the best pose obtained by redocking and the actual binding mode of ligand to ib2y.pdb. Futhermore, an RMSD of 0.0185 Å was obtained by redocking betulin to ib2y.pdb and the actual binding mode of ligand to ib2y.pdb. This is satisfactory with regard of less than 2 Å threshold was usually used to assess successful docking [13]. Binding energy and Ki of ligand to HPA and RPA model was shown in table 1 of the suplementary material. Binding energy is vary between -1.92 to -6.66 kcal/mol in RPA and -3.06 to -8.42kcal/mol in HPA. In general betulin is calculated to be the strongest binding to alpha amylase both in RPA ($E_{binding}$ -6.66 kcal/mol, Ki 13.12 µM) and HPA ($E_{binding}$ -8.42 kcal/mol, Ki 0.67µM). These docking results verify that betulin is more efficient ligand and more affinity of all towards alpha amylase

in RPA model and HPA. Interestingly, betulin binds stronger in HPA than RPA.

Betulin-alpha amylase complex was shown bellow **(Figure 1. A_1 and B_1).** Further analysis shows that betulin has vanderwaals interaction with ASN 115, ASN 152, ARG 170, ASP 179, HIS 213 (Figure 1 B_2) and hydrogen bond 2.22Å and 2.32 Å with ASP368 in RPA. This interaction is different between betulin and HPA (vanderwaals interaction with ASN 100, ASN137, ARG 158, ASP167, ASP 197, HIS201, and hydrogen bond 2.44Å with ASP 300) (Figure 1 A_2). Betulin has an interaction with the catalytic site both in HPA and RPA. It means that betulin could be a potential inhibitor of alpha amylase. The ligand position diferences in alpha amylase are due to gab presence between RPA and HPA sequence.

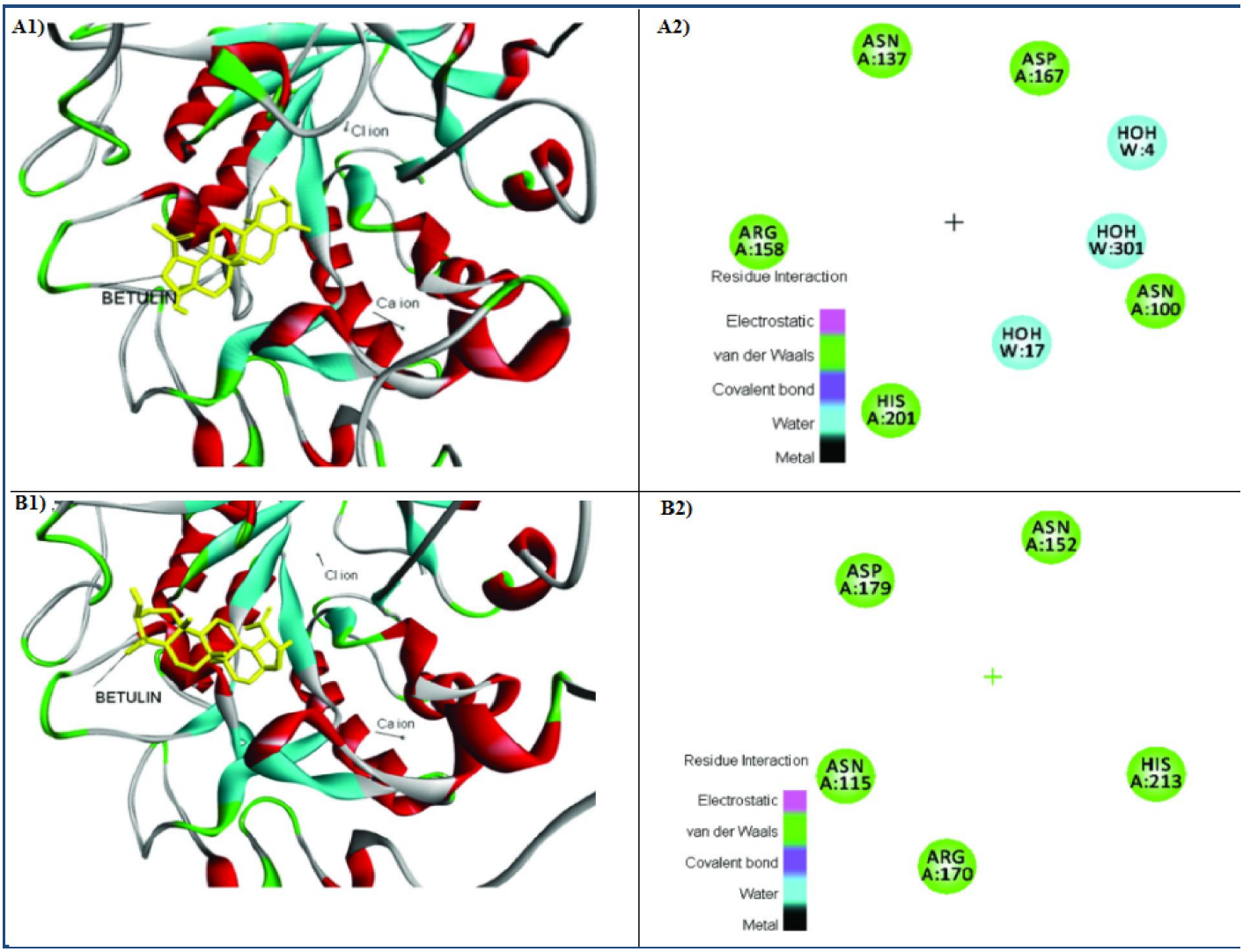

Figure 1: betulin-human pancreatic alpha amylase complex **A1)** betulin-rat pancreatic alpha amylase complex; **B1)** Two dimensional diagram shows van der waals interaction between betulin (plus sign) and ASN100 (1.02Å), ASN137 (1.02Å), ARG158 (1.02Å), ASP167 (1.02Å), and HIS201 (1.02Å), and water interaction with H2O (W:4), (W:17), and (W:301) in human pancreatic alpha amylase **(A2).** Two dimensional diagram shows van der waals interaction between betulin (plus sign) and ASN115 (1.02Å), ASN152 (1.02Å), ARG170 (1.02Å), ASP179 (1.02Å), and HIS213 (1.02Å) in rat pancreatic alpha amylase **(B2).**

In order to get an approximation of the possible effectiveness of betulin as potential inhibitor to alpha amylase, docking score was obtained for the betulinic acid. Betulin is a derivate of betulinic acid. From the experimental study, betulinic acid, compound of aqueous extract *S cumini's* show 98% inhibitory activity to porcine pancreatic alpha amylase with non-competitive manner **[14].** Molecular docking of Betulin has a smaller $E_{binding}$ and Ki value (($E_{binding}$ -6.66 kcal/mol, Ki 13.12 µM to RPA and ($E_{binding}$ -8.42 kcal/mol, Ki 0.67µM to HPA) than betulinic acid ($E_{binding}$ -6.44 kcal/mol, Ki 18.97 µM to RPA and $E_{binding}$ -7.08 kcal/mol, Ki 6.48 µM to HPA). Futhermore, betulinic acid and betulin shows the same interaction to amino acid residue of alpha amylase **Table 1 (see supplementary material).** It suggests that betulin could be potential inhibitor with non-competitive pattern like betulinic acid. In comparison, acarbose had Ki around 2.6 µM **[15]**, which suggesting that betulin could be potentially better than acarbose, but this assumption still remains to be verified.

Conclusion:

Overall, betulin is the most potential α-amylase inhibitor compound in *Ruellia tuberosa*. It suggests the inhibition of

pancreatic alpha amylase both in rat and human. The shortening of α-amylase residue in rat enzyme should be highlighted, as it may produce effect in the case of ki, $E_{binding}$, and the interaction between ligand and enzyme. The approximity based on the ki suggesting that betulin is more potential as a inhibitor rather than acarbose with non-competitive pattern inhibition, but this assumption must be verified *in vitro*.

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Supplementary material:

Figure 1: Results of the online blastP alignment for the pancreatic alpha-amylase sequences of human and rat *(Rattus norvegicus*) enzymes. Conserved regions are shown in yellow, gap are shown in pink.

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      Score          Expect       Method        Identities    Positives      Gaps

891 bits(2303)      0.0      Compositional   419/496(84%)  457/496(92%)  3/496(0.6%)
                             matrix adjust.

Human   1     QYSPNTQQGRTSIVHLFEWRWVDIALECERYLAPKGFGGVQVSPPNENVAIYNPFRPWWE   60
              QY P+T  GRT+IVHLFEWRW DIA ECERYLAPKGFGGVQVSPPNEN+ I NP RPWWE
Rat     16    QYDPHTADGRTAIVHLFEWRWADIAKECERYLAPKGFGGVQVSPPNENIIINNPSRPWWE   75

Human   61    RYQPVSYKLCTRSGNEDEFRNMVTRCNNVGVRIYVDAVINHMCGNAVSAGTSSTCGSYFN   120
              RYQP+SYK+C+RSGNE+EF++MVTRCNNVGVRIYVDAVINHMCG+  SAGT STCGSYFN
Rat     76    RYQPISYKICSRSGNENEFKDMVTRCNNVGVRIYVDAVINHMCGSGNSAGTHSTCGSYFN   135

Human   121   PGSRDFPAVPYSGWDFNDGKCKTGSGDIENYNDATQVRDCRLTGLLDLALEKDYVRSKIA   180
              +R+F AVPYS W FND KC   +G+I NYNDA QVR+CRL+GLLDLAL+KDYVR+K+A
Rat     136   PNNREFSAVPYSAWYFNDNKC---NGEINNYNDANQVRNCRLSGLLDLALDKDYVRTKVA   192

Human   181   EYMNHLIDIGVAGFRLDASKHMWPGDIKAILDKLHNLNSNWFPAGSKPFIYQEVIDLGGE   240
              +YMN+LIDIGVAGFRLDA+KHMWPGDIKA+LDKLHNLN+ WF  GS+PFI+QEVIDLGGE
Rat     193   DYMNNLIDIGVAGFRLDAAKHMWPGDIKAVLDKLHNLNTKWFSQGSRPFIFQEVIDLGGE   252

Human   241   PIKSSDYFGNGRVTEFKYGAKLGTVIRKWNGEKMSYLKNWGEGWGFMPSDRALVFVDNHD   300
               IK S+YFGNGRVTEFKYGAKLGTVIRKWNGEKMSYLKNWGEGWGF+P+DRALVFVDNHD
Rat     253   AIKGSEYFGNGRVTEFKYGAKLGTVIRKWNGEKMSYLKNWGEGWGFVPTDRALVFVDNHD   312

Human   301   NQRGHGAGGASILTFWDARLYKMAVGFMLAHPYGFTRVMSSYRWPRQFQNGNDVNDWVGP   360
              NQRGHGAGGASILTFWDAR+YKMAVGFMLAHPYGFTRVMSSYR  R FQNG DVNDW+GP
Rat     313   NQRGHGAGGASILTFWDARMYKMAVGFMLAHPYGFTRVMSSYRRTRNFQNGKDVNDWIGP   372

Human   361   PNNNGVIKEVTINPDTTCGNDWVCEHRWRQIRNMVIFRNVVDGQPFTNWYDNGSNQVAFG   420
              PNNNGV KEVTINPDTTCGNDWVCEHRWRQIRNMV FRNVV+GQPF NW+DNGSNQVAF
Rat     373   PNNNGVTKEVTINPDTTCGNDWVCEHRWRQIRNMVAFRNVVNGQPFANWWDNGSNQVAFS   432

Human   421   RGNRGFIVFNNDDWSFSLTLQTGLPAGTYCDVISGDKINGNCTGIKIYVSDDGKAHFSIS   480
              RGNRGFIVFNNDDW+ S TLQTGLPAGTYCDVISGDK+NGNCTG+K+ V  DGKAHFSIS
Rat     433   RGNRGFIVFNNDDWALSSTLQTGLPAGTYCDVISGDKVNGNCTGLKVNVGSDGKAHFSIS   492

Human   481   NSAEDPFIAIHAESKL                                              496
              NSAEDPFIAIHA+SKL
Rat     493   NSAEDPFIAIHADSKL                                              508
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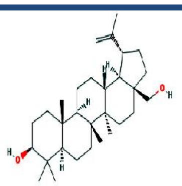
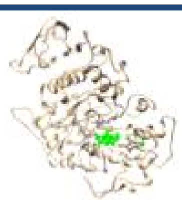
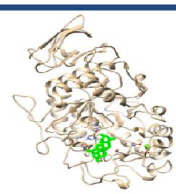
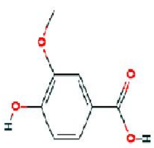
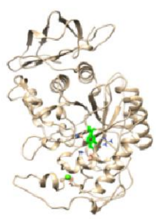
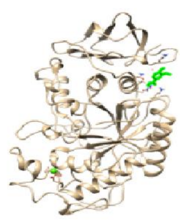
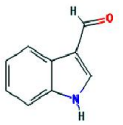
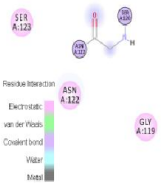
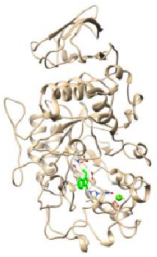
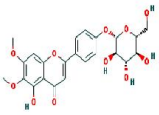
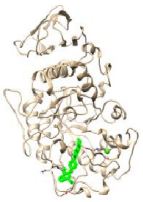
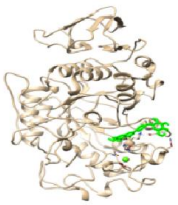
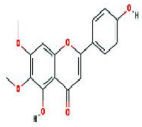
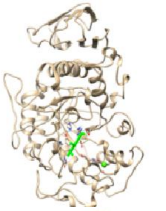
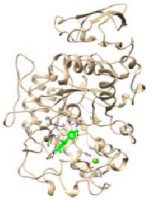
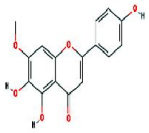
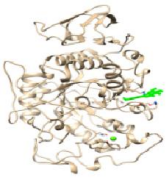
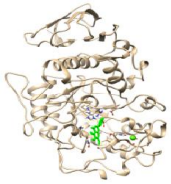
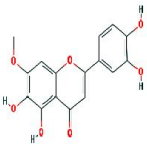
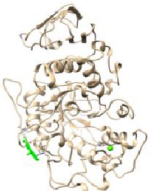
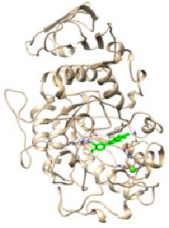
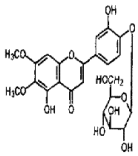
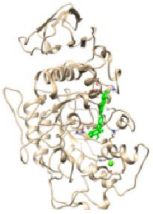
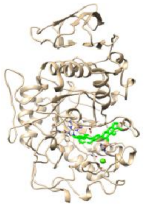
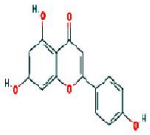
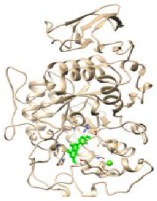
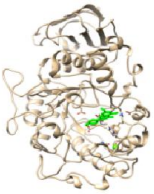
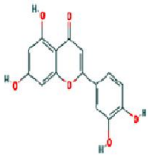
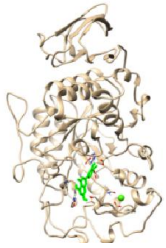
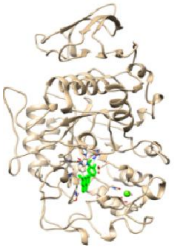
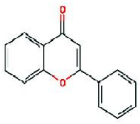
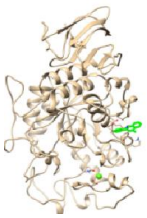
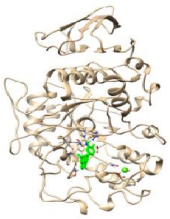
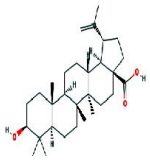
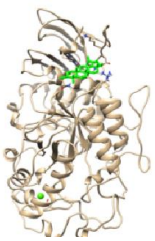
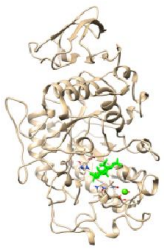
Table 1: Datas of binding energy, inhibition constant, structure of ligand-RPA and HPA complex (ligand was shown in green), and type of residue interaction

Phytochemical name & molecular formula & pubchem compound id	Molecular structure	Binding energi (kcal/mol)		Inhibition constanta (ki)		Ligand-RPA complex		Ligand-HPA complex	
		Rat	Human	Rat	Human	Visualization	Type of residue interaction	Visualization	Type of residue interaction
Betulin $C_{30}H_{50}O_2$ CID 72326		-6.66	-8.42	13.12 µM	0.67 µM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213 hydrogen bond: ASP368		Van der waals: ASN100 ASN137 ARG158 ASP167 ASP 197 HIS201 Hydrogen bond: ASP 300
Vanilic acid C8H8O4 CID 8468		-3.17	-3.66	4750 µM	2.07 mM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213 MET214 hydrogen bond: GLN16 ARG239		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 hydrogen bond: LYS35

Indole-3-carboxaldehyde C_9H_7NO CID 10256		-4.21	-4.06	816 µM	1.06 mM		Electrotsatic: GLY119 SER120 ASN122 SER123 GLY176 Covalent bond: SER120 ASN122 hydrogen bond: ASP248		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201
Cirsimarin $C_{23}H_{24}O_{11}$ CID 159460		-3.42	-4.81	3120 µM	296.73 µM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213 hydrogen bond: GLN78		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 hydrogen bond: GLY239,GLY239 HIS201
Cirsimaritin $C_{17}H_{14}O_6$ CID 188323		-4.95	-5.62	235.61µM	76.4µM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213 Hydrogen bond: GLN78 GLU245		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 Hydrogen bond: GLN63 ASP197
Sorbifolin $C_{16}H_{12}O_6$		-4.67	-4.77	377.12µM	318.41µM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 Hydrogen bond: ASP197
Pedalitin $C_{16}H_{12}O_7$ CID 31161		-3.95	-4.41	1270 µM	580.49µM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213 Hydrogen bond: ARG35 ARG71 GLU93 THR129		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 Hydrogen bond: LYS200 ASP300
Cirsiliol 4'-glucoside $C_{23}H_{24}O_{12}$		-1.92	-3.06	39460 µM	5600 µM		Van der waals: ASN115 ASN152 ARG170 ASP179 HIS213 Hydrogen bond: HIS317 GLY320		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 Hydrogen bond: ASP197
Apigenin $C_{15}H_{10}O_5$ CID 5280443		-5.1	-5.55	181.54 µM	85.14 µM		Van der waals: ASN 115 ASN 152 ARG170 ASP 179 hydrogen bond 213 Hydrogen bond: SER 175		Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 Hydrogen bond: LYS 200
Luteolin $C_{15}H_{10}O_6$ CID 5280445		-4.79	-5.75	308.99 µM	61.09 µM		Van der waals: ASN 115 ASN 152 ARG170 ASP 179 HIS 213 bond: SER 175		Van der waals: TYR 342 ILE 367 ASN 380 ASP381 TRP 382 Covalent bond: PRO 360 ASN 362 Electristatic: GLY 359 PRO 360 ASN 362 LYS 368

Flavone $C_{15}H_{10}O_2$ CID 10680		-6.0	-5.72	39.89 µM	64.46 µM		Van der waals: ASN 115 ASN 152 ARG170 ASP 179 HIS 213 Hydrogen bond: LYS 255		Hydrogen bond: ASP197 HIS 299 Van der waals: ASN100 ASN137 ARG158 ASP167 HIS201 Hydrogen bond: GLN 63
Betulinic acid $C_{30}H_{48}O_3$ CID 64971		-6.44	-7.08	18.97 µM	6.48 µM		Van der waals: ASN 115 ASN 152 ARG170 ASP 179 HIS 213		Van der waals: ASN100 ASN137 ARG158 ASP167 ASP 197 HIS201 ASP 300