ORIGINAL ARTICLE

Discovery Phytomedicine 2019, Volume 6, Number 3: 138-142

Experimental analysis of isolated compounds of *Borreria hispida* (L) in the context of antioxidant.

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ABSTRACT

Borreria hispida comprises an effective potential source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. This study aimed to gain information by molecular docking of biologically active compounds of *Borreria hispida* with Glutathione reductase (GR), Urate oxidase(UO), Protein-tyrosine kinase 2-β (PTK-2β) and Peroxiredoxin-5(PRDX5) proteins target that are responsible for antioxidant activity and also correlate the relation by previous literature in vitro antioxidant analysis. Molecular docking analysis of the compounds was done by Schrodinger. Furthermore ADME properties of the isolated compounds were evaluated with QikProp. A mixed range of docking score was found during molecular docking by Schrodinger where the in vitro study showed moderate antioxidant activity. They also satisfy the Lipinski rule to sow the drug-like properties. Due to its superior docking score, it could be an effective GR, UO, PTK-2β and PRDX5 inhibitors. Furthermore studies are required to detect GR, UO, PTK-2β and PRDX5 inhibitory activity of isolated compounds from *Borreria hispida*.

Keywords: *Borreria hispida*, Antioxidant activity, Molecular docking, ADME.

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Cite This Article: Tareq, A.M., Farhad, S., Chakraborty, S., 2019. Experimental analysis of isolated compounds of Borreria hispida (L) in the context of antioxidant. *Discovery Phytomedicine* 6(3): 138-142. DOI:10.15562/phytomedicine.2019.107

INTRODUCTION

Plants have always been the primary source of medicine in Bangladesh and currently becoming quite popular around the world, as people fight to remain healthy in the concept of pollution and chronic diseases. There is a comprehensive belief that green medicines are better, healthier and safer than synthetic ones. Though the recovery may be a bit on the slower side, the therapeutic use of medicinal plant is on the rise due to its reduced ability to cause adverse effects.[1-3] Antioxidants are molecules which resist oxidation reactions. Oxidation reactions can produce free radicals, which leads to chain reactions harming the cells of organisms.[4] The primary characteristic of an antioxidant is its potential to counter free radicals. These free radicals can oxidase proteins, nucleic acids, lipids, DNA and can bring in degenerative diseases. Human body possesses different enzyme systems to counter free radicals, such as vitamin B, vitamin C, and beta-carotene. As human body is not able to produce these vitamins, so they are required to be supplied from outside. Researchers are now working on natural antioxidants and pure natural compounds of plant source which are known to have antioxidant like properties.

Borreria hispida belongs to Rubiaceae family which is used as antieczemic, antibacterial and also in some cardiovascular diseases. It is consumed as vegetables too. *B.hispida* also showed antioxidant, analgesic and anti-inflammatory activity.[5-7] The aim of the present study is to find the potential inhibitor for antioxidant activities of isolated compounds of *Borreria hispida* and also correlated the previous study of *in vitro* antioxidant activity by Shajiselvin, C. 2010.

METHODS

PASS Prediction

The isolated compounds of *Borreria hispida* was allowed to predict the antioxidant activity by using PASS online server.[8] PASS online server predict the activity as probable of activity (P_a) and probable of inactivity (P_i).

Preparation of Ligand

Borreria hispida has several isolated compounds[9] while we selected 4 for molecular docking, namely, Ursolic acid, Beta sitosterol, Isorhamnetin and 1-amino-3-ethoxypropan-2-ol. All the structures were downloaded in 2D SDF format from PubChem database. By using force filed OPLS3 in LigPrep, the energy minimization was done to convert 3D structure from 2D structures.

Preparation of Protein

As antioxidant protein, proline-rich tyrosine kinase 2 (PDB ID: 3FZS),[10] glutathione reductase (PDB ID: 3GRS) (11) human peroxiredoxin 5(PDB ID: 1HD2)[12] and urate oxidase (PDB ID: 1R4U)[13] was saved in PDB format from Protein

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data bank.[14] Protein preparation Wizard of Maestro version 11.1 was used for refining the structure while the removal of water and optimization of H-bond was done. Minimization of heavy atom molecule at RMSD (0.30Å) by using force filed OPLS3.

Grid Generation and Molecular Docking

Glide generation of Maestro version 11.1 was used to generate the receptor grid for interaction between protein-ligand while OPLS3 were used as force field and all other parameters in defaults. A specific bounding box was set for every protein to evaluate the docking experiments. Standard Precision was followed for docking experiments while flexible ligand sampling was used. The best score for each interaction with protein-ligand was noted as docking score.[15]

ADME analysis

Lipinski's rule for oral drug-properties was used. QikProp of Maestro V11.1 was used for prediction of ADME properties while it is a quick, accurate, easy-to-use (16, 17).Rule of five are: Molecular weight (acceptable range: 500), Hydrogen bond donor (acceptable range: ≤5), Hydrogen bond acceptor (acceptable range: ≤10), High lipophilicity (expressed as LogP, acceptable range: <5), Rotatable Bond ≤ 10

RESULTS

Pass prediction

PASS prediction of isolated compounds of *Borreria hispida* shown in Table 1. Among the 4 compounds, Isorhamnetin show the highest probability of activity (P_a=0.814) while ursolic acid P_a = 0.408.

Molecular docking analysis

The molecular docking result was summarized in Table 2 and 2D interaction figure shown in Figure 1-4. The proline-rich tyrosine kinase 2

Table 1 Pass prediction of Ursolic acid, Beta sitosterol, Isorhamnetin and 1-amino-3-ethoxypropan-2-ol for antioxidant activity

	Antioxidant	
Compound name	**P_a**	**P_i**
Ursolic acid	0.408	0.011
Beta sitosterol	0.177	0.072
Isorhamnetin	0.814	0.003
1-amino-3-ethoxypropan-2-ol	-	-

Table 2 Docking results of Ursolic acid, Beta sitosterol, Isorhamnetin and 1-amino-3-ethoxypropan-2-ol for antioxidant activity.

	Docking score (Kj/mol)			
Compound name	**3FZS (PTK-2β)**	**3GRS (GR)**	**1HD2 (PRDX5)**	**1R4U (UO)**
Ursolic acid	-2.706	-4.584	-	-
Beta sitosterol	-2.62	-3.625	-3.144	-2.681
Isorhamnetin	-5.031	-5.78	-	-4.048
1-amino-3-ethoxypropan-2-ol	-3.937	-4.26	-3.582	-4.376

Table 3 ADME properties by QikProp (MW, HBD, HBA, Log P and ROTBs)

	Lipinski's rule of five				
Compound name	**MW[1]**	**HBD[2]**	**HBA[3]**	**Log P[4]**	**ROTB[5]**
Ursolic acid	456.711	2	3	7.3	1
Beta sitosterol	414.718	1	1	9.3	6
Isorhamnetin	316.265	4	7	1.9	2
1-amino-3-ethoxypropan-2-ol	119.164	2	3	-1.1	4

[1]Molecular weight (acceptable range: 500), [2] HBD-Hydrogen bond donor (acceptable range: ≤5), [3] HBA-Hydrogen bond acceptor (acceptable range: ≤10), [4]High lipophilicity (expressed as LogP, acceptable range: <5), [5] ROTB-Rotatable Bond ≤ 10.

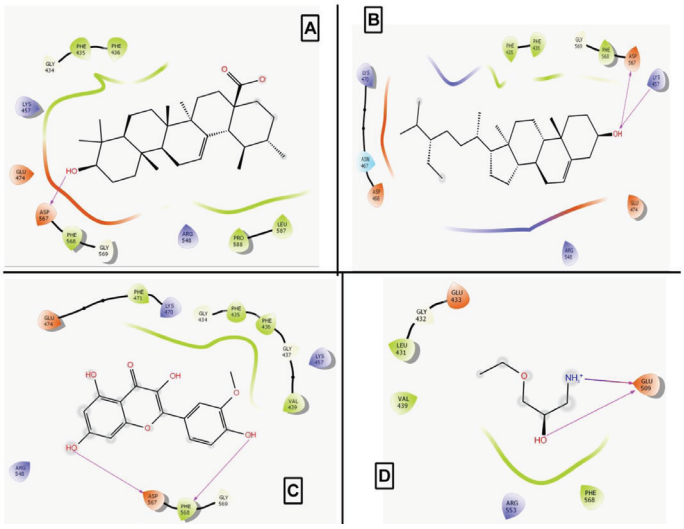

Figure 1 Docking results of ursolic acid (A), beta sitosterol (B), isorhamnetin (C) and 1-amino-3-ethoxypropan-2-ol (D) with proline-rich tyrosine kinase 2 (3FZS) for antioxidant effect. The colors represent the residue (or species) type: Red-acidic, Green-hydrophobic, darker gray-metal atoms, Purple-basic, Blue-polar, and Light gray-other. Interactions with the protein are marked with lines between ligand atoms and protein residue: Solid pink—H-bonds to the protein backbone, Dotted pink-H-bonds to protein side chains, Green—pi-pi stacking interactions, Orange-pi-cation interactions.

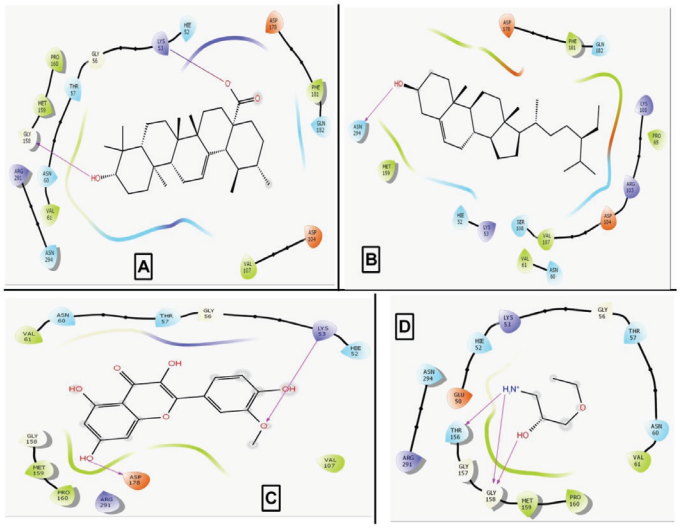

Figure 2 Docking results of ursolic acid (A), beta sitosterol (B), isorhamnetin (C) and 1-amino-3-ethoxypropan-2-ol (D) with Glutathione reductase (3GRS) for antioxidant effect. The colors represent the residue (or species) type: Red-acidic, Green-hydrophobic, darker gray-metal atoms, Purple-basic, Blue-polar, and Light gray-other. Interactions with the protein are marked with lines between ligand atoms and protein residue: Solid pink—H-bonds to the protein backbone, Dotted pink-H-bonds to protein side chains, Green—pi-pi stacking interactions, Orange-pi-cation interactions.

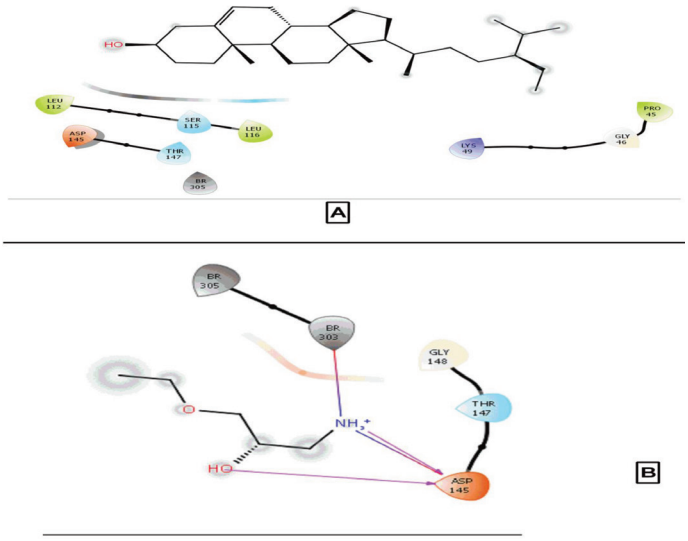

Figure 3 Docking results of beta sitosterol (A) and 1-amino-3-ethoxypropan-2-ol (B) with peroxiredoxin 5(PDB ID: 1HD2).for antioxidant effect. The colors represent the residue (or species) type: Red-acidic, Green-hydrophobic, darker gray-metal atoms, Purple-basic, Blue-polar, and Light gray-other. Interactions with the protein are marked with lines between ligand atoms and protein residue: Solid pink—H-bonds to the protein backbone, Dotted pink-H-bonds to protein side chains, Green—pi-pi stacking interactions, Orange-pi-cation interactions.

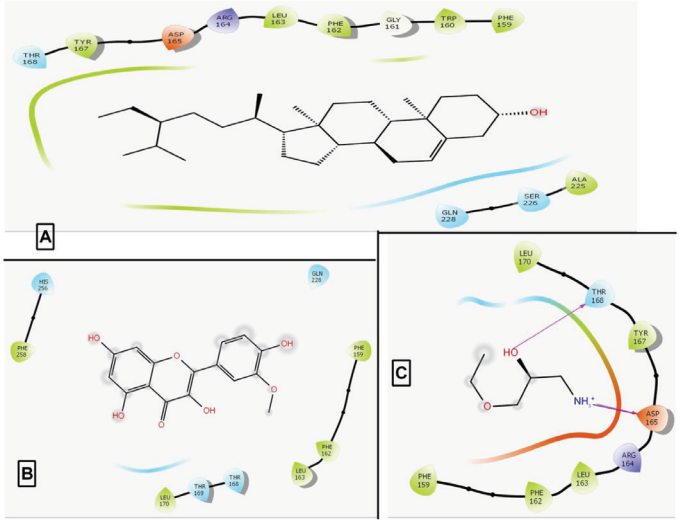

Figure 4 Docking results of beta sitosterol (A), isorhamnetin (B) and 1-amino-3-ethoxypropan-2-ol (C) with urate oxidase (1R4U) for antioxidant effect. The colors represent the residue (or species) type: Red-acidic, Green-hydrophobic, darker gray-metal atoms, Purple-basic, Blue-polar, and Light gray-other. Interactions with the protein are marked with lines between ligand atoms and protein residue: Solid pink—H-bonds to the protein backbone, Dotted pink-H-bonds to protein side chains, Green—pi-pi stacking interactions, Orange-pi-cation interactions.

(3FZS) interact with 4 compounds while the isorhamnetin (-5.031 Kj/mol) showed the maximum lower binding affinity by interacting with ASP 567 and PHE 568 (H-bonding). The other compounds namely, 1-amino-3-ethoxypropan-2-ol, ursolic acid and beta sitosterol showed -3.937, -2.706 and -2.62 Kj/mol respectively. Glutathione reductase (3GRS) showed a similar manner docking score while the isorhamnetin (-5.78 Kj/mol) exhibited the highest binding affinity by H-bond with LYS 53 and ASP 178, followed by ursolic acid isorhamnetin (-4.584), beta sitosterol (-3.625) and 1-amino-3-ethoxypropan-2-ol (-4.26). Among the four compounds, two of them were interacting with peroxiredoxin 5(PDB ID: 1HD2). 1-amino-3-ethoxypropan-2-ol (-3.582) and beta sitosterol (-3.144) had a similar manner binding affinity. 1-amino-3-ethoxypropan-2-ol interacts by one salt bridge and two H-bond with ASP 145. Urate oxidase (1R4U) interact with three out of four compounds while the 1-amino-3-ethoxypropan-2-ol (-4.376) and isorhamnetin (-4.048) demonstrated similar binding activity, followed by beta sitosterol -2.681 Kj/mol. 1-amino-3-ethoxypropan-2-ol interact by Two H-bond and one salt bridge with THR 168 and ASP 165 respectively.

ADME analysis

The ADME properties of Ursolic acid, Beta sitosterol, Isorhamnetin and 1-amino-3-ethoxypropan-2-ol for antioxidant activity were shown in Table 3. All the compounds satisfy the Lipinski rule whereas only one rule (Log P) was violated by ursolic acid and beta sitosterol which could be considered for drug like prosperities.

In vitro antioxidant activity

The previous literature review of Shajiselvin C. et. al. 2010[5] investigate the potential antioxidant activity of methanolic extract of *Borreria hispida* by total antioxidant activity test; reducing ability by FRAP assay and Total phenolic content. The extract showed IC_{50}= 160 µg/ml in total antioxidant activity, followed by reducing ability IC50 = 65 µg/ml and total phenolic content 4.8 mg/g of Catechol.

CONCLUSION

It could be concluded that the bioactive compound of *Borreria hispida* showing a potential affectivity upon the antioxidant protein which are aligned with the previous in vitro finding of antioxidant activity. Therefore it is suggested that the isolated compounds could be formulated as a drug for oxidative stress whereas it does not show any toxicity level in ADME analysis and a predicting a potential antioxidant activity in PASS prediction. Further studies are recommended to evaluate the specific compounds for antioxidant activity.

Figures legend:

Figure 1: Docking results of ursolic acid (A), beta sitosterol (B), isorhamnetin (C) and 1-amino-3-ethoxypropan-2-ol (D) with proline-rich tyrosine kinase 2 (3FZS) for antioxidant effect. The colors represent the residue (or species) type: Red-acidic, Green-hydrophobic, darker gray-metal atoms, Purple-basic, Blue-polar, and Light gray-other. Interactions with the protein are marked with lines between ligand atoms and protein residue: Solid pink—H-bonds to the protein backbone, Dotted pink-H-bonds to protein side chains, Green—pi-pi stacking interactions, Orange-pi-cation interactions.

Figure 2: Docking results of ursolic acid (A), beta sitosterol (B), isorhamnetin (C) and 1-amino-3-ethoxypropan-2-ol (D) with Glutathione reductase (3GRS) for antioxidant effect. The colors represent the residue (or species) type: Red-acidic, Green-hydrophobic, darker gray-metal atoms, Purple-basic, Blue-polar, and Light gray-other. Interactions with the protein are marked with lines between ligand atoms and protein residue: Solid pink—H-bonds to the protein backbone, Dotted pink-H-bonds to protein side chains, Green—pi-pi stacking interactions, Orange-pi-cation interactions.

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