# *In Silico* Study of the Cardioactive Constituents of *Tinospora crispa*

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**ABSTRACT:** *Tinosporacrispa*is a well-known medicinal plant utilized for hypertension. A number of studies have irrefutably established this characteristic, and there have been attempts to narrow down the possible plant constituents responsible. One of the most efficient ways of carrying out this search is the employment of *in silico* methods, which constitute molecular docking and interaction studies with particular endogenous macromolecular targets (assumed as receptors) using the plant compounds. Drug compound optimization can also be assisted by *in silico* pharmacokinetic and chemical analyses. In this study, protein and drug databases are used as the source of target and ligand libraries, respectively. AutodockVina and Discovery Studio are used for docking and interaction studies. Afterward, 6 cardioactive compounds are identified, and their potential mechanisms of action are elucidated. Later on, SwissADME and ProTox-II servers are used to assess the chemical and pharmacokinetic properties.

**Keywords:** *Tinosporacrispa*, antihypertensive, cardioactive, *in silico*, molecular docking

## INTRODUCTION

*Tinosporacrispa* (L) Hook. f. & Thomson is a climber plant with established medicinal properties belonging to the genus *Tinospora* of the Menispermaceae family. *T. crispa* is commonly known in Bangladesh as Guloncho/Gulonchi, and its use in local ethnopharmacy has been vividly documented.[1-3] The cardioactivity of the plant has been investigated in various studies. Mokkhasmit *et al.* demonstrated that in anesthetized dogs, administration of an alcoholic extract of the stem yields a simultaneous increase in heart rate and diminishes blood pressure.[4] Two compounds affecting the contractility of the heart, cycloeucalenone and cycloeucalenol, were isolated later by Kongkathip *et al.*[5] Administration of the N-butanolic fraction obtained by extraction from the stem of the plant resulted in a reduction in mean arterial pressure with a simultaneous increase in heart rate in murine models, indicating the hypotensive activity of the extract.[6] In a subsequent study by the same group, chromatographic and HPLC analysis of the aforementioned fraction resulted in the isolation of five cardioactive compounds: viz- Uridine, Tyramine, Salsolinol, Adenosine, and Higenamine.[7] This study presented the first evidence of bioactivity of these compounds. *In vivo* experimentation on murine models suggested that the activity of Salsolinol, Tyramine and Higenamine was exerted through interaction with the α and β adrenoceptors, and that of Adenosine and Uridine was mediated by the A2 and P2 purinergic receptors.[8] These investigations, beyond doubt, established the antihypertensive properties of *T. crispa*. However,

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investigations up to this point have mostly been conducted with particular plant parts, most commonly the stem of the plant, in animal models. *T. crispa* is rich in phytoconstituents, and more than 65 compounds have thus far been isolated from the plant.[9] Further experimentation in this field focusing on compound-specific assaying of the phyto-constituents for cardio activity is therefore warranted.

In this study, we aim to employ *in silico* techniques to assess whether the variety of compounds available in the plant of interest are capable of exerting significant cardiac activity in computational models, as well as elucidate their possible mechanisms with the hopes of finding novel compounds that will serve as potential compounds of interest for future pre-clinical *in vitro* and *in vivo* studies. The human A2a adenosine receptor, human P2YA receptor, α2A adrenergic receptor, and β1 adrenergic receptor were employed as the macromolecular targets, the first, the third, and the fourth for their effect on contractility, the second for its vasodilatory action.[10-13] Target interactions, drug-likeness, chemical and pharmacokinetic properties of the compounds are also assessed *in silico*.

## MATERIALS AND METHODS

**Construction and preparation of macromolecule and ligand libraries.** Initially, 34 plant constituents with well-defined 3D configurations were downloaded in .sdf format from Pubchem and Zinc Databases.[14,15] The ligands were then optimized in PyRx.[16]

The crystallographic structures of the target macromolecules were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank in .pdb format.[18] PyMol software was used to clean the structures.[19] Servers UniProtKB and CastP were used to identify the active sites.[19,20]

**Virtual screening by molecular docking and interaction study.** For each protein, the PyRx software was used to perform docking studies on the various constituents.[16] The macromolecules were loaded into the software one by one, the active site residues were marked, and the target grid was set so that it covered all active site residues in their entirety. For ligands with greater binding affinity, Biovia Discovery Studio was used to study the ligand-macromolecule interactions.[21]

**Drug likeness prediction.** Drug-likeness based on Lipinski's rule, Ghose rule, Veber Rule, Egan Rule, and Muegge Rule were calculated using SwissADME server.[22-27]

**ADMET studies.** Absorption, distribution, metabolism and excretion of the screened phytochemicals were predicted using the Swiss ADMEserver.[27] Oral toxicity of the screened compounds was predicted via the ProTox II server, which uses the Globally Harmonized System of labeling of chemical compounds (GHS).[28]

**Medicinal chemistry studies.** Structural alerts, namely Pan Assay Interference Structures (PAINS) and Brenk were obtained for the screened phytoconstituents from the SwissADME server, the first checking for promiscuity and the second for toxicity, instability, or dye-like properties.[29,30] The synthetic accessibility on a scale of 1 to 10 (1 being the most accessible and 10 being the least accessible) as well as the violation of lead-likeness were also assessed using the SwissADME server.[31]

## RESULTS AND DISCUSSION

A list of 34 compounds was prepared for the initial *in silico* and molecular docking study, and it is presented in table 1**.**

The target library is comprised of four targets or receptor molecules, also known as macromolecules. These are human A2a adenosine receptor, human P2YA receptor, α2A adrenoceptor, and β1 adrenoceptor. Their PDB IDs, reference (standard) ligands, active site residues where a ligand or drug molecule may bind, and the docking grid are presented in table 2. The 3D structures of the targets are shown in figure 1.

*In silico* docking study with AutodockVina revealed that 14 out of the 34 screened compounds showed a greater binding affinity (BA) to the target human A2a adenosine receptor (2YDO) compared to the standard adenosine (BA= -8.1 Kcal/mole).

Inspecting the interactions in Biovia Discovery Studio, it was found that only Berberine (BA= -11 Kcal/mole), Diosmetin (BA= -9.5 Kcal/mole), and Apigenin (BA= -9.3 Kcal/mole) showed interactions with the desired amino acid residues within the target molecule. The other compounds with better binding compared to adenosine failed to bind to the active sites. Praman *et al*. has hinted at the possibility that the crude extract of *T. crispa* may exert its effect through the modulation of this pathway.[8] These findings also present a strong probability of this and warrant further research into the field.

**Table 1. Phytochemical library.**

| Sl. No. | PubChem ID | Compound name | Reference |
|---|---|---|---|
| 1 | 2353 | Berberine | 32 |
| 2 | 102335359 | Higenamine | 7 |
| 3 | 5280537 | Moupinamide | 33 |
| 4 | 6453733 | N Acetyl Anonaine | 33 |
| 5 | 9994897 | N Caffeoyl Tyramine | 33 |
| 6 | 6440659 | N Cis Feruloyl Tyramine | 34 |
| 7 | 5372945 | Paprazine | 35 |
| 8 | 91588 | Salsolinol | 13 |
| 9 | 5610 | Tyramine | 7 |
| 10 | 101558917 | 2-O- LactoylBorapetoside B | 36 |
| 11 | 101558918 | 6'-O- LactoylBorapetoside B | 36 |
| 12 | 21636047 | Borapetol A | 34 |
| 13 | 21636044 | Borapetol B | 34 |
| 14 | 21636215 | Borapetoside A | 34 |
| 15 | 21636042 | Borapetoside B | 34 |
| 16 | 15934414 | Borapetoside C | 34 |
| 17 | 10556637 | Borapetoside D | 34 |
| 18 | 124578182 | Borapetoside E | 34 |
| 19 | 21625636 | Borapetoside F | 34 |
| 20 | 101683477 | Rumphioside A | 34 |
| 21 | 101683478 | Rumphioside B | 34 |
| 22 | 5316860 | Syringin | 34 |
| 23 | 101558920 | Tinocrispol A | 36 |
| 24 | 5280443 | Apigenin | 33 |
| 25 | 5281612 | Diosmetin | 37 |
| 26 | 5281617 | Genkwanin | 37 |
| 27 | 44258221 | Luteolin 4' Methyl Ether 7 Glucoside | 37 |
| 28 | 190 | Adenine | 7 |
| 29 | 6083 | Adenosine | 13 |
| 30 | 6029 | Uridine | 7 |
| 31 | 5354503 | Beta Sitosterol | 33 |
| 32 | 24984905 | Makisterone C | 33 |
| 33 | 5280794 | Stigmasterol | 33 |
| 34 | 21594790 | Cycloeucalenone | 5 |

**Table 2. Macromolecular targets.**

| Sl. | Target Name | PDB ID | Reference/ Standard ligand | Active site residues | Docking grid | |
|---|---|---|---|---|---|---|
| | | | | | Center | Dimensions (Angstrom) |
| 1 | Human A2a adenosine receptor | 2YDO | Adenosine | THR88, PHE168, ASN181, HIS250, ASN253, THR256, ILE274, SER277, HIS278. | X: -28.7130<br>Y: 7.7541<br>Z: -25.9510 | X: 15.8168<br>Y: 22.6246<br>Z: 17.2052 |
| 2 | Human P2YA receptor | 4XNW | MRS2500 | CYS42A, LEU44A, LYS46A, TYR110A, TYR111A, ASP204A, ARG287A, GLN291A. | X: 21.0599<br>Y: 20.5608<br>Z: 8.4131 | X: 26.3336<br>Y: 19.8995<br>Z: 19.1993 |
| 3 | α2A adrenoceptor | 6KUX | RS 79948 | TYR109A, VAL114A, ILE190A, SER200A, PHE390A, TYR394A, PHE412A. | X: -3.2397<br>Y: -6.2809<br>Z: -19.7854 | X: 20.4669<br>Y: 19.7141<br>Z: 15.1608 |
| 4 | β1 adrenoceptor | 3ZPQ | Cyano-pindolol | ASP121A, VAL122A, VAL125A, THR126A, PHE201A, ALA206A, SER211A, SER212A, SER215A, VAL309A, ASN310A, ASN313A, ASN329A, TYR333A. | X: -22.4723<br>Y: -12.1674<br>Z: 28.5367 | X: 23.5424<br>Y: 16.1974<br>Z: 21.6568 |

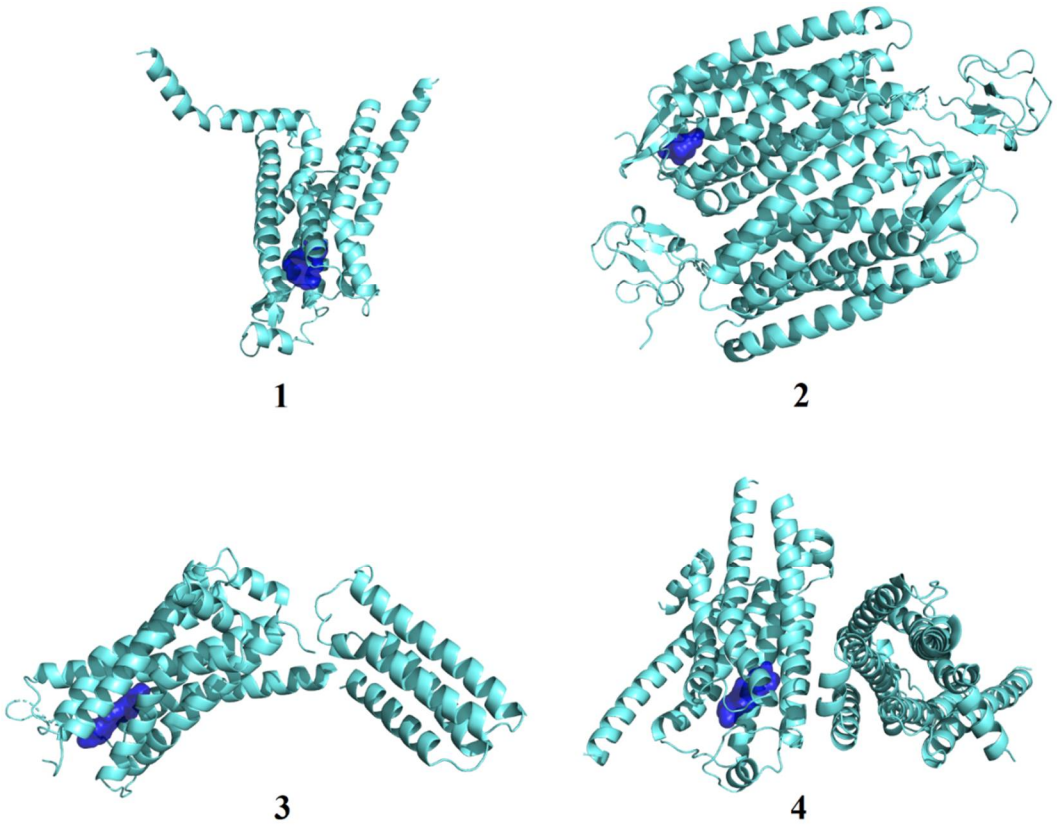



**Figure 1.** Macromolecular targets human A2a adenosine receptor **(1)**, human P2YA receptor **(2)**, α2A adrenoceptor **(3)**, and β1 adrenoceptor **(4)**.

The binding phenomena of the screened compounds with amino acid residues within the assumed receptor molecules are presented in table 3. Target interactions are presented in figure 2.

From table 3, it has been observed that the major binding forces are hydrogen bonds, hydrophobic interactions and other forces.

**Table 3. Binding affinity and interaction of compounds with human A2a adenosine receptor (2YDO)**.

| Compound | Binding affinity (Kcal/mole) | Ligand-macromolecule interactions | |
|---|---|---|---|
| | | Binding Force | Amino acid residue as part of the receptor |
| Adenosine (standard) | -8.1 | Hydrogen bonds | GLU169, ASN253, ASN181, THR88, HIS250 |
| Berberine | -11 | Hydrogen bonds | ASN181 |
| | | Hydrophobic interactions | ALA63, ILE66, PHE168, LEU249, ILE274 |
| | | Others | MET270 |
| Diosmetin | -9.5 | Hydrogen bonds | THR88, ASN181 |
| | | Hydrophobic interactions | PHE168, LEU249, ILE274, LEU85 |
| Apigenin | -9.3 | Hydrogen bonds | ALA63 |
| | | Hydrophobic interactions | ILE274, PHE168, LEU249, LEU85 |

GLU: Glutamic acid, ASN: Asparagine, THR: Threonine, HIS: Histidine, ILE: Isoleucine, TYR: Tyrosine, LEU: Leucine, MET: Methionine, PHE: Phenylalanine, ALA: Alanine

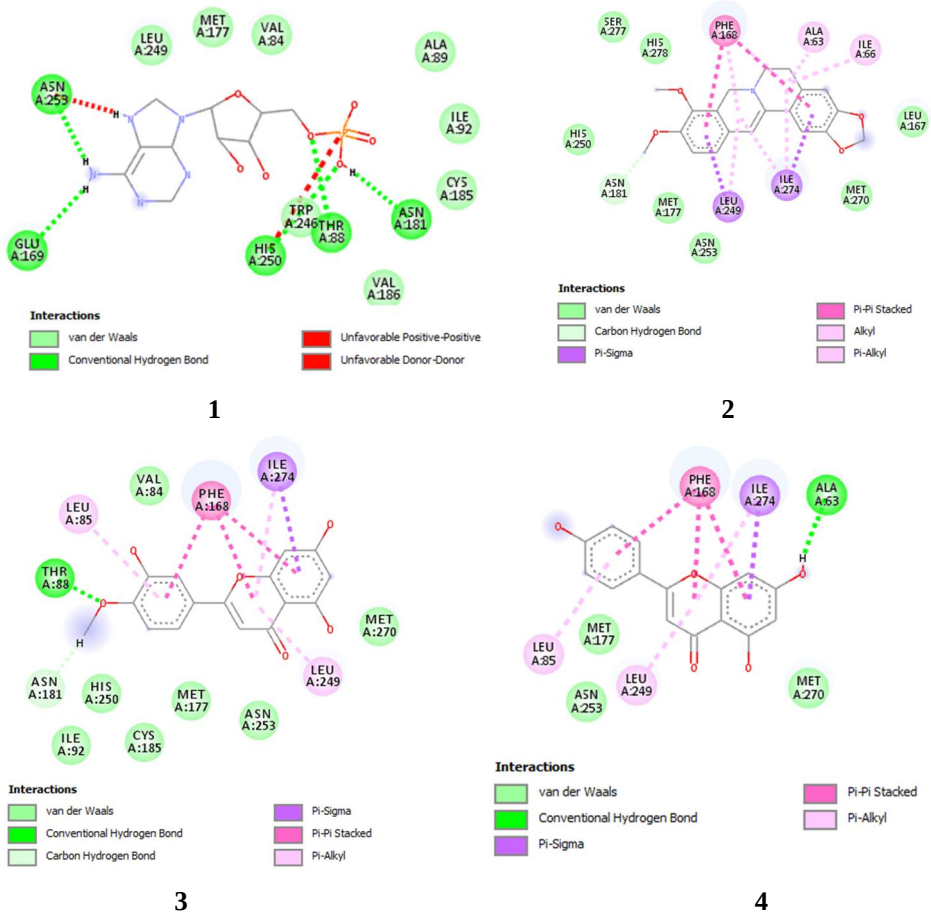


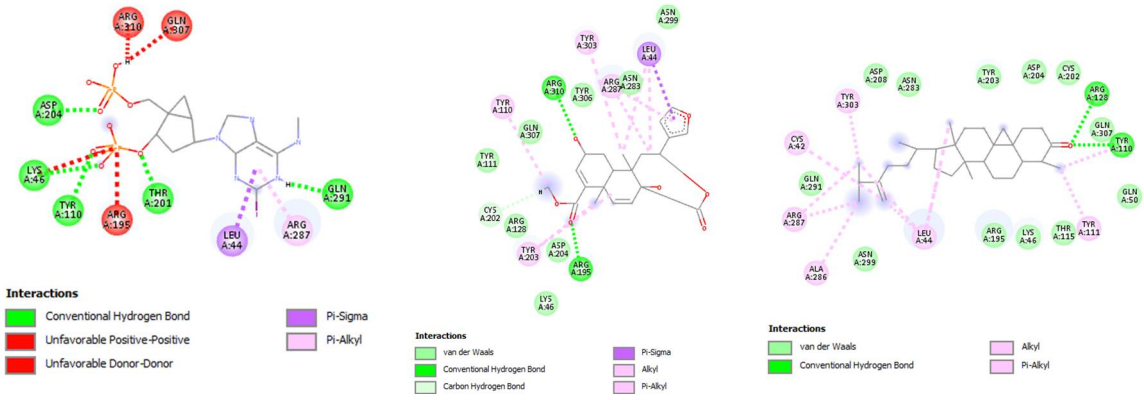

**Figure 2.** Interactions of ligands adenosine **(1)**, berberine **(2)**, diosmetin **(3)**, and apigenin **(4)** with human A2a adenosine receptor.

For docking studies with the second target, human P2YA Receptor (4XNW)**,** MRS2500 (PubChem ID: 44448831) was considered the reference standard as it was bound to the crystallographic structure as the ligand. Two compounds, Cycloeucalenone (BA= -9.8 Kcal/mole) and Tinocrispol A (BA= -8.7 Kcal/mole) showed greater binding affinity compared to the -8.5 Kcal/mole displayed by the standard. These compounds also displayed desired interactions with the active site of the target protein. No interaction with the binding site was shown by the other screened compounds. The binding phenomena of the screened compounds with amino acid residues within the assumed receptor molecules are presented in table 4, from which it has been observed that the major binding forces are hydrogen bonds and hydrophobic interactions. Target interactions are presented in figure 3.

**Table 4. Binding affinity and interaction of compounds with human P2YA receptor (4XNW)**.

| Compound | Binding affinity (Kcal/mole) | Macromolecule-ligand interactions | |
|---|---|---|---|
| | | Binding Force | Amino acid residue as part of the receptor |
| MRS2500 (Standard) | -8.5 | Hydrogen bonds | GLN291A, LYS46A, TYR110A, ASP204A, THR201A |
| | | Hydrophobic interactions | LEU44A, ARG287A |
| Cyclo-eucalenone | -9.8 | Hydrogen bonds | TYR110A, ARG128A |
| | | Hydrophobic interactions | LEU44A, CYS42A, ARG287A, ALA286A, TYR110A, TYR111A, TYR303A |
| Tinocrispol A | -8.7 | Hydrogen bonds | ARG195A, ARG310 |
| | | Hydrophobic interactions | ARG287A, LEU44A, TYR110A, TYR203A, TYR303A, ASN283A, CYS202A |

GLN: Glutamine, LYS: Lysine, TYR: Tyrosine, ASP: Aspartic acid, LEU: Leucine, ARG: Arginine, CYS: Cysteine, ALA: Alanine, THR: Threonine, ASN: Asparagine




**1** **2** **3**

**Figure 3.** Interactions of MRS2500 (**1**), Tinocrispol A (**2**), and Cycloeucalenone (**3**) with human P2YA receptor.

Therefore, a total of 5 compounds (Berberine, Diosmetin, Apigenin, Cycloeucalenone, and Tinocrispol A) were found from tables 3 and 4 as strong candidates for the purinergic receptor systems compared to the reference ligands, which could be investigated further. These compounds are, therefore, potential purinergic system modulators and may provide similar activity as those of adenosine and uridine.[8]

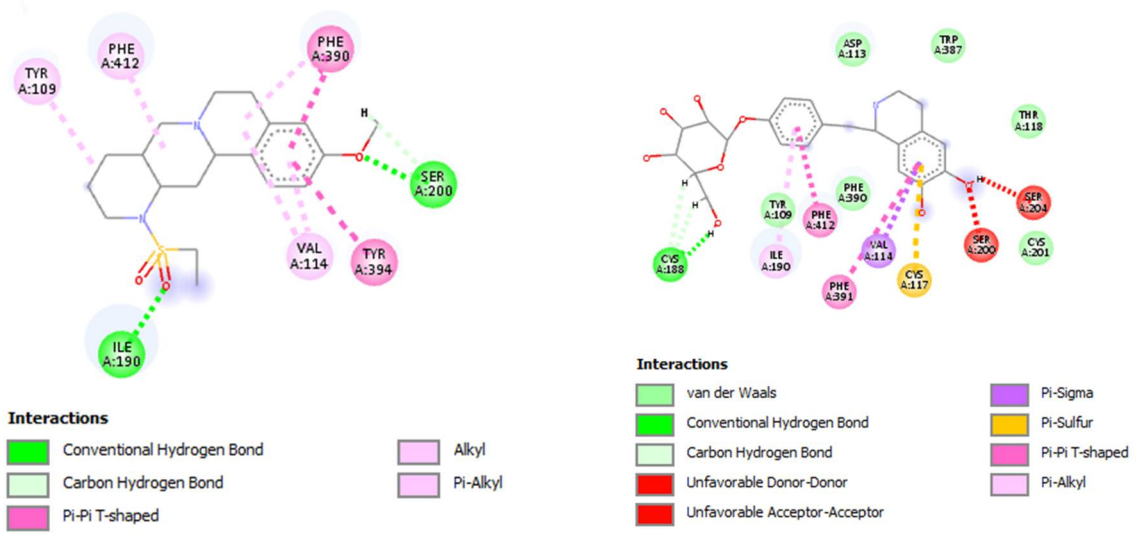
RS 79948 (PubChem ID:9908992) was utilized as the reference standard for the third target macromolecule α2A adrenergic receptor (6KUX), which showed a binding affinity of -8.6 Kcal/mole. Five compounds displayed a higher affinity compared to the standard, of which the following three: Higenamine (BA= -9.2 Kcal/mole), Apigenin (BA= -8.8 Kcal/mole), and Diosmetin (BA= -8.7 Kcal/mole) bound to the active site of the protein, in addition to the standard. The other screened compounds showed no interaction with the binding site. Praman *et al.* suggested that the positive inotropic effect of *T. crispa* extract on isolated rat atria may be mediated through the α- adrenoceptor.[8] The *in silico* approach generates data that supports this idea, but further clarification should be pursued employing appropriate and highly accurate *in vitro* and *in vivo* techniques. The binding phenomena of the screened compounds with amino acid residues within the assumed receptor molecule are presented in table 5. Target interactions are presented in figure 4.

From table 5, it has been observed that the major binding forces are hydrogen bonds, hydrophobic interactions, electrostatic force and other forces.

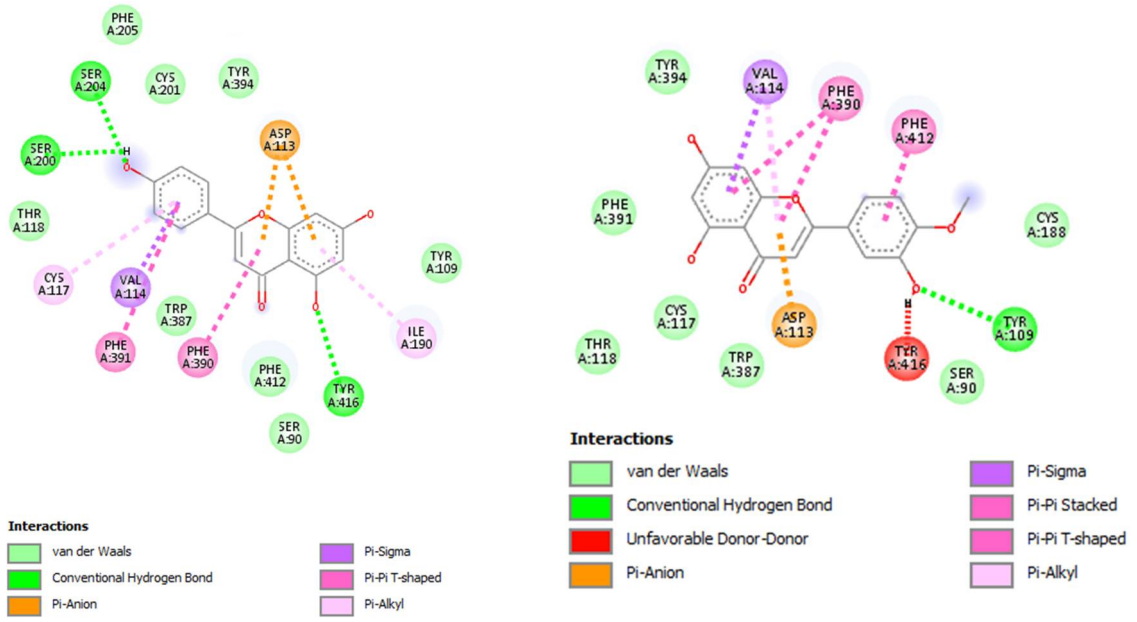
**Table 5. Binding affinity and interaction of compounds with α2A adrenoceptor (6KUX).**

| Compound | Binding affinity (Kcal/mole) | Macromolecule-ligand interactions | |
|---|---|---|---|
| | | Binding Force | Amino acid residue as part of the receptor |
| RS 79948 (Standard) | **-8.6** | Hydrogen bonds | ILE190A, SER200 |
| | | Hydrophobic interactions | PHE390A, TYR394A, VAL114A, TYR109A, PHE412 |
| Higenamine | **-9.2** | Hydrogen bonds | CYS188 |
| | | Hydrophobic interactions | VAL114A, PHE412A, PHE391A, ILE190 |
| | | Others | CYS117 |
| Apigenin | **-8.8** | Hydrogen bonds | SER200A, SER204A, TYR416 |
| | | Hydrophobic interactions | VAL114A, PHE390A, PHE391A, ILE190A, CYS117A |
| | | Electrostatic | ASP113A |
| Diosmetin | **-8.7** | Hydrogen bonds | TYR109A |
| | | Hydrophobic interactions | VAL114A, PHE412A, PHE390 |
| | | Electrostatic | ASP113A |

ILE: Isoleucine, SER: Serine, CYS: Cysteine, PHE: Phenylalanine, TYR: Tyrosine, VAL: Valine, ASP: Aspartic acid




**1** **2**




**3** **4**

**Figure 4.** Interaction of RS 79948 **(1)**, Higenamine **(2)**, Apigenin **(3)**, and Diosmetin **(4)** with α2A Adrenoceptor.

For the β1 adrenergic receptor (3ZPQ), Cyanopindolol (PubChem ID: 155346) was considered as the reference standard. A total of 9 compounds was predicted to have greater affinity compared to the -6.7 Kcal/mole of Cyanopindolol. Through interaction studies, it was found that the standard was bound to the active site. Among the screened compounds with greater binding affinity; Higenamine (BA= -9.1 Kcal/mole), Berberine (BA= -8.7 Kcal/mole), and Diosmetin (BA= -7.5 Kcal/mole) also succeeded to interact with the desired binding amino acids. The binding phenomena are presented in table 6. Target interactions are presented in figure 5.

It was observed from the interaction studies that not all potential compounds with greater binding affinity compared to the standard were able to interact with the macromolecular targets as desired. Only 21% of the screened constituents for the A2a adenosine receptor were able to bind the active sites and hence were selected for further investigation. For the human P2YA receptor, α2A adrenoceptor, and β1 adrenoceptor, the percentages were respectively 20%, 60%, and 33%, respectively. A total of 6 compounds, viz - Apigenin, Berberine, Cycloeucalenone, Diosmetin, Higenamine, and Tinocrispol A were found to have potential purinergic and adrenergic activity (figure 6). Among them, Apigenin and Diosmetin are flavones in nature, Berberine and Higenamine are alkaloids, Tinocrispol A is a diterpene, and Cycloeucalenone is a triterpene. The antihypertensive potential, therefore, did not seem limited to a particular chemical class of compounds belonging to the plant; rather, different classes of compounds synergistically confer beneficial effects such as antihypertensive activity. The drug-likeness of the above-mentioned six compounds predicted by the SwissADME server is presented in table 7.

The drug-likeness of the compounds on the basis of rules of thumb based on various properties yielded satisfactory results. Lipinski rule and Veber rule were complied with by all 6 compounds.[22,24] All compounds except Cycloeucalenone also complied with the Ghose, Egan, and Muegge rules.[23,25,26] Prediction on the basis of these rules acts merely as a guide, and further testing is warranted to assess these predictions in real-world scenarios. However, these predictions provide a focused starting point for drug compound optimization.

**Table 6. Binding affinity and interaction of compounds with β1 adrenoceptor (3ZPQ).**

| Compound | Binding affinity (Kcal/mole) | Macromolecule-ligand interactions | |
|---|---|---|---|
| | | Binding Force | Amino acid residue as part of receptor |
| Cyano-pindolol (Standard) | **-6.7** | Hydrogen bonds | ASN310A, ASN313A, ASN329A |
| | | Hydrophobic interactions | PHE325A |
| Higenamine | **-9.1** | Hydrogen bonds | GLY98A, ASN310A, THR203A, ASN313A, ASN329A, PHE201A |
| | | Hydrophobic interactions | PHE201A, PHE325A, VAL326A |
| Berberine | **-8.7** | Hydrogen bonds | TRP330A, TYR333A, PHE201A, ASN310A |
| | | Hydrophobic interactions | PHE325A, VAL326A |
| Diosmetin | **-7.5** | Hydrogen bonds | TYR333A, ASN310, PHE201A |
| | | Hydrophobic interactions | PHE201, PHE325, VAL326A |

ASN: Asparagine, PHE: Phenylalanine, GLY: Glycine, THR: Threonine, VAL: Valine, TRP: Tryptophan, TYR: Tyrosine

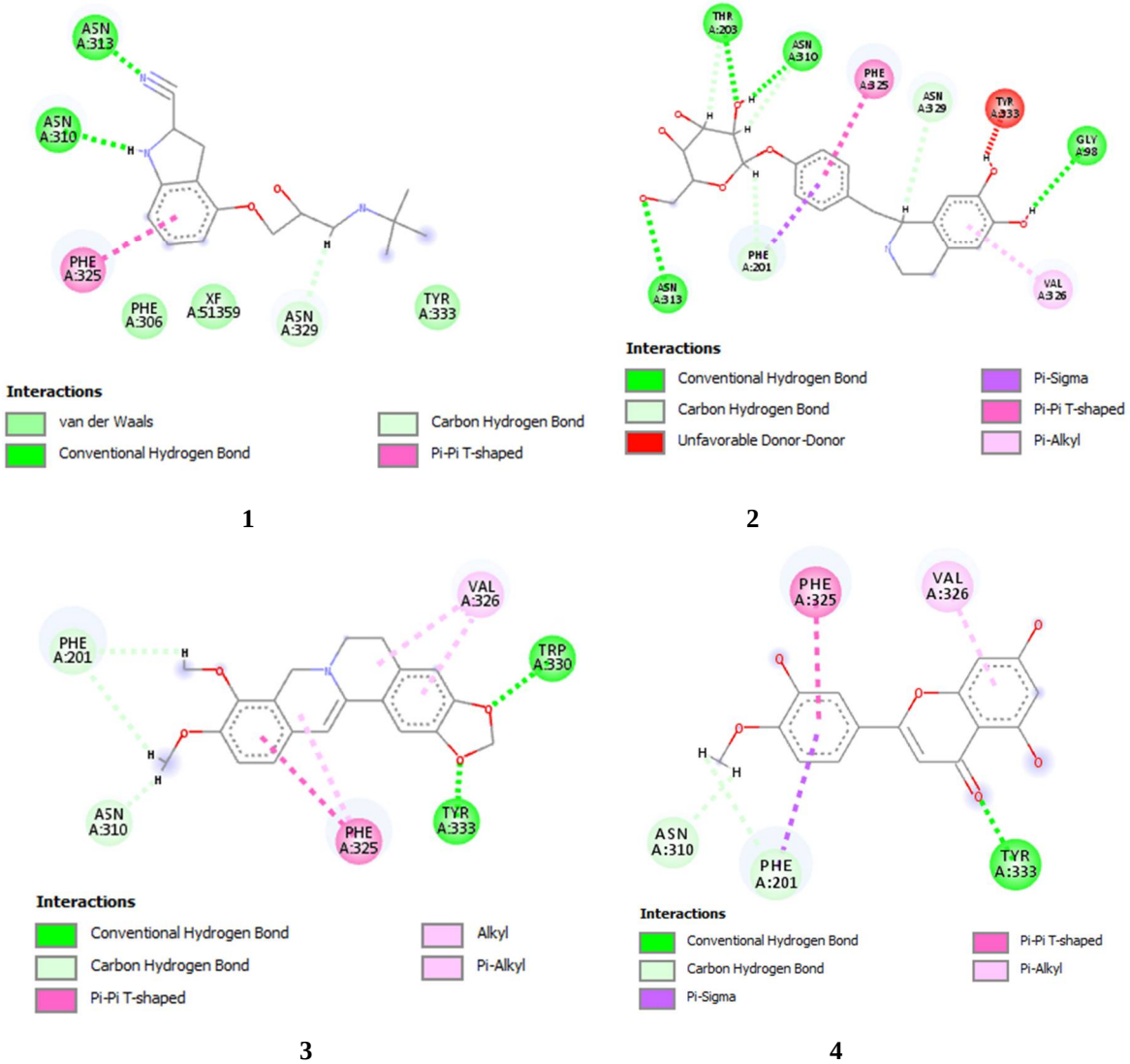



Figure 5. Interactions of Cyanopindolol (1), Higenamine (2), Berberine (3), and Diosmetin (4) with β1 adrenoceptor.

**Table 7. Drug likeness prediction**.

| Compound | Lipinski rule | Ghose rule | Veber rule | Egan rule | Muegge rule |
|---|---|---|---|---|---|
| Apigenin | Yes | Yes | Yes | Yes | Yes |
| Berberine | Yes | Yes | Yes | Yes | Yes |
| Cycloeucalenone | Yes | No | Yes | No | No |
| Diosmetin | Yes | Yes | Yes | Yes | Yes |
| Higenamine | Yes | Yes | Yes | Yes | Yes |
| Tinocrispol A | Yes | Yes | Yes | Yes | Yes |

Yes: Predicted to be drug like molecule, No: Predicted to be non-drug like molecule.

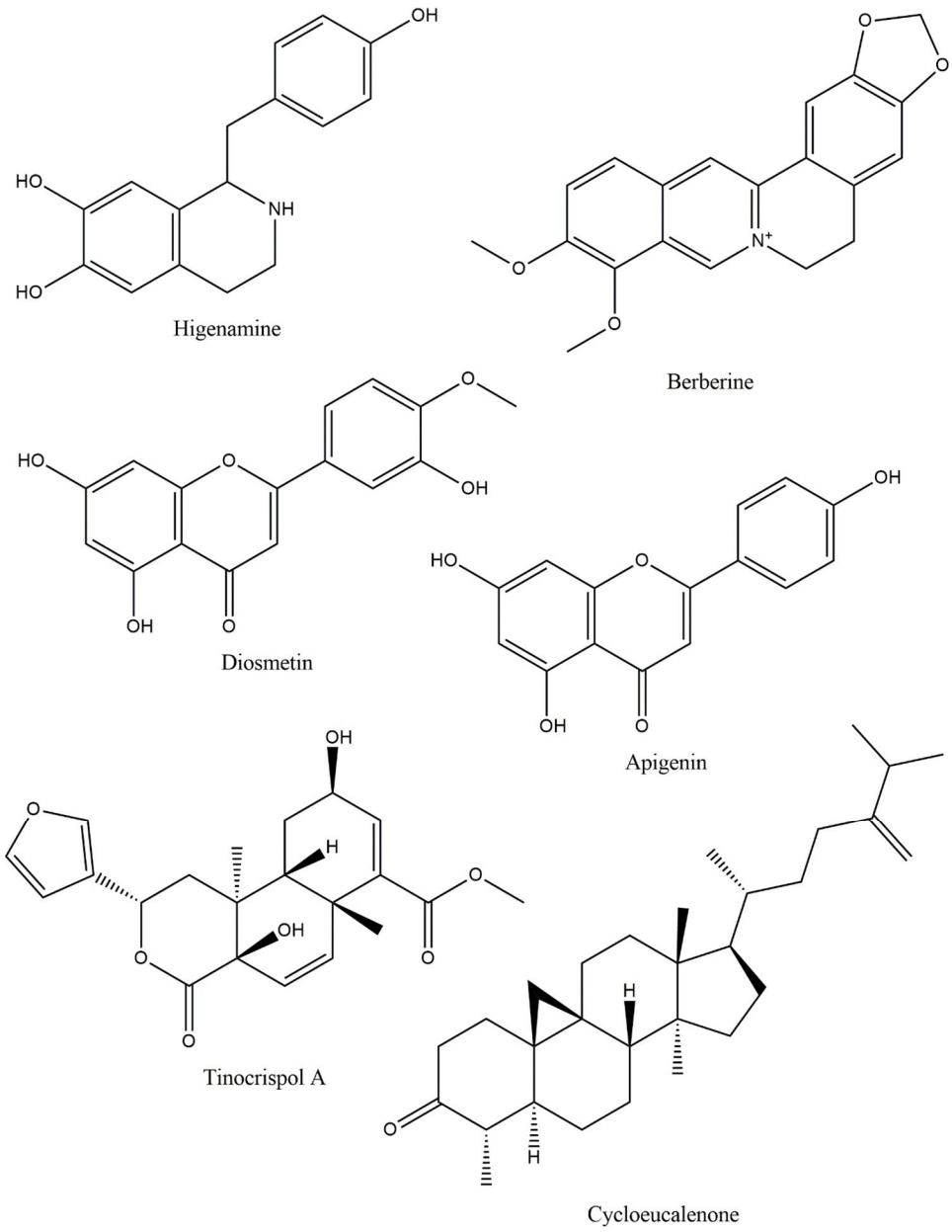



Figure 6. Probable cardioactive compounds from *T. crispa*.

The predicted ADME properties are presented in table 8.

**Table 8. Predicted ADME properties**.

| Compound | GI absorption | BBB permeant | P-gp substrate | log $K_p$ (cm/s) | Cytochrome P450 inhibition | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1A2 | 2C19 | 2C9 | 2D6 | 3A4 |
| Apigenin | High | No | No | -5.8 | Yes | No | No | Yes | Yes |
| Berberine | High | Yes | Yes | -5.78 | Yes | No | No | Yes | Yes |
| Cyclo-eucalenone | Low | No | No | -2.05 | No | No | No | No | No |
| Diosmetin | High | No | No | -5.93 | Yes | No | Yes | Yes | Yes |
| Higenamine | High | No | Yes | -6.36 | No | No | No | Yes | No |
| Tinocrispol A | High | No | Yes | -7.37 | No | No | No | No | No |

The predicted toxicity parameters are presented in table 9.

**Table 9. Predicted toxicity of the compounds.**

| Compound | Predicted $LD_{50}$ (mg/kg) | Toxicity class | Hepato-toxicity | Carcino-genecity | Immuno-toxicity | Muta-genicity | Cyto-toxicity |
|---|---|---|---|---|---|---|---|
| Apigenin | 2500 | V | NA | NA | NA | NA | NA |
| Berberine | 1000 | IV | NA | A | A | A | A |
| Cyclo-eucalenone | 5000 | V | NA | NA | A | NA | NA |
| Diosmetin | 3919 | V | NA | NA | NA | NA | NA |
| Higenamine | 3350 | V | NA | NA | NA | NA | NA |
| Tinocrispol A | 500 | IV | NA | NA | A | NA | NA |

A: Active, NA: Inactive

Class I: fatal if swallowed ($LD_{50} \leq 5$), Class II: fatal if swallowed ($5 < LD_{50} \leq 50$), Class III: toxic if swallowed ($50 < LD_{50} \leq 300$), Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$), Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$), Class VI: non-toxic ($LD_{50} > 5000$)

All of these 6 candidates except for Cycloeucalenone were predicted to have a high gastrointestinal absorption in the virtual ADMET studies. Berberine was found to be the only BBB permeant compound in this study. Cycloeucalenone and Tinocrispol A were the only two compounds predicted to not interact with the CYP enzymes. In terms of the toxicity, no compound obtained a Class III label which implies that it is toxic if swallowed or any class above it. Hepatotoxicity was displayed by none of the compounds. Berberine was predicted to be possibly associated with carcinogenicity. Berberine, Cycloeucalenone, and Tinocrispol A; were all implicated in immunotoxicity. In the case of mutagenicity and cytotoxicity, Berberine was the only compound implicated. A general pattern can be assumed here that Berberine would require a greater degree of caution if ever administered *in vivo.* The medicinal chemistry simulations from SwissADME are presented in table 10.

**Table 10. Compound properties pertaining to medicinal chemistry.**

| Compound | Number of PAINS alerts | Number of Brenk alerts | Leadlikeness violations | Synthetic accessibility |
|---|---|---|---|---|
| Apigenin | 0 | 0 | 0 | 2.96 |
| Berberine | 0 | 1 | 1 | 3.14 |
| Cycloeucalenone | 0 | 1 | 2 | 6.18 |
| Diosmetin | 0 | 0 | 0 | 3.05 |
| Higenamine | 1 | 1 | 0 | 2.62 |
| Tinocrispol A | 0 | 2 | 1 | 5.4 |

It was found that no other compound except Higenamine had raised 1 PAINS alert due to a particular catechol-like structure. It was also found in this study that Higenamine showed interactions with both the α- and β-adrenoceptors. Therefore, it may possibly exert its action through multiple receptors. Literature also supports Higenamine acting through these adrenergic receptors.[13] Brenk alerts were raised by Berberine, Cycloeucalenone, Higenamine, and Tinocrispol A. This corresponds to the toxicity predictions for Berberine, Cycloeucalenone, and Tinocrispol A. Higenamine, Apigenin, and Diosmetin also showed no lead-likeness violations, which suggested that they could be potential lead compounds. This potential was also reflected in their synthetic accessibility. These compounds showed accessibility scores of 2.62, 2.96, and 3.05, respectively. Among the six potential compounds, Cycloeucalenone had shown the lowest accessibility score of 6.18.

The compounds Apigenin, Berberine, Cycloeucalenone and Diosmetin have previously been indicated to have cardioprotective activities *in vitro* and *in vivo*. Apigenin has been observed to provide therapeutic relief in ischemic or myocardial injury in several experimentations.[41,42] The molecule has also shown activity in hypertension and cardiac hypertrophy through the modulation of cytokines and NADPH oxidase-propagated ROS generation.[43] Berberine, a compound of considerable interest in Chinese traditional medicine, has long been known for its antiarrhythmic and vasodilatory activities, as well as its negative chronotropic and positive inotropic effects.[44] Cycloeucalenone has previously been reported as being mildly cardiotonic.[5] Diometin alleviates oxidative stressand functions as an apoptosis inhibitor in cardiac hypoxia, thereby providing cardioprotective activity in myocardial ischemia.[45,46] In high-fat diet-fed rats, diosmetin was found to ameliorate hypertension and dyslipidemia.[47] Overall, these and several other compounds of *T. crispa* show great promise as cardioprotective agents, and therefore further extensive assessment of these compounds is warranted.

## CONCLUSION

In this study, the investigation of a common medicinal plant of Bangladesh had been undertaken to glean information on probable phytoconstituents playing roles in hypertension. The compounds which have been identified through this study can be isolated, purified, and tested on a biological model to gain insight into their activities in a real-world scenario. If found active, they can also be further modified and optimized as suggested by their synthetic accessibility. The *in silico* study can thus facilitate the shrinking of the drug candidates for research on biological models.

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