Screening of potential anti-HIV compounds from *Achyranthes aspera* extracts for SARS-CoV-2: An insight from molecular docking study

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**Abstract:** Till date millions of people are already infected by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). Situation is getting worse day-by-day due to lack of efficient Drug or Vaccine. It is already established that few compounds of *Achyranthes aspera* extract have significant anti-HIV activity. Present study examines inhibitory efficiency of 8 anti-HIV compounds of *A. aspera* extract against SARS-Cov-2. Angiotensin converting enzyme-2 (ACE-2) of Human body, RNA-dependent RNA polymerase (RdRp) and Main protease (Mpro) of SARS-CoV-2 have been chosen as targets for this study. The binding energies and inhibition constant values of these 8 compounds have been compared with the three drugs (Chloroquine, Hydroxychloroquine and Remdesivir). Most of the compounds showed good binding energies and inhibition constant values compared to the drugs. Excretion and Toxicity (ADMET) profile also reflected that the hits from our analysis are safe. According to the molecular docking study, among these 8 anti-HIV compounds, Oleanolic acid has the highest binding affinity and low inhibition constant value with the three proteases. So, we believe that this study will help in therapeutic efforts against SARS-CoV-2.

**Keywords:** SARS-CoV-2; *Achyranthes aspera*; Mpro; ACE-2; RdRp; Molecular docking
1. Introduction:

World Health Organization (WHO) declared a pandemic situation after spreading a new strain of Coronavirus (SARS-CoV-2) around the globe in late December 2019[1-3]. Common symptoms like flu, headache, cold, dihedra and some major cases lung or brain failure are shown by infected patients[4]. Due to high transmission rate of this disease, hundreds of millions lives of approximately 188 countries are already affected[5]. Day by day, the number of asymptomatic patients are increasing and worsen the problem more[6]. Global economy and Health care systems of different countries are in question mark due to this pandemic threat. SARS-CoV-2, a fairly large RNA virus consists of a membrane, spike glycoprotein in the outer surface, envelope and nucleocapsid proteins. Spike protein of this deadly virus interact with the angiotensin-converting enzyme-2 (ACE-2) receptors of host cell to enter in the host cell [7]. Viral replication and transcription are immediately started after entering in the host cell with the functional proteins like main protease (Mpro), RNA-dependent RNA polymerase (RdRp) etc[8]. Many studies showed that Mpro is the key for SARS-CoV-2 replication and it is a potent target for potential inhibitor drugs[9, 10]

In this pandemic situation, scientists are also trying their best to develop an efficient strategy against COVID 19, but till now neither any potentially active drug nor any vaccine is available in the market to combat with SARS-CoV-2[11]. Recently Few drugs like Hydroxychloroquine, Remdesivir, Chloroquine become a new hope to the researchers, but none of these are 100% able to fight against COVID 19[5, 12]. Secondary metabolites available in different types of tropical plants have already drawn great attention due its efficiency to combat against different types of viral diseases[13-16]. Few scientists already claimed that leave extracts of Ocimum sanctum and Azadirachta indica have significant inhibitory activity against SARS-CoV-2[17]. According to the previous reports, extracts from different parts of the various medicinal plants have shown anti-HIV activity and these compounds are different stages of clinical development[18-20]. One of the such kind of plant is Achyranthas aspera. Few scientists already established anti-HIV activity of various compounds present in this plant[21].

In this study, we have selected 8 anti-HIV compounds of Achyranthas aspera and virtually checked their binding efficiency with ACE2 protein of human body, Mpro, and RdRp proteins of SARS-CoV-2 by Molecular Docking study (Figure 1). The binding efficiency of these compounds have been compared with the three FDA approved traditional drugs (Chloroquine, Hydroxychloroquine and Remdesivir). Furthermore, we have checked Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET)
profile of these compounds. We believe this study will be very much helpful to the other scientists to develop efficient drugs against SARS-CoV-2.

Figure 1. Molecular Docking Study

2. Methodology:
Structures of Mpro (PDB ID: 6LU7), RdRp (PDB ID: 6M71) and ACE2 (PDB ID: 6M0J) were downloaded from Protein Data Bank (http://www.rcsb.org). The ‘sdf’ files of 8 anti-HIV compounds and drugs were downloaded from the PubChem (National Library of Medicine) and then files were converted to respective pdb files. The structures of anti-HIV compounds are illustrated in Figure 2. All the protein structures were cleaned by removing hetero-atoms and water molecules using UCSF Chimera[22]. Autodock Vina package [23] was used for docking between the anti-HIV compounds and the best binding sites of the proteins. Docked structures and the active sites interaction were visualized by Discovery Studio Visualizer v20.1.0.19295 [24]. We have used pkCSM database [25] for the Toxicity and ADME studies of the 8 anti-HIV compounds. Ramachandran plot was drawn with the help of Discovery Studio Visualizer v20.1.0.19295.
Figure 2. Structures of the anti-HIV compounds present in Achyranthes aspera
3. Results and discussions:

Table 1 represents the details of 8 anti-HIV compounds viz PubChem CID, Molecular weight, Molecular formula etc. Another important fact is also reflected in Table 1 that which plant parts are responsible for the availability of these compounds.

| Name of the compounds | PubChem CID | Molecular Weight (g/mol) | Molecular Formula | Source Plant | Source Plant Parts | References |
|-----------------------|-------------|--------------------------|-------------------|--------------|-------------------|------------|
| α-Spinasterol         | 5281331     | 412.7                    | C_{29}H_{48}O     | Achyranthes aspera | Stem | [16, 21] |
| Asarone               | 636822      | 208.25                   | C_{12}H_{16}O_{3} |              | Leaves            |            |
| β-Sitosterol          | 222284      | 414.7                    | C_{29}H_{50}O     |              | Stem              |            |
| Ecdysone              | 19212       | 464.6                    | C_{27}H_{44}O_{6} |              | Root              |            |
| Oleanolic acid        | 10494       | 456.7                    | C_{30}H_{48}O_{3} |              | Root              |            |
| Sapogenin             | 23265676    | 490.7                    | C_{30}H_{50}O_{5} |              | Seed              |            |
| Spathulenol           | 92231       | 220.35                   | C_{15}H_{24}O     |              | Leaves            |            |
| Stigmasta-5,22-dien-3-ol | 53870683 | 412.7                    | C_{29}H_{48}O     |              | Seed              |            |

The molecular docking results are recorded in Figure 3 which represents the Binding affinities of 8 anti-HIV compounds (Figure 3a) and 3 drugs against ACE2, Mpro and RdRp. The more negative binding energy and smaller value of inhibition constant (Ki) (Table 2) implies best docking score[26]. Highest inhibitory activity achieved by Oleanolic acid (OA) with binding energy values of -8.9, -7.6 and -7.4 Kcal/mol against ACE2, Mpro and RdRp respectively. So, highest binding affinity was shown by OA with ACE2 receptor. Lowest Ki values of OA in comparison to all the compounds with the three proteins clearly indicated its highest activity against SARA-CoV-2.
Figure 3. Docking score of (a) 8 anti-HIV compounds of *Achyranthes aspera* and (b) 3 FDA approved drugs with 3 proteins.

Remdesivir showed highest inhibitory activity against SARA-CoV-2 among the three drugs. Our study showed that most active compound OA has more negative binding affinity value with ACE2 (-8.9 Kcal/mol) and RdRp (-7.4 Kcal/mol) than Remdesivir which showed the binding affinity value of -7.5 Kcal/mol with ACE2 and -7.3 Kcal/mol with RdRp. Other compounds also showed comparable values of binding affinity and Ki values with the drugs. This information indicated that though other compounds showed its inhibitory efficiency against SARS-CoV-2, OA is the most active compound to fight against this deadly virus.

**Table 2.** Inhibition constant of docked structure of the selected anti-HIV compounds and drugs with ACE2, Mpro and RdRp protease of SARS-CoV-2

| Name of the compounds/Drugs | ACE2  | Mpro  | RdRp  |
|-----------------------------|-------|-------|-------|
| α-Spinasterol               | 2.27  | 5.29  | 17.23 |
| Asarone                     | 66.47 | 130.55| 130.55|
| β-Sitosterol                | 1.62  | 12.30 | 4.47  |
| Ecdysone                    | 7.41  | 12.30 | 14.56 |
| Oleanolic acid              | 0.30  | 2.69  | 3.77  |
3.1. Prediction of toxicity:

Toxicity studies are very crucial for the compounds to determine the tolerability towards human body. All compounds have negative hERGI inhibition activity. The LD50 values of the 8 examined compounds fall in between 1.6 to 2.6 (mol/kg) while the chronic oral rat toxicity (LOAEL) values vary in between 0.85 to 2.11 (log mg/kg_bw/day). None of the compounds showed AMES toxicity and most of the compounds did not show hepatotoxicity and Skin Sensitisation. T. pyriformis and minnow toxicity values are also available in Table 3.

Table 3. Toxicity prediction of different anti-HIV compounds extracted from Achyranthes aspera

| Compound                  | AMES toxicity | Max. tolerated dose (human) | hERG I inhibitor | hERG II inhibitor | Oral Rat Acute Toxicity (LD50) (mol/kg) | Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day) | Hepatotoxicity | Skin Sensitisation | T.Pyriformis toxicity (log ug/L) | Minnow toxicity (log mM) |
|---------------------------|----------------|-----------------------------|------------------|------------------|----------------------------------------|-------------------------------------------------|----------------|------------------|--------------------------------|--------------------------|
| α-Spinasterol             | No             | -0.664                      | No               | Yes              | 2.54                                   | 0.872                                           | No             | No               | 0.433                           | -1.675                   |
| Asarone                   | No             | 0.792                       | No               | No               | 1.939                                  | 2.114                                           | No             | Yes              | 1.48                            | 0.786                    |
| β-Sitosterol              | No             | -0.621                      | No               | Yes              | 2.552                                  | 0.855                                           | No             | No               | 0.43                            | -1.802                   |
| Ecdysone                  | No             | -0.612                      | No               | No               | 2.63                                   | 1.516                                           | No             | No               | 0.294                           | 2.124                    |
| Oleanolic acid            | No             | 0.203                       | No               | No               | 2.349                                  | 2.085                                           | Yes            | No               | 0.285                           | -0.823                   |
3.2. Prediction of ADME:

ADME studies are also very important to determine the pharmacodynamic parameter of the selected anti-HIV compounds. According to the study of pharmacokinetic properties, it was found that 8 anti-HIV compounds were effectively absorbed by the gastro-intestinal part with low blood brain-barrier (BBB) permeability value and these compounds also not affect CYP2D6 substrate, CYP1A2, CYP2C19 (except Spathulenol), CYP2C9, CYP2D6, CYP3A4 inhibitors. Caco2 and Skin permeability values of these compounds vary in the range of 0.59 to 1.9 and -3.8 to -1.9 units respectively. Table 4 represents ADME toxicity table.

Table 4. ADME table of different anti-HIV compounds extracted from Achyranthes aspera

| Compound       | Caco2 permeability | Skin Permeability | P-glycoprotein substrate | P-glycoprotein I inhibitor | P-glycoprotein II inhibitor | BBB Permeability | CNS permeability | CYP 2D6 substrate | CYP 3A4 substrate | CYP 1A2 inhibitor | CYP 2C9 inhibitor | CYP 2D6 inhibitor | CYP 3A4 inhibitor | Total Clearance | Renal OCT 2 substrate |
|----------------|-------------------|-------------------|--------------------------|----------------------------|----------------------------|------------------|------------------|-------------------|------------------|------------------|------------------|-------------------|------------------|------------------|------------------|
| Sapogenin      | No                | -0.888            | No                       | No                         | 2.395                      | 1.462            | No               | No                | No               | 0.304            | 1.486            |                   |                  |                  |                  |
| Spathulenol    | No                | 0.077             | No                       | No                         | 1.687                      | 1.39             | No               | Yes               | 1.153            | 1.266            |                   |                  |                  |                  |
| Stigmasta-5,22-dien-3-ol | No            | -0.664           | No                       | Yes                        | 2.54                       | 0.872            | No               | No                | No               | 0.433            | -1.675          |                   |                  |                  |                  |
| enin          | Spath ulenol | Stigmasta-5,22-dien-3-ol |
|--------------|--------------|--------------------------|
|              | 1.388        | 1.213                    |
| -2.141       | -2.783       |                          |
| No           | No           | No                       |
| No           | Yes          | Yes                      |
| No           | Yes          | Yes                      |
| 0.6          | 0.771        |                          |
| -2.447       | -1.652       |                          |
| No           | Yes          | No                       |
| Yes          | No           | No                       |
| Yes          | No           | No                       |
| Yes          | No           | No                       |
| No           | No           | No                       |
| No           | No           | No                       |
| 0.895        | 0.618        |                          |
| No           | No           | No                       |
The binding interactions of OA and ACE2, Mpro and RdRp are represented by Fig 4a, 4b and 4c respectively. Major interactions that are responsible to bind the proteins are electrostatic and Van der Waal type. The residues corresponding proteins, involved to bind the active compound OA are shown on the right panel of Figure 3(a-c) figures where the left panels (3D-contour) of these Figures 3(a-c) indicates the donor and acceptor sites that are engaged in H-bonding. The docking representation of OA with ACE-2 (Figure 4a) shows strong binding affinity with multiple interactions.

**Figure 4.** Docked structure of OA and SARS-CoV-2 proteins (a) ACE2; (b) Mpro (c) RdRp

Another descriptor of binding affinity of OA (highest docking score) with the three proteases was studied by Ramachandran Plot in Figure 5. Entire plot reflects that maximum residues are
lying in parallel β-sheet in all the docked structures. Few numbers of parallel and flexible α-sheets are also there.

**Figure 5.** Ramachandran plot of OA with (a) ACE2 (b) Mpro (c) RdRp
4. Conclusion:

The present work deals with the virtual screening of 8 anti-HIV compounds from Achyranthes aspera in comparison with three potentially active drugs against SARS-CoV-2. Docking scores and inhibitory constant values obtained from molecular docking studies of 8 anti-HIV compounds are comparable with three potentially active drugs like Chloroquine, Hydroxychloroquine and Remdesivir. Out of the 8 anti-HIV compounds, highest binding affinity was shown by Oleanolic acid against the three proteins (ACE2, Mpro and RdRp) and with ACE2, OA showed highest binding energy in comparison to other two proteins. ADMET analysis revealed that the compounds are safe for human body. Hence OA could be a potential therapeutic option for further consideration of drug development to combat SARS-CoV-2.

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