



## Docking Studies of HIV-1 Reverse Transcriptase and HIV-1 Protease with Phytochemicals of *Carissa Carandas* L.

Reetika Singh<sup>1</sup>, Abhigyan Nath<sup>2</sup>, Bechan Sharma<sup>1,\*</sup>

<sup>1</sup>Department of Biochemistry, University of Allahabad, Allahabad-211002, Uttar Pradesh, India

<sup>2</sup>Department of Biochemistry, Pandit Jawaharlal Nehru Memorial Medical College Raipur, Chhattisgarh, India

### Abstract

**Background:** *Carissa carandas* L. is a well-known wild fruit plant distributed through-out the India and also present in other countries. The fruits are rich in nutrients and minerals. A number of medicinally important phytochemicals such as carrisone, carindone, carandinol, lupeol, scopoletin, stigmasterol,  $\beta$ -sitosterol, myo-inositol,  $\beta$ -amyrin, Des-n-methylnoracronycine etc. have been reported from the extract of this plant. Being safe and cost effective molecules, the activity of phytochemicals against HIV-1 enzymes needs to be screened.

**Objective:** The aim of this study was to screen the potent phytochemical of *C. carandas* against human immunodeficiency virus-1 using docking method.

**Methods:** Total nine compounds viz. carandinol, caridone, carrisone, lupeol, p-coumaric acid, gallic acid, rutin, scopoletin and ursolic acid were used for *in-silico* study towards drug development against human immunodeficiency virus-1 reverse transcriptase (HIV-1RT; PDB ID: 1REV) and human immunodeficiency virus-1 protease (PDB ID:1EBY) using Autodock software.

**Results:** The qualitative characterization of the extracts showed the presence of a number of phytochemicals such as phenolics, flavonoids, alkaloids, terpenoids, terpenes, steroids, glycosides etc. Carandinol was observed as most effective anti-HIV-1 molecule having lowest binding energy and small inhibition coefficient. Another compound, p-coumaric acid, showed least effectiveness against human immunodeficiency virus- 1 reverse transcriptase or human immunodeficiency virus-1 protease showing highest binding energy and inhibition coefficients among all the evaluated phytochemicals.

**Conclusion:** The *in-silico* study demonstrated that some phytoconstituents of *C. carandas* exhibit potential anti-human immunodeficiency virus -1 activity and hence can be optimized to develop as a drug candidate in future.

**Corresponding author:** Bechan Sharma, Department of Biochemistry, University of Allahabad, Allahabad-211002, Uttar Pradesh, India, Cell:+91-9415715639, Email: [bechansharma@gmail.com](mailto:bechansharma@gmail.com)

**Keywords:** *Carissa carandas*, Phytochemicals, HIV-1RT, HIV-1protease, Anti HIV-1Drugs

**Received:** May 11, 2019

**Accepted:** May 28, 2019

**Published:** May 29, 2019

**Editor:** Dr Shivaji Kashinath Jadhav, MapMyGenome India Limited, Hyderabad Previous experience, Sandor Life Sciences Pvt Ltd/ NIMR, Indian Council of Medical Research, Goa, NIRRH ICMR Mumbai, India.

## Introduction

Human immunodeficiency virus (HIV) is one of the most austere and deadly infectious viruses with disastrous concerns [1, 2]. Infections with HIV caused Acquired Immune Deficiency Syndrome (AIDS) and it has been considered as a pandemic disease [1, 3]. HIV-1 is more common and high infectious than HIV-2. HIV-1 has the ability to mutate and change their genetic makeup over the time like many other viruses. There are many genetically distinct subgroups derived from the main type of HIV. HIV-1 is predominant type of HIV and 95% of total HIV infectious person infected with HIV-1. HIV-1 and HIV-2 viruses are genetically different from each other by almost 55% [4]. HIV-1 has four subtypes placed in different groups such as M, N, O and P. Group M is the most dominant and infectious in nature and this group have several strains such as A,B, C, D, F, G, H, J, K and Circulating Recombinant Forms (CRFs). CRFs are hybrid viruses formed as a result of combination of the genetic materials of two subtypes [5, 6]. HIV has infected to about 70 million people all over the world since the beginning of the epidemic and has caused mortality to about 50% of the infected population. Till the end of 2017, about 37 million people were reported to harbour HIV-1 infection [7]. About 40 million peoples are suffering from HIV infection and Africa region is a serious victim with 25 million peoples. Every year about 2.4 million deaths occurred due to HIV and AIDS [4]. According to the National Aids Control Organisation (NACO, India) 2015 reports, in adults HIV occurrence in India was about 0.26% (0.22%–0.32%). HIV prevalence was about 0.30% in males and 0.22% in females in India. Among the Indian states, Manipur has shown the highest estimated adult HIV prevalence of 1.15% and some states have HIV prevalence below 0.20% [8].

Reverse transcriptase (RT) is most important enzyme for the retroviruses to complete their life cycle [9]. Reverse transcriptase (HIV-1 RT) contains 1000 amino acids and is an asymmetric heterodimer. The larger subunit known as p66 contains 560 amino acid long and performing all the enzymatic activities of the RT [10]. Two active sites namely polymerase and ribonuclease H have been found on larger subunit (p66).

The smaller subunit p51 contains 440 amino acids and it is deliberated to stabilize the heterodimer but this unit also may take part in t-RNA primer binding [10, 11, 12]. The HIV-1RT catalyses replication of viral genome (mRNA) converting it into proviral DNA (cDNA) which gets integrated into the human genome present into the nucleus of an infected cell. The integration is catalysed by HIV-1 integrase. The ribonuclease H-domain of the larger subunit (p66) of HIV-1 RT degrades retroviral RNA while synthesising cDNA [10, 12]. Most of the anti-HIV-1 drugs have targeted the RT so as to block cDNA synthesis and hence further sequence of reactions concerning retroviral replication.

HIV-1 protease is another key enzyme which is essential for the HIV life-cycle [13]. The protease cleaves the newly translated chain and generates required protein components to assemble the infectious HIV virions [13, 14]. HIV virions become uninfected without operative HIV protease [15, 16]. Thus, the inhibition of the activity of HIV-1 or the mutation of the active site residues of HIV protease may disrupt the packaging and maturation of the viruses [17]. Therefore, HIV protease becomes one of the main targets towards design and development of many new HIV-1 drugs in addition to the reverse transcriptase and integrase enzymes [18].

Various synthetic drugs are in practice against reverse transcriptase such as nucleoside / nucleotide reverse transcriptase inhibitors (Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, Tenofovir, Zidovudine) and non-nucleoside reverse transcriptase inhibitors (NNRTI) (efavirenze, nevirapine, delavirdine and etravirdine). The drugs against HIV-1 protease include saquinavir, ritonavir, indinavir, and nelfinavir. The applications of these synthetic drugs are reported to develop drug resistance in HIV and also the emergence of the new species of the virus [19, 20]. The approved anti HIV synthetic drugs are effective in the early phase but during the course of time the efficacy starts decreasing. Therefore, it is a necessity to develop a cost-effective, potential anti-HIV drug with easy availability from the other sources rather than the synthetic source. The natural compounds may prove as the promising alternative options to develop a new

anti-HIV drug with negligible side effects and higher efficacy. Various phytochemicals such as curcumin, geranin, gallotannin, tiliroside, kaempferol-3-o-glucoside and trachelogenin etc. have been evaluated for the *in-silico* analysis against HIV-1 [21]. The aim of molecular docking is to estimate the realistic binding geometries of a proposed ligand with a target with known target site. Characterization of the binding site, appropriate orientation of the ligand into the binding site and estimation of the strength of interaction of ligand-receptor complex is the main task of the molecular docking. Recently most of the drugs in the market are either derived from the natural sources or semi-synthetic in nature [21]. Some analogues of the natural compounds for some new targets in HIV-1 may be developed as the new anti HIV drugs. Since metals and metal-complexes have been used to cure the several critical diseases, therefore, metal-based therapy may be the next step to treat the HIV/AIDS [22]. The use of phytochemicals to be explored as anti-HIV agents could be another viable option.

*Carissa carandas* Linn. (family; Apocynaceae) is commonly known as Karonda. *C. carandas*, commonly present in India and other Asian countries [23]. Fruits and leaves are known for presence of various pharmacologically important phytochemicals [24]. The extract of different parts of this plant is used for the cure of various ailments. From the extracts of different parts of *C. carandas* few potential phytochemicals named as carandinol, carrinsone, carindone, lupeol etc. were identified and isolated [25, 26]. In the present paper, we have presented the qualitative characterization of different extracts of leaves and fruits of *C. carandas* using solvents of varying polarities such as hexane, ethanolic, methanolic, and aqueous. Using some known molecules from this plant as ligands and HIV-1 RT and HIV-1 protease as targets, the *in-silico* analysis has been carried out in order to assess their prospective applications as effective anti-HIV-1 regimen in future.

## Materials and Methods

### Collection of Plant Materials

Mature fruits and healthy leaves of *C. carandas* were collected during November from the Pratapgarh district of Uttar Pradesh, India. Plant materials were

washed under running tap water followed by double distilled water and dried under shade condition for 2 h in the hot air oven at 45°C before grinding. The dried material was used to make powder using electrical grinder.

### Preparation of Plant Extracts and Qualitative Characterization of Phytochemicals

Extracts were prepared using Soxhlet apparatus in different solvents (ethanol, methanol, hexane and double distilled water) by the method described by Singh and Kumari [27]. Phytochemical screening was performed using the standard methods of Harborne 1973 [28, 29].

### Compounds Used for Molecular Docking Study Against HIV-1 RT and HIV-1 Proteases

Total nine phytochemicals of *C. carandas* were used for docking study. The details of the used compounds are mentioned in the Table 1.

### Average Linkage Between the Compounds

We used hierarchical clustering facility of ChemMine (webserver) ChemMine tools: an online service for analyzing and clustering small molecules (Nucleic Acids Research, 39 (2011) W486-W491) for clustering of all the phyto-compounds. Atom pair structural descriptors and average linkage method were used for the agglomerative hierarchical clustering with tanimoto coefficient as the similarity measure.

### Molecular Docking Methods of Phytochemicals with HIV-1 Molecule

The three dimensional structures of HIV reverse transcriptase (PDB ID: 1REV) and HIV protease (PDB ID: 1EBY) were retrieved from protein data bank at resolutions of 2.6 Å and 2.29 Å, respectively [30]. The ligand molecules were retrieved from PubChem database [31]. Docking studies were performed by AutoDock [32]. For docking experiments with AutoDock, the ligands were preprocessed by addition of Gasteiger partial charges and by merging of non-polar hydrogen atoms with the help of AutoDock tools. Autogrid program was used to create the Affinity maps with 0.375 spacing. Each docking experiment was obtained from ten different runs and simulations were performed using the Lamarckian genetic algorithm (LGA). The docking experiments by AutoDock were both focused on the

Table 1. Compounds used for docking study

Compound name	Common name	Pubchem CID no.	Molecular formula	Chemical name (IUPAC name)
Carindone	Not any	101316738	$C_{31}H_{44}O_6$	7,7'-bis(2-hydroxypropan-2-yl)-1',4'a,5,9a-tetramethylspiro[3a,6,7,8,9,9b-hexahydrobenzo[g][1]benzofuran-2,3'-5,6,7,8-tetrahydro-4H-naphthalene]-2',3,4-trione
Carrisone	Carrison	5086419	$C_{15}H_{27}O_2$	7-(2-hydroxypropan-2-yl)-1,4a-dimethyl-3,4,5,6,7,8 hexahydronaphthalen-2-one
Carandinol	Not any	102202376	$C_{30}H_{52}O_2$	(3R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-5a,5b,8,8,11a,13b-hexamethyl-3-propan-2-yl 2,3a,4,5,6,7,7a,9,10,11,11b,12,13,13a-tetradecahydro-1H cyclopenta[a]chrysene-3,9-diol
Lupeol	Fagarasterol, Clerodol, Monogynol B,	259846	$C_{30}H_{50}O$	1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13bhexadecahydrocyclopenta[a]chrysen-9-ol
Scopoletin	Gelseminic acid	5280460	$C_{10}H_8O_4$	7-hydroxy-6-methoxychromen-2-one
Ursolic acid	Malol, Prunolurson,	64945	$C_{30}H_{48}O_3$	(1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl 2,3,4,5,6,6a,7,8,8a,10,11,12, 13, 14b-tetradecahydro-1H-picene-4a-carboxylic acid
p-caumaric acid		637542	$C_9H_8O_3$	(E)-3-(4-hydroxyphenyl)prop-2-enoic acid
Rutin	Quercetin 3 Rutinoside	5280805	$C_{27}H_{30}O_{16}$	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2 yl]oxychromen-4-one
Gallic acid	Gallate; Benzoic acid,	370	$C_7H_6O_5$	3,4,5-Trihydroxybenzoic acid

docking site on the predefined binding site by ligand TB9 (9-chloro-TIBO) in 1REV and by BAE369 in 1EBY.

## Results

### Phytochemical Screening

Qualitative characterization of the leaf and fruit extracts showed the presence of various phytochemicals. *C. carnadas* extracts showed the presence of carbohydrates, fat, oil, phenols, flavonoids, alkaloids, terpenoids, steroids, glycosides, saponins, glycosides, and tannins (Table 2). Some compounds were present in very little amount and some were in excess. Ethanolic and methanolic extracts showed the presence of most of the phytochemicals while hexane and aqueous extracts displayed lesser amount of phytochemicals.

### Average Linkage Between the Compounds

The average linkages have been shown in the hierarchical study of all nine compounds (Fig. 1). Rutin and carrinsonne are more structurally similar. Carandinol and lupeol are similar but derived from the ursolic acid. Scopoletin and p-coumaric acid are similar to each other and structurally derived from the gallic acid.

### Docking Study of the Compounds Against 1REV and 1EBY

Molecular docking was performed to analyze the inhibitory action of each phytochemical with both

HIV-1 reverse transcriptase (1REV) and HIV-1 protease (1EBY). To find the best binding pose of ligand at the active site of protein, the binding energy was calculated by adding the final intermolecular energy and torsional free energy. Our study revealed that Carandinol, Carindone and Carrinsonne docked against HIV-1 reverse transcriptase and HIV-1 protease showed maximum binding with binding energy; the values being 10.11, -8.85, -8.71 kcal/mole and -10.99, -9.38 and -7.23 kcal/mole, respectively.

Carnadinol was most effective compound against both 1REV (Table 3) and 1EBY. Rutin was least effective against 1REV while p-coumaric acid was least effective against 1EBY. 1REV contains several active sites. The active site of 1REV comprises leucine, glycine, tryptophan, tyrosine valine, phenylalanine, and alanine etc. Carandinol dominantly binds with LEU100, Val106, Val179, ILE180, TYR181, TYR188, PHE 227, LEU234, TYR318 and inhibits the activity of 1REV. While p-coumaric acid has least binding affinity toward the active sites and it only binds with few amino acids like GLN23, LYS 22, PRO59, ASP76, PHE61, TRP24, GLU399 and showed least inhibition. Molecular docking between the ligands and target molecule has been clearly demonstrated in Figure 2.

## Discussion

The plant extracts of *C. carandas* showed the presence of many phytochemicals. Phytochemical

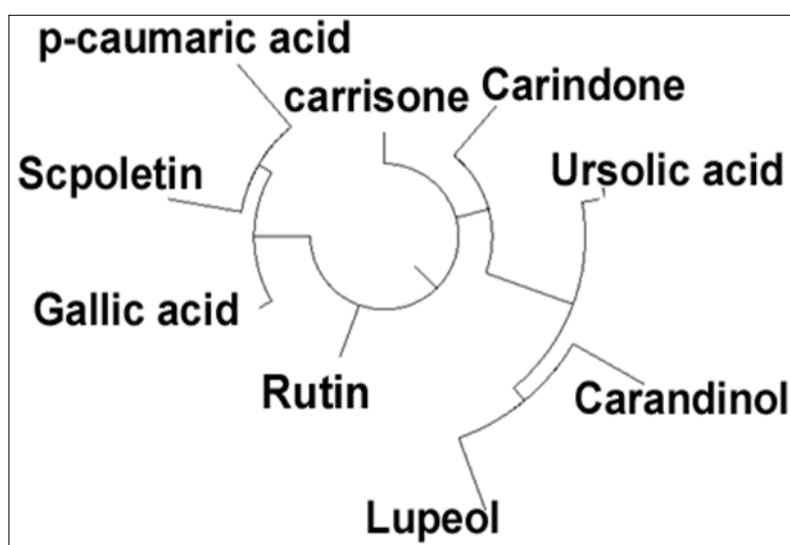


Figure 1. Hierarchical clustering of all the phyto-compounds present (Average linkage method)

Table 2. Efficiency ranking and molecular docking data of phytochemicals against 1REV

Ligands	Binding energy (Kcal/mole)	KI	Intermolecular energy (Kcal/mole)	Internal energy (Kcal/mole)	Torsional energy (Kcal/mole)	Efficiency ranking
Carandinol	-10.11	39.14nM	-11.00	-0.16	0.89	1
Carindone	-8.85	324.25nM	-10.05	-0.52	1.19	2
Carinsone	-8.71	409.19nM	-9.31	0.25	0.6	3
Lupeol	-7.54	2.97uM	-8.14	-0.16	0.6	4
Ursolic acid	-7.47	3.37uM	-8.36	0.33	0.89	5
Gallic acid	-6.58	14.94uM	-11.36	-8.78	4.77	6
p-coumaric acid	-4.64	395.5uM	-5.84	-0.08	1.19	7
Scopoletin	-4.88	265.12uM	-5.48	-0.23	0.6	8
Rutin	-4.41	585.02uM	-9.18	-4.67	4.77	9

KI= Binding affinity index, 1REV= HIV-1 reverse transcriptase

Table 3. Efficiency ranking and molecular docking data of phytochemicals against 1EBY

Ligands	Binding energy (Kcal/mole)	KI	Intermolecular energy	Internal energy	Torsional energy	Efficiency ranking
Carandinol	-10.99	8.87nM	-11.88	0.21	0.89	1
Carindone	-9.38	133.79nM	-10.57	0.52	1.19	3
Carinsone	-7.23	5.0uM	-7.83	-0.26	0.6	6
Lupeol	-10.24	31.14nM	-10.84	-0.26	0.6	2
Ursolic acid	-8.37	738.3nM	-9.26	0.07	0.89	4
Gallic acid	-8.01	1.35uM	-12.78	-5.2	4.77	5
p-coumaric acid	-4.7	357.15uM	-5.9	-0.08	1.19	9
Scopoletin	-5.67	69.4uM	-6.27	-0.13	0.6	8
Rutin	-6.28	24.76uM	-11.06	-4.84	4.77	7

KI= Binding affinity index, 1EBY= HIV-1 integrase

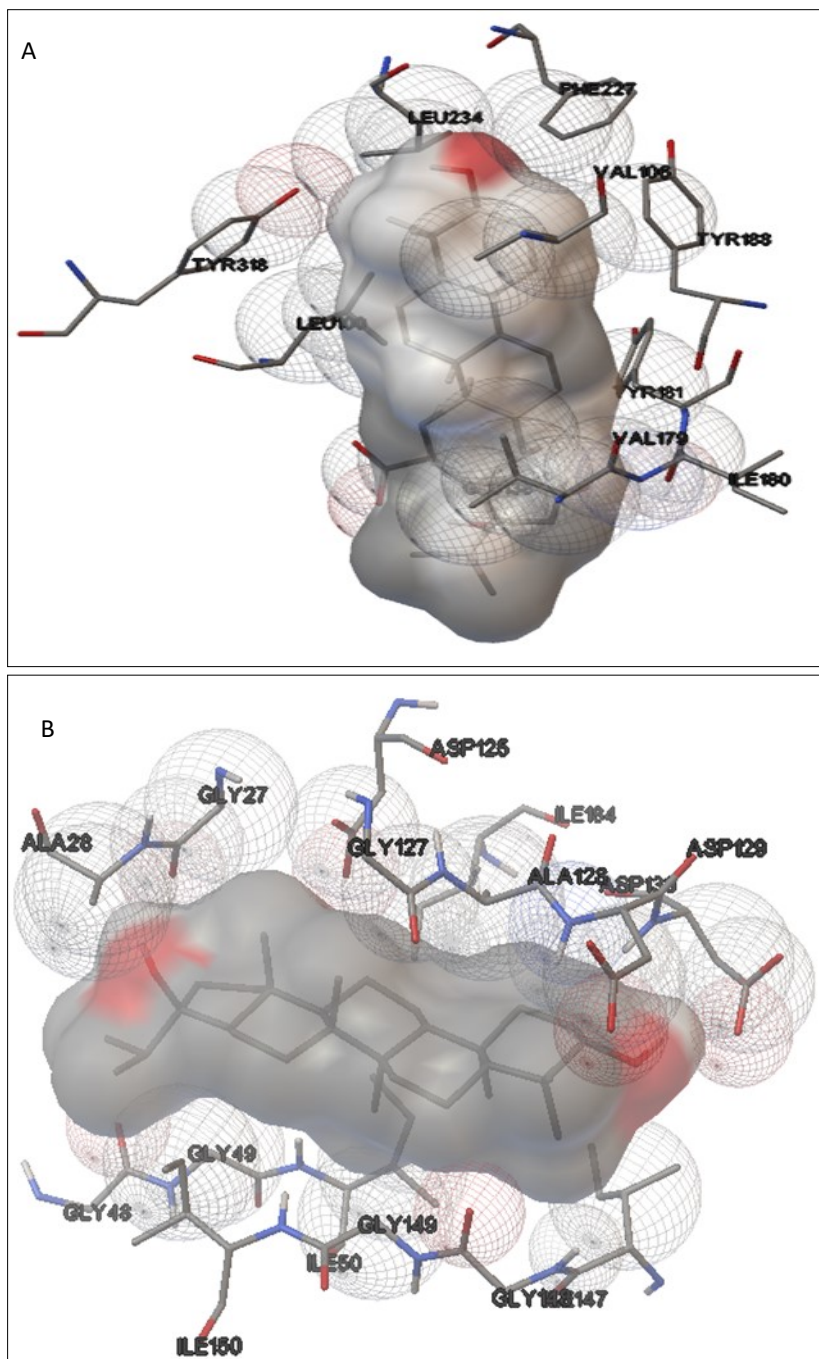


Figure 2. Protein-ligand interactions: (a) Indicates interaction between HIV-1 RT and carandinol (b) Indicates interaction between HIV-1 protease and carandinol

screening of the leaves and root extracts of this plant has been reported by Sawant and Godghate [19]. Qualitative characterization has also been reported in other plants and presence of phytochemicals has been also reported in other plants [29, 33].

Carandinol showed the highest inhibition activity and rutin the least inhibition activity against both 1REV and 1EBY. The inhibition potential of ligand depends upon the active sites of molecules and structure of ligands. Molecular docking was also performed by other worker against HIV-1 RT [21] and HIV-1 integrase [34] and reported the significant anti-HIV activity of phytochemicals. *In-silico* analysis of Dolichin A and B against HIV-RT was also evaluated [35].

### Conclusions

From the present study several compounds were screened in the leaf and fruit extracts. In this work, molecular docking was performed by phytocompounds with both HIV-1 reverse transcriptase and HIV-1 protease. Based on the 3D structure obtained from PDB, the molecular docking was used to produce the binding pose of best docked structure. Total nine compounds were used for molecular docking and among all the carandinol exhibited to be the most potential inhibitor against 1REV and 1EBY. Based on the molecular docking results, it was evident that carandinol possessed lowest binding energy of -10.11 kcal/mole with HIV-1 reverse transcriptase and -10.99 kcal/mole with HIV-1 protease. Inhibition of 1REV and 1EBY proved that the plant extract of *C. carandas* had the anti-HIV potential. Hence, it can be exploited to develop more effective and potential anti-HIV drugs.

### Acknowledgements

RS is grateful to DST-New Delhi for financial support in the form of N-PDF (PDF/2016/000061). BS is grateful to UPCST-Lucknow for financial support in the form of a research grant.

### Conflicts of Interest

Authors declare that they do not have any conflicts of interest.

### Contribution

RS and BS conceived the idea on subject of research mentioned in the manuscript. RS performed the phytochemical characterization. RS and AN performed the docking study. All the authors have contributed in preparation and editing of the manuscript.

### References

1. Weiss RA. (1993) How does HIV cause AIDS. *Science*. 260, 5112
2. Douek DC, Roederer M, Koup RA. (2009) Emerging Concepts in the Immunopathogenesis of AIDS, *Annu Rev Med*. 60, 471.
3. Sharma B. (2014) Oxidative stress in HIV Patients receiving Antiretroviral therapy. *Current HIV-Research*. 12, 13-21.
4. Website: [www.aidsmap.com /HIV-1 and HIV-2/ pages/ 1322970](http://www.aidsmap.com/HIV-1-and-HIV-2/pages/1322970). NAM Aidsmap, accessed July 2017.
5. Hemelaar J. (2012) The origin and diversity of the HIV-1 pandemic. *Trends in Molecular Medicine*, 18, 182-192
6. HIV sequence database (2017) HIV Circulating Recombinant Forms (CRFs), accessed on April 21 2018.
7. Website: WHO (2016) HIV report, accessed on 30<sup>th</sup> April 2018.
8. Website: National Aids Control Organisation, India, report (2015), accessed on 30<sup>th</sup> April 2018.
9. Boyer PL, Coffin JM, Delviks-Frankenberry KA et al. (2008) HIV-1 reverse transcriptase connection subdomain mutations reduce template RNA degradation and enhance AZT excision. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10943–10948.
10. Giridhar R, Prajapati DG, Ramajayam R, Yadav MR. (2009) The search for potent, small molecule NNRTIs: A review. *Bioorganic Medicinal Chemistry*. 17, 5744–5762,
11. Anderson KS. Reverse transcription of the HIV-1 pandemic, *The FASEB Journal*. 2007; 21: 3795–3808.
12. Herschorn A, Hizi. Retroviral reverse transcriptases (other than those of HIV-1 and murine leukemia



- virus): A comparison of their molecular and biochemical properties. *Virus Res.* 2008; 134: 203–20.
13. Gu W-G, Zhang X, Yuan JF.(2014) Anti-HIV Drug Development through Computational Methods. *The AAPSJ*, 16, 674-80.
  14. Huang X, Britto MD, Kear-Scott JL, Boone CD, Rocca JR, et al.(2014) The role of select subtype polymorphisms on HIV-1 protease conformational sampling and dynamics. *Journal of Biological Chemistry*, 289, 17203–17214.
  15. Kohl NE, Emini EA, Schleif WA, et al. (1988) Active human immunodeficiency virus protease is required for viral infectivity. *Proceeding of National Academy of Science U.S.A.* 85, 4686–90.
  16. Krausslich HG, Ingraham RH, Skoog MT, Wimmer E, Pallai PV et al. (1989) Activity of purified biosynthetic proteinase of human immunodeficiency virus on natural substrates and synthetic peptides". *Proceeding of National Academy of Science U.S.A.* 86, 807–11.
  17. Seelmeier S, Schmidt H, Turk V, von der Helm K. (1988) Human immunodeficiency virus has an aspartic-type protease that can be inhibited by pepstatin A. *Proceeding of National Academy of Science U.S.A.* 85, 6612–6.
  18. McPhee F, Good AC, Kuntz ID, Craik CS. (1996) Engineering human immunodeficiency virus 1 protease heterodimers as macromolecular inhibitors of viral maturation. *Proc. Natl. Acad. Sci. U.S.A.* 93, 11477–81
  19. Clercq ED. (2009) Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV. *International Journal of Antimicrobial Agent.* 33, 307-320.
  20. Flexner C. (2007) HIV drug development: the next 25 years. *Nature Reviews*, 6, 959- 66.
  21. Seal A, Aykkal R, Babu RO, Ghosh M. (2011) Docking study of HIV-1 reverse transcriptase with phytochemicals. *Bioinformation.* 5, 430-9
  22. Singh R, Sharma B. (2018) Metal-Based Therapy in Traditional and Modern Medicine Systems in Biomedical Applications of Metals, 195-211. [https://doi.org/10.1007/978-3-319-74814-6\\_9](https://doi.org/10.1007/978-3-319-74814-6_9).
  23. Sawant RS, Godghate AG. (2013) Comparative studies of phytochemical screening of *Carissa carandas* Linn. *Asian Journal of Plant Science Research.* 3, 21-25.
  24. Panda D, Panda S, Pramanik K, Mondal S. (2014) Karonda (*Carissa* spp.): An Underutilized Minor Fruit Crop with Therapeutic and Medicinal Use. *International Journal of Economic Plants*, 1, 009-014.
  25. Begum S, Syed SA, Siddiqui BS, Sattar SA, Choudhary MI. (2013) Carandinol: First isohopanetriene from the leaves of *Carissa carandas* L. and its cytotoxicity against cancer cell lines. *Phytochemistry Letters.* 6, 91–95.
  26. Ganapaty S, Bharath CH, Laatsch H. (2010) Des-N-Methylnoracronycine from the roots of *Carissa conjesta* Wight. *International Journal of Green Pharmacy*, 186-188.
  27. Singh R, Kumari N. (2015) Comparative determination of phytochemicals and antioxidant activity from leaf and fruit of *Sapindus mukorossi* Gaertn. – A valuable medicinal tree, *Industrial Crops and Products.* 73, 1-8.
  28. Harborne JB. (1973) A guide to Modern techniques of plant analysis; phytochemical methods, London. Chapman and Hall Ltd., 49-188.
  29. Singh R, Kumari N, Gangwar M and Nath G. (2015) Qualitative characterization of phytochemicals and *in vitro* antimicrobial evaluation of leaf extract of *Couroupitaguianensis* Aubl. – a threatened medicinal tree. *International Journal of Pharmacy and Pharmaceutical Sciences.* 7, 212-215.
  30. Berman HM, Westbrook J, Feng Z et al. (2000) The Protein Data Bank. *Nucleic Acids Research.* 28, 235-242.
  31. Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Bryant SH. (2009) PubChem: a public information system for analyzing bioactivities of small molecules. *Nucleic Acids Research.* 37, W623-W633.
  32. Morris GM, Huey R, Lindstrom W et al. (2009) AutoDock4 and AutoDockTools4: Automated

- Docking with Selective Receptor Flexibility. *Journal of Computational Chemistry*.30, 2785-2791, doi: 10.1002/jcc.21256.
33. Singh R, Kumari N, Nath G.(2016)Free radical scavenging activity and antimicrobial potential of leaf and fruit extracts of *Sapindus mukorossi* Gaertn. against clinical pathogens. *International Journal of Phytomedicine*. 8, 22-28.
34. Chaniad P, Wattanapiromsakul C, Pianwanit S, Tewtrakul S. (2016) Anti-HIV-1 integrase compounds from *Dioscorea bulbifera* and molecular docking study. *Pharmaceutical Biology*. 54, 1077-1085.
35. Auxilia LR, Sundari TM, Daniel RR.(2013) Molecular Docking Studies of Dolichin A and B, Pterocarpans from Horsegram (*Macrotyloma uniflorum*) against HIV Replication Enzymes. *International Journal of Computer Application*. 75, 19-23