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In silico Molecular docking studies of *Enicostemma axillare*-leaves against HIV

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Abstract

Introduction and Aim: Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plants drug discovery continues to provide new and important leads against various pharmacological targets including Cancer, HIV/AIDS, Alzheimer's, Malaria, etc,. Several natural products drugs of plant origin have either recently been introduced to the United States markets, including art ether, galatamine, nitisinone, and tiotropium or currently involved in late phase clinical trials. Realising the importance of HIV/AIDS, knowing the immense value of Indian medicine plants and attempt was made to study the anti viral properties of these plants, to investigate the antiviral, phytochemical screening and molecular docking of *Enicostemma axillare*-Leaves against HIV.

Materials and Methods: The phytochemical analysis, antiviral, GC-MS analysis and molecular docking studies Molecular docking studies of *Enicostemma axillare*-Leaves against HIV.

Results: The phytochemical study was done, the anti-HIV property of the plants was evaluated based on its ability to inhibit the HIV reverse transcriptase enzyme and several plants have shown potential HIV inhibitory activity in-vitro. The high intensity signals obtained by GC-MS against methanolic extract of *Enicostemma axillare* contain fifteen spectrum of compounds. The compound 2-chloro ethyl linoleate was docked with HIV receptor (1W5V) and the dock score was 75.567.

Conclusions: The results in the present study suggest that *Enicostemma axillare* leaf can be used in treating HIV. From the results obtained, it could be concluded that the herb exhibited excellent antiviral activity. Hence the objective of antiviral activity was satisfied. Such type of plant extract can find wide application in the pharmaceutical industries.

Keywords: Anti-Viral Activity; Anti-HIV Inhibitory Assay; Phytochemical Evaluation; Bioactive Compounds; Molecular Docking Studies

Introduction

Human immunodeficiency virus (HIV), the causative agent for acquired immunodeficiency syndrome (AIDS) belongs to family of Retroviridae. There is no satisfactory or curative treatment for this disease Acquired immunodeficiency syndrome caused by the human immunodeficiency virus (HIV), results in life-threatening opportunistic infections and malignancies. HIV leads to the destruction and functional impairment of the immune system, subsequently destroying the body's ability to fight against infections [1].

In 1981, the first patient diagnosed with acquired immunodeficiency syndrome (AIDS) in the USA was reported. The growing number of people living with HIV has constantly been detected the world over and in particular from the three main continents, Asia, South America and sub-Saharan Africa [2].

HIV infection is not a cause for termination of employment. As with many other illnesses, persons with HIV-related illnesses should be allowed to work for as long as they are medically fit for available, appropriate work.

Sexual behaviour is central to the epidemic spread of HIV and acquired immunodeficiency syndrome (AIDS). As of January 1992, an estimated 71% of HIV infection around the world was due to heterosexual behavior and 15% was due to homosexual behavior; only a relatively small proportion was due to intravenous drug use [3].

The symptoms of AIDS are primarily the result of conditions that do not normally develop in individuals with healthy immune systems. Most of these conditions are opportunistic infections caused by bacteria, viruses, fungi and parasites that are normally controlled by the elements of the immune system that HIV damages [4].

People with AIDS also have an increased risk of developing various cancers such as Kaposi's sarcoma, cervical cancer and cancers of the immune system known as lymphomas. Additionally, people with AIDS often have systemic symptoms of infection like fevers, sweats (particularly at night), swollen glands, chills, weakness, and weight loss. The specific opportunistic infections that AIDS patients develop depend in part on the prevalence of these infections in the geographic area in which the patient lives [5].

Antiretroviral drugs used in the treatment of HIV infection includes lamivudine, abacavir, zalcitabine, dideoxycytidine, tenofovir disoproxil fumarate, emtricitabine and stavudine.However Many different side effects are associated with the use of anti-HIV drugs. The occurrence of side effects plays a large role in adherence to drug regimens, which in turn can impact the development of drug resistance. Side effects such as nausea and diarrhea which affect drug absorption can also contribute to drug resistance.

Current anti-retroviral therapies available for symptomatic treatment of AIDS are quite expensive or unaffordable by common men and are associated with rapid emergence of drug resistance. Therefore, urgent need for new anti-HIV/AIDS drug is a global concern. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Anti-HIV inhibitory activity is widely distributed in nature in the form of medicinal plants. In this present study, the phytochemical evaluation and antiviral potentiality and molecular docking of predominant hit compounds with receptors of selected medicinal plants.

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanic phytochemica, biological and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including ether, galantamine nitisinone and tiotropium, or are currently involved in late-phase clinical trials [6].

Medicinal plants are widely studied looking for new compounds possessing anti-HIV activity. Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them.

A variety of natural products have been found to inhibit unique enzymes and proteins crucial to the life cycle of HIV including efficient intervention with the reverse transcription process, virus binding, and the integrase [7-9].

Till date, numerous papers have been published on the activity of various medicinal plants against the human immunodeficiency virus. Scientists from the developed and developing countries are in the quest for a natural product that will prove to be a therapeutic option against this deadly virus.

Enicostemma axillare is a perennial herb with sessile lanceolate leaves. The white flowers are arranged in clusters. This tropical genus is widely distributed in South America, Africa, and Asia. The plant is bitter, acrid, thermogenic, digestive, carminative, stomachic, laxative, anthelmintic, anti- inflammatory, liver tonic, urinary astringent, depurative, revulsive, antiperiodic and is useful in dyspepsia, flatulence, colic, helminthiasis, abdominal ulcers, hernia, constipation, dropsy, swellings, vitiated conditions of kapha and vata, hepatopathy, glycosuria, leprosy, skin diseases, pruritus, intermmitant fever and malaise. The plant is locally applied in snake bites [10-12].

Based on the importance of HIV/ AIDS, and knowing the immense value of Indian medicinal plants the present study was carried out to study the antiviral properties of certain Indian Medicinal plants against HIV virus.

Materials and Methods

Preparation of plant Extracts

The plant sample *Enicostemma axillare*- (Leaves) were washed, shade-dried, powdered and extracted in Hexane, Methanol, Ethanol, Chloroform, Petroleum either and stored at 4 °C until they were processed for biological evaluation. The solvent from the extract was removed under reduced pressure at 40 °C. The solid was used in antiviral assay after dissolving in Dimethyl-Sulphoxide (DMSO) taken into account that the maximum concentration of DMSO in the test solution should not exceed one percent.

Preliminary Phytochemical Screening

Various extracts collected from the shade-dried powdered leaves and seeds of all three plants were tested for identification of its active chemical constituents [13,14] .

Test for alkaloids

To the small quantity of test solution, a few drops of diluted HCl was added and filtered. The filterate may be tested carefully with various alkaloidal reagents such as,

a. Mayor's reagent – Creamy precipitate
b. Dragondroff's reagent – Orange brown precipitate
c. Hager's reagent – Yellow precipitate
d. Wager's reagent – Reddish brown precipitate

Test for proteins and free amino acids

Dissolve small quantities of test solution in a few ml of water and treated with

Millon's reagent – appearance of red colour shows the presence of proteins and free amino acids.

Ninhydrin reagent – appearance of purple colour shows the presence of proteins and free amino acids.

Biuret test – Equal volume of 5% of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink colour shows the presence of proteins and free aminoacids.

Tests for anthraquinone glycosides

Borntrager's test: The small quantity of test solution was boiled with dil. Sulphuric acid and filtered. Benzene or ether is added to the filterate and shaken well. An organic layer separates. To this ammonia is added. The layer becomes pink to red. It indicates the presence of anthraquinone glycosides.

Test for flavonoids

Shimoda's test: The small quantity of test solution is dissolved in alcohol, to that piece of magnesium followed by conc. HCl drop was added and heated. Appearance of magneta colour shows the presence of flavonoids.

Test for tannin and phenolic compounds

The small quantity of test solution was taken separately in water and tested for the presence of phenolic compounds and tannins with

1. Diluted ferric chloride solution (5%) – violet colour
2. 1% solution of gelatin containing 10% NaCl – white precipitate
3. 10 % lead acetate solution – white precipitate.

Test for Carbohydrates

The small quantity of test solution was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates.

a. **Molisch' test:** The filtrate was treated with 2-3 drops of 1% alcoholic alpha napthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube.
b. **Fehling's test:** The filtrate was treated with 1 ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Test for saponins

The small quantity of test solution was diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The presence of saponin was indicated by the formation of 1 cm layer of foam.

Test for phytosterol

Liebermann Burchard test: The small quantity of test solution was dissolved in few drops of dry acetic acid; 3 ml of acetic anhydride was added followed by few drops of conc. Sulphuric acid. Appearance of bluish green colour indicates the presence of Phytosterols.

Test for triterpenes

To the small quantity of test solution, add few Tin pieces and 3 drops of Thionyl chloride and appearance of violet or purple color indicates the presence of triterpenes.

HIV-RT Inhibition assay: (www.roche-applied-science.com)

General recommendations for the quantification of the inhibitory effect of reverse transcriptase inhibitors, a reverse transcriptase (e.g. reverse transcriptase, HIV-1, recombinant) used in conjunction with the colorimetric Reverse Transcriptase Assay. For the quantification of the inhibitory effect of reverse transcriptase inhibitors in the current procedure, all steps of the Reverse Transcriptase Assay, including the RT reaction, can be performed directly in the MP modules supplied with the kit. Inhibitory activity of reverse transcriptase inhibitors is calculated as percentage inhibition as compared to a sample that does not contain an inhibitor. Therefore, an HIV-1-RT calibration curve is not required.

Additional reagents required

Reverse Transcriptase, HIV-1, recombinant

Reverse Transcriptase inhibitors

Assay procedure

- 4–6 ng recombinants HIV-1-RT, diluted in lysis buffer (20 µl/well) was added in a separate reaction tube. Lysis buffer with no HIV-1-RT should be used as a negative control.
- 20 µl of RT inhibitors diluted in lysis buffer was added to 20 µl reaction mixture (solution 3a or 3b) per reaction tube and incubate for 1 hour at 37 °C.
- Enough foil bags were opened for the number of MP modules to be used. Put them into the frame in the correct orientation. (The correct fitting ensures a tight support of the MP modules). MP modules are ready to use and need not be rehydrated prior to addition of the samples.
- 60 µl samples were transferred into the wells of the MP modules. The MP modules were covered with a cover foil and incubated for 1 hour at 37 °C.
- Remove the solution completely. Rinse 5 times with 250 µl of washing buffer per well (solution 6) for 30 seconds each and remove washing buffer carefully.
- Add 200 µl of anti-DIG-POD working dilution (200 mU/ml, solution 5a) per well, cover the MP modules with a cover foil and incubate for 1 hour at 37 °C.
- Remove the solution completely. Rinse 5 times with 250 µl of washing buffer (solution 6) for 30 seconds per well each and remove washing buffer carefully.
- Add 200 µl of ABTS substrate solution (solution 7) per well and incubate at +15 to +25 °C until color development (green color) is sufficient for photometric detection (10–30 minutes).
- Using a micro plate (ELISA) reader, the absorbance of the samples was measured at 405 nm (reference wavelength: approx. 490 nm).

Note: Shaking of microplates at 250 rpm during incubation with substrate solution can be employed to shorten the incubation period, but is not essential. If shaking is not carried out, gently tap on the side of the microplate was done before measuring absorbance to ensure a homogeneous distribution of the colored reaction product.

Gas Chromatography-Mass spectroscopy (GC-MS)

The value of the technique is that it requires only microgram amount of material, that it can provide an accurate molecular weight and that it may yield a complex fragmentation pattern which is often characteristic of (and may identify) hat particular compound.

Mass spectroscopy, in essence, consists of degrading trace amounts of an organic compound and regarding the fragmentation pattern according to mass. The ample vapors diffuses in to the low pressure system of the mass spectrometer here it is ionized with sufficient energy to cause fragmentation of the chemical bonds. The resulting positively charged ions are accelerated in a magnetic field which disperses and permits relative abundance measurements of ions of given mass-to-charge ratio. The resulting record of ion abundance versus mass constituents, the mass spectral graph, which thus consists of a series of lines of various intensity at different mass units. In many cases, some of the parent compound will survive. The vaporization process will be recorded as a parent ion.

Those compounds which are too involatile to vaporize in the MS instrument converted to trimethyl silyl ethers, methyl esters, or similar derivatives[15]

Mass spectroscopy is frequently used in conjunction with GLC and the combined operation provides to go for a qualitative and quantitative identification of the many structurally complex components that may be present together in a particular plant extract.

Instrument Description

GC-Fisons instruments (Automated)

Model : GC 8000 Series

Column : DBSMS size 0.25u ID X 0.25u X 30 m

Injection temperature – 25 °C

Temperature programming:

Time	Rate / Min	Temp (°C)
0	6	*50*
45	0	*250*

MS : Fisons instrument

Molecular docking

Retrieval of 3d structure for HIV

STEP1: Visited the website www.Google.com and in the search column entered RCSB and clicked the search button.

STEP2: The RCSB homepage was displayed.

STEP3: In the search column entered as HIV, then a list of receptors were displayed.

STEP4: From the list, structure of HIV was selected and then

3D structure of receptor was saved as 1w5v.pdb file format.

Selection of Ligand

Step 1: Enter into google and type pubchem compound

Step 2: Pubchem home page was displayed.

Step 3: In search box enter into a compound names.

Step 4: Select the structure for the compounds in .mol file format

Step 5: Load the structure into Discovery studio 2.1

Docking Process

Before beginning the docking, it is necessary to specify a binding site of the receptor. Ligandfit uses a method based on protein shape searching for cavities. Often the largest cavity is part of the ligand-binding site.

STEP 1: In the Tools Explorer, selected the "protein report and utilities" by expanding it,

STEP 2: Clicked the protein report. So that a text window was displayed with all the information about the PDB file.

STEP 3: Clicked the "Split all" under the "protein report and utilities" to split the receptor into protein and non-protein parts. Non-protein parts were deleted.

STEP 4: Under the "Binding site" from Tools Explorer, "Define protein molecule as Receptor" was selected.

STEP 5: Selected the "Find sites from Receptor cavities" under the Binding site.

STEP 6: A list of binding sites was opened in the hierarchy view.

STEP 7: Load the 3-D Structure of Ligand

STEP 8: Select the Receptor – Ligand interaction Protocol

STEP 9: Set the Parameters

STEP 10: Click RUN Button for docking process.

STEP 11: Analysis the Results.

STEP 12: From the results choose least Dock Score Value.

Result and Discussion

In the present study, the Antiviral activity, Phytochemical studies, GC-MS and Molecular docking of selected plant extracts have been investigated and the results were documented under various segments.

Traditional medicines are used by about 60 per cent of the world's population. Many medicinal plants produce a variety of compounds of known therapeutic properties

All the Extracts obtained from the medicinal plants were evaluated for their antiviral activity against HIV-reverse transcriptase enzyme, and the following results were obtained. The Hexane extract of *Enicostemma axillare* inhibited HIV-RT Table:1 A study with nineteen Chinese medicinal herbs was conducted by Collins *et al.* (1997) [15] to evaluate their anti -HIV activity by virtue of their inhibition of the interaction of the HIV-1 gp120 and CD4 receptors and inhibition of the HIV RT activity.

Similarly Shuwen Liu *et al.* [16] found that the extracts of two herbs, Prunella vulgaris and *Rhizoma cibotte*, showed potent HIV inhibitory activity.

Veljko Veljkovic *et al.* [17] Stated that Flavonoid compounds represent an important natural source of antiretrovirals for AIDS therapy due to their signicant anti-HIV-1 activity and low toxicity.

S.No	SOLVENT	HIV-RT Inhibition Assay
1	*Hexane extract*	Pos
2	Methanol extract	Neg
3	Ethanol extract	Neg
4	Chloroform extract	Neg
5	Petroleum ether extract	Neg

Table 1: Antiviral activity of *Enicostemma axillare*

Petroleum ether extract of *Enicostemma axillare* found to possess Steriods, Sugars, Alkaloids, and Phenolic groups, Flavones, Saponins, Tannins and Flavonones. Ethanol extract had all the secondary metabolites except Catachin, Saponins, and Anthroquinone glycosides. Methanol extract did not show the presence of Anthroquinone glycosides and aminoacids. Hexane extract consists of Steriods, Sugars, and Phenolic groups, Flavones, Tanins and Flavanones. In Chloroform extract most of the components were absent except Triterpenes, sugars, Alkaloids, Catachin, Anthroquinone glycosides and aminoacids Table 2.

Constituents	Petroleum ether	Ethanol	Methanol	Hexane	Chloroform
Steroids	+	+	+	+	+
Triterpenes	-	+	+	-	-
Sugars	+	+	+	+	-
Alkaloids	+	+	+	-	-
Phenolic groups	+	+	+	+	+
Flavones	+	+	+	+	+
Catachin	-	-	-	-	-
Saponins	+	-	+	-	+
Tannins	+	+	+	+	+
Anthroquinone glycosides	-	-	-	-	-
Amino acids	-	+	-	-	-
Flavonones	+	+	+	+	+

Table 2: Preliminary Phytochemical screening of various extracts of the leaves of *Enicostemma axillare*

Raveendra Retnam and John de britto[18] investigated that the phytochemical analysis of a medicinal plant *Enicostemma axillare (Lam.)* for the identification of alkaloids, steroids, terpenoids, and flavonols by Gas Chromatography. Their study threw light on various alkaloids, steroids, terpenoids, and flavonols present in this medicinal plant which was proven by the present phytochemical studies which reveals the presence of Steroids,Flavones,Tannins and Flavonones. The detected plant compounds were the bioactive molecules which exhibit antiviral property against HIV and HBV.

The predominant components present in all the extracts of *Enicostemma axillare* are steroid, Phenolic groups, Flavones, Tannins and Flavonones

It is well known that many pharmacologically active components in herbal medicines are volatile chemical compounds and therefore Gas chromatography is very important in the analysis of herbal medicines. In the present study the plant extracts were subjected to GC-MS for the identification of spectrum of compounds. Only Hexane extracts of *Enicostema axillare* was subjected for this analysis.

The high intensity signals obtained by GC-MS against methanolic extract of *Teprosia uniflora* pronounced to contain spectrum of compounds such as -Propyl-Cis-Bicyclo(3,2,0)Hept-6-En-2-One,6-Octen-1-Ol,3,7-Dimethyl, Ethanoe,1(2,4,6-TrimethylPhenyl), 1(2H)Naphthalenone,3,4 Dihydro-6-Methoxy, 2-Propenoic Acid,3(3-Hydroxy Phenyl),Methyl Est, 2-FuranCarboxaldehyde,5(2-Furanyl Methyl), CycloDoDecane Methanol, 2-Chloro Ethyl Linoleate, 5,8,11-HeptaDecatrien-1-Ol, 1-Decanol.2-Hexyl, 3-Methyl-2(2-Oxopropyl)Furan,Z,Z-6,28-HeptatriactonTadien-2-One,CycloHexanol,4Ethyl-4-Methyl-3(1-Methylethyl), CycloPentanone,2-(Methylpropyl), CycloHepta Decanol.The previous investigation carried out by Fyhrquist *et al.* (2002) found to be corelated with the present GC MS analysis of *Enicostemma axillare* plant extracts (Table 3).

S. no	Compound name	Molecular Formula
1	Propyl-Cis-Bicyclo(3,2,0)Hept-6-En-2-One	$C_{10}H_{14}O$
2	6-Octen-1-Ol,3,7-Dimethyl	$C_{10}H_{20}O$
3	Ethanoe,1(2,4,6-TrimethylPhenyl)	$C_{11}H_{14}O$
4	1(2H) Naphthalenone, 3,4 Dihydro-6-Methoxy	$C_{11}H_{12}O_2$
5	2-Propenoic Acid,3(3-Hydroxy Phenyl),Methyl Est	$C_{10}H_{10}O_3$
6	2-FuranCarboxaldehyde,5(2-Furanyl Methyl)	$C_{10}H_8O_3$
7	CycloDoDecane Methanol	$C_{13}H_{26}O$
8	2-Chloro Ethyl Linoleate	$C_{20}H_{35}O_2Cl$
9	5,8,11-HeptaDecatrien-1-OL	$C_{17}H_{30}O$
10	1-Decanol.2-Hexyl	$C_{16}H_{34}O$
11	3-Methyl-2(2-Oxopropyl) Furan	$C_8H_{10}O_2$
12	Z,Z-6,28-HeptatriactonTadien-2-One	$C_{37}H_{70}O$
13	CycloHexanol,4 Ethyl-4-Methyl-3(1-Methylethyl)	$C_{12}H_{24}O$
14	CycloPentanone,2-(Methylpropyl)	$C_9H_{16}O$
15	CycloHepta Decanol	$C_{17}H_{34}O$

Table 3: Gas Chromatographic Analysis of Methanolic extracts of Plant

Similar studies by Proestos *et al.* (2006) [19] on *Enicostemma axillare* explained that GC–MS can be used for characterization of different phenolics as trimethylsilyl derivatives. The antioxidant capacity was determined, in dried plants and in their methanol extracts, with the Rancimat test using sunflower oil as substrate. Both pulverized plants and extracts showed antioxidant capacity. Total phenolic content in the extracts was determined spectrometrically applying the Folin- Ciocalteu assay. It ranged from 2.9 to 28.2 mg gallic acid/100 g dry sample.

From the present investigation, *Enicostemma axillare*, was observed to have anti HIV activity. The compounds therefore were isolated from the plant extracts and specific hit compounds (ligands), docked against the receptors of respective viruses to check their antiviral binding activity.

The compound 2-chloro ethyl linoleate (CEL) from *Enicostemma axillare* was docked with HIV receptor (1W5V) and the dock score was 75.567. The hit compound cycloheptodecanol from *Enicostemma axillare*was docked with HIV receptor (1W5V) and the dock score was 53.288. (Figures 1,2,2a,3 & 3a)

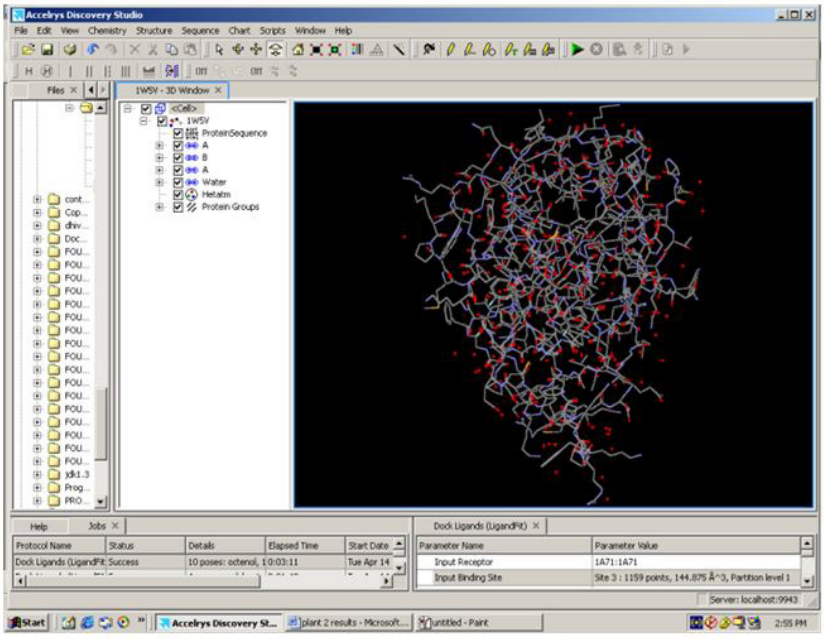


Figure 1: Structure of HIV (1W5V) receptor

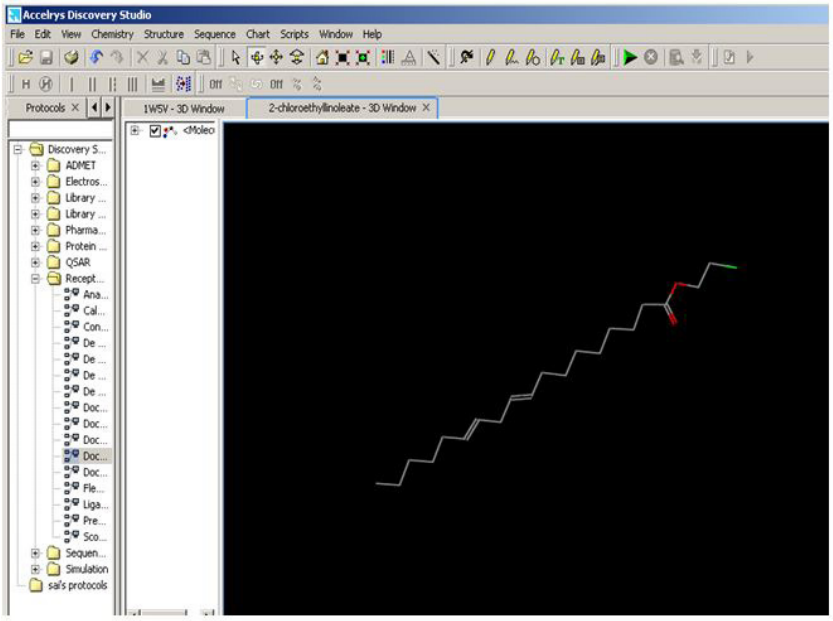
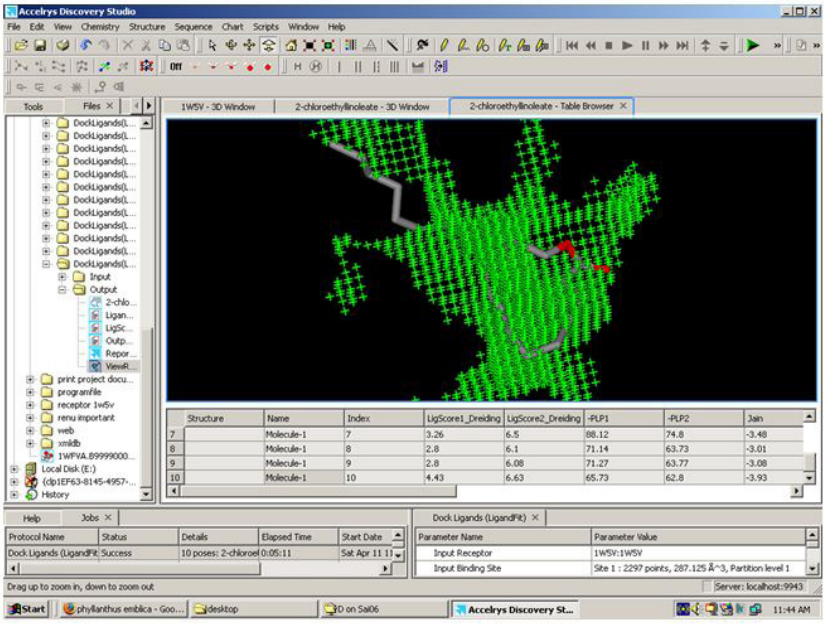


Figure 2: Structure of chloro ethyl linoleate first hit compound from *Enicostemma axillare*



	LigScore1_Dreiding	LigScore2_Dreiding	-PLP1	-PLP2	Jain	-PMF	LigScore Warning	DOCK_SCORE
1	3.57	6.37	79.19	74.03	-3.84	84.98	unfilled valencies:...	79.116
2	3.2	6.64	92.12	86.83	-4.01	81.67	unfilled valencies:...	77.911
3	3.34	6.24	81.93	74.09	-4.29	86.15	unfilled valencies:...	76.87
4	3.34	6.24	81.83	74.03	-4.3	86.09	unfilled valencies:...	76.865
5	3.89	6.89	83.55	72.52	-3.95	84.36	unfilled valencies:...	76.043
6	3.22	6.5	88.67	74.85	-3.47	80.76	unfilled valencies:...	75.783
7	2.8	6.1	71.35	64.04	-3.04	76.06	unfilled valencies:...	75.648
8	2.82	6.11	71.49	64.17	-3.08	76.38	unfilled valencies:...	75.644
9	3.13	6.18	79.69	74.36	-4.04	78.18	unfilled valencies:...	75.574
10	4.4	6.62	65.69	62.53	-3.96	92.65	unfilled valencies:...	75.567

Figure 2a: Docked view of chloro ethyl linoleate from *Enicostemma axillare* with HIV (1W5V) receptor

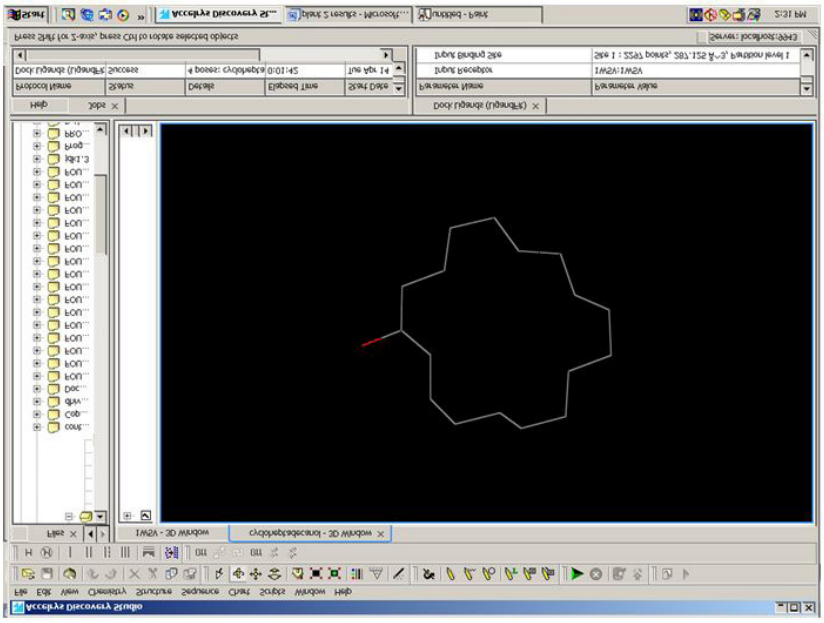
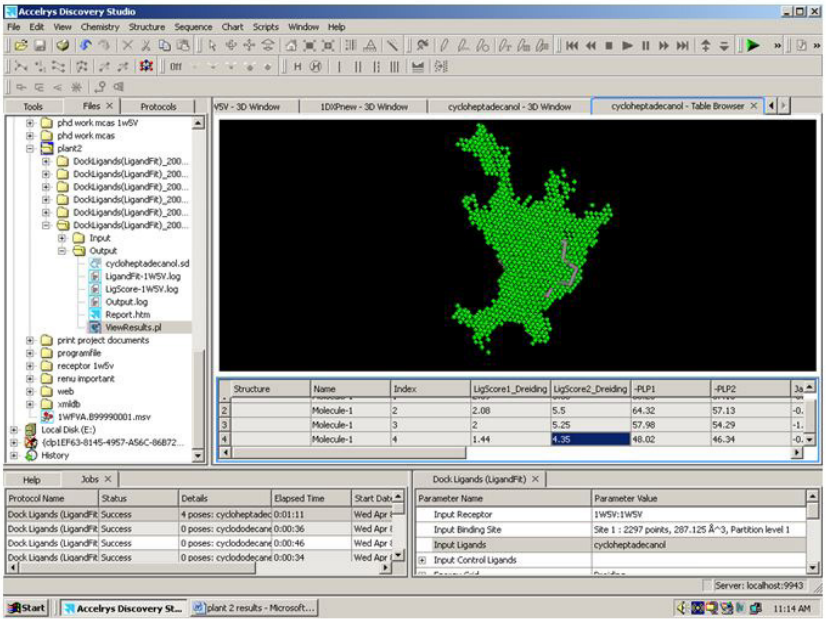


Figure 3: Structure of Cycloheptadecanol (second hit compound from *Enicostemma axillare*)



	_Dreiding	-PLP1	-PLP2	Jain	-PMF	LigScore Warning	DOCK_SCORE	LF
1		63.81	59.91	-0.69	60.88	unfilled valencies:...	59.106	0
2		47.14	45.56	-1.2	75.2	unfilled valencies:...	58.496	0
3		60.66	54.68	-0.56	59.01	unfilled valencies:...	56.833	0
4		51.7	51.52	-1.09	68.1	unfilled valencies:...	53.288	0

Figure 3a: Docked view of cyclo hepta decanol (second hit compound from *Enicostemma axillare*) with HIV receptor (1w5v)

Shailza Singh *et al.* (2007) [20] studied the chemokine receptor CXCR4 which is the receptor for several chemokines and major co-receptor for X4 Human Immunodeficiency Virus type-1 strains entry into cell.

Subsequently, Harnett *et al.* (2005) [21] screened the extracts made from Sutherlandia frutescens (L.) R. Br (Fabaceae) and *Lobostemon trigonus* (Boraginaceae) as identified by the Botany Department, University of Port Elizabeth to detect if any of the extracts inhibited the human immunodeficiency virus (HIV).

Conclusion

Viral diseases, such as acquired immunodeficiency syndrome (AIDS), respiratory viral diseases and hepatitis, are the leading causes of death in humans worldwide, despite the tremendous progress in human health care and medicine. The lack of effective therapies and/or vaccines for several viral infections and the rapid emergence of new drug-resistant viruses have urged a growing need for developing new and effective chemotherapeutic agents to treat viral diseases. Recent advances in the understanding of both the cellular and molecular mechanisms of virus replication have provided the basis for novel therapeutic strategies. Several hundred natural products have been isolated for screening and identifying antiviral activity and some have been shown to have great medicinal value in preventing and/or ameliorating viral diseases in preclinical and clinical trials. There are innumerable potentially useful medicinal plants and herbs waiting to be evaluated and exploited for therapeutic applications against genetically and functionally diverse virus families.

These natural active compounds, which contain more characteristics of high chemical diversity and biochemical specificity than standard combinatorial chemistry, offer major opportunities for finding novel lead structures that are active against a wide range of assay targets. In addition, natural products that are biologically active in assays are generally small molecules with drug-like properties capable of being absorbed and metabolized by the body. Hence, the development costs of producing orally active medicines are likely to be much lower than that of biotechnological products or most compounds produced to date from combinatorial chemistry. Therefore, natural products, including traditional medicinal plants (herbs), offer great promise as potentially effective new antiviral drugs.

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