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Analysis of phytocomponents in the methanolic extract of *Eupatorium triplinerve by GC-MS method*

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Abstract

Objective: To characterize the phytochemical constituents of *Eupatorium triplinerve* using GC – MS and to study the ability of the metabolites to serve as an antagonist to caspase 3 receptors to ascertain its anticancer properties.

Methods: Ten grams of the powdered sample was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethanol and methanol respectively. n-Hexane and Chloroform did not elute much of the compounds. The methanol fraction of the *Eupatorium triplinerve* was taken for GC-MS analysis. The analysis was carried out on a GC Clarus 500 GC system with a column packed with Elite – 1 (10% dimethyl poly siloxane, 30 x 0.25 mm ID x 1 EM df), the compounds are separated using with Helium as carrier gas at a constant flow 1ml/min. sample extract (2 µL) injected into the instrument was detected by Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. The important compounds obtained from GC-MS were further studied *in silico* to study its anticancer activity by docking with caspase 3 receptor of four important metabolites neophytadiene, nitrocyclohexane, octadecane and tetradecanoic acid.

Results: The GC MS analysis provided peaks of eleven different phytochemical compounds namely hexadecanoic acid (14.65%), 2,6,10-trimethyl,14-ethylene-14-pentadecne (9.84%), Bicyclo[4.1.0]heptane, 7-butyl- (2.38%), Decanoic acid, 8-methyl-, methyl ester (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1,14-tetradecanediol (6.78%), Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (19.18%) and 2-hydroxy-3-[(9E) -9-octadecenoyloxy] propyl(9E)-9-octadecenoate (8.79%). From the docking assay it was found that nitrocyclohexane and neophytadiene compounds exhibited good docking score.

Conclusion: The bioactive compounds in the methanolic extract of *Eupatorium triplinerve* have been screened using this analysis. Isolation of individual components would however, help to find new drugs.

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INTRODUCTION

In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the World Health Organization (WHO) states that around 85 – 90% of the world's population consumes traditional herbal medicines (1). Plants are capable of synthesizing an

overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors and fragrances.

Eupatorium triplinerve Vahl is familiarly known as Ayappana belongs to Asteraceae family. It is a slender herb with narrow lanceolate leaves and large number of pedicelled flower-heads at the top of the branch. The methanolic extract of *E triplinerve* is reported to have hepatoprotective effect and antioxidant effect against carbon tetracholoride induced hepatotoxicity in rats (2), while the ethanolic extract had analgesic effect in inflammatory model of pain (3), antibacterial and antifungal activity (4), antiseptic and in the treatment of various ulcers and haemorrhages (5). Although the plant is used in Ayurvedic medicine for the treatment of ailments there are no reports on the constituents that are responsible for the therapeutic effect. With this background the present study was aimed to identify the phytoconstituents present in *E triplinerve* using GC-MS analysis.

The anticancer activity of the metabolites obtained as a result of GC-MS analysis were analyzed by docking with caspase3 receptor which is responsible for apoptosis. The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein cleaves and activates caspases 6 and 7; and the protein itself is processed and activated by caspases 8, 9, and 10 (6).

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh plants of *E. triplinerve* were collected from the natural habitats of Tiruchirappalli, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and other adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Plant sample extraction

Plant sample extraction and Column chromatography

Ten grams of powdered sample was extracted with 50 mL methanol overnight and filtered through ash less filter paper with sodium sulphate (2 g). The crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethanol and methanol respectively. n-Hexane and Chloroform did not elute much of the compounds. The methanol fraction of the *Eupatorium triplinerve* was taken for GC-MS analysis.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS analysis was carried out on a GC Clarus 500 Perlin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrophotometer (GC – MS) instrument employing the following conditions: column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1 injector temperature 250 ^{o}C; ion-source temperature 280 ^{o}C. The oven temperature was programmed from 110 ^{o}C (isothermal for 2 min). with an increase of 10 C/min, to 200 ^{o}C then 5 ^{o}C/min to 280 ^{o}C, ending with a 9 min isothermal at 280 ^{o}C. Mass spectra were taken at

70 eV; a scan interval of 0.5s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Molecular docking

Preparation of ligands

The secondary metabolites obtained from GC-MS analysis were used for docking. The two-dimensional structures of the ligands were generated using the ACD/ChemSketch tool. The data are converted and saved in mol format and is used for docking analysis.

Protein Data Bank (PDB)

Source: www.rcsb.org

The PDB is the single, global archive for information about the 3D structure of biomacromolecules and their complexes, as determined by X-ray crystallography, NMR spectroscopy and cryo-electron microscopy. Caspase 3 receptor was downloaded from Protein data bank with the specific resolution and the PDB id is 2X70.

Docking

Auto-dock (modeling and simulation application was used for the study which focuses on optimizing the drug discovery process. The mechanism for ligand placement is based on fitting points. Fitting points are added to hydrogen bonding groups on the protein and ligand. A molecular mechanics like scoring function which includes terms of hydrogen bonds is employed by DS to rank the docked posses. The docking algorithm was also accessed in order to know the binding sites and the number of rotatable bonds of the ligand.

RESULTS

The identified compounds of the leaves of *E triplinerve*, their retention indices, percentage composition, chemical structure and activities are given in Table 1. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results showed the presence of hexadecanoic acid (14.65%), 2,6,10-trimethyl, 14-ethylene-14-pentadecne (9.84%), bicycle[4.1.0]heptanes (2.38%), decanoic acid (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1,14-tetradecanediol (6.78%), octadecanoic acid (19.18%) and 2-hydroxy-3-[(9E)-9-octadecenoyloxy]propyl(9E)-9-octadecenoate (8.79%). The spectrum profile of GC-MS confirmed the presence of 10 major components with retention time15.084, 15.75, 16.2,16.40,16.96,17.15,18.38,19.986,20.148 and 21.619 respectively (Figure 1). The individual fragmentation of the components is illustrated in (Figures 2A-2J).

The 3D structure of compounds namely Neophytadine, Nitrocyclohexane, Octadecane and Tetadecanoic acid which were present in notable amounts in the extract were docked with Caspase 3 receptor. The results of interaction with these compounds are shown in Fig 3. All amino acid residues are displayed as ball and stick and the receptor as a ribbon. The interaction between them is given in table 2.

DISCUSSION

In the present study, the GC-MS analysis of the methanolic extract of *E triplinerve* showed the presence of ten compounds. In terms of percentage amounts hexadecanoic acid, tetradecanoic acid and octadecanoic acid were predominant in the extract. These three major compounds have all shown to have

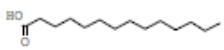
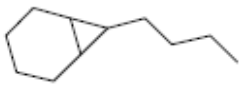
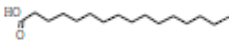
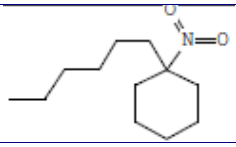
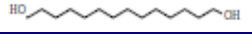
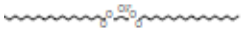
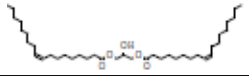
hypocholesterolemic activity, antioxidant and lubricating activity. Anticancer and antiproliferative are shown by tetradecanoic acid and 2,6,10,-trimethyl,14-ethylen-14-pentadecne, while 1-hexyl-1-nitrocyclohexane and 1,14-tetradecanediol other compounds show antimicrobial and anti-inflammatory activities.

There is growing awareness in correlating the phytochemical components and their biological activities [7,8,9]. *E triplinerve* is a plant used in Ayurvedic medicine however there are no reports on the thorough phytochemical analysis of the plant. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities.

Docking is the process of fitting together of two molecules in 3-dimensional space. Docking allows the scientist to virtually screen a database of compounds and predicts the strongest binders based on various scoring function. It explores ways in which two molecules such as drug and receptor together and dock to each other well. The molecules binding to a receptor, inhibits function, and acts as a drug [10]. Verlinde and Hol, [11], suggested that when a drug binds to a target in molecular modeling and molecular design software, the lower the energy value the higher is the affinity of the drug. Keeping this in this view, it is clear from the results (table-4) that nitrocyclohexane and neophytadiene have a higher affinity towards the receptor, since they produce a lower energy value while interacting with the receptor.

Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

Table 1: Phytocomponents identified in the methanolic leaf extract of *Eupatorium triplinerve* by GC-MS

No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structure	Nature of compound	Activity
1	15.084	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	14.65		Fatty acid	Antioxidant, cancer preventive, nematicide, hypercholesterolemic, Lubricant
2	15.75	2,6,10-trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278	9.84		Olefins	Antiproliferative
3	16.20	Bicyclo[4.1.0]heptane, 7-butyl-	C_7H_{12}	96.170	2.3		Alkane	Activity not known
4	16.401	Decanoic acid, 8-methyl-methyl ester	$C_{10}H_{22}O_{11}$	172.26	3.86		Fatty acid	Flavor Nematicide Pesticide
5	16.96	1-undecanol	$C_{11}H_{24}O$	172.30	7.82		Fatty alcohol	Flavor, perfumery
6	17.15	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	24.61		Fatty acid	Antioxidant, hypocholesterolemic, nematicide, hemolytic, 5-alpha reductase inhibitor
7	18.38	1-hexyl-1-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.31	2.09		Ketone	Antioxidant, antimicrobial, anti-inflammatory
8	19.986	1,14-tetradecanediol	$C_{14}H_{30}O_2$	230.39	6.78		Alcoholic	Antimicrobial
9	20.148	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{18}H_{34}O_2$	282.46	19.18		Fatty acid	Hypocholesterolemic, antiarthritic, nematicide, 5-alpha reductase inhibitor, antiacne, hepatoprotective
10	21.619	2-hydroxy-3-[(9E) -9-octadecenoyloxy]propyl(9E)-9-octadecenoate	$C_{39}H_{72}O_5$	620.98	8.79		Ester	No activity reported

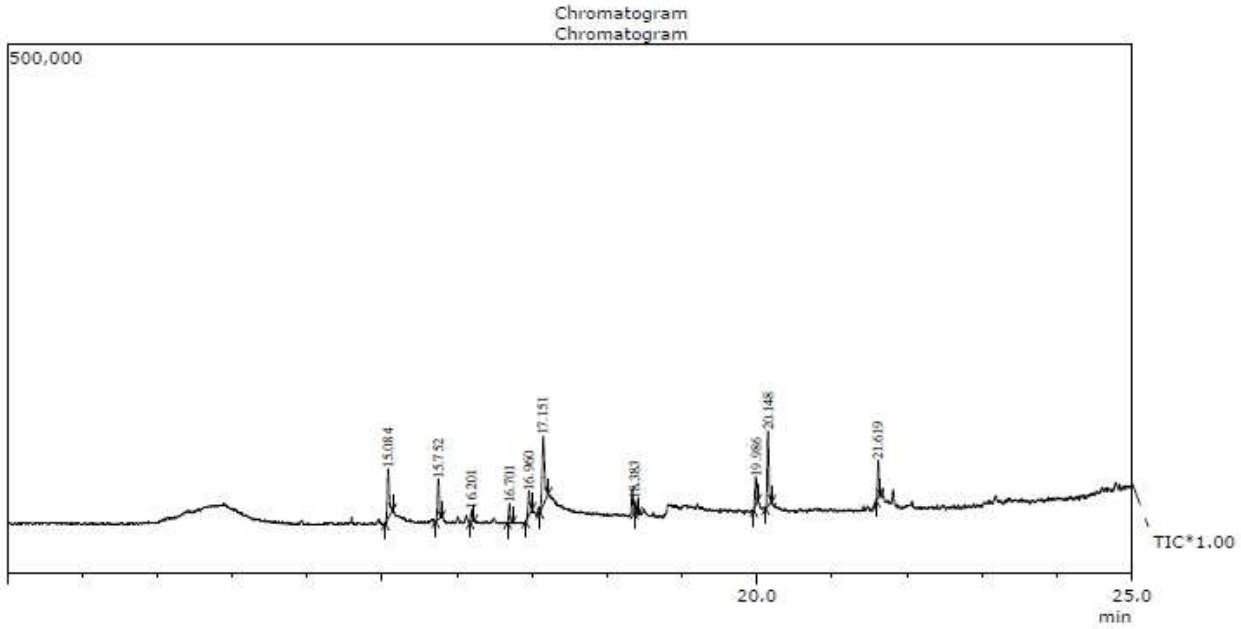


Fig 1: GC-MS Chromatogram of methanolic extract of *Eupatorium triplinerve*

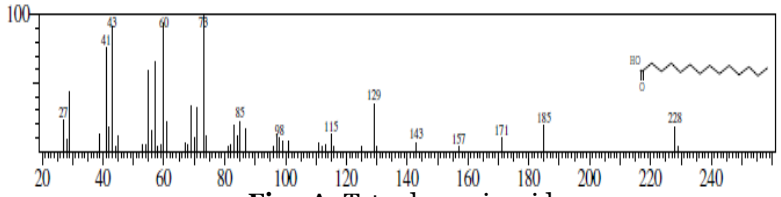


Fig 2A: Tetradecanoic acid

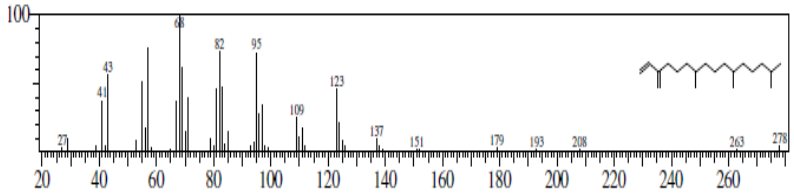


Fig 2B: 2,6,10-trimethyl,14-ethylene-14-pentadecne

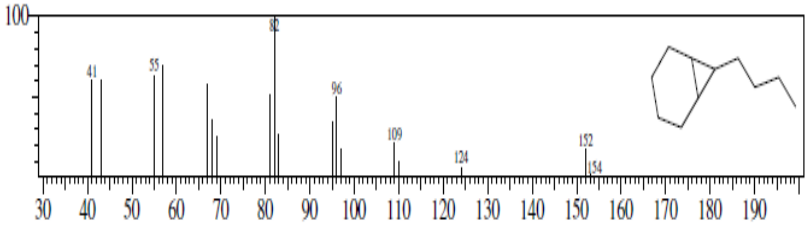


Fig 2C: Bicyclo[4.1.0]heptane, 7-butyl-

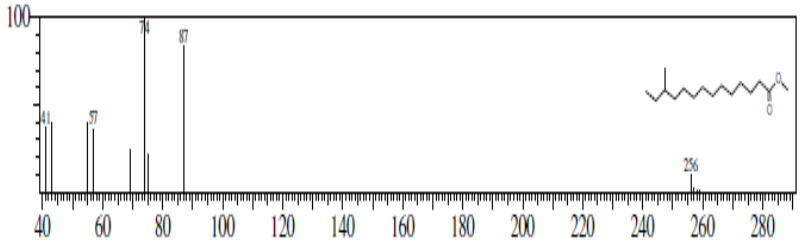


Fig 2D: Decanoic acid, 8-methyl-, methyl ester

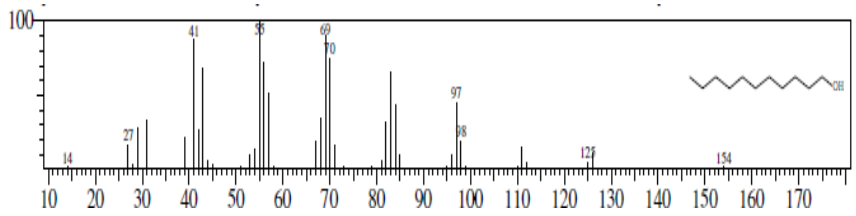


Fig 2E: 1-undecanol

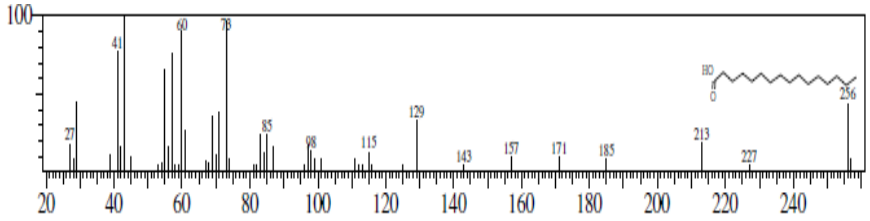


Fig 2F: Hexadecanoic acid

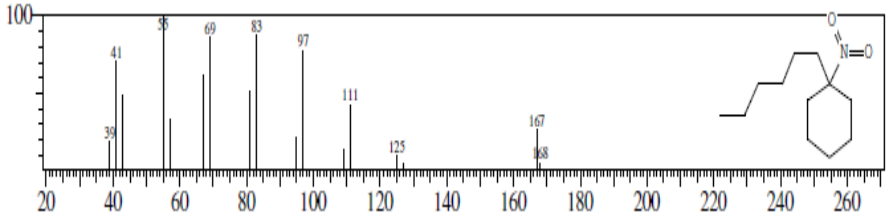


Fig2G: 1-hexyl-1-nitrocyclohexane

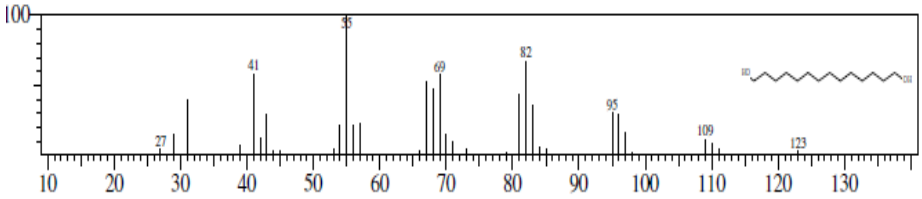


Fig 2H: 1,14-tetradecanediol

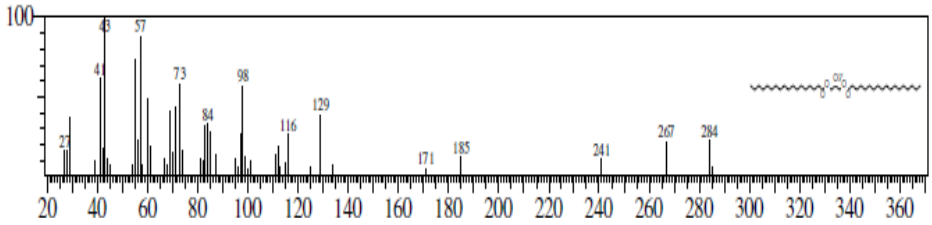


Fig 2I: Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester

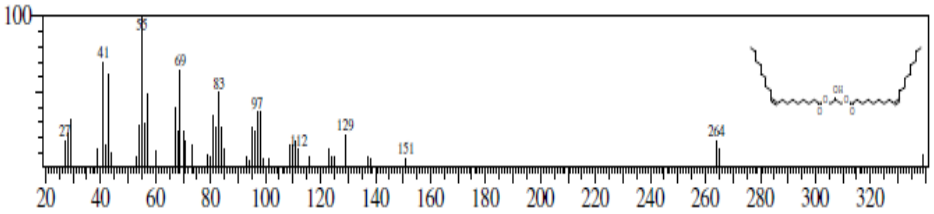
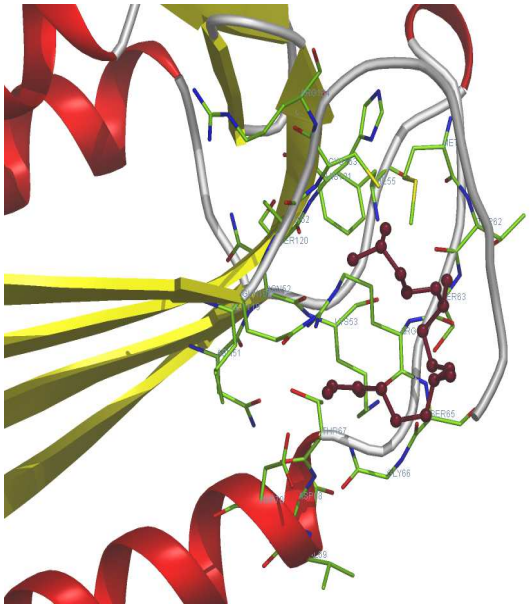


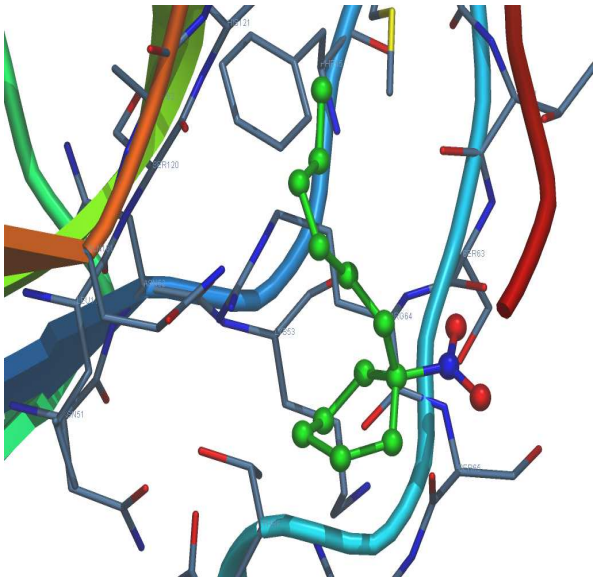
Fig 2J: 2-hydroxy-3-[(9E) -9-octadecenoyloxy]propyl(9E)-9-octadecenoate

Table 2: Docking score of docking between caspase 3 receptor with various inhibitors

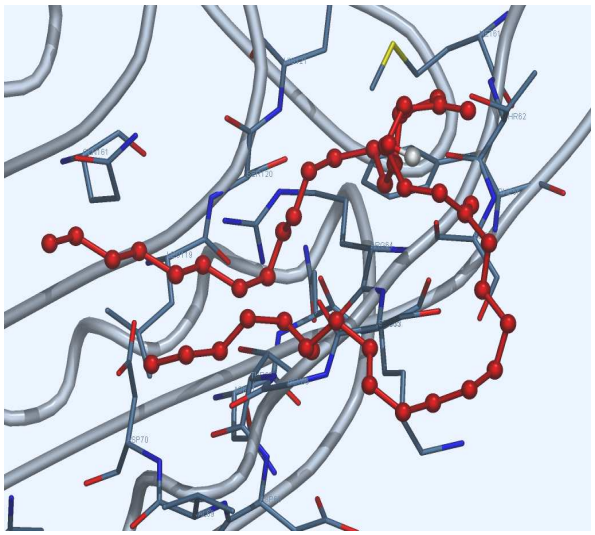
Ligand	Docking score (kcal/Mol)
Neophytadiene	-2.32 kcal/mol
Nitrocyclohexane	-3.07 kcal/mol
Octadecane	+2.03 kcal/mol
Tetradecanoic acid	-1.82 kcal/mol



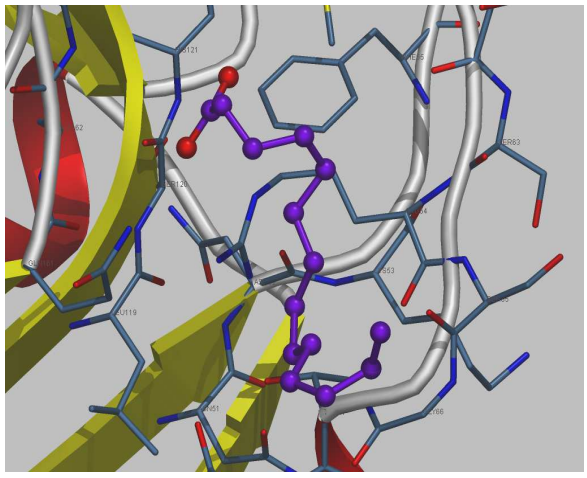
A Neophytadine



B Nitrocyclohexane



C Octadecane



D Tetadecanoic acid

Figure 3: Interaction of drug-receptor complexes. The docked complexes are A- Neophytadiene, B- Nitrocyclohexane, C- Octadecane and D- Tetradecanoic acid

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