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Molecular Docking Studies of *Indigofera Tirunelvelica* Sanjappa against *Hepatocarcinomo* Receptors

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Abstract

Objective: Hepatocarcinomo keeps on being an overall executioner, in spite of the huge measure of research and quick advancements seen amid the previous decade. Since it is normally trusted that many are preventable, there is dire need to recognize regular meds as viable hepatoprotective specialists. Normal items recognized and disengaged from plants have assumed an imperative job in disclosure of medications against liver infections. **Methods:** *In silico* docking systems are being utilized to explore the correlatively at the sub-atomic dimension of a ligand and a protein target. In the present investigation, four ligands which have been disengaged and distinguished from the ethanolic concentrate of the entire plant of *Indigofera tirunelvelica* Sanjappa are docked with two novel hepatocacrcinomo receptors, Hepatitis B X and Heme Oxygenase I. **Results:** Out of the four phytochemical constituents separated and distinguished from the ethanolic concentrate of the entire plant of *Indigofera tirunelvelica*, phytol ligand uncovered the best wellness score contrasted and the other three ligands. **Conclusion:** This present examination induced that phytol could be a viable potential inhibitor against Hepatitis B X and Heme Oxygenase I receptor and could be assessed as hepatoprotective medication particle.

Keywords
Indigofera tirunelvelica, *In silico*, Autodock, Hepatocacrcinomo receptors

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INTRODUCTION

The liver plays a bewildering exhibit of fundamental capacities in the support, execution and controlling homeostasis of the body. It has extraordinary ability to detoxicate dangerous substances and integrate helpful standards [1]. Liver sicknesses stay one of the significant dangers to general wellbeing and are an overall issue [2]. They are mostly caused by synthetic substances like acetaminophen, overabundance utilization of liquor, diseases and immune system issue. The vast majority of the hepatotoxic synthetic compounds harm liver cells for the most part by

actuating lipid peroxidation and other oxidative harms [3]. Tragically, customary or engineered drugs utilized in the treatment of liver illness are deficient and now and again cause genuine symptoms. Along these lines, it is important to scan for elective medications for the treatment of liver infection with more viability and security and to enhance, enlarge, or supplant as of now utilized medications [4]. Hence, look for more current medications with least symptoms acquired from conventional prescriptions proceeds. Logical investigations accessible on therapeutic plants demonstrate that promising phytochemicals can be produced for some medical issues [5]. Computational Biology and Bioinformatics have the potential not just of accelerating the medication disclosure process yet decreases the expenses, and furthermore of changes the manner in which drugs are structured. One such technique is the docking of the medication particle with the objective receptor [6]. Sub-atomic docking ponders are utilized to decide the collaboration of two particles and to locate the best introduction of ligand which would frame a complex with generally least vitality. The little particle, known as ligand normally fits inside protein's pit which is anticipated by the pursuit calculation. These protein depressions end up dynamic when interacted with any outer mixes and are in this way called as dynamic destinations. In the present examination sub-atomic docking investigations of the four phytochemical constituents segregated and distinguished from the ethanolic concentrate of the entire plant of *Indigofera tirunelvelica* Sanjappa have been completed utilizing two novel target receptors of hepatocarcinoma, Hepatitis B X and Heme Oxygenase I by utilizing the Autodock.

MATERIALS AND METHODS

Collection of Plant Materials:

The whole plant of *Indigofera tirunelvelica* Sanjappa was collected from Sathankulam (Formerly this place was in Tirunelveli District), of Thoothukudi district of Tamil Nadu, India. This plant was identified and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. A voucher specimen (BSI/SRC/5/23/ 18/Tech) has been deposited at Herbarium of Botanical Survey of India, Coimbatore.

Instruments and Chromatographic Conditions:

GC-MS analysis of the extracts was carried out on a GCMS Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 mm x 0.2 5mm ID x 1 µMdf, composed of 100 % Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99. 999 %) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1); injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

Identification of Photochemical Constituents:

Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The mass spectrum of the unknown component was compared with that of the known components stored in the NIST library. The name, molecular weight and structure of the four phytochemical constituents 2-Methyl-Z, Z-3,13-octadecadienol, Phytol, Heptadecenal and Hexadecanoic acid isolated and identified from the ethanolic extract of whole plant of *Indigofera tirunelvelica* Sanjappa were ascertained by GC-MS analysis [7] and are presented in Table.1.

Potential Targets and Binding Site:

The 3D structures of hepatic cancer potential drug targets such as Hepatitis B X (317H), Heme Oxygenase I (1N3U) receptors were retrieved from PDB database [8]. The active sites in these receptors were determined based on the ligands in the crystallized structures. The interactions and the affinities between the phytochemical constituents and receptor were predicted by using Autodock docking program [9].

Ligand Generation:

The 2D structures of phytochemical constituents from the ethanolic extract of whole plant of *Indigofera tirunelvelica* Sanjappa were drawn in ACD-Chemsketch[10] and their SMILES notations were obtained. The 3D structures were obtained and converted into SDF files by using 'Online SMILES convertor and Structure file generator' server [11].

Molecular docking

In silico study was carried out using autodock vina 1.1.2 software. A computational ligand target docking approach was to analyze the structural complex of the 2-Methyl-Z, Z-3,13-octadecadienol, Phytol, Heptadecenal and Hexadecanoic acid (ligand) with the target (Hepatitis B X (317H) and Heme Oxygenase I (1N3U) receptors) in order to understand the specificity of the protein targets. During the selection of the ligand Lipinski's rule 5 was applied. Lipinski's rule 5 was essential to pharmacological industries to increase the activity

and selection of ligand as well as drug like properties. Grid: a computational principle for determining actively favorable binding site on molecules of identified structure. A scoring grid was computed using the ligand structure to reduce the computation time. ADMET helps us to know the toxicity bases [Computational Resource for Drug Discovery].

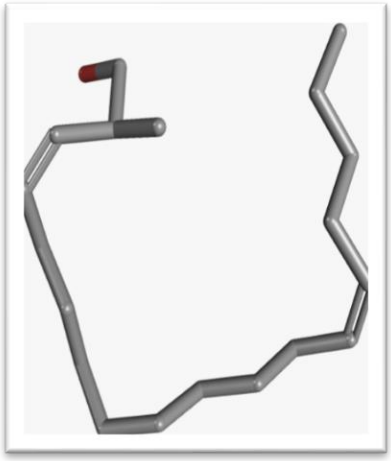
Prediction of Ligand- Receptor Interactions:

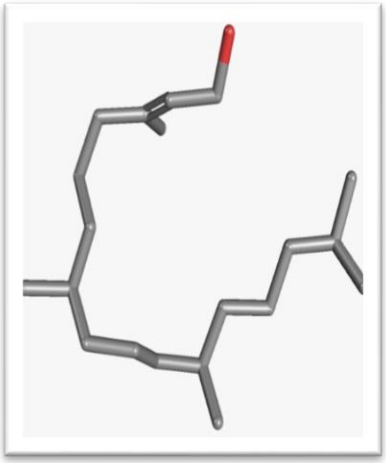
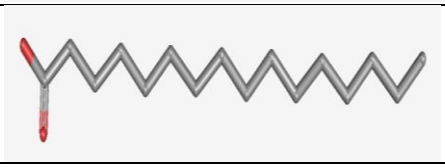
The interactions between the four phytochemical constituents isolated and identified from the ethanolic extract of whole plant of *Indigofera tirunelvelica* Sanjappa, and the two novel receptors as docked complexes were analyzed by the Pymol[12].

RESULTS AND DISCUSSION

Differing homeostatic systems are influenced if liver capacity is debilitated, with conceivably genuine outcomes. Around 20, 000 passings happen each year because of liver sicknesses. Hepatocellular carcinoma (HCC) is one of the ten most basic tumors on the planet with more than 2, 50,000 new cases every year. HCC all the more frequently emerges on infection prompted liver cirrhosis, hence delineating a model of illness movement from unending irritation to malignancy and permitting structure of new methodologies focusing on key focuses at each progression of the ailment. In this manner in the present examination two novel receptors, Hepatitis B X and Heme Oxygenase I were chosen as a potential medication focuses of Hepatocellular carcinoma. 3D structures of Hepatitis B X and Heme Oxygenase I were resolved and the atomic docking investigations of the four phytochemical constituents disengaged and recognized from the ethanolic concentrate of entire plant of *Indigofera tirunelvelica* have been performed. The receptors, Hepatitis B X and Heme Oxygenase I were considered as the potential medication focuses of HCC and their 3D structures were recovered from Protein Databank (Figure 1) and their coupling locales were resolved. The Docking program, from Autodock was utilized to determine restricting surface of the receptors and the phytochemical constituents in SDF arrange. The docking was completed with the span of 6.5 A0 at the site of docking. 3D structures of the four phytochemical constituents separated and distinguished from the ethanolic concentrate of entire plant of *Indigofera tirunelvelica* are displayed in Table. 1.

Table 1: Molecular formula, Molecular weight and 3D Structures of the four phytochemical constituents isolated and identified from the ethanolic extract of whole plant of *Indigofera tirunelvelica* Sanjappa.

S. No.	Ligands	Molecular Formula and Weight	3D Structure
1.	2-Methyl-Z, Z-3,13-octadecadienol	Molecular Formula:$C_{19}H_{36}O$ Molecular Weight: 280.496g/mol	

2.	Phytol	Molecular Formula: $C_{20}H_{40}O$ Molecular Weight: 296.539g/mol	
3.	Heptadecenal	Molecular Formula: $C_{17}H_{32}O$ Molecular Weight: 252.442g/mol	
4.	Hexadecanoic acid	Molecular Formula: $C_{16}H_{32}O_2$ Molecular Weight: 256.43 g/mol	

The docking communications between the coupling site amino acids of Hepatitis B X and Heme Oxygenase I and the four ligand particles are exhibited in Table. 2. Phytol is observed to be a best docking ligand for both the Hepatitis B X and Heme Oxygenase I (Figure 2 and 3). The aftereffects of hydrogen holding and hydrophobic collaborations of ligand particles with Hepatitis B X and Heme Oxygenase I are exhibited in Table. 3.

Table 2: Docking score of the four phytochemical constituents isolated and identified from the ethanol extract of from the ethanolic extract of whole plant of *Indigofera tirunelvelica* with Hepatitis B X and Heme oxygenase I.

S.NO	Ligands	Binding Energy	
		Hepatitis B X	Heme Oxygenase I
1	2-Methyl-Z, Z-3,13-octadecadienol	-4.96	-4.45
2	Phytol	-6.16	-5.97
3	Heptadecenal	-5.4	-4.5
4	Hexadecanoic acid	-4.73	-5.31

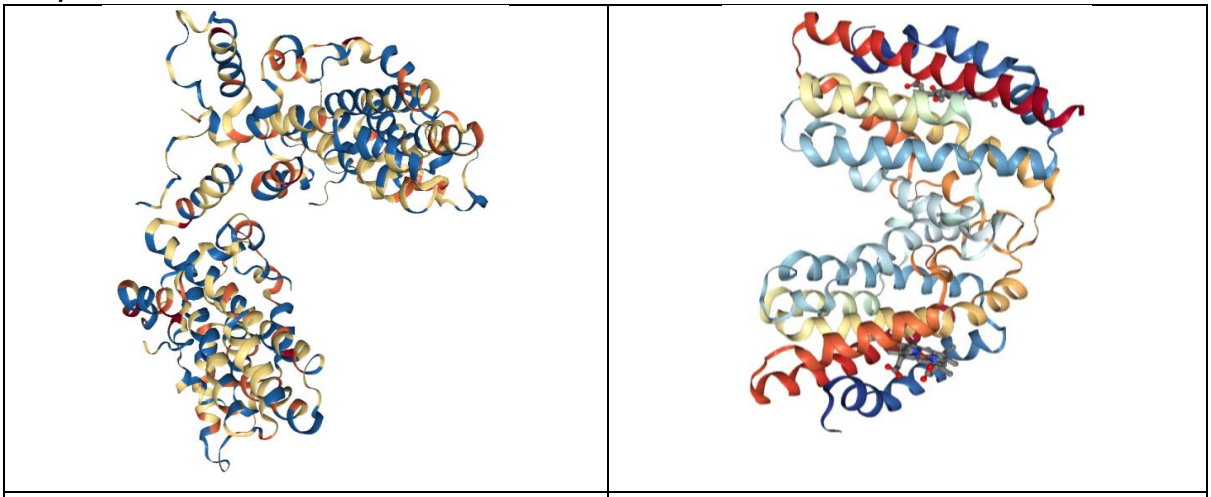
Table 3: Ligand Efficiency, Hydrogen bonding and Hydrogen bond distance of the four phytochemical constituents isolated and identified from the ethanolic extract of whole plant of *Indigofera tirunelvelica* with Hepatitis B X and Heme Oxygenase I.

S.NO	Phyto constituent	Ligand Efficiency		Hydrogen bond		H-Bond Distance	
		Hepatitis B X	Heme Oxygenase I	Hepatitis B X	Heme Oxygenase I	Hepatitis B X	Heme Oxygenase I
1	2-Methyl-Z, Z-3,13-octadecadienol	-4.96	-0.22	:2-Methyl-Z, Z-3,13-octadecadienol:H56 - D:GLN57:O	:2_Methyl_octadecadienol:H56 - B:GLU29:OE1	2.20	2.000810

S.NO	Phyto constituent	Ligand Efficiency		Hydrogen bond		H-Bond Distance	
		Hepatitis B X	Heme Oxygenase I	Hepatitis B X	Heme Oxygenase I	Hepatitis B X	Heme Oxygenase I
2	Phytol	-6.16	-0.25	: Phytol:H61 C:GLN99:OE1	B: ARG136:NH2 - :Phytol:O1 : Phytol:H61 - B:THR135:O : Phytol:H61 - B:ARG136:O	2.18	3.10 2.12 2.57
3	Heptadecenal	-5.4	-0.25	Only Hydrophobic interactions	B: SER142:OG - :Heptadecenal:O1	-	3.04
4	Hexadecanoic acid	-4.73	-0.33	:Hexadecanoic acid:H50 - D:GLU64:OE1 C:LYS96:NZ - :Hexadecanoic acid:O1	B:ARG183:HE - :Hexadecanoic acid:O2 :Hexadecanoic acid:C17 - B:HIS25	1.92 2.65	3.01 3.68

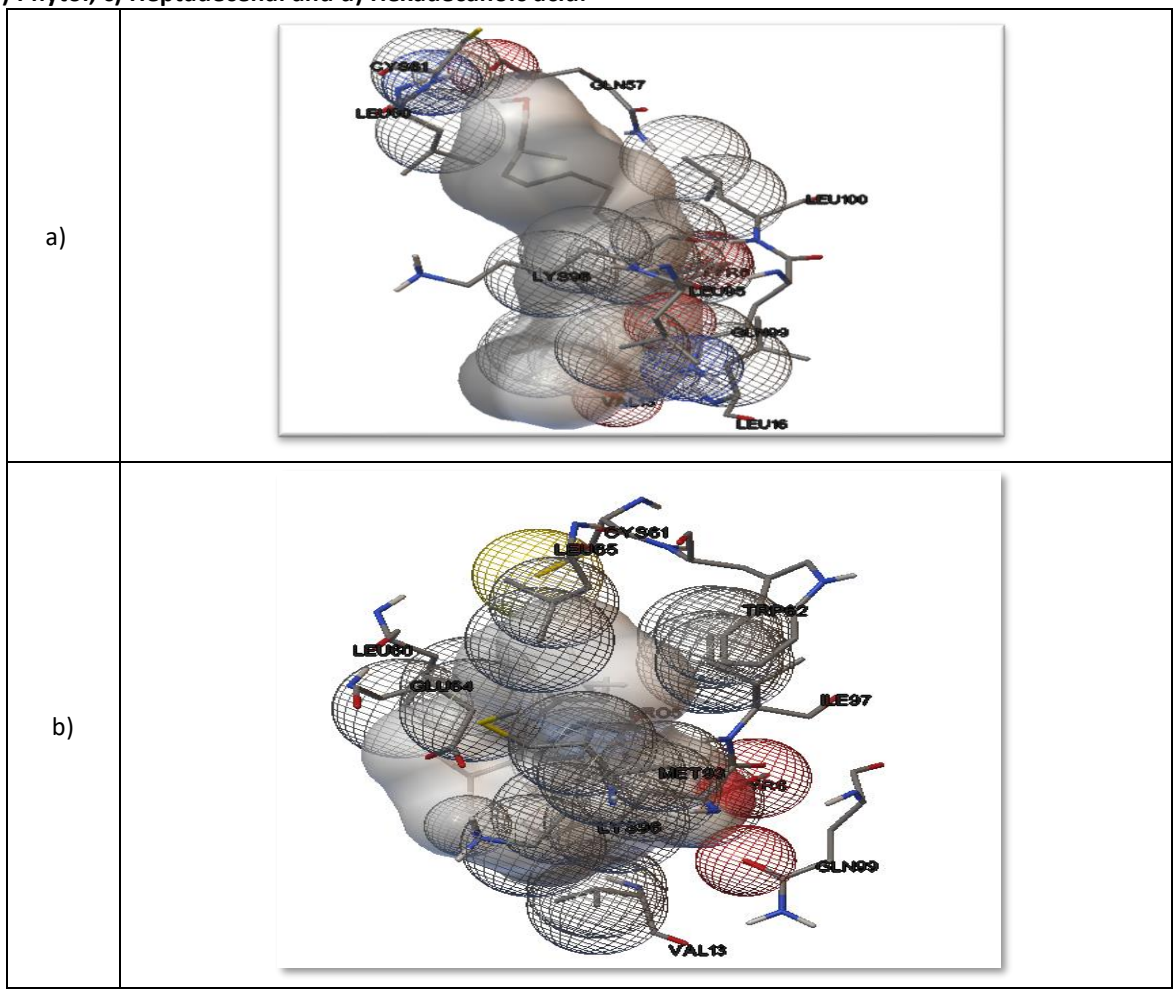
Hepatitis B X assumes a fundamental job in hepatitis contamination and a requirement for Hepatitis B X focusing on medications rendered it as an objective for our study [13]. The most noteworthy docking collaborations score was watched for Phytol (- - 6.16 kJ/mol) with the Hepatitis B X receptor. The mixes, 2-Methyl-Z, Z-3,13-octadecadienol, Heptadecenal and Hexadecanoic corrosive display less authoritative inside Hepatitis B X dynamic site. The best docking associations of phytol is supported by the arrangement of hydrogen bond with His61 and Gln99. The authoritative of outstanding phytochemical constituents which showed the docking score extending from - 5.4 kJ/mol to - 4.73 kJ/mol. Table 2: Docking score of the four phytochemical constituents disconnected and recognized from the ethanolic concentrate of entire plant of *Indigofera tirunelvelica* with Hepatitis B X and Heme oxygenase I. It is seen that the NH gathering of the amino corrosive and the carbonyl gathering present in the phyto substance constituents support the hydrogen bond communications. The discoveries visualize that amid the plan of novel hepatoprotective exacerbates, the preserved amino acids, histidine (H) and Glutamine (N)) are to be considered for upgrading the hepatoprotective action of the phyto compound constituents against Hepatitis B X. The most astounding docking communications score (- 5.97 kJ/mol) was watched for Phytol with the Heme Oxygenase I receptor. Other three ligands exhibit less restricting connection inside the Heme Oxygenase I dynamic site. The best docking communications of Phytol is supported by the arrangement of hydrogen security with ARG136, H61 and THR135. It is seen that the NH gathering of the amino corrosive and the carbonyl gathering present in the phytochemical constituents support the hydrogen bond communications. The discoveries visualize that amid the plan of novel hepatoprotective exacerbates, the rationed amino acids must be considered for improving the hepatoprotective movement of the phytochemical constituents against Heme Oxygenase I. Heme Oxygenase items, the acceptance of this chemical or its synergist action by either common or manufactured mixes may speak to a powerful procedure to intercede in liver carcinogenesis and other hepatic disorders[14].

Figure 1: 3D view of Target Proteins – (a) Hepatitis B (1QGT) Receptors and (b) Heme Oxygenase I (1N3U) Receptors



(a)	(b)

Figure. 2: Hydrogen bonding of phytochemical constituents isolated and identified from the ethanolic extract of whole plant of *Indigofera tirunelvelica* with Hepatitis B X. a) 2-Methyl-Z, Z-3,13-octadecadienol, b) Phytol, c) Heptadecenal and d) Hexadecanoic acid.



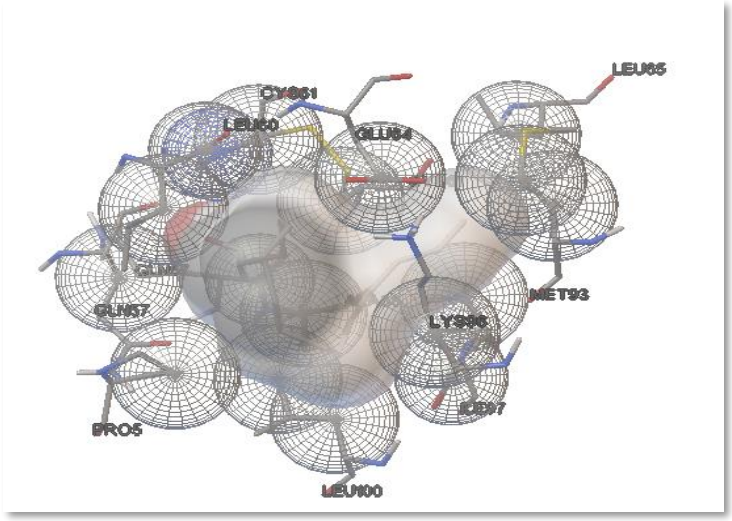
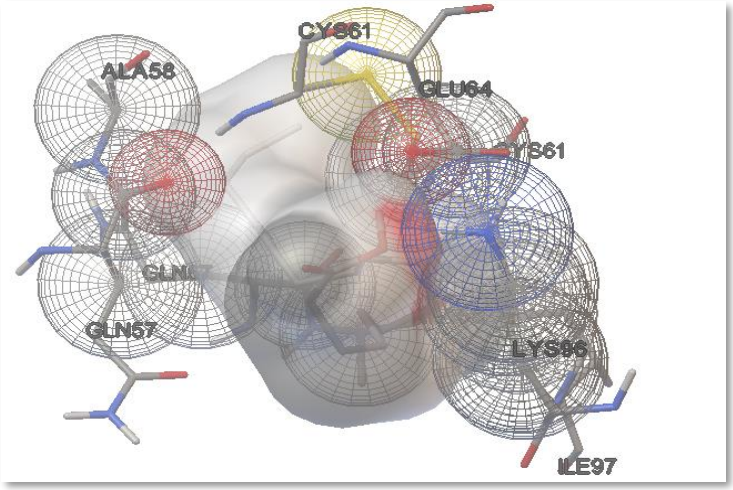
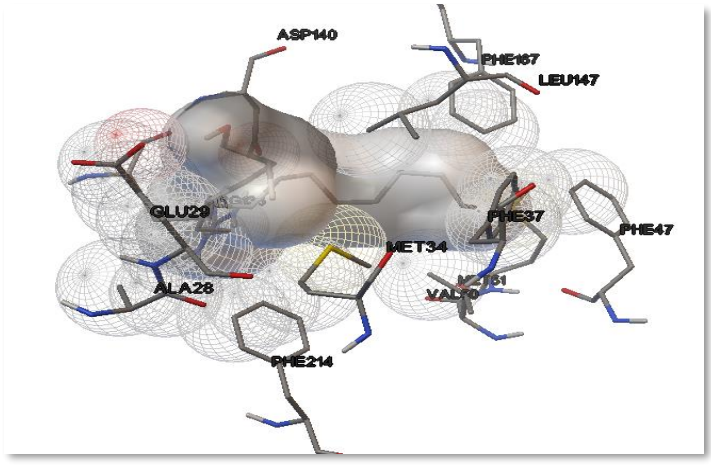
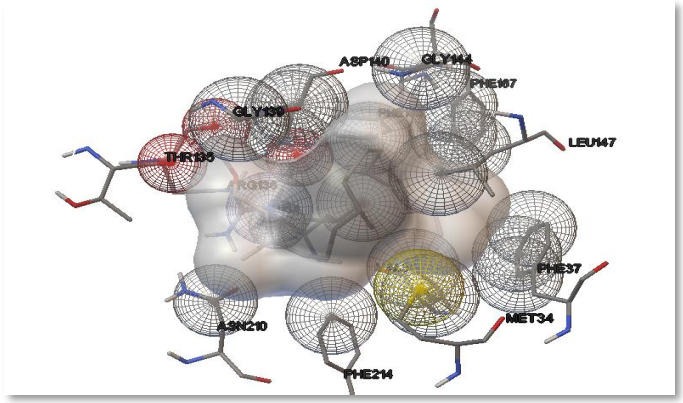
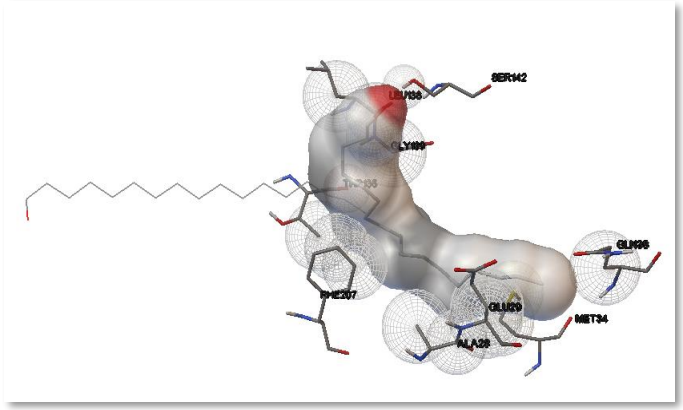
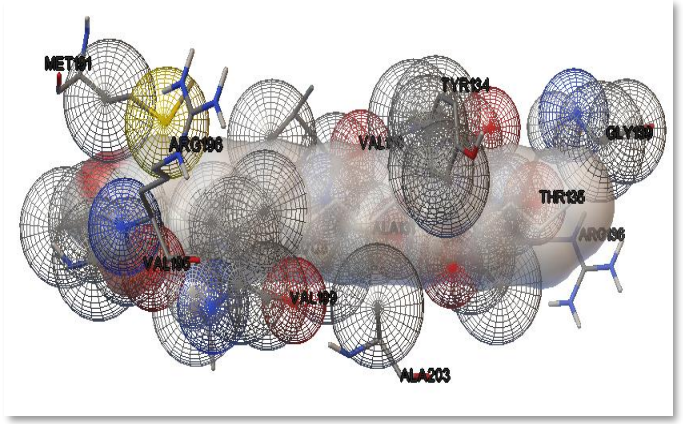
c)	
d)	

Figure. 3: Hydrogen bonding of phyto chemical constituents isolated and identified from the ethanolic extract of whole plant of *Indigofera tirunelvelica* with Heme Oxygenase I a) 2-Methyl-Z, Z-3,13-octadecadienol, b) Phytol, c) Heptadecenal and d) Hexadecanoic acid.

a)	

b)	
c)	
d)	

CONCLUSION

The advancement of novel mixes with natural action is an earnest need. The atomic docking study uncovered that the coupling introductions of the phytochemical constituents from the ethanolic concentrate of entire plant of *Indigofera tirunelvelica* Sanjappa with the dynamic site of the objective proteins, Hepatitis B X and Heme Oxygenase I. The present examination affirms that phytochemical constituents with fascinating natural properties and basic assorted variety may fill in as significant lead tranquilize possibility for the treatment of liver ailments. This investigation may give a knowledge to abuse of medications from phytochemical constituents against hepatocarcinomo receptors of various sorts in closeness to future.

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