Original Article

Anti-Oxidant Studies, GC-MS Analysis Of Phytoconstituents From Evolvulus Nummularius And Molecular Docking Interactions With Target Proteins In NAFLD.

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DOI: 10.47750/pnr.2022.13.S05.385

Abstract

Evolvulus nummularius Linn is medicinal plant, belonging to the family Convolvulaceae, also known as Round leaf bind weed reported from the tropical countries, with ethno-botanical values and is familiar for its ethno medicinal applications. It comprises of a series of phytochemicals such as oleanolic acid, ursolic acid, saponins, triterpenoids etc. The study was carried out to explore and characterize the bioactive components of methanolic extract of E. nummularius Linn. The leaves were collected, shade dried, pulverized and extracted by soxhlation with methanol as a solvent. The crude extract was analysed for secondary metabolites and characterized using Gas Chromatography – Mass Spectroscopy. Some of the compounds were analysed for their bioactivity through insilico molecular docking studies. The insilico molecular docking was performed to confirm its binding interactions against target proteins. The results have revealed the presence of many nphytoconstituents and identification of target receptors against Non Alcoholic Fatty Liver(NAFLD) and will create a way for several ailments which lead to the development of novel drugs. However, additional studies are required to achieve its exploration of bioactivity and toxicity.

Keywords: Evolvulus nummularius, GC-MS, insilico, phytoconstituents, NAFLD, receptors.

1.INTRODUCTION

NAFLD is defined as steatosis in the hepatocytes with or without necrosis andinflammation with cause other than alcohol intake. NAFLD is rapidly becoming a worldwide health problem due to high calorie intake along with a sedentary lifestyle[1,2]. Traditional drugs are gaining a massive interest than synthetic and chemical drugs as they are eco-friendly, lack of adverse effects and more natural. As the medicinal plants contain various valuable phytoconstituents[3], they show distinctive potential in the treatment and cure of different ailments. In different systems of Indian medicine, about 80000 species of medicinal plants have been used as traditional medicines for the treatment of many diseases. Various bioactive compounds exhibit stimulating properties like anticancer, anti inflammatory, anti oxidant, antibacterial, hepatoprotective[4] etc. The plant based medicines are prepared from the crude extract which comprises of the complex mixture of different phytoconstituents. These phytoconstituents have complex and unique structures that are used in treating different prolonged and contagious diseases. Many plant species possess a wide range of bioactive secondary metabolites, but only a small percentage of them were examined to provide the significant source of bioactive agents. Preliminary screening of the herbal extracts by spectrophotometric and chromatographic methods[5] provides a basic information about the chemical and pharmacological activities. For detecting the functional groups and identification of the secondary metabolites in the medicinal plant, Fourier-transform infrared (FTIR)[6] and gas chromatography-mass spectrometry (GC–MS)[7] techniques are being used now-a-days[8]. GC-MS study provides faster and effective interpretation by detecting various compounds such as alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, amino acids, steroids, esters etc., and requires only a minute volume of plant extract. The present study involves GC-MS technique for the detection and identification of phytochemical compounds present in the plant, *Evolvulus nummularius* Linn**[9]**. Due to the poor pharmacokinetic properties, many drugs have got rejected to enter into the market that may afford huge loss to the pharmaceutical companies. Computational predictive models play a vital role in the selecting the methods involved in pharmaceutical research[10], and also used in *insilico* prediction of pharmacological, pharmacokinetic and toxicological activities[11,12] . Now-a-days, the most effective and inexpensive method for testing and designing the drugs is Molecular Docking which provides the information about the drug receptor interactions for predicting the binding capacity of the ligand to the target receptors. Therefore the present study focuses on the identification of the bioactive compounds in the methanolic leaf extract of *Evolvulus nummularius* by GC-MS analysis[13].

2.MATERIALS AND METHODS:

2.1.Collection and identification of plant: The leaves of *Evolvulus nummularius* Linn were collected in and around Tirumala hills, Andhra Pradesh. The specimen of the leaves was authenticated with Reg No. 0817 by Dr. K.Madhava Chetty, Asst.Professor, Dept. of Botany, S V University, Tirupati, Andhra Pradesh.

2.2.Processing of plant material: The leaves of *Evolvulus nummularius* were washed, rinsed properly and shade dried for more than 2 weeks to dry them completely. The dried leaves were then pulverized to powder by using a blender and then sieved for fine powder. Then the sample was stored in an airtight container for sfurther use.

2.3.Physicochemical Parameters: Different physicochemical parameters like total ash, water soluble ash, acid insoluble ash, p^H and extractive values were carried as per WHO guidelines to find out the quality of the raw material. The results obtained were given in the Table 1.

2.4.Preparation of methanolic extract: The powdered plant material of 500gms is subjected to extraction[14] in soxhlet apparatus with 2000ml methanol at 35^0C for 72 hrs. Then, it was filtered using Whatman No.1 filter paper and concentrated under rotary evaporator. The extract obtained was hygroscopic, so stored in an airtight container for further studies. The extracts were formulated as suspension using 1% saline as, suspending agent. Later, the extract obtained was tested for the presence of the phytoconstituents by preliminary phytochemical screening. The extractive values were given in Table 2.

2.5.Phytochemical Screening: To find the therapeutic effects in the medicinal plants, screening of the chemical constituents is essential for useful information. Preliminary phytochemical screening was performed to find the presence or absence of certain phytochemicals. The extract obtained was subjected to various qualitative tests to identify different phytoconstituents. The results of preliminary phytochemical screening of the methanolic extract of *E.nummularius* was tabulated in Table 3.

2.6.Anti-oxidant Studies:

2.6.1.DPPH Assay: The 2,2-diphenyl, 1,1-picrylhydrazyl (DPPH) solution was prepared freshly by mixing 1.97mg of DPPH in 50ml of methanol. Serial dilution technique was used to prepare various concentrations of extracts of 50-250µg/ml[15]. The extract (sample) solution of 0.2ml was taken at different concentrations and compared with ascorbic acid (standard or positive control). The reaction mixture was shaken vigorously and then incubated at 37^0C for 60min in the darkness to subside. The purple colour appeared initially will disappear which indicates the free radical scavenging activity. The absorbances of these reaction mixtures were measured with the help of an UV Visible spectrophotometer at a wavelength of 517nm. The percentage of DPPH radical scavenging activity was calculated by the following formula:

$$\text{Scavenging activity\%} = \frac{\text{Absorbance}^{\text{control}} - \text{Absorbance}^{\text{sample}}}{\text{Absorbance}^{\text{control}}} \times 100$$

2.6.2.H_2O_2 Radical Scavenging Activity:

Hydrogen Peroxide radical scavenging activity was determined as per the method of Ruch et al.,.

Aliquots of 0.1ml of the extracts(50-250µg/ml) was taken into the eppendorf tubes and the volume was make upto 0.4ml with 50mM of phosphate buffer of p^H 7.4[16]. This is followed by the addition of 0.6ml of 2mM H_2O_2 solution. The reaction mixture was swirled and after 10min, the absorbance was measured at 230nm with Ascorbic acid as the positive control. The scavenging ability of the extract by H_2O_2 was calculated by the following equation:

$$\text{H2O2 scavenging activity \%} = [(A0 - A1)/A0] \times 100$$

A0 = Absorbance of control, A1 = Absorbance of sample.

2.7.Gas Chromatography-Mass Spectroscopy(GC-MS) Analysis:

GC-MS analysis of the methanolic leaf extract of *Evolvulus nummularius* Linn was performed by using Thermo GCTrace Version: 5.0, Thermo MS DSQ II. This had a DB 35-MS Capillary Standard non-polar column of 30mm X 0.25mm dimensions and Helium is used as a Carrier gas at 1.0ml/min. The injector was operated at a temperature of 250^0C and the temperature in the oven was programmed as 60^0C for 15 min and then increased to 280^0C for 3 min gradually[15]. GC-MS instrument was attached to Willey and NIST libraries to detect the different constituents and the comparison of peak retention time, chemical formula, molecular weight and mass spectral fragmentation patterns and the results obtained were given in Table 2.

2.8.Molecular Docking Studies:

The pathogenesis [17] of NAFLD involves a variety of receptors[18] in the progression of the disease which includes Sterol Regulatory Element Binding Protein-1c(SREBP-1c)[19,20], Carbohyrate Response Element Binding protein (ChREBP), Peroxisome Proliferator Activated Receptors[21,22]
(PPAR α, β, γ), Liver X Receptor(LXR)[23], Farsenoid X Receptor (FXR)[24] etc., These nuclear receptors have been docked against the phytoconstituents present in the plant extract that supports the present activity. Ursolic acid and Oleanolic acid

identified through GC-MS were likely to be more efficient against Non Alcoholic Fatty Liver Disease. So, the structures of the compounds were obtained from Pubchem database (https ://pubch em.ncbi.nlm.nih.gov/) as sdf file and converted into Mol, PDBQT and PDB file formats using OPEN BABEL software . For the confirmation of the study[25], optimisation of three dimensional (3D)[26] structures of phytocompounds is necessary and structure based molecular docking[27] has been performed for studying the protein ligand interactions against NAFLD[28] by using Ursodiol as a reference. The target proteins are obtained from Research Collaboratory for Structural Bioinformatics(RCSB) Protein Data Bank (https ://www.rscb.org) with the help of Autodock Software.

3.RESULTS

Table 1 Physicochemical Properties Of Evolvulus Nummularius Linn

S.No	Parameters	*Evolvulus nummularius* Linn leaf
1	Odour	Characteristic
2	Colour	Light green
3	Taste	Slightly bitter
4	Moisture %	2.2
5	Ash Content %	9.1
6	Extarctive value %	10.8
7	p^H	5.8

Table 2: Extractive Values Of The Methanolic Leaf Extract

S.No	Extract	Result
1	Yield	10.8
2	Colour	Yellowish browm
3	Nature	Semisolid

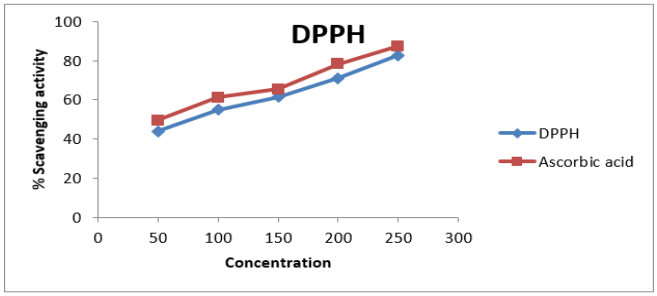
Table 3. Preliminary Phytochemical Screening Of Methanolic Leaf Extract Of *Evolvulus Nummularius*. The Marks + And – Indicate Present And Absent Respectively.

S.No	Phytochemical tests	Inference
1	Alkaloids a) Mayers test b) Wagners test	Present(+)
2	Carbohydrates a) Molisch test b) Benedicts test	Absent(-)
3	Steroids a) Salkowski test b) Liebermann – Burchard test	Present(+)
4	Glycosides a) Modified Borntrager test b) Legal test	Present(+)
5	Tannins a) Gelatin test b) Ferric chloride test	Present(+)
6	Resins a) Hydrochloride test b) Copper acetate test	Absent(-)
7	Triterpenoids a) Salkowski test	Present(+)
8	Saponins a) Foam test	Present(+)
9	Flavonoids a) Shinoda test b) Lead acetate test	Present(+)
10	Phenols a) Salkowski test	Present(+)

Phytochemical Screening: The phytochemical study of the methanolic leaf extract of *Evolvulus nummularius* had revealed a variety of phytochemicals which includes alkaloids, steroids, glycosides, tannins, triterpenoids, saponins, flavonoids and phenols.

Table 4: Dpph Radical Scavenging Activity

S.No	Concentration µg/ml	% RSA (Extract)	% RSA (Standard)	IC 50
1	50	44.03	49.54	51.23
2	100	55	61.25	62.86
3	150	61.53	65.47	68.24
4	200	71.15	78.23	77.45
5	250	82.69	87.42	89.37



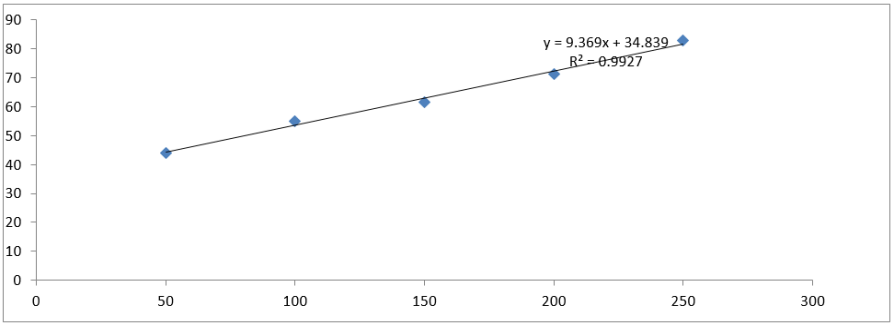
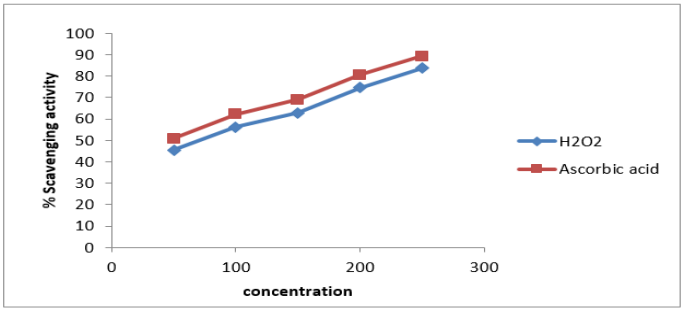


Fig 1 DPPH Free Radical Scavenging Activity Of *Evolvulus Nummularius* Linn Extract. IC 50 Value Was Calculated And Was Found To Be 69.83

Table 5 H_2O_2 Radical Scavenging Activity:

S.No	Concentration µg/ml	% RSA (Extract)	% RSA (Standard)	IC 50
1	50	45.42	50.72	34.89
2	100	56.25	62.24	48.25
3	150	62.84	68.92	54.65
4	200	74.55	80.51	67.15
5	250	83.75	89.27	85.26



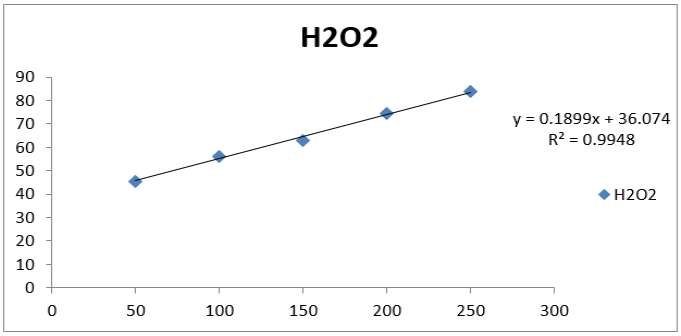


Fig 2: Radical Scavenging Activity Of Hydrogen Peroxide By The Extract Of *Evolvulus Nummularius* **.** IC50 Value Was Measured And Calculated As 58.04

Gas Chromatography-Mass Spectroscopy(GC-MS) Analysis:

The GC-MS chromatogram of methanolic leaf extract of *Evolvulus nummularius* recorded number of peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, chemical formula, molecular weight and mass spectral fragmentation patterns that are described by National Institute of Standards and Technology (NIST) Library. Overall the structures of 15 compounds have been identified in the methanolic leaf extract of *Evolvulus nummularius* given in Table 2 along with their Retention Time. The phytoconstituents that are identified are Ursolic acid, Ethanoic acid, Benzene,(methyl sulfinyl) methyl, N-methoxy-N-methyl acetamide, Ethyl-2-hydroxybenzyl sulfone, 3-methoxy 2,5-dimethyl pyrazine, Ribitol, Ursolic acid, Oxime, methoxy-phenyl-, Nickel, nitrosyl[1,2,3,4,5-pentamethyl-

2,4-cyclopentadien-1-yl]-, Oxirane, 2,2-diphenyl-, 2-Furancarboxaldehyde, 5-methyl-, 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-,8aH-2,4a-methanonaphthalen-8a-ol, octahydro-1,1,5,5-tetramethyl-, Bicyclo[2.2.1]hept-2-en-7-ol, 7-(4-methoxyphenyl)-.

S.No	Retention Time	Name of the Compound	Formulae	Molecular Weight	SI	RSI	Probability
1	3.81	Oleanolic acid	$C_{30}H_{48}O_3$	456.7	897	916	76.19
2	9.36	Ethanoic acid	$C_2H_4O_2$	60	674	863	22.34
3	9.6	Benzene, (Methyl sulfinyl) methyl	$C_8H_{10}OS$	154	786	856	42.58
4	9.88	N-methoxy-N-methyl acetamide	$C_4H_9NO_2$	103	714	751	31.16
5	10.00	Ethyl-2-hydroxybenzyl sulfone	$C_9H_{12}O_3S$	200	771	813	38.05
6	10.69	3-methoxy 2,5-dimethyl pyrazine	$C_7H_{10}N_{20}$	138	569	713	21.53
7	10.95	Ribitol	$C_{19}H_{20}O_5$	328	650	656	39.04
8	11.18	Ursolic Acid	$C_{30}H_{48}O_3$	456.7	879	901	72.81
9	11.52	Oxime, methoxy-phenyl-	$C_8H_9NO_2$	151	757	771	77.61
10	11.75	Nickel, nitrosyl[1,2,3,4,5-pentamethyl-2,4-cyclopentadien-1-yl]-	$C_{10}H_{15}NNiO$	223	654	674	60.34
11	12.04	Oxirane, 2,2-diphenyl-	$C_{14}H_{12}O$	196	627	722	44.24
12	12.76	2-Furancarboxaldehyde, 5-methyl-	C_6H_6O2	110	461	787	19.79
13	27.84	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O2$	336	755	768	6.25
14	33.08	8aH-2,4a-methanonaphthalen-8a-ol, octahydro-1,1,5,5-tetramethyl-	$C_{15}H_{26}O$	222	769	786	18.61
15	34.08	Bicyclo[2.2.1]hept-2-en-7-ol, 7-(4-methoxyphenyl)-	$C_{14}H_{16}O_2$	216	612	657	16.42

Table 6. Retention Time (Min), Chemical Formula, Molecular Weight, Matching Factors To Mass Spectrometry SI(Match Factor) And RSI(Reverse Match Factor) And Probabilities Identified In The Methanolic Leaf Extract Of *Evolvulus Nummularius* By **Gas Chromatography – Mass Spectrometry**

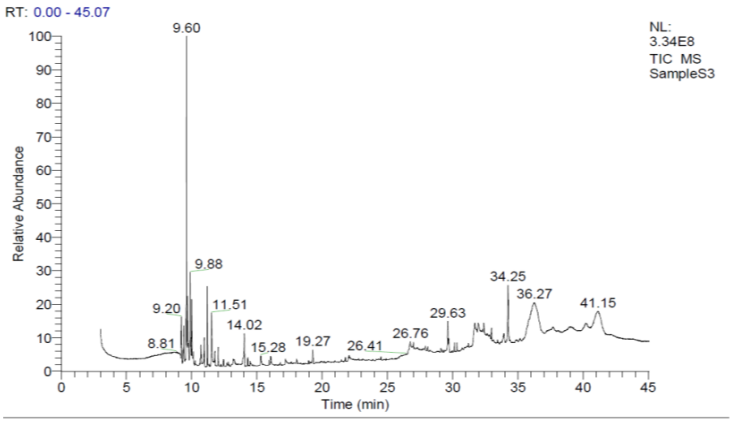


Fig 3: Chromatogram Showing The Identified Phytocompounds With Retention Time On X-Axis And Relative Abundance On Y-Axis Through GC-MS.

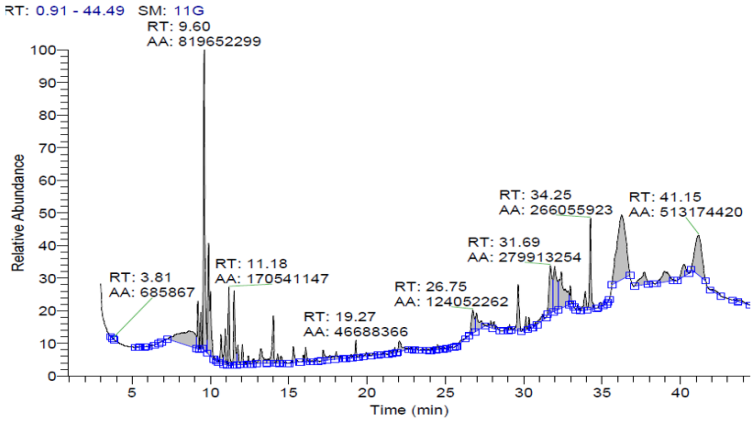
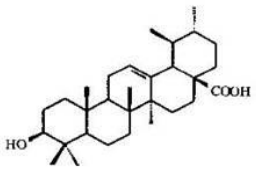
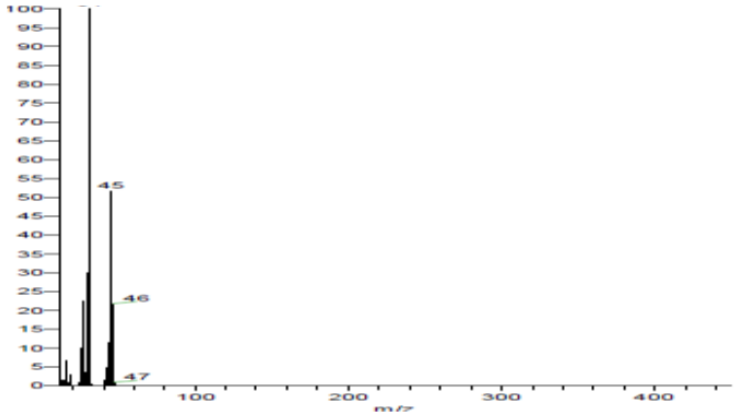


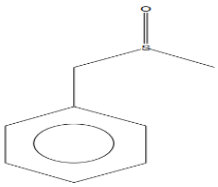
Fig 4: Chromatogram Showing The Identified Phytocompounds And Area With Retention Time On X-Axis And Relative Abundance On Y-Axis Through GC-MS.

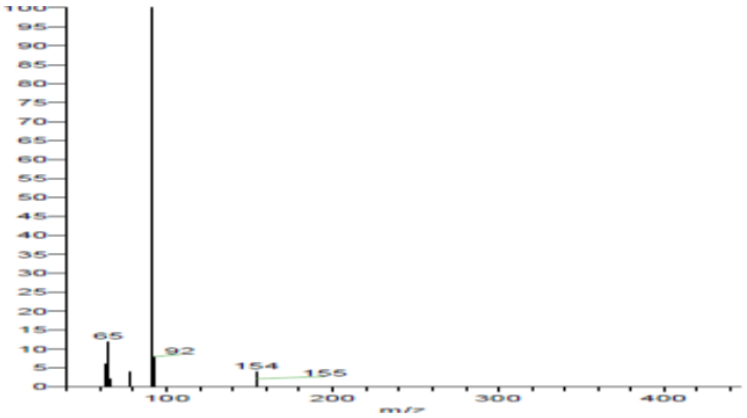
Altogether, 15 Compounds Were Isolated By Using GC-MS Technique. The Best Identified Compounds Based On The Area And Area% Were Notified And Given In The Above Chromatogram. The Area, Area % Is Given In The Table 7.

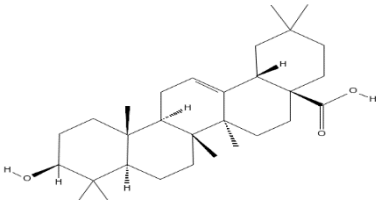
S.No	Retention time	Compound	Molecular formula	Molecular weight	Area	Area%
1	3.81	Ursolic acid	$C_{30}H_{48}O_3$	456.7	685867	0.03
2	9.60	Benzene, (Methyl sulfinyl) methyl	$C_8H_{10}OS$	154	819652299	36.9
3	11.18	Oleanolic acid	$C_{30}H_{48}O_3$	456.7	170541147	76.79
4	19.27	Oxirane, 2,2-diphenyl-	$C_{14}H_{12}O$	196	46688366	2.10
5	26.75	2-Furancarboxaldehyde, 5-methyl-	C_6H_6O2	110	124052262	5.50
6	31.69	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O2$	336	279913254	12.60
7	34.25	8aH-2,4a-methanonaphthalen-8a-ol, octahydro-1,1,5,5-tetramethyl-	$C_{15}H_{26}O$	222	266055923	11.98
8	41.15	Bicyclo[2.2.1]hept-2-en-7-ol, 7-(4-methoxyphenyl)-	$C_{14}H_{16}O_2$	216	513174420	23.10

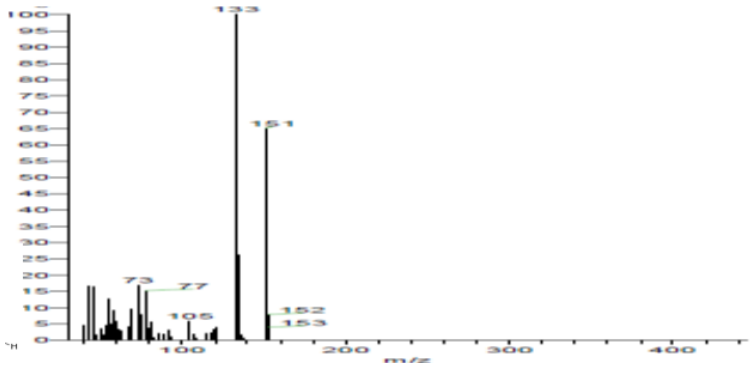


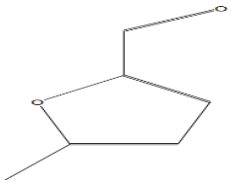


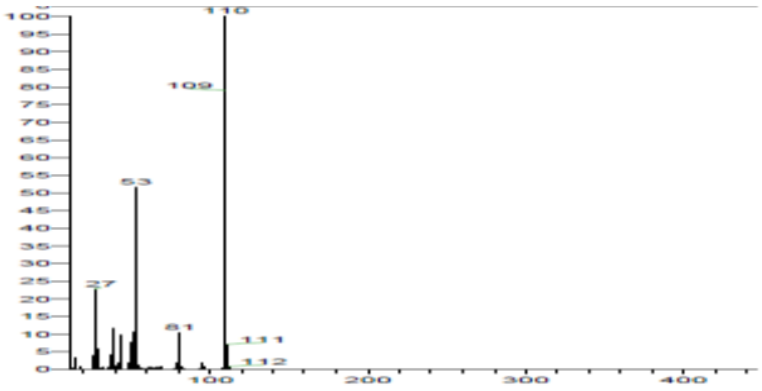








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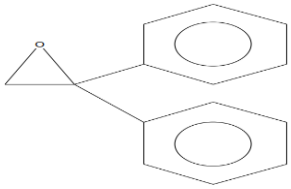

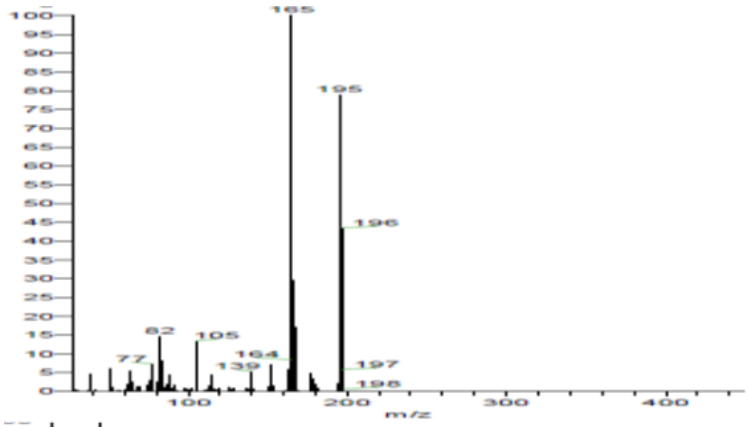
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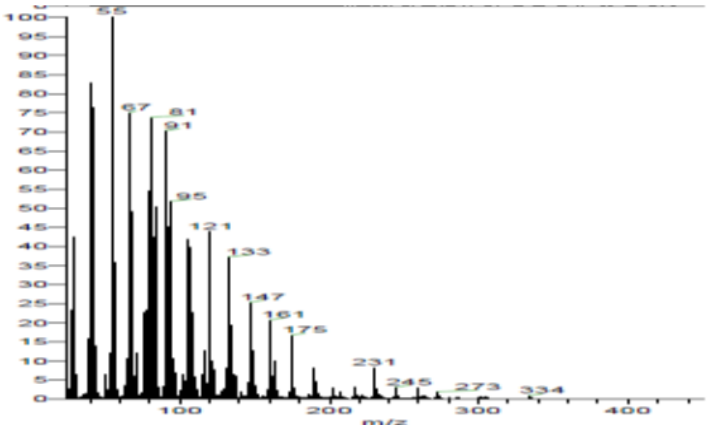
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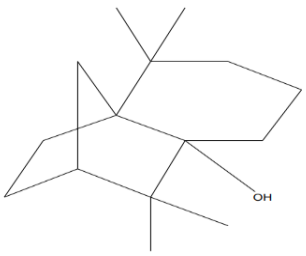
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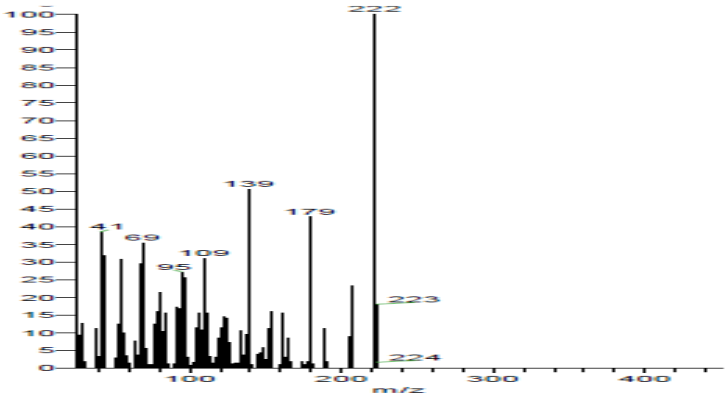
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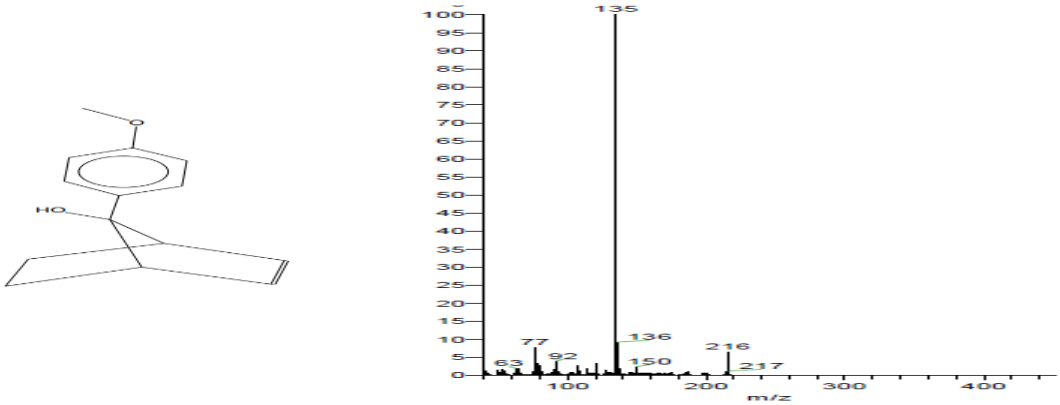


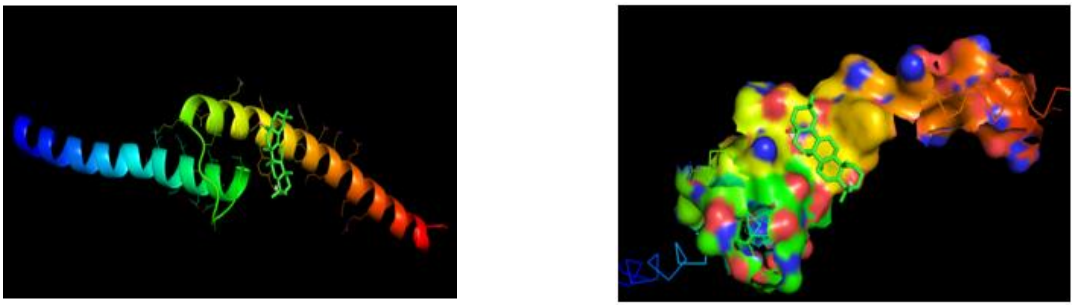
Fig 5: Chemical Structures Of The Major Bioactive Compounds Eluted In The Methanolic Leaf Extract Of E.Nummularius.

Molecular Docking Studies:

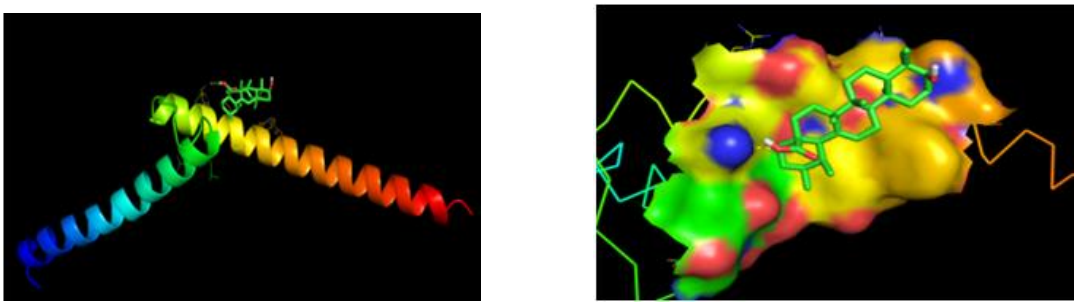
The pathogenesis[15] of NAFLD involves a variety of receptors[16] in the progression of the disease which includes Sterol Regulatory Element Binding Protein-1c(SREBP-1c)[17,18], Carbohyrate Response Element Binding protein (ChREBP), Peroxisome Proliferator Activated Receptors[19,20]
(PPAR α, β, γ), Liver X Receptor(LXR)[21], Farsenoid X Receptor (FXR)[22] etc., These nuclear receptors have been docked against the phytoconstituents present in the plant extract that supports the present activity. Ursolic acid and Oleanolic acid identified through GC-MS were likely to be more efficient against Non Alcoholic Fatty Liver Disease. So, the structures of the compounds were obtained from Pubchem database (https ://pubch em.ncbi.nlm.nih.gov/) as sdf file and converted into Mol, PDBQT and PDB file formats using OPEN BABEL software . For the confirmation of the study[23], optimisation of three dimensional (3D)[24] structures of phytocompounds is necessary and structure based molecular docking[25] has been performed for studying the protein ligand interactions against NAFLD[26] by using Ursodiol as a reference. The target proteins are obtained from Research Collaboratory for Structural Bioinformatics(RCSB) Protein Data Bank (https ://www.rscb.org) with the help of Autodock Software. The relative strengths of the binding interactions, binding energy, interacting residues and the type of interactions were determined by using AutoDOCK programme that are mentioned in the table 8.

Name of the Receptor	Name of ligand	Binding Energy (Kcal/mol)	Interacting Residues
SREBP1 (PDB ID: 1AM9)	Oleanolic Acid	-6.8	1 HB - LYS 365, 1 HB - TYR 369
SREBP1 (PDB ID: 1AM9)	Ursolic Acid	-7.7	1 HB - LYS 365
SREBP1 (PDB ID: 1AM9)	Ursodeoxycholic acid	-6.3	2 HB - LYS 365, 1 HB - HIS 375
PPAR alpha (PDB ID: 2REW)	Oleanolic Acid	-8.5	1 HB - LEU 410, 1 HB - LEU 414,
PPAR alpha (PDB ID: 2REW)	Ursolic Acid	-9.3	1 HB - SER 414
PPAR alpha (PDB ID: 2REW)	Ursodeoxycholic acid	-7.2	1 HB - THR 288, 1 HB - THR 307
PPAR beta (PDB ID: 1Y0S)	Oleanolic Acid	-8.9	1 HB - TYR 326, 1 HB - TYR 473
PPAR beta (PDB ID: 1Y0S)	Ursolic Acid	-8.8	1 HB - THR 447
PPAR beta (PDB ID: 1Y0S)	Ursodeoxycholic acid	-7.7	1 HB - LYS 319, 1-HB- HIS 323, 1-HB - THR 447.
PPAR Gamma (PDB ID: 6DHA)	Oleanolic Acid	-8.2	1 HB - GLN 454
PPAR Gamma (PDB ID: 6DHA)	Ursolic Acid	-7.8	1 HB - LYS 336
PPAR Gamma (PDB ID: 6DHA)	Ursodeoxycholic acid	-7.5	1 HB - ARG 288
FXR (PDB ID: 1OT7)	Oleanolic Acid	-8.4	1 HB - GLU 331
FXR (PDB ID: 1OT7)	Ursolic Acid	-8.4	1-Hb with ASN-441, 2Hb with ARG-392
FXR (PDB ID: 1OT7)	Ursodeoxycholic acid	-7.4	4 HB - GLU 331, ASN 334, LYS 335, ILE 332
ChREBP (PDB ID: 5F74)	Oleanolic Acid	-9.2	1 HB - LYS 51, 1 HB - LYS 122, 1 HB - ASN 124
ChREBP (PDB ID: 5F74)	Ursolic Acid	-9.0	1 HB - LYS 122, 1 HB - ASN 124
ChREBP (PDB ID: 5F74)	Ursodeoxycholic acid	-8.1	1 HB - ASN 123, 2 HB-ARG 128, 1 HB - ARG 129, 1 HB - ARG 58, 1 HB - TYR 130, 1 HB - LYS 51
LXR (PDB ID: 1UHI)	Oleanolic Acid	-7.2	1 HB - ARG 387
LXR (PDB ID: 1UHI)	Ursolic acid	-7.9	2 HB - TYR 468, 3 HB - ARG 464
LXR (PDB ID: 1UHI)	Ursodeoxycholic acid	-6.5	1 HB - ARG 373, 2 HB - ASN 377

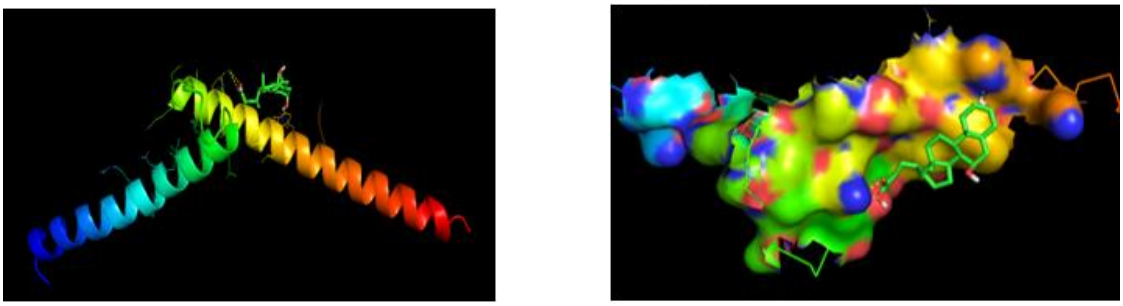
Table 8. Molecular Docking Of The Phytoconstituents Identified Through GC-MS Along With The Standard With The Binding Energy, Binding Residues And The Type Of The Interactions.



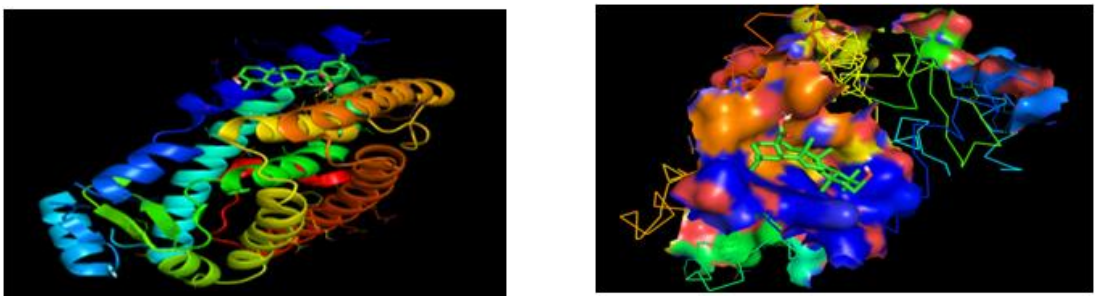
Oleanolic Acid (Mode-1) In The Binding Pocket Of SREBP1 (PDB ID: 1AM9) With Affinity -6.8 (Kcal/Mol)



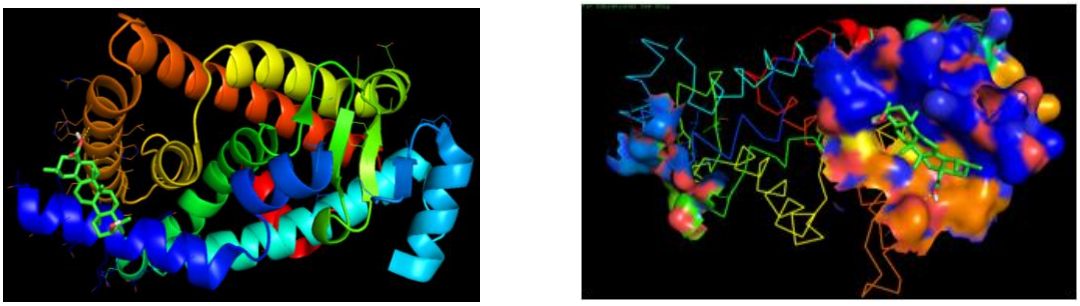
Ursolic Acid (Mode-1) Interacting With SREBP1 (PDB ID: 1AM9) With Affinity -7.7 (Kcal/Mol)



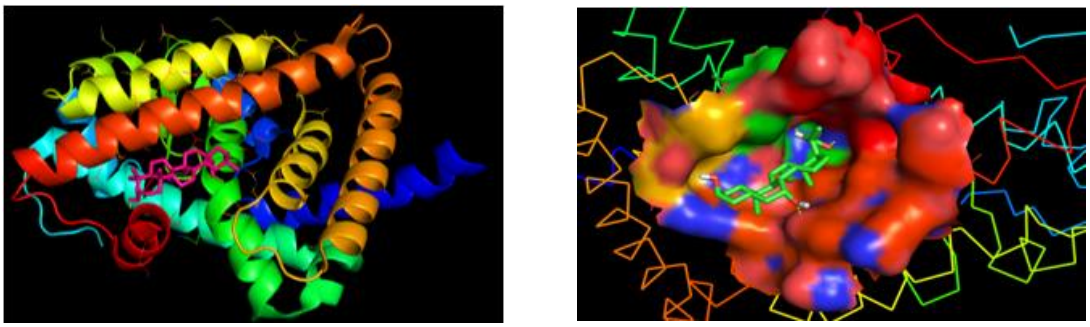
Ursodeoxycholic Acid In The Binding Pocket Of SREBP1 (PDB ID: 1AM9) With Affinity -6.3 (Kcal/Mol)



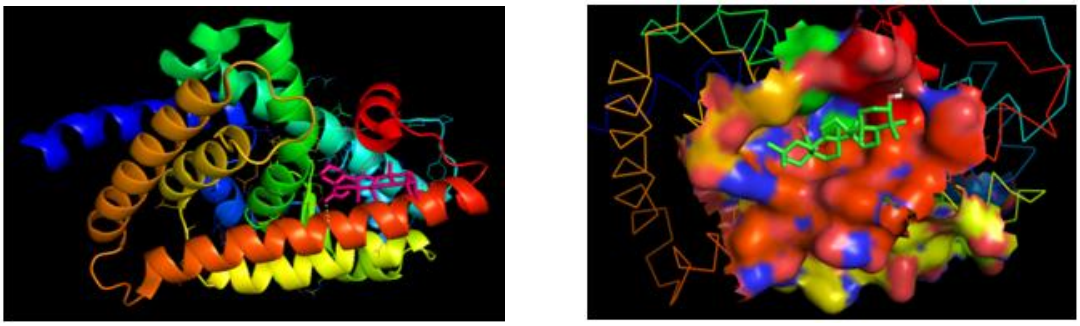
Oleanolic Acid In The Binding Pocket Of PPAR Alpha (PDB ID: 2REW) With Affinity 8.5(Kcal/Mol)



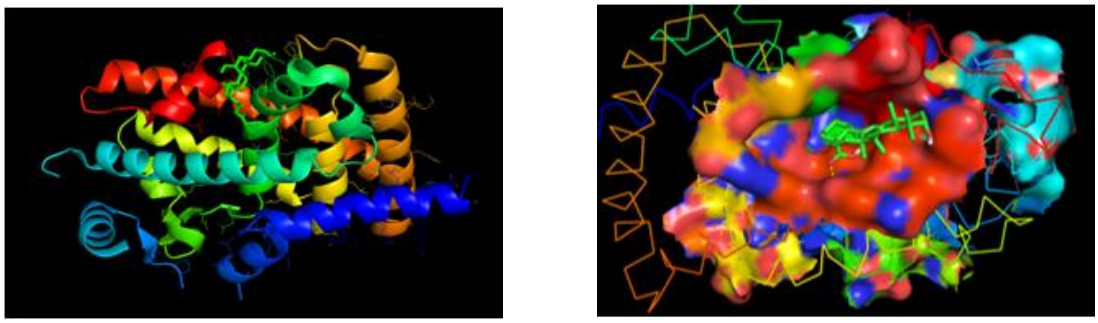
Ursolic Acid In The Binding Pocket Of PPAR Alpha (PDB ID:2REW) With Affinity -9.3 (Kcal/Mol)



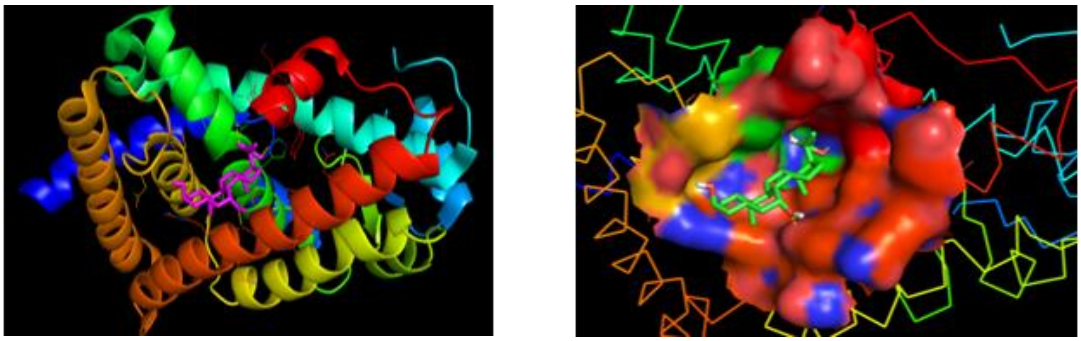
Ursodeoxycholic Acid In The Binding Pocket Of PPAR Alpha (PDB ID: 2REW) With Affinity -7.2 (Kcal/Mol)



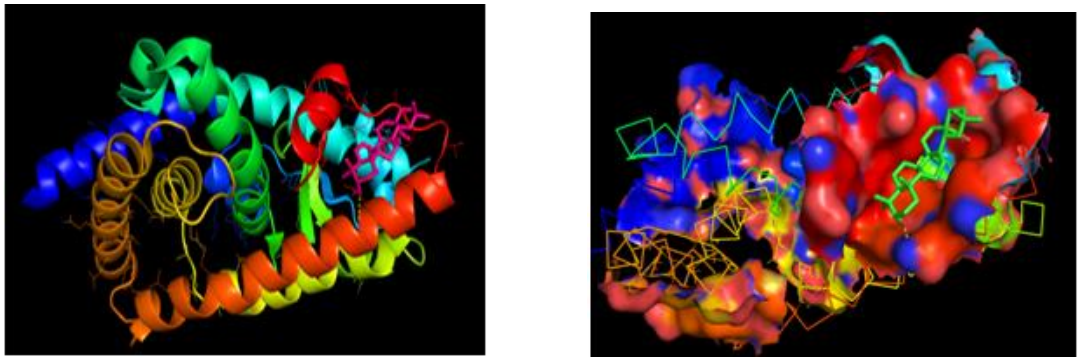
Oleanolic Acid In The Binding Pocket Of PPAR Beta (PDB ID: 1Y0S) With Affinity -8.9 (Kcal/Mol)



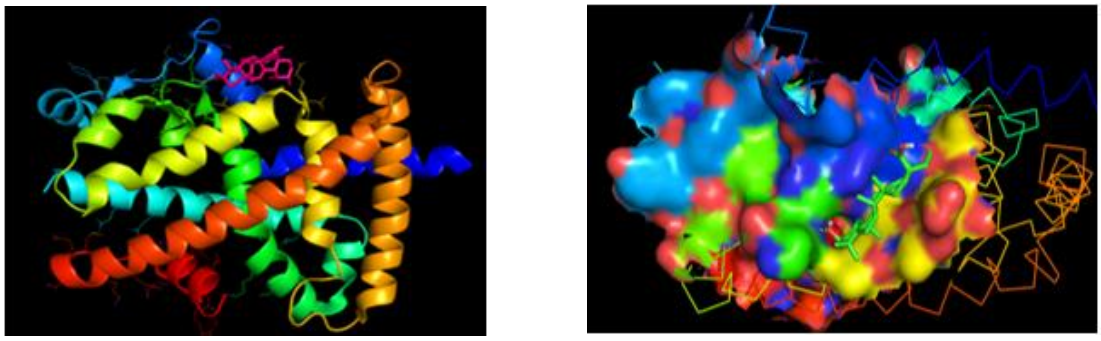
Ursolic Acid In The Binding Pocket Of PPAR Beta (PDB ID: 1Y0S) With Affinity -8.8 (Kcal/Mol)



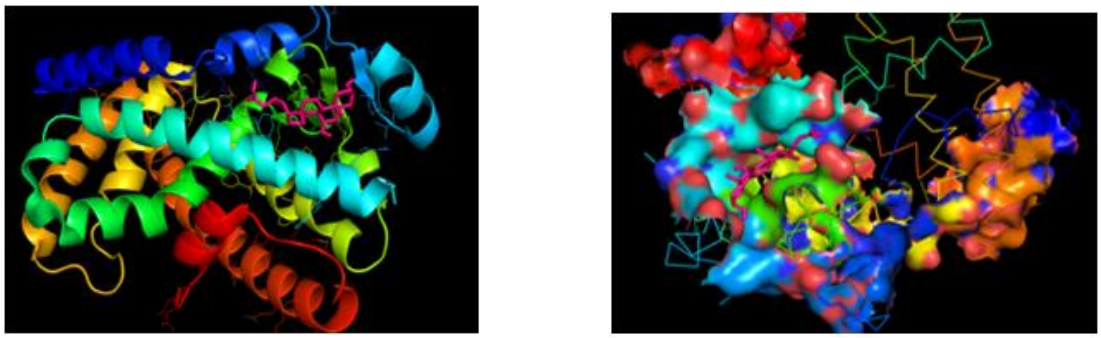
Ursodeoxycholic Acid In The Binding Pocket Of PPAR Beta (PDB ID: 1Y0S) With Affinity -7.7 (Kcal/Mol)



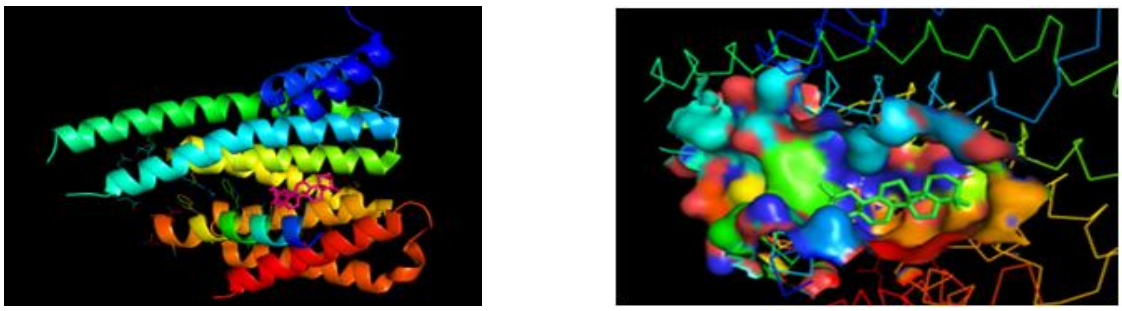
Oleanolic Acid In The Binding Pocket Of PPAR Gamma (PDB ID: 6DHA) With Affinity -8.2 (Kcal/Mol)



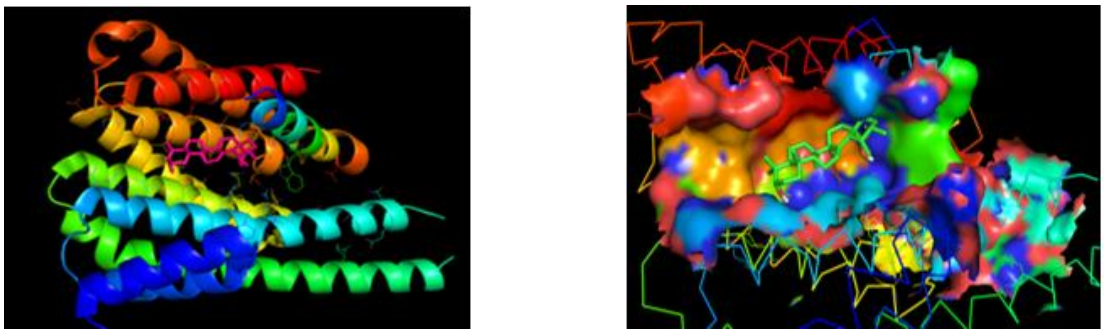
Ursolic Acid In The Binding Pocket Of PPAR Gamma (PDB ID: 6DHA) With Affinity -7.8 (Kcal/Mol)



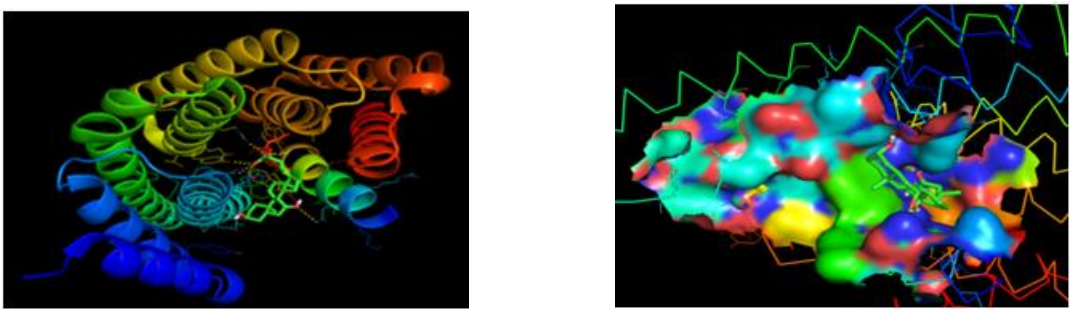
Ursodeoxycholic Acid In The Binding Pocket Of PPAR Gamma (PDB ID: 6DHA) With Affinity -7.5 (Kcal/Mol)



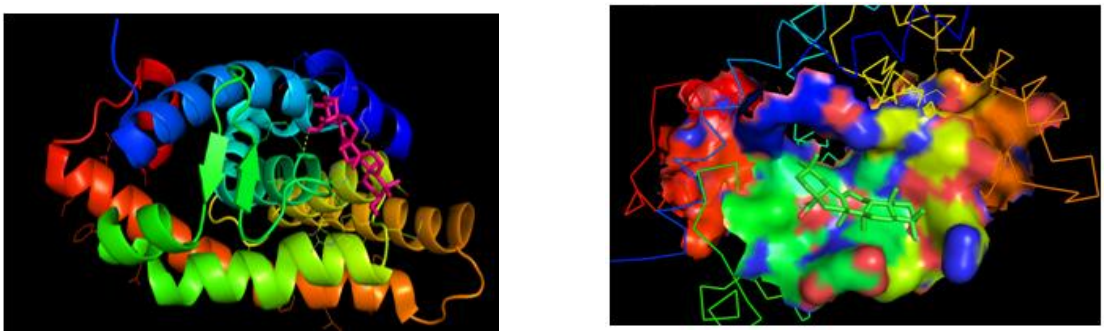
Oleanolic Acid In The Binding Pocket Of Chrebp (PDB ID: 5F74) With Affinity -9.2 (Kcal/Mol)



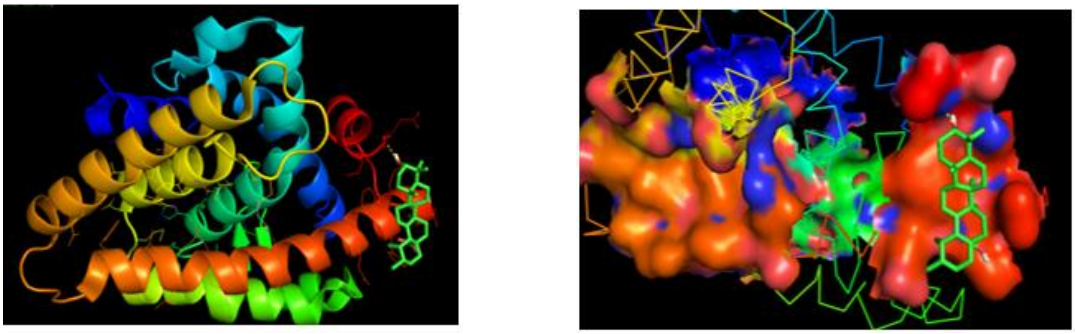
Ursolic Acid In The Binding Pocket Of Chrebp (PDB ID: 5F74) With Affinity -9.0 (Kcal/Mol)



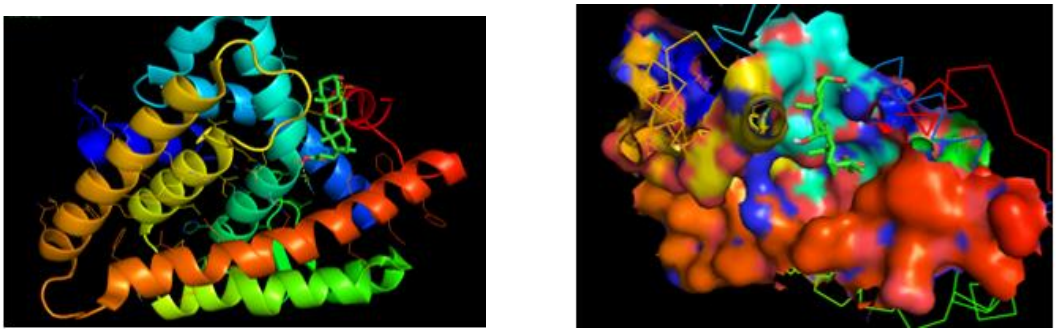
Ursodeoxycholic Acid In The Binding Pocket Of Chrebp (PDB ID: 5F74) With Affinity -8.1 (Kcal/Mol)



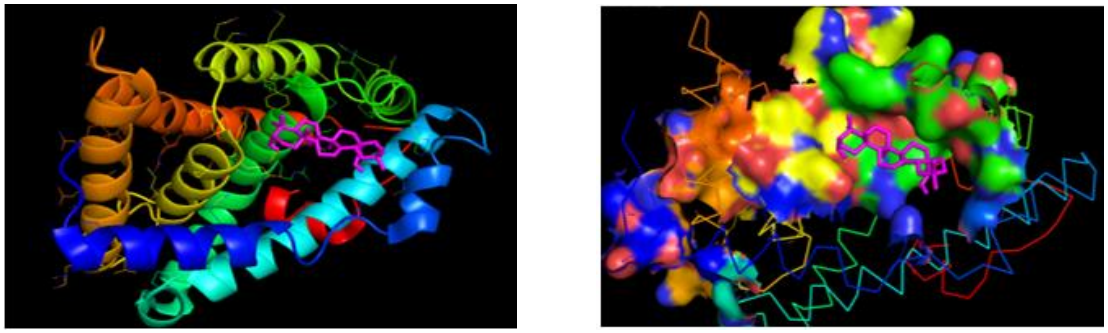
Oleanolic Acid In The Binding Pocket Of LXR (PDB ID: 1UHI) With Affinity -7.2 (Kcal/Mol)



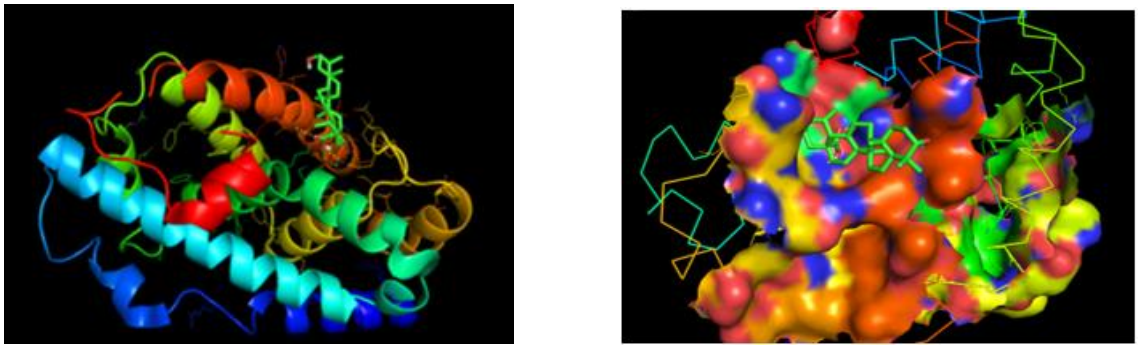
Ursolic Acid In The Binding Pocket Of LXR (PDB ID: 1UHI) With Affinity -7.9 (Kcal/Mol)



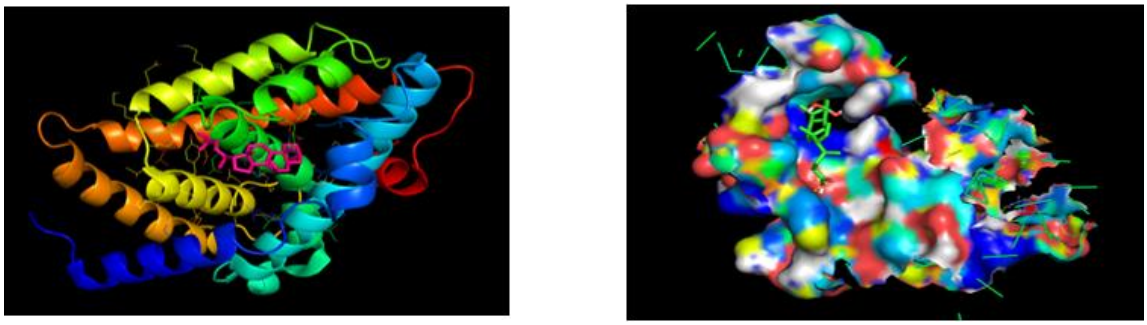
Ursodeoxycholic Acid Interacting With LXR (PDB ID: 1UHI) With Affinity -6.5 (Kcal/Mol)



Oleanoloic Acid In The Binding Pocket Of FXR (PDB ID: 1OT7) With Affinity -8.4 (Kcal/Mol)



Ursolic Acid In The Binding Pocket Of FXR (PDB ID: 1OT7) With Affinity -8.4 (Kcal/Mol)



Ursodeoxycholic Acid In The Binding Pocket Of FXR (PDB ID: 1OT7) With Affinity -7.4 (Kcal/Mol)

Fig 6: Gives The Pictorial Representation Of The Ligands Present In The Plant Which Have Been Docked Against The Target Receptors.

4.DISCUSSION:

Non Alcoholic Fatty Liver Disease is a rapidly developing serious health problem that ranges from hepatic steatosis to nonalcoholic steatohepatitis and cirrhosis, can also lead to fibrosis, cirrhosis and hepatocellular carcinoma[29]. The treatment for this is limited, so, an attempt was made to know the molecular receptors involved in the pathogenesis of NAFLD. *Evolvulus nummularius* Linn is a perennial herb belonging to the family Convolvulaceae, has been selected for the study. The plant shows characteristic odour with light green colour and slightly bitter taste. The methanolic extract of the plant was prepared and subjected for the preliminary phytochemical screening[30] which shows the presence of different phytoconstituents like alkaloids, saponins, flavonoids, tannins etc., These were found to exhibit beneficial role due to the presence of anti-oxidant properties. The methanolic leaf extract of *Evolvulus nummularius* shows the significant anti-oxidant activity with both DPPH and Hydrogen Peroxide radical assays. In DPPH scavenging activity, the percentage inhibition was found to be 69.83% at 250µg/ml with Ascorbic acid as a positive control. The methanolic leaf extract shows increase in the scavenging activity with an average inhibitory concentration of 58.04% at different concentrations. Thus, the extract shows favourable anti-oxidant potential. Due to the presence of the various phytoconstituents like flavonoids, triterpenoids, alkaloids, saponins etc., the plant can be used in the treatment of NAFLD.

In GC-MS study, many compounds have been identified which shows different area and area%. Ursolic acid, Ethanoic acid, Benzene,(methyl sulfinyl) methyl, N-methoxy-N-methyl acetamide, Ethyl-2-hydroxybenzyl sulfone, 3-methoxy 2,5-dimethyl pyrazine, Ribitol, Ursolic acid, Oxime, methoxy-phenyl-, Nickel, nitrosyl[1,2,3,4,5-pentamethyl-2,4-cyclopentadien-1-yl]-, Oxirane, 2,2-diphenyl-, 2-Furancarboxaldehyde, 5-methyl-, 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro-,8aH-2,4a-methanonaphthalen-8a-ol, octahydro-1,1,5,5-tetramethyl-, Bicyclo hept-2-en-7-ol, 7-(4-methoxyphenyl)-. and of them, two compounds, Ursolic acid and Oleanolic acid which are potent against NAFLD were docked against the receptors. The receptors include SREBPs, PPARα, PPARβ, PPARγ, ChREBPs, FXR, LXR[31,32] etc., The binding energies and the type of interactions were given in the Table 2.The affinities varies for different receptors like Oleanolic Acid shows more affinity for SREBPs with binding energy of -6.8 Kcal/mol, Oleanolic Acid for PPARα with -8.5, PPARβ for Ursolic acid with -8.8, PPARγ for Ursolic Acid with -7.8, ChREBPs for Ursolic Acid with -9.0, FXR shows same affinity for both Ursolic Acid and Oleanolic Acid with -8.4, LXR for Oleanolic Acid with -7.2.

Thus, our present study shows the presence of various phytochemicals in the preliminary screening, exhibited the anti-oxidant potential and also the ligands possess affinity towards the target receptors in molecular docking.

5.CONCLUSION:

From the present study, it was concluded that the methanolic extract of *Evolvulus nummularius* contain different compounds and the compounds identified through GC-MS were docked. The affinities and the binding interactions revealed that the main ingredients from the methanolic extract of *Evolvulus nummularius* Linn have predicted the proteins compared to the native ligands of those. Furthermore, Ursolic acid shows high affinity towards ChREBPs with binding energy of -9.0kcal/mol. These regulatory mechanisms were likely to be the targets for the modulation of lipid metabolism and inflammation in the treatment of NAFLD. However, further studies on this relevance to NAFLD are needed.

ACKNOWLEDGEMENT:

The authors wish to express sincere thanks to Dr.V.Chitra for support and motivation and Dr.K.Peera, Research Associate, DBT, S.V.University, Tirupati for his help in GC-MS studies.

AUTHOR CONTRIBUTION: All authors are contributed for the study, concept and design of the work.

DECLARATIONS

Ethics Approval: Not Applicable

Consent to participate: Yes . All authorsconsent to participate.

Consent for publication: Yes. All authors have contributed to the latest version of the manuscript for its submission.

Competing interests: The authors declare no competing interests.

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