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Potential Activity Of The Components Of *Madhuca longifolia* As A Hepatoprotective Agent Through In Vitro And In Silico Docking Technique Against The Orphan Nuclear Receptors.

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ABSTRACT

Hepatotoxicity is an adverse complication of most prescribed drugs. Studies on Madhuca longifolia have proven to be used for various medical treatments. The aim of the current study is to predict the inhibitory effect of the active ingredients of the components of M. longifolia against the toxic receptor and its pharmacological properties. The in vitro analysis was carried out in the seed oil to know its beneficial effect on total phenolic content, DPPH assay, catalase activity and peroxidase activity. The chosen toxic receptors are apo human pregnane X receptor, nuclear bile acid receptor FXR, constitutive and rostane receptor, LXR and Nf-kB through molecular docking techniques. 3D structures of the receptors and ligands were obtained from RCSB data bank and Corina molecular network. Docking experiments were carried out on Patch Dock server and the docked complexes were analyzed using Pymol molecule viewer which predicts the bond length and interacting residue of ligand-protein complexes. The seed oil showed the beneficial effect in in vitro studies. MiSaponin A and MiSaponin B shown an effective interaction with all receptors compared to all other ligands. We predicted that MiSaponin A and MiSaponin B has an effective potential in treating the hepatotoxicity.

Keywords: Hepatotoxicity, Madhucalongifolia, Insilico Docking, Computational Methods, In vitro

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INTRODUCTION

Dealing with natural medicine from the natural plants is an oldest form of practice in humankind. The knowledge of medicinal plants was been used much effectively against human illness through its part like barks, leaves, fruits and flowers. The experienced results by the human have paid the way for the high usage of medicinal plant until the discovery of modern medicines. Herbal plants are still used as the mainstream for about 75-80% of the world's population in developing countries as health care supplements. The usage of medicinal plants is still been increased due to the minimal efficacy of synthetic drugs [1].

Madhucalongifolia Syn.M.indica(Sapotaceae) is an important tropical tree present largely in the forest areas of north Indian and central plains which is not well known. It is also called as mahuwa, mahua, mohulo, or Ilupallrvippachettu. It's a fast growing tree which grows to the height of 20 meters and it is an ever green tree which matures and starts bearing even at 8-15years that can yield fruits for upto 60 years. It is mostly found in the moist tropical forests of western India from Konkan southwards to Travancore, Deccan that is common in Ceylon, Carnatic and upper Burma[2]. This can yield various important products from its seed, bark, fruits, flower, leaves and seed oil. The mahua butter was named after its butter characteristics in oil[3]. This mahua butter is being characterized by its rich content of phytosterols, the major tocopherol isomer and α and β isomers. Madhucalongifolia have the various important activities like pharmacological, antioxidant, analgesic, anticancer, anticonvulsant, immune modulatory and heap to protective activity. The outcome of these studies would results in providing a convincing support for its later clinical use in present day medicine[4]. The various disease that can be treated with the different parts of this tree are tuberculosis, rheumatoid arthritis, cholera, influenza piles, helminthiasis, low semen count, headache, infections. Besides these it can be used as a blood purifier and as a counteragent to poison. The flower is edible and is mainly used to make syrup for medicinal purposes. It is an essential drink used during celebrations by both men and women. The oil of M. longifolia seeds has a refractive index of 1.452 which is rich in fatty acids like palmitic, stearic, oleic, linolenic[5].The ethanolic extract of Madhuca longifolia can treat the toxicity induced in rats which was found to normalize the elevated serum urea and creatinine that was reduced significantly by its treatment at different doses of M. longifolia extract. That demonstrated the efficacy of Madhuca longifolia in treating the toxicity against the hepto and renal damage [6].

Liver plays an important role in the maintenance of the homeostasis of body. The important role of liver is supplying nutrient, fighting against the pathogens and other metabolic pathways. Hepatotoxicity is the chemical driven damages caused in the liver[7].Hepatotoxicity is caused due to the oxidative stress that leads to metabolic disorder. It is also caused due to multi-dimensional dysfunction or xenobiotics which is caused by the hepatotoxic chemical that will cause lipid peroxidation, oxidative damage and mitochondrial dysfunction[8].Some pharmaceutical drugs like chlorpromazine, halothane, isoniazid and amoxicillin-clavulanate are documented to cause liver damage. Liver Tox is an online database that provides the accurate up-to-date information about the causes, diagnosis, frequency, severity of liver injury. From the case report on hepatotoxicity, it was reported that about 353 out of 671 marketed drugs are known to cause liver damage. Only 47% of drugs are reported to negotiate causing hepatotoxicity. There is a large category of therapeutical drugs which are known to cause big threat to the functioning of liver. Diclofenac and Troglitazone are the most common pharmaceutical drug-induced hepatotoxic drugs. Diclofenac is a non steroidal inflammatory drug that form quinine intermediates on binding to the protein and nucleic acid. Oxidative stress is further caused by diclofenac through redox cycling of reacting oxidation species. [9].

There are numerous plants in curing hepatotoxicity, but major problem is the lack of understanding its ability. So to understand the activity of the active components of the plants, a computational technique called docking is performed. This helps in discovering new drugs within a short period of time and of less cost. Docking will help to predict the interactions between the ligand and receptor. The interacted ligand and receptor having more bonds will be the best interacting molecule. Auto dock is the most common docking site that helps in predicting the interaction between the ligand and the receptor[10].The aim of the docking method is to predict the experimental bonding and affinities of the small molecules with the receptors. This is now mostly used as a computation tool in drug designing. Pose prediction, affinity and virtual screening are the main goals of the docking process. A successful docking will able to explain the binding of the native ligand to the receptor. A search algorithm and scoring are the basic rules in the docking methodology to know the ligand interactions and confirmations[11]. Describing the ligand receptor interaction correctly is major challenge of docking that is useful in prospective drug designing and studies. Binding of ligands to the receptor

and understanding their affinities will help to know the efficient binding of them in drug designing. The binding site of ligand to the protein can be clearly explained by docking technique[12]. The aim of current study is to understand the affinity of active components of Madhucalongifolia to the hepatotoxic receptors.

MATERIALS AND METHOD

In silico analysis on seed, bark and fruit of Madhuca longifolia

Receptors

The five receptors used are Apo Human Pregnane X, Nuclear bile acid receptor FXR, Constitutive and rostane, LXRalpha and Nf-kB receptors. These receptors are activated during hepatotoxicity. The structure of all the receptors was obtained from PDB. The PDB id of the used receptor is mentioned in Table 1.

Table 1: Used receptors for molecular docking

S.No.	Receptor	PDB Id.
1	Apo Human Pregnane X Receptor	1ILG
2	Nuclear bile acid receptor FXR	1OSH
3	Constitutive androstane receptor	1XNX
4	LXRalpha	5AVI
5	Nf-kB	1NFK

Ligands

About twenty nine compounds were selected from different parts of Madhuca longifolia. The ligands were obtained from seed, bark, fruit and nut shell. The canonical smile of each ligand was obtained from Pubchem which was submitted to corina molecule net to obtain its structure. The characteristics of the ligands used are given in Table 2.

Table 2: Characteristics of the ligand

Molecule Name	Molecule formula	Molecular weight (g/mol)	PubChem compound Id.
Ethylcinnamate	C11H12O2	176.215	637758
Sesquiterine alcohol	C27H46O11	564.654	91754221
α-terpeneol	C10H18O	154.253	17100
α- and β- amyrin acetates	C94H154O5	1364.261	71597151
n-Hexacosanol	C26H54O	382.717	68171
Quercetin	C15H10O7	302.238	5280343
Dihydroquercetin	C15H12O7	304.254	471
β-sitosterol	C29H50O	414.718	222284
Quercitin- 3-glucoside	C20H19O12	463.371	5280804
Icosonoic acid	C20H40O2	312.538	10467
Octadeca 9,12 dienoic acid	C18H32O2	280.452	5280450
Octadec-9-enoic acid	C18H34O2	282.468	445639
Tetradecanoic acid	C14H28O2	228.376	11005
Hexadecanoic acid	C16H32O2	256.430	985
Octadecanoic acid	C18H36O2	284.484	5281

2 amino propanoic acid	C3H7NO2	89.094	5862
4 methyl 2(phenyl methoxy carbonyl amine) pentanoic acid	C14H19NO4	265.309	74840
2-6 diaminohexanoic acid	C6H14N2O2	146.190	5962
2 amino 4 methyl sulfanylbutanoic acid	C5H11NO2S	149.208	6137
Pyrrolidine-2 carboxylic acid	C5H9O2	115.132	145742
2 amino 3 hydroxypropanoic acid	C3H7NO3	105.093	5951
2 amino-3 hydroxybutanolic acid	C4H9NO3	119.120	6288
3,5,7 trihydroxy -2-(3,4,5 trihydroxyphenyl) chromen-4-one	C15H10O8	318.237	5281672
2(3-4 dihydroxyphenyl)-5,7 dihydroxy-3	C21H20O12	464.379	5280804
Mi-saponin A	C58H94O27	1223.363	179637
Mi-saponin B	C63H102O31	1355.475	51136518
2 amino butanedoicacid	C4H7NO4	133.103	5960
2 amino-3-[2amino-2carboxy	C6H12N2O4S2	240.292	67678
2 amino acetic acid	C2H5NO2	75.067	750
2 amino 3 methyl pentanoic acid	C6H13NO2	131.175	6306

Insilico Docking

Patch dock online server was used for performing insilico docking. The ligands and receptors were submitted to the online server to obtain the docked complex. The images were visualized using PyMol software.

Analysis Of Docked Complex

PyMol software is used for identifying the interaction between the ligands and receptors. The interactions were identifies along with its bond length, Area, Score and ACE. The atoms in the ligands and amino acid residues in the receptors were labeled.

Invitro analysis of the Madhuca longifolia oil

Preparation of extract

Commercially available Madhuca longifolia seed oil was purchased from Tamil Traders Pvt. Ltd, Coimbatore, Tamil Nadu, India. For the preparation of extract the oil was subjected to serial dilution in the ratio 1, 1:2, 1:4, 1:8, 1:16 and 1:32. Group A consist of oil only, group B consist of oil with the dilution of 1:2, group C consist of oil with the dilution 1:4, group D consist of oil with dilution 1:8, group E consist of oil with dilution 1:16, group F consist of oil with dilution 1:32.

DPPH Assay

In DPPH Assay 1ml of extract was mixed with 0.5ml of DPPH(0.2mM) and the absorbance was measured at 520nm against blank which consisted of distilled water and DPPH.

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Catalase Test

In Catalase test, 1ml of hydrogen peroxidase and 1ml of 0.01M phosphate buffer saline of pH 7.5 is mixed with 1 ml of extract and its absorbance is measured at 240nm against blank consisting of distilled water , hydrogen peroxide and phosphate buffer saline.

Peroxidase Test

In, Peroxidase test 0.5ml of extract is mixed with 1.5ml of pyrogallol to which 0.5ml of hydrogen peroxide is added. Absorbance for every 30 seconds till 3 minute is measured at 430nm against blank consisting of distilled water, pyrogallol and hydrogen peroxide.

Total Phenolic Content

In total phenolic content 1ml of extract is mixed with 1ml of sodium carbonate and 1 ml of folin reagent and the absorbance is measured at 763nm against blank consisting of distilled water, folin reagent and sodium carbonate.

RESULTS

In silico analysis on seed, bark and fruit of Madhuca longifolia

Interaction of ligands With 1ILG Complex

The interaction of the receptor 1ILG with the ligands hasshowed hydrogen bond which is represented in Table 3. The ligands like Aspartic acid, Cystine, Glycine, Leucine, MiSaponin A, MiSaponin B, Myricetin, Myristicacid, Oleic acid, Quercetin, Serine, Threonine, Sesquiterene alcohol, Dihydroquercetin, Quercetin-3-glucoside has showed potential interaction with the docked complex of 1ILG. The Score, Area, ACE and bonds of the docked complex with 1ILG is given in Table 3.

Table 3: Score, Area, ACE and bonds of the docked complex with 1ILG

Ligand	Score	Area	ACE	Bonds		
				Residue	Atom	Length
Alpha alanine	1976	208.90	-77.73	NIL		
Arachidonic acid	4544	672.60	-295.44	NIL		
Aspartic acid	2144	232.90	-80.43	SER-247	O5	2.9
				SER-247	O4	2.5
Cystine	3490	390.00	-182.74	GLU-321	H20	2.3
Glycine	1850	192.40	-67.24	LEU-206	H8	1.8
Isoleucine	2706	292.00	-81.51	NIL		
Leucine	4562	467.90	-114.84	GLN-285	O8	3.6
Linoelic acid	4598	572.20	-256.41	NIL		
Lysine	2872	317.70	-91.74	NIL		
Methionine	2694	289.60	-112.41	NIL		
Misaponin A	7422	1378.00	-715.13	GLN-285	O62	2.9
				ASP-310	O15	3.1
				SER-208	O34	2.3
				SER-247	O72	2.5
				ALA-312	H	2.8
				GLY-314	O84	3.1
				LEU-209	H	1.2
				LEU-209	O34	1.4
Misaponin B	8888	1226.30	-522.73	PRO-175	H	1.2
				PHE-172	H	3.3

				THR-165	O93	2.8
				CYS-301	H	2.4
				ARG-303	O16	3.5
				ARG-303	O15	1.7
				ARG-303	O91	2.3
				ARG-303	O8	2.2
				ARG-303	O92	3.3
Myricetin	4836	481.00	-139.16	SER-208	H26	2.6
				ARG-410	O7	2.1
				SER-247	O21	2.8
				MET-243	H31	2.5
Myristic acid	4246	457.30	-188.05	GLU-295	H44	1.9
Oleic cid	5020	626.20	-230.13	HIS-407	O19	2.4
Palmitic acid	4146	506.60	-200.13	NIL		
Proline	2216	236.70	-127.48	NIL		
Quercetin	4126	470.20	-165.73	HIS-407	O17	3.5
				SER-247	O20	3.1
Serine	1984	217.20	-97.37	LYS-210	O4	3.4
Stearic acid	4634	514.50	-223.11	NIL		
Threonine	2178	240.10	-72.35	ASP-205	H14	2.9
Alpha amyrin acetate	5864	737.20	-279.19	NIL		
Alpha terpeneol	3306	373.20	-102.21	NIL		
Ethylcinnmate	3572	390.10	-96.28	NIL		
Sesquiterene alcohol	6272	797.40	-201.05	LEU-209	H73	2.1
				ARG-410	O29	1.9
				ARG-410	O27	2.9
				SER-208	C26	1.9
Beta sitosterol	6256	736.30	-244.87	NIL		
Dihydroquercetin	4360	461.00	-108.84	SER-208	H30	2.8
				HIS-407	O10	2.3
				GLN-285	H32	2.4
				GLN-285	O20	3.3
1-hexacosanol	5734	657.90	-271.84	NIL		
Quercetin -3-glucoside	5640	622.70	-168.50	HIS-407	O10	2.4
				SER-208	H39	2.5
				GLN-285	O26	3.4
				SER-247	H50	3.0

The 1ILG receptor showed greater hydrogen bond interaction with MiSaponin A and and MiSaponin B(Fig 1). The amino acid residues and their corresponding bond length in MiSaponin A are GLN285 -2.9, ASP310-3.1, SER208-2.3, SER247-2.5, GLY314-3.1, ALA312-2.8, LEU209-1.2, LEU209-1.4 and in MiSaponin B are PRO175-1.2, PHE172-3.3, THR165-2.8, CYS301-2.4, ARG303-3.5, ARG303-3.5, ARG303-1.7, ARG303-2.2, ARG303-2.3, ARG303-3.3.

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• FIG 1 a. DOCKED COMPLEX OF 1ILG WITH MI SAPONIN A b. DOCKED COMPLEX OF 1ILF WITH MI SAPONIN B

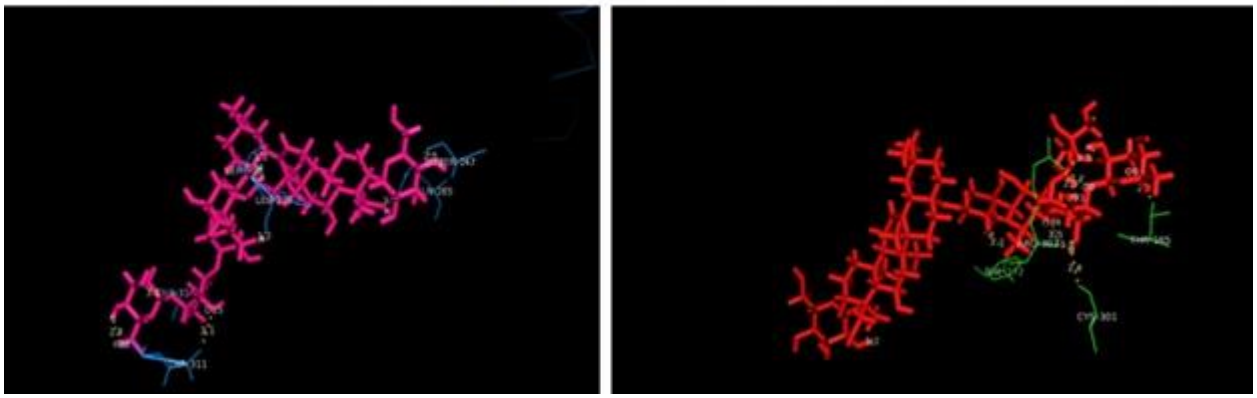

Fig 1: Docked complex of ILG with MiSaponin A and MiSaponin B

Docked complex of Receptor (Blue) and Ligand (Pink) with bonding (Yellow); b. Docked complex of receptor(Green) and Ligand(Red) with bonding(Yellow)

Interaction Of Ligands With 1OSH Complex

The interaction of the receptor 1OSH with the ligands has showed hydrogen bond that is given in Table 4. The ligands like Alpha alanine, Cystine, Isoleucine, Methionine, MiSaponin A, MiSaponin B, Myricetin, Quercetin, Serine, Alpha amyrin acetate, Alpha terpeneol, Beta sitosterol and Dihydoquercetinhas shown potential interaction with the docked complex of 1OSH. The Score, Area, ACE and bonds of the docked complex withn1 OSH is given in Table 4.

Table 4: Score, Area, ACE and bonds of the docked complex with 1OSH

Ligand	Score	Area	ACE	Bonds		
				Residue	Atom	Length
Alpha Alanine	2108	222.30	-93.83	THR-292	O4	3.5
Arachidonic Acid	5044	59940	-192.70	NIL		
Aspartic Acid	2344	266.60	-115.53	NIL		
Cystine	3588	399.20	-248.59	TYR 365	O12	2.1
					H26	2.5
Glycine	1766	198.9	-78.58	NIL		
Isoleucine	2746	293.90	-106.12	LEU 291	H20	1.8
				ALA 295	O8	3.0
Leucine	4658	521.40	-210.14	SER 336	O8	3.0
Linoelic Acid	5764	676.20	-234.07	NIL		
Lysine	3098	331.60	-147.09	NIL		
Methionine	2906	305.20	-168.98	LEU 291	H19	2.0
MiSaponin A	7670	1134	-488.73	HIS 348	H	2.6
MiSaponin B	8014	920.80	11.23	ASP 398	H	1.8
				GLN 400	H	3.3
				HIS 449	H	3.3
				TYR 401	O89	3.1
				GLU 471	H	2.9
				GLU 471	O32	3
				GLU 471	O32	3.2
				TYR 365	H26	2.4
Myricetin	4464	500.90	-185.54	LEU 291	H28	2.1
				TYR 365	H26	2.4
				SER 336	H33	3.4

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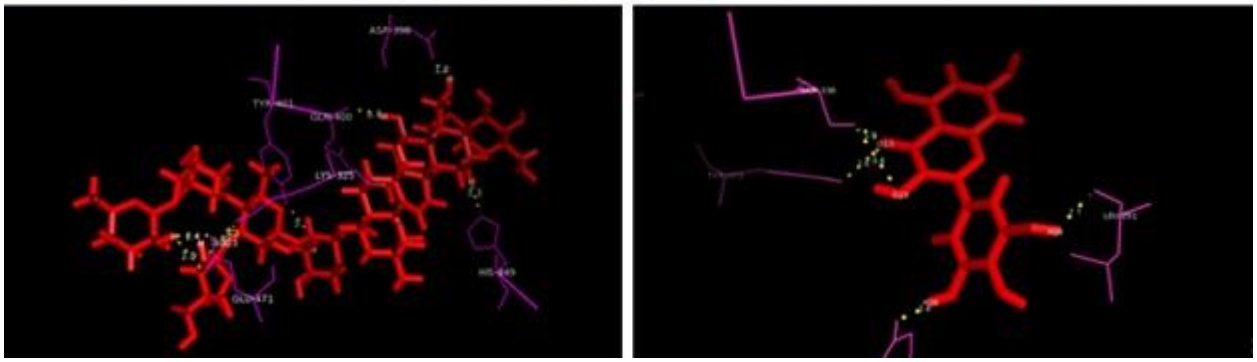
				TYR 373	O13	2.8
				TYR 373	O13	1.9
Myristic Acid	4370	521.0	-195.93	NIL		
Olenic Acid	5994	695.90	-253.39	NIL		
Palmitic Acid	4586	550.80	-217.71	NIL		
Proline	2364	271.30	-118.10	NIL		
Quercetin	5702	637.10	-368.63	THR 292	O21	3.6
				HIS298	O19	3.4
Serine	2158	231.40	-100.87	THR 292	O7	2.8
Stearic Acid	4734	594.0	-222.32	NIL		
Threonine	2352	263.80	-114.86	NIL		
Alpha Amyrin Acetate	5932	761.80	-322.89	TYR 365	O26	3.5
Alpha Terpeneol	3276	360.80	-171.62	TYR365	O11	2.9
Ethylcinnmate	3878	433.20	-161.40	NIL		
Sesquiterene Alcohol	6190	752.90	-298.57	NIL		
Beta Sitosterol	5862	714.70	-273.51	TYR 365	H67	3.5
Dihydroquercetin	4464	495.20	-214.94	TYR 373	O19	2.3
				TYR 373	O10	3.0
1 Hexocosanol	5672	732.70	-97.72	NIL		
Quercetin -3-Glucoside	5598	642.0	-276.52	NIL		

The 1OSH receptor showed greater hydrogen bond interaction with MiSaponin B and Myricetin(Fig 2). The amino acid and corresponding bond length in MiSaponin B are GLN100-3.3, HIS449-3.3, TYR401-3.1, GLU471-2.9, GLU471-3, GLU471-3.2, TYR365-2.4 and in Myricetin are LEU291-2.1, TYR365-2.4, SER336-3.4, TYR373-2.8 and TYR373-1.9.

FIG 2 a. DOCKED COMPLEX OF 1OSH WITH MI SAPONIN B b. DOCKED COMPLEX OF 1OSH WITH MYRICETIN



Docked complex of Receptor (Violet) and Ligand (Red) with bonding (Yellow)

Fig 2: Docked complex of 1OSH with MiSaponin B and Myricetin

Interaction Of Ligands With 1XNX Complex

The interaction of receptor 1XNX with the ligands has showed hydrogen bond that is represented in Table 5. The ligands like Alpha alanine, Aspartic acid, Cystine, Glycine, Isoleucine, Linoleic acid, Lysine, Methionine, MiSaponin A, MiSaponin B, Myricetin, Oleic acid, Proline, Quercetin, Serine, Steric acid, Threonine, Sesquiterene alcohol, Dihydroquercetin and Quercetin-3-Glucoside has showed potential interaction with the docked complex of 1XNX.the Score, Area, ACE and bonds of the docked complex with 1XNX is given in Table 5.

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Table 5: Score, Area, ACE and bonds of the docked complex with 1XNX

Ligand	Score	Area	ACE	Bonds		
				Residue	Atom	Length
Alpha alanine	1986	220.80	-51.74	ASP-238	H11	2.6
				ARG-156	O4	3.0
				PRO-157	H12	2.3
Arachidonic acid	5402	632.00	-187.98	NIL		
Aspartic acid	2478	265.20	-67.31	GLN-159	O9	2.5
				ARG-156	O5	2.9
				GLN-159	H14	2.2
Cystine	3406	360.20	-201.81	ASN-175	H24	3.6
				ASN-175	O12	3.4
Glycine	1634	184.90	-42.77	ARG-156	O4	3.5
Isoleucine	2828	296.10	-119.06	SER-315	H22	1.9
				SER-315	O8	1.9
Leucine	4538	521.90	-200.57	NIL		
Linoleic acid	4456	492.20	-165.31	ASN-258	O20	2.7
				ASN-258	O19	1.9
Lysine	2812	308.20	-87.94	ASP-238	H22	2.3
				ARG-156	O9	3.4
				GLN-159	H23	2.1
				GLN-159	O8	3.5
Methionine	2828	309.20	-133.22	SER-315	H20	2.2
				GLY-261	H19	2.2
				GLN-314	O8	2.7
Misaponin A	8464	1024.40	-173.57	ASN-258	O10	3.1
				LYS-260	O80	3.2
				ASP-140	H	1.8
				LYS-257	O23	2.3
Misaponin B	8490	1014.20	-202.82	SER-311	O81	2.8
				ASN-226	H	2.9
				THR-224	H	2.1
				GLN-223	O31	3.3
				GLN-223	O28	2.9
				GLN-223	O23	3.0
				GLN-223	O33	1.7
				HIS-155	H	1.7
Myricetin	4194	434.80	-194.17	ASN-175	O21	2.2
Myristic acid	4216	433.50	-174.50	NIL		
Oleic acid	5186	598.50	-148.89	GLU-266	O19	3.5
Palmitic acid	4260	475.90	-195.76	NIL		
Proline	2356	257.00	-90.39	PRO-157	H14	2.1
				GLN-159	O7	3.3
Quercetin	4054	448.00	-179.77	GLY-261	H31	1.5
				SER-315	O20	3.1
				GLN-314	O10	3.1
				ARG-312	O10	2.2
				GLN-314	O19	3.5
				SER-315	O17	3.1
Serine	2190	236.00	-61.04	GLN-159	H12	2.3
Stearic acid	5018	580.80	-178.80	GLY-354	O20	2.5

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Threonine	2446	260.40	-56.65	PRO-157	H17	2.2
				ARG-156	O8	3.4
				ARG-156	O6	3.0
				HIS-241	H15	2.3
Alpha amyrin acetate	5676	744.70	-374.80	NIL		
Alpha terpeneol	3276	341.40	-137.88	NIL		
Ethylcinnmate	3538	434.30	-133.37	NIL		
Sesquiterene alcohol	5898	796.00	-398.39	LEU-340	H71	2.1
				ASN-175	O30	2.2
Beta sitosterol	5190	661.70	-206.49	NIL		
Dihydroquercetin	4130	442.70	-179.20	LYS-235	H33	2.3
				ALA-239	O21	3.3
				ASN-175	O19	2.2
1-hexacosanol	5908	686.00	-183.87	NIL		
Querectin-3-glucoside	5420	624.40	-278.98	LYS-235	H49	2.7

The receptor 1XNX showed greater hydrogen bond interaction with MiSaponin B and Quercetin (Fig 3). The amino acid and corresponding bond length in MiSaponin B are SER311-2.8, ASN226-2.9, THR224-2.1, GLN223-3.3, GLN223-2.9, GLN223-3.0, GLN223-1.7, HIS155-1.7 and in Quercetin are GLY261-1.5, SER315-3.1, GLN314-3.1, ARG312-2.2, GLN314-3.5 and SER315-3.1.

FIG 3 a. DOCKED COMPLEX OF 1XNX WITH MI SAPONIN B b. DOCKED COMPLEX OF 1XNX WITH QUERCETIN

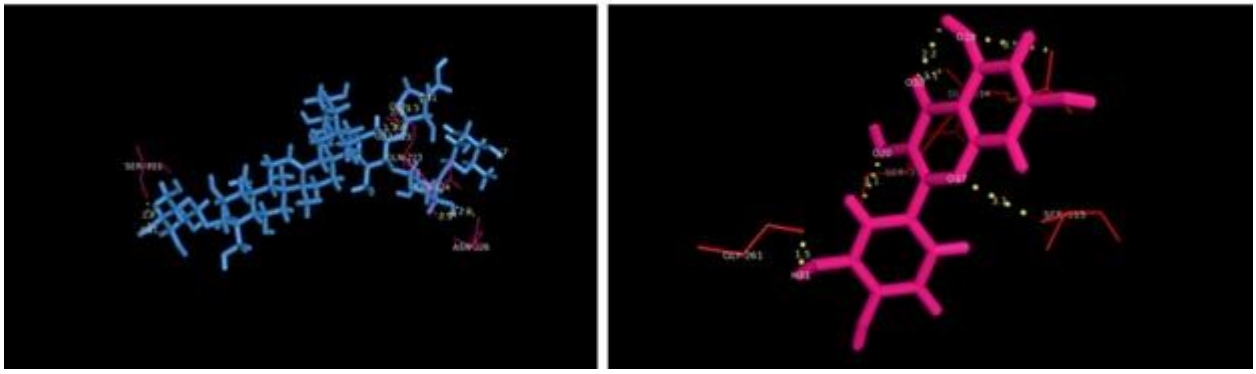

Fig 3: Docked complex of XNX with MiSaponin B and Quercetin

Docked complex of Receptor (Pink) and Ligand (Blue) with bonding (Yellow); b.Docked complex of receptor(Red) and Ligand(Pink) with bonding(Yellow)

Interaction Of Ligands With 5AVI Complex

The interaction between receptor 5AVI and the ligands has showed the hydrogen bond which is mentioned in Table 6. The ligands like Alpha alanine, Aspartic acis, Cystine, Glycine, Lysine, Methionine, MiSaponin A, MiSaponin B, Myricetin, Palmitic acid, Proline, Quercetin, Serine, Threonine, Alpha terpeneol, Sesquiterene alcohol, Dihydroquercetin and Quercetin-3-Glucoside has showed potential interaction with the docked complex of 5AVI. The Score, Area, ACE and bonds of the docked complex with 5AVI is given in Table 6.

Table 6: Score, Area, ACE and bonds of the docked complex with 5AVI

Ligand	**Score**	**Area**	**ACE**	**Bonds**		
				Residue	**Atom**	**Length**
Alpha alanine	2136	224.40	-11.69	GLY 382	H13	2.2

				LYS 410	O4	3.1
Arachidonic acid	5932	710.80	-190.65	NIL		
Aspartic acid	2498	275.50	1.61	ARG 415	O5	3.4
				LYS 410	O9	3.2
				GLU 379	H15	2.6
Cystine	3832	435.90	-53.49	GLU 379	H26	1.6
				MET 409	H20	2.5
				SER 413	H20	2.4
				GLN 382	H18	3.3
				ALA 367	O5	2.9
Glycine	1790	192.30	-13.85	SER 413	O4	3.2
				ASP 368	H8	2.0
Isoleucine	2720	295.90	-100.99	NIL		
Leucine	4918	514.20	-195.84	NIL		
Linoleic acid	5578	636.00	-169.72	NIL		
Lysine	3260	359.50	-19.50	GLN 382	O9	3.5
Methionine	3122	349.20	-42.46	GLU 379	H20	2.3
Misaponin A	8856	1333.70	-452.82	SER 419	H	2.2
				THR 416	H	2.2
				SER 422	O34	3.2
				PRO 370	H	2.8
				GLN 375	O83	3.1
				GLN 375	H	1.8
Misaponin B	9946	1431.70	-308.13	THR 302	O68	2.8
				ARG 305	O41	2.9
				ARG 305	O19	2.7
				ASP 353	H	2.5
				GLN 222	O93	2.6
				GLN 222	H	1.3
Myricetin	4566	488.70	-229.66	SER 264	O7	3.2
Myristic acid	4952	597.20	-88.71	NIL		
Oleic acid	5698	624.40	-166.35	NIL		
Palmitic acid	5362	591.00	-135.40	LYS 317	O17	3.4
				LYS 317	O17	3.2
				ARG 305	O18	1.5
Proline	2454	276.30	-12.90	LYS 410	O7	3.1
Quercetin	4588	545.60	27.11	GLU 348	H28	2.4
				GLN 375	H29	2.1
				GLN 375	H29	2.4
				ARG 344	O19	2.4
				GLN 375	O10	3
				GLU 341	O10	2.1
				ASP 368	O21	3.1
				ASP 367	O21	3.3
Serine	2252	228.10	-11.03	GLU 379	H11	2.5
				LYS 410	O4	2.6
				ARG 415	O7	3.3
				MET 409	H13	2.3
Staeric acid	5514	620.20	-168.89	NIL		
Threonine	2484	274.70	-2.05	GLU 379	H15	2.1
				LYS 410	O6	2.9
				SER 413	O8	2.2
Alpha amyrin acetate	6420	775.50	-386.83	NIL		

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Alpha terpeneol	3464	386.20	-27.75	MET 409	H29	2.0
				SER 413	O11	2.6
Ethylcinnmate	3892	456.10	-17.14	NIL		
Sesquiterene alcohol	5976	756.90	-126.91	THR 302	H71	2.8
				MET 298	H71	2.2
				SER 264	H77	2.6
				SER 264	O33	2.1
Beta sitosterol	6216	758.30.	-371.17	NIL		
Dihydroquercetin	4670	483.80	-230.99	THR 302	O21	3
				THR 302	O23	1.2
1-hexacosanol	6418	826.10	-131.01	NIL		
Quercetin-3-glucoside	5702	637.10	-368.63	HIS 421	O10	2.7
				HIS 421	O19	3.4
				ILE 295	H50	2.4
				THR 302	O29	3.2

The receptor 5AVI showed greater hydrogen bond interaction with Cystine, MiSaponin A, MiSaponin B and Quercetin (Fig 4). The amino acid residues and bond length in Cystine are GLY379-1.6, MET409-2.5, SER413-2.4, GLN382-3.3, ALA367-2.9; in MiSaponin A are SER419-2.2, THR416-2.2, SER422-3.2, PRO370-2.8, GLN375-3.1, GLN375-1.8; in MiSaponin A are THR302-2.8, ARG305-2.9, ARG305-2.7, ASP353-2.5, GLN222-2.6, GLN222-1.3 ; in Quercetin are GLU348-2.4, GLN375-2.1, GLN375-2.4, GLN375-3.0, ARG344-2.4, GLY341-2.1, ASP368-3.1 and ASP367-3.3 .

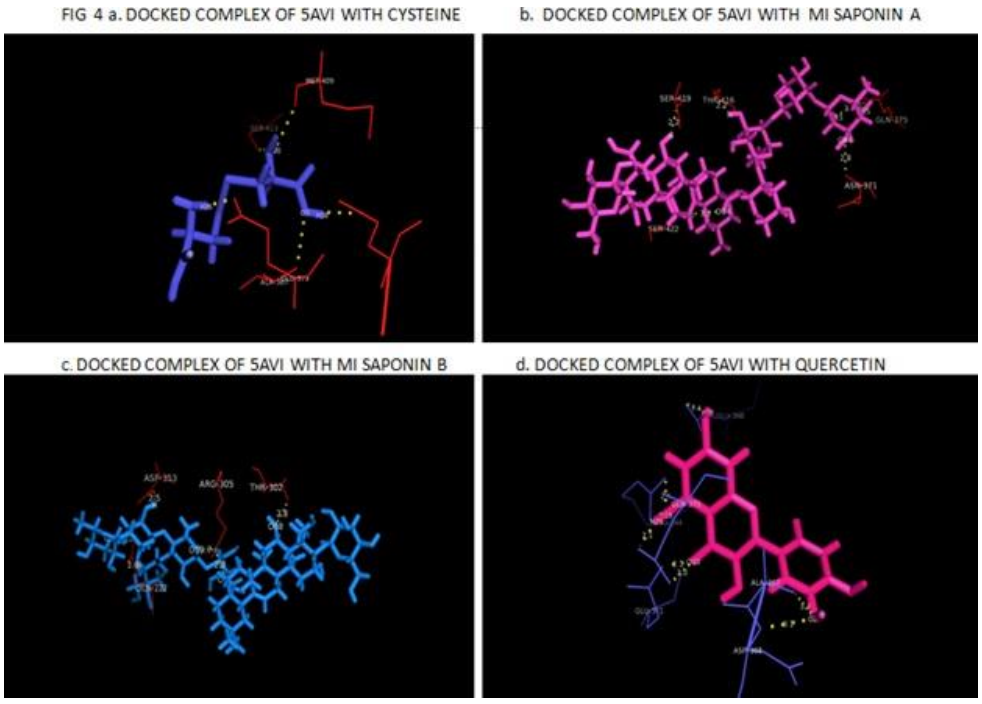

Fig 4: Docked complex of 5AVI with (a) Cystine, (b) MiSaponin A, (c) MiSaponin B and (d) Quercetin

Docked complex of Receptor (Red) and Ligand (Blue) with bonding (Yellow); b.Docked complex of receptor(Red) and Ligand(Pink) with bonding(Yellow); c. Docked complex of Recptor (Red) and Ligand (Blue) with bonding (Yellow); d. Docked complex of Receptor (Blue) and Ligand (Pink) with bonding (Yellow)

Interaction Of Ligands With 1NFK Complex

The interaction of receptor 1NFK with the ligands has showed hydrogen bond interaction that is represented in Table 7. The ligands like Arachidonic acid, Cystine, Isoleucine, Leucine, Lysine, Methionine, MiSaponin A, MiSaponin B, Myricetin, Oleic acid, Palmitic acid, Quercetin, Serine, Threonine, Alpha terpeneol, Sesquiterene alcohol, Beta sitosterol, Dihydroquercetin, 1-Hexacosanol, Quercetin-3-glucodise and Stearic aicd has showed potential interaction with the docked complex of 1NFK. The Score, Area, ACE and bonds of the docked complex with 1NFK is given in Table 7.

Table 7: Score, Area, ACE and bonds of the docked complex with 1NFK

Ligand	Score	Area	ACE	Bonds		
				Residue	Atom	Length
Alpha Alanine	2154	233.10	-55.65	NIL		
Arachidonic Acid	5560	699.70	-128.46	ARG-305	O21	2.7
				DA-5	H54	2.4
				DA-5	O22	1.4
				ARG-305	O21	2.0
Aspartic Acid	2480	293.20	-46.63	NIL		
Cystine	3508	420.70	-194.19	CYS-145	O13	2.7
				CYS-145	O12	2.1
				DT-8	H25	2.3
Glycine	1782	194	-40.35	DC-9	H8	2.5
Isoleucine	2966	325.70	-79.65	LYS-272	H20	2.4
				GLN-306	O8	2.1
Leucine	5130	550.70	-92.40	ARG-305	O12	2.8
				DA-5	O11	2.0
				DA-5	O11	2.7
				LYS-272	O2	2.6
				DA-6	H30	2.6
Linoelic Acid	5042	603.42	-131.85	NIL		
Lysine	5130	550.70	-92.40	HIS-141	O9	2.8
Methionine	2902	338.80	-152.38	DT-8	H20	2.6
				DT-8	H20	2.4
				LYS-145	O7	1.4
MiSaponin A	9492	1328.1	-167.76	ASN-247	O83	2.6
				ASN-247	H	2.1
				LYS-74	O24	2.3
				LYS-74	H	2.3
				LYS-74	H	1.4
				LYS-76	O71	2.0
				LYS-76	O75	0.7
MiSaponin B	8868	1363.4	-112.78	SER-246	O71	2.4
				ASN-247	H	2.1
				LYS-241	O83	1.3
				LYS-272	O81	1.2
				LYS-272	O82	1.6
				DG-2	H	2.6
				LYS-74	033	2.2
				GLU-73	H	1.1
				GLU-73	H	2.5
				LYS-76	O93	1.9

Myricetin	4352	494.60	11.4	LYS-272	O22	2.2
				ARG-305	O23	1..5
				LYS-249	O8	2.7
				LYS-249	O7	1.7
Myristic Acid	4750	557..6	-158.63	NIL		
Oleic Acid	5420	665.3	-303.59	DG-3	O19	2.8
Palmitic Acid	5088	602.50	-76.73	ARG-305	O18	2.7
				DA-5	O18	2.5
Proline	2478	281.3	-53.80	NIL		
Quercetin	4346	539.20	-127.07	DT-8	H31	2.8
				LYS-145	O20	2.2
Serine	2200	236.30	-58.54	DT-8	H14	2.2
				TYR-57	O7	2.7
Threonine	2452	275.50	-63.28	DA-6	O6	2.4
				DA-5	H14	2.3
				LYS-272	O6	2.7
Alpha Amyrin Acetate	6316	783.00	-61.88	NIL		
Alpha Terpenol	3558	413.10	-68.76	LYS-145	O11	2.1
Ethyl Cinnamate	3930	452.30	-130.93	NIL		
Sesquiterene Alcohol	6826	860.40	-41.63	SER-240	O34	2.5
				ARG-54	O35	2.6
				SER-240	O27	2.8
				ARG-305	O32	2.3
Beta Setosterol	5976	761.00	-102.38	DA-5	O25	2.4
				LYS-272	O25	1.2
DehydroQuercetin	4338	511.80	-83.27	LYS-145	O22	2.5
				LYS-145	O21	2.0
				DT-8	H32	2.0
1-Hexocosanol	7306	877.80	-123.28	LYS-241	O27	2.3
Quercetin- 3-Glucoside	5630	648.40	33.10	SER-240	O33	3.1
				ARG-247	O32	2.4
				ARG-247	H48	2.5
				DA-5	O30	2.2
				DG-4	H50	2.7
				ASP-271	C9	3.1
				ARG-305	O10	0.5
				DG-4	H40	2.3
				LYS-249	O18	1.3
Stearic Acid	5748	676.40	-106.62	LYS-272	O19	2.8
				LYS-241	O20	2.1

The receptor showed greater interaction with Leucine, MiSaponin A, MiSaponin B and Quercetin-3-glucoside (Fig 5). The amino acid residues and the bond length in Leucine are ARG305-2.8, DA5-2.0, DA5-2.7, LYS272-2.6, DA6-2.6; in MiSaponin A are ASN247-2.6, ASN247-2.1, LYS74-2.3, LYS74-2.3, LYS74-1.4, LYS76-2.0. LYS76-0.7; in MiSaponin B are SER246-2.4, ASN247-2.1, LYS241-1.3, LYS272-1.2, DG2-2.6, LYS272-1.6, LYS74-2.2, GLU73-1.1, GLU73-2.5, LYS76-1.9; in Quercetin-3-glucoside are SER240-3.1, ARG247-2.4, ARG247-2.5, DA5-2.2. DG4-2.7, ASP271-3.1, ARG305-0.5, DG4-2.3 and LYS249-1.3.

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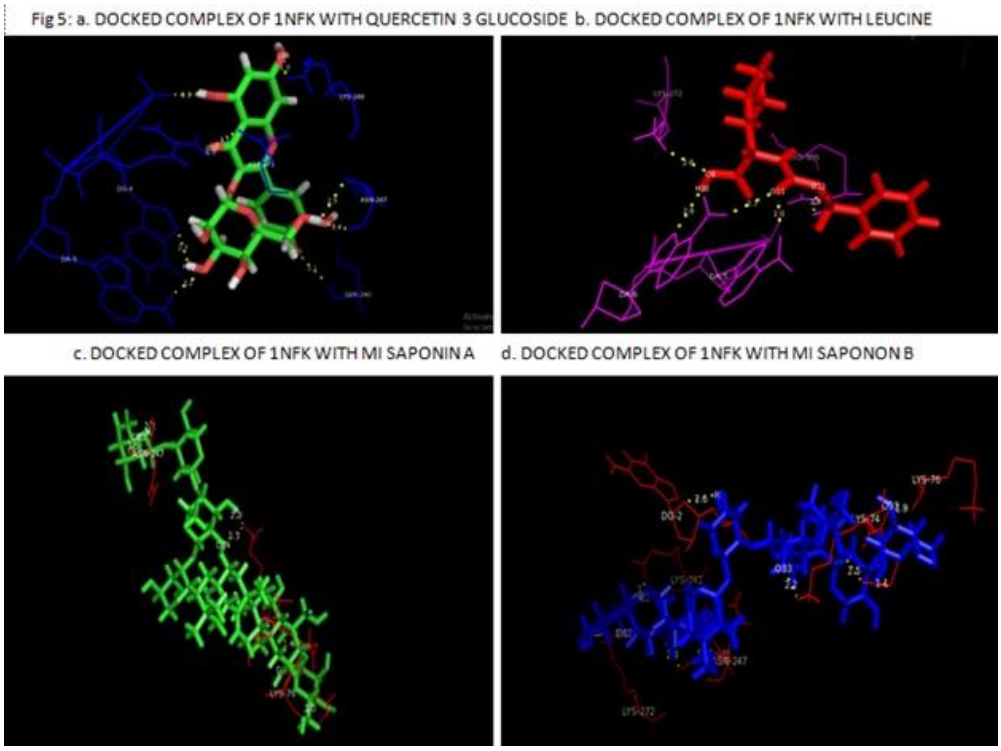

Fig 5: Docked complex of 1NFK with (a) Quercetin-3-glucoside, (b) leucine, (c) MiSaponin A and (d) MiSaponin B

Docked complex of Receptor (Blue) and Ligand (all colours) with bonding (Yellow); b. Docked complex of receptor(Violet) and Ligand(Red) with bonding(Yellow); c. Docked complex of Receptor (Red) and Ligand (Green) with bonding(Yellow); d. Docked Complex of Receptor (Red) and Ligand (Blue) with bonding (Yellow)

Invitro analysis of the Madhuca longifolia oil

Potential activity of Madhucalongifolia oil in Dpph Assay

In this assay (Fig 6), Group A showed 1.74% inhibition which shows that it has better potential effect. The graph shows decrease in the % inhibition as increase in the ratio of extract. The ratio of the extract is directly proportional to activity assay.

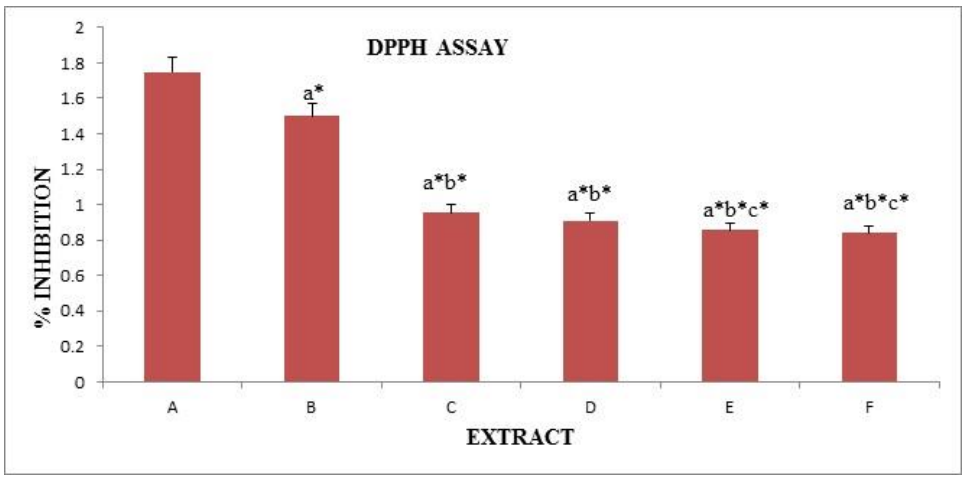

Fig 6: Potential activity of Madhuca longifolia oil on DPPH assay

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Each value represents the mean ± SD of six rats. Comparisons were made as follows: a-Group-Avs groups B, C, D, E, F; b- Group-B vs Group-C, D, E, F; c-Group-C vs Group-D, E, F; d-Group-D vs Group-E, F; Group-E vs Group-F. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

Potential activity of Madhuca longifolia oil inCatalase Test

In this assay (Fig 7), group A consisting of oil showed high catalase activity than other groups. Group A showed 0.577±0.014. There is a sharp decrease in the catalase activity as increase in dilution of the extract

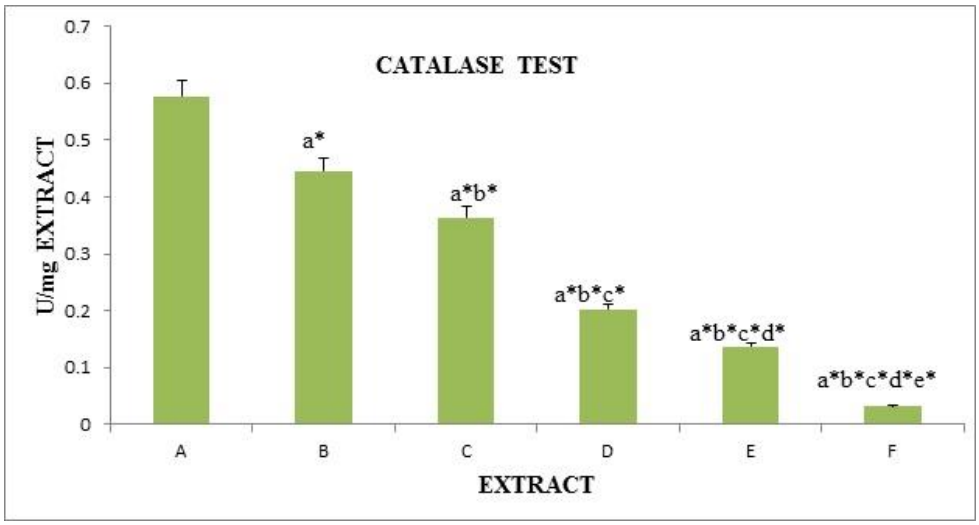

Fig 7: Potential activity of Madhuca longifolia oil on catalase assay

Each value represents the mean ± SD of six rats. Comparisons were made as follows: a-Group-Avs groups B, C, D, E, F; b- Group-B vs Group-C, D, E, F; c-Group-C vs Group-D, E, F; d-Group-D vs Group-E, F; Group-E vs Group-F. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

Potential activity of Madhuca longifolia oil inPeroxidase Test

In this study (Fig 8), group A showed peroxidase activity of 0.834±0.007 which is maximum among other groups. The graph shows decrease in the peroxidase activity with increase in dilution of extract.

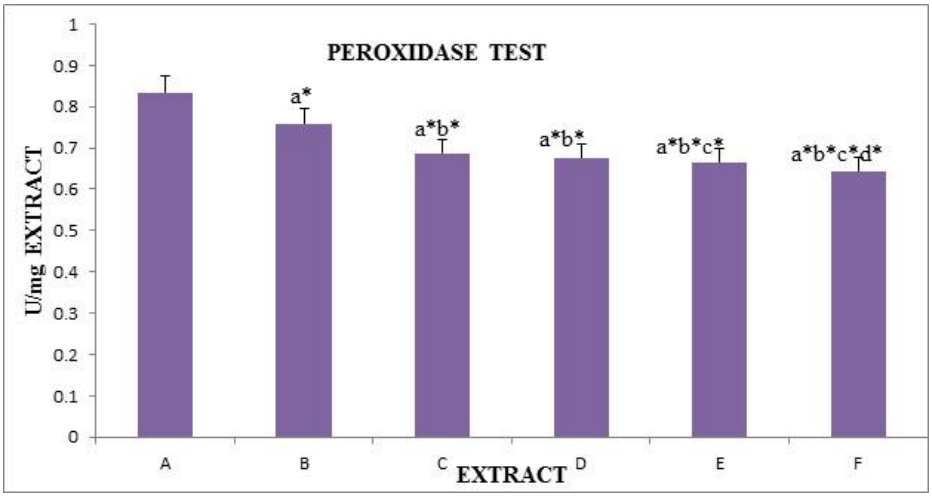

Fig 8: Potential activity of Madhuca longifolia oil on Peroxidase assay

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Each value represents the mean ± SD of six rats. Comparisons were made as follows: a-Group-Avs groups B, C, D, E, F; b- Group-B vs Group-C, D, E, F; c-Group-C vs Group-D, E, F; d-Group-D vs Group-E, F; Group-E vs Group-F. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

Potential activity of Madhuca longifolia oil inTotal Phenolic Content

In this study (Fig 9), the total phenolic content of group A is 2.613±0.039; of group B is 1.492±0.004; of group C is1.403±0.004; of group D is 1.205±0.003; of group E is 1.151±0.006; of group F is 1.138±0.002. The graph shows decrease in total phenolic content with increase in dilution of extract.

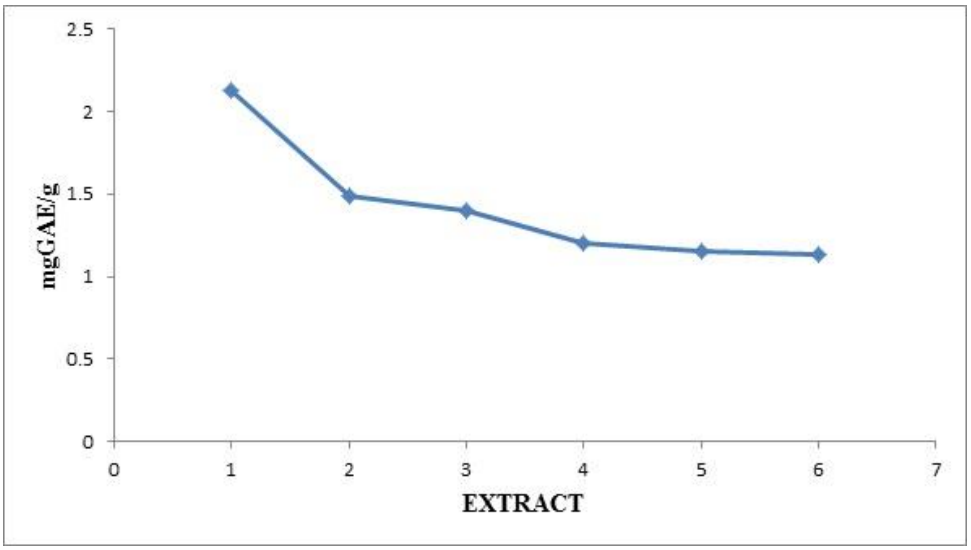

Fig 9: Potential activity of Madhuca longifolia oil on total phenolic content

Each value represents the mean ± SD of six rats. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

DISCUSSION

Madhuca longifolia has been proved to have an effective protectiveness against hepatotoxicity. The receptors that get activated during hepatotoxicity like 1ILG, 1OSH, 1XNX, 5AVI and 1NFK were docked with the ligands from the M.longifolia that has shown a potential interaction which predicts the beneficial effect M.longifoliain treating hepatotoxicity. The receptors that when activated causes hepatotoxicity that showed high interaction with the ligands on the M.longifolia[13]. The ligands that showed high interaction with all the receptors is MiSaponin B which has a high hydrogen bonding and high oxygen interaction followed by MiSaponin A, Myricetin, Quercetin, Leucine and Cystine. The activation of 1OSH suppresses autophagy and causes defects in metabolic targets outside of bile[14].These nuclear receptors function to detect the presence of toxic foreign substance and regulate the proteins involved in detoxification. Thus to conclude MiSaponin B can be used preferentially as the main ligand from M.longifolia for treating hepatotoxicity by their good interactions with the receptors which get activated during hepatic diseases. Saponin is a natural amphile that is used as an adjuvant for drug delivery which consist of hydrophobic aglycone and hydrophilic glycine[15]. Saponin is reported to have beneficial effect as anti-apoptosis, angiogenic and antioxidant activity[16–18].

The in vitro assay of the oil of Madhuca longifolia has showed its beneficial activity which demonstrates its antioxidant activities. During chemical reactions some free radical are produced in the form of reactive oxygen species. The accumulation of this reactive oxygen species will cause oxidative stress in the system by the formation of toxic metabolites from these free radicals. Naturally these free radicals are scavenged by the antioxidant enzyme present in the body [19]. The antioxidant enzyme like catalase will degrade the free radicals hydrogen peroxidase by converting them into non-toxic compound like water and

oxygen [20]. The peroxidase will also degrade the hydrogen peroxidase and detoxify the toxic metabolites [21]. DPPH will help in enhancing the antioxidant properties [22]. In our result the oil alone has showed potential result than the serial dilution. Our result demonstrates the activity of the oil of Madhuca longifolia as antioxidant agent.

CONCLUSION

Hepatotoxicity is a chemical driven liver disorder. The current study was done to know the inhibitory activity of the ligands towards the receptors that causes hepatotoxicity. The hepato inhibitory activity of the active components of Madhucalongifolia was taken for the study. Docking was performed to know the interactions between the ligand and the receptor. From the present study, the docked complex of MiSaponin B had shown the maximum affinity toward the receptor than any other ligands. It had shown maximum greater hydrogen bond interaction with the receptor. MiSaponin B binds to the receptor and thus prevents the binding of the hepatotoxic chemical to the receptor and thus prevents hepatotoxicity. So our present study concludes that MiSaponin B has higher effectiveness in the treatment of hepatotoxicity and the in vitro activity demonstrated the antixodant activity of the oil of Madhuca longifolia. This can then be further experimented through in vivo models and can be studied by gene expression studies.

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