CHAPTER-5

RESULT & DISCUSSION

Index

5.1 Phytochemical screening and proximate analysis:

5.1.1 Phytochemical screening

Table 5.1Phytochemical Screening

Chemical Class	Test name	Aghedo extract	Garcincia ext	Curry leaves extract	Guggul extract
Carbohydrate	**Molisch's test**	-	+	+	-
Proteins	**Biuret test**	-	+	+	+
	Millon's test	-	-	+	+
	Precipitation	-	-	+	+
	test				
Amino acids	**Ninhydrin test**	-	+	+	+
Steroids	**Salkowaski reaction**	-	-	+	+
	Libermann – Burchard reaction	-	-	+	+
Cardiac glycosides	**Legal's test**	-	-	-	+
	Libermann's test	-	-	-	+
Saponin Glycosides	**Foam test**	+	+	-	-
Flavanoids	**Fluorescence test**	-	+	+	+
Alkaloids	**Dragendorff's test**	+	-		
	Mayer's test	+	-	-	-
	Hager's test	+	-	-	+-
	Wagner's test	+	-	-	+
Tannins and Phenolics	**Ferric chloride test**	-	+	+	-
	Lead acetate test	-	+	+	-

5.1.2 Organoleptic evaluation:

Table 5.2 organoleptic evaluation

Parameters	Aghedo extract	Garcinia Extract	Curry leaves extract	Guggul extract
Colour	Light brown	Off White	Dark brown	Off white
Odour	Characteristic	Characteristic	Characteristic	Pungent
Taste	Bitter	Sour	Sweet	Astringent

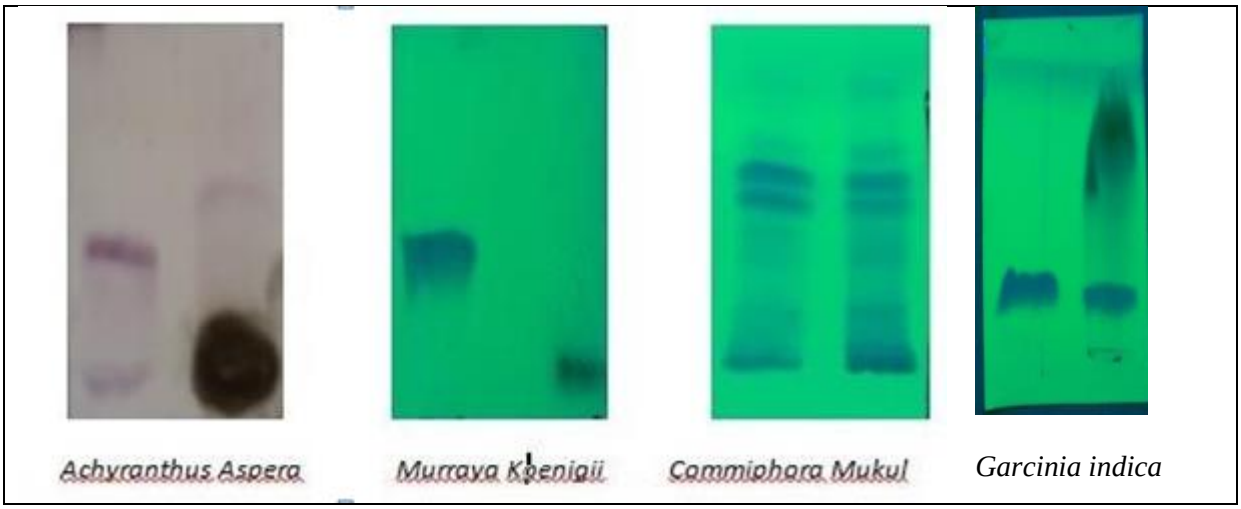
5.1.3 Microbial Analysis:

Table 5.3 Microbial Analysis

Parameter	Aghedo extract	Garcinia extract	Curry Leaves extract	Guggul extract
Total microbial count(CFU/gm)	340	Complies	3698	3568
(Limit)	NMT 10,000 CFU/GM			
Yeast and Mould Count(CFU/gm)	28	Complies	25	Less than 10
(Limit)	NMT 100CFU/GM			
E.coli.	Absent	Absent	Absent	Absent

5.1.4 Thin Layer Chromatography:

Table 5.4 TLC of extracts



5.1.5 Determination of moisture content:

Table 5.5 LOD results for extracts

Plants	Moisture content (Loss on drying) (% w/w)
Aghedo extract	4.99 %
Garcinia extract	1.74 %
Curry leaves extract	2.0 %
Guggul extract	1.6 %

5.1.6 Determination of Heavy metals:

Table 5.6Heavy metal results for extracts (from COA)

Heavy Metals	Standard Values	Aghedo extract	Garcinia extract	Curry leaves extract	Guggul extract
Arsenic	NMT 1.0 ppm	Complies	Complies	Complies	Complies
Mercuric (ppm)	NMT 0.1 ppm	Complies	Complies	Complies	Complies
Lead (ppm)	NMT 3.0 ppm	Complies	Complies	Complies	Complies
Cadmium(ppm)	NMT 1.0 ppm	Complies	Complies	Complies	Complies

5.1.7 Detemination of secondary metabolites:

5.1.7.1 Determination of total phenolic content:

Calibration data for gallic acid

Table 5.7Calibration data for Gallic acid

Concentration of Gallic acid (µg/ml)	Absorbance
4	0.267
6	0.412
8	0.544
10	0.684
12	0.818

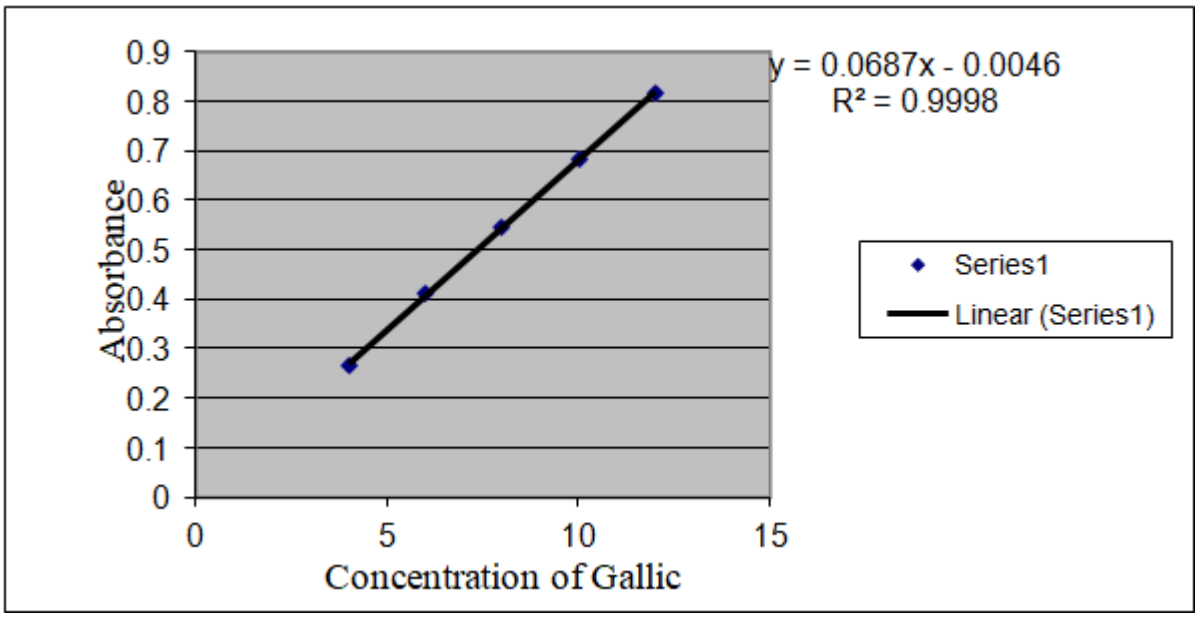


Figure 5.1 Calibration graph of Gallic acid

Phenolic compounds like Gallic acid reacts with folin ciocalteu reagent and forms blue coloured complex and measured the absorbance of this blue coloured complex at 550nm. The total phenolic content was expressed in terms of % of gallic acid

Results for total phenolic content:

Table 5.8Total phenolic content for all plant extracts

Plant Extract	Total phenolic content
Aghedo	0.77%
Garcinia	0.59%
Curry leaves	5.197%
Guggul	4.03%

5.1.7.2 Determination of Total Tannin content:

Table 5.9Calibration data for Tannin content

Concentration (µg/ml)	Absorbance
1	0.196
2	0.262
3	0.314
4	0.373
5	0.425
6	0.489

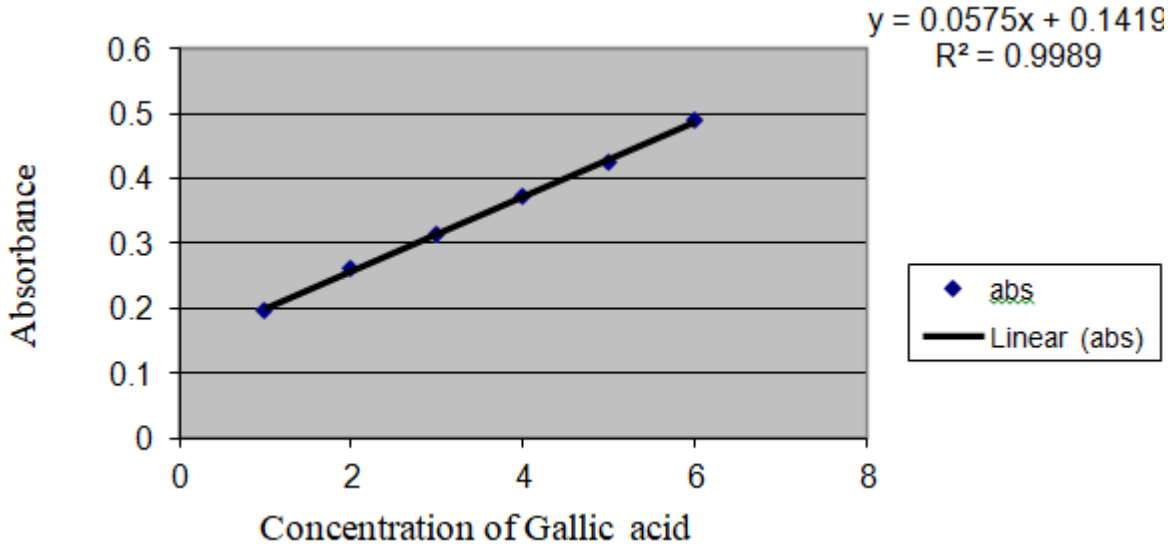


Figure 5.2Calibration plot of gallic acid for tannin content

Results for Total Tannin content:

Table 5.10Total Tannin content

Plant Extract	Total Tannin content
Aghedo	5.2%
Garcinia	40.70%
Curry leaves	21.74%
Guggul	0.33%

5.1.7.3 Determination of Total Flavanoid content:

Table 5.11Absorbance of Quercetin

Concentration of Quercetin (µg/ml)	Absorbance
10	0.126
20	0.252
30	0.363
40	0.477
50	0.596

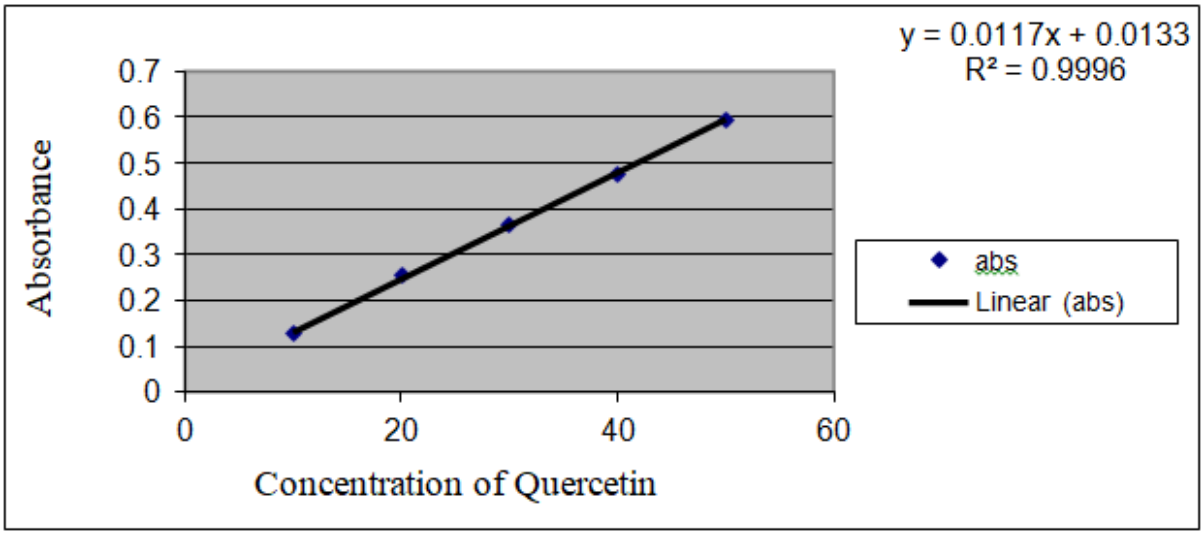


Figure 5.3Calibration plot of Quercetin

Result of total Flavanoid content:

Table 5.12Total Flavanoid Content

Plant extract	Total Flavanoid content
Aghedo	0.37%
Garcinia	0.67%
Curry leaves	4.816%
Guggul	6.055%

5.2 Tablet Formulation:

5.2.1 Preformulation data for tablet:

Table 5.13Preformulation Data

Herbal Extracts	Bulk density	Tapped density	Hausner's ratio	Angle of repose	Compressibility Index
Aghedo	0.612	0.77	0.78	34.02	21.33
Garcinia	0.31	0.34	1.10	45.00	9.36
Curry leaves	0.50	0.61	0.817	26.56	18.30
Guggul	0.58	0.68	0.898	34.59	10.07
Blend(Extracts+Excipients	0.61	0.70	0.88	32.45	12.03

5.2.2 Development of Tablet Formulation:

Here Tablet was prepared by Direct Compression method. Direct compression method is the best suitable method for developing polyherbal tablet.

In wet granulation method the mass became very sticky and in dry granulation the mass became too much hard difficult for disintegrating the tablet.

Syloid is having good adsorbant property. Therefore It is widely used in formulating polyherbal tablet. Here With the use of Syloid and without Syloid , comparison was made by appearing tablet.

Table 5.14 Tablet Formulation

Achyranthus aspera ext.	50mg
Commiphora wightii ext	150mg
Morraya koinigi ext.	50mg
Garcinia indica ext	150mg
Avicel PH 102	100mg
Styloid	16mg
SSG	42mg
Cross carmellose sodium	30mg
Talc	6mg
Methyl peraben	6mg
Total	600mg

5.2.3 Evaluation Parameters for tablet:

Table 5.15Evalution of tablets

Appearance	Complies
Hardness	4.5 kg
Disintegration	9 mins
Friability	>1 %

5.2.4 Stability Studies:

Table 5.16Stability Data

Sr.No.	Physiological Parameters	Storage condition (Room Temperature)				
		0 Day	15 days	30 days	02 Months	03 Months
1	Appearance	Complies	Complies	Complies	Complies	Complies
2	Hardness(Kg)	4.5	4.5	4.3	4.3	4.3
3	Disintegration time (Minutes)	9	10	10	12	13
4	Friability (%)	0.8	0.8	0.85	0.85	0.85

5.3 HPLC Analytical method development and validation:

5.3.1 HPLC Method development:

5.3.1.1 Method parameters:

Table 5.17HPLC Method Parameter

Column	Hyperchrom ODS BP C18 (Size: 250*4.6 mm,5µ)
Flow rate	1.0 ml/min
Detection wavelength	222 nm
Mobile Phase	Ortho Phosphoric acid 0.1 % in Water : Methanol(5:95) It was filtered through 0.45 µm Nylon filter and sonicated for 5 min.
Injection Volume	20 µl through rheodyne manual injector.
Temperature	Ambient
Retention Time	2.8 min for Gallic acid and 9.9 min for Oleanolic acid

5.3.1.2 Isoabsorptive point (Wavelength selection)

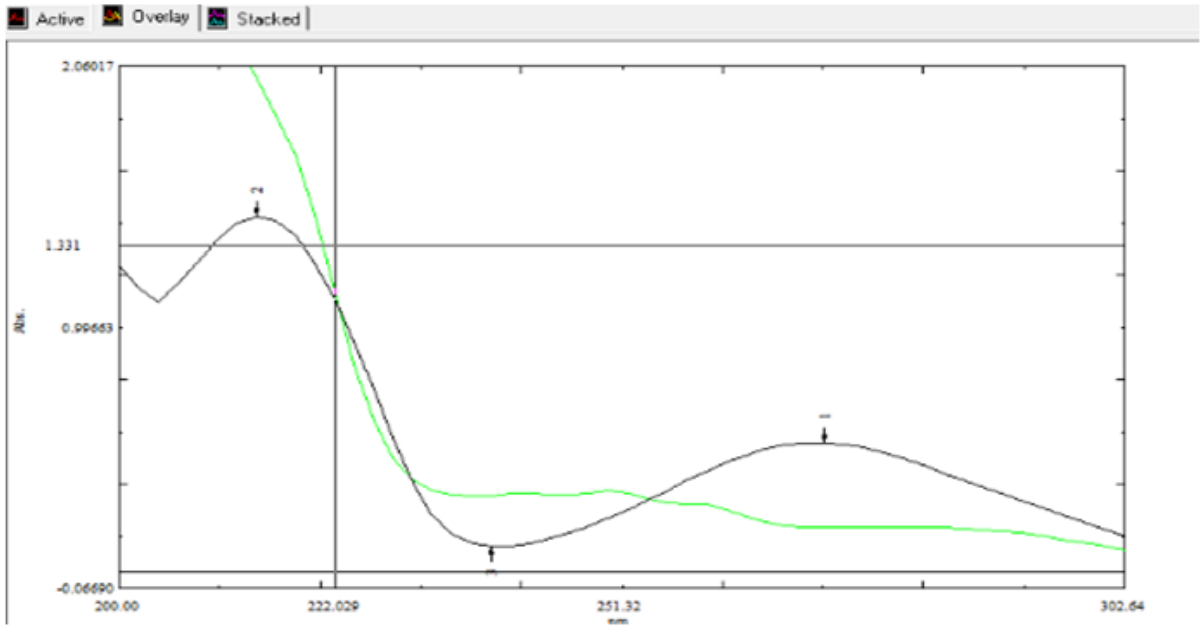


Figure 5.4 Overlay spectra of Overlay spectra for both markers GA and OA

Scanning of Gallic acid standard and Oleanolic acid standard were run by UV Visible spectroscopy and both the markers were intercept at 222 nm. Therefore 222nm was selected as detection wavelength for further study.

5.3.1.3 System Suitability Parameters:

After various trials the mobile phase 0.1 % Orthophosphoric acid and methanol with the ratio of 5:95 would give a good resolution and sharp peak. The below chromatogram pass the system suitability parameters such as tailing factor, theoretical plates and resolution.

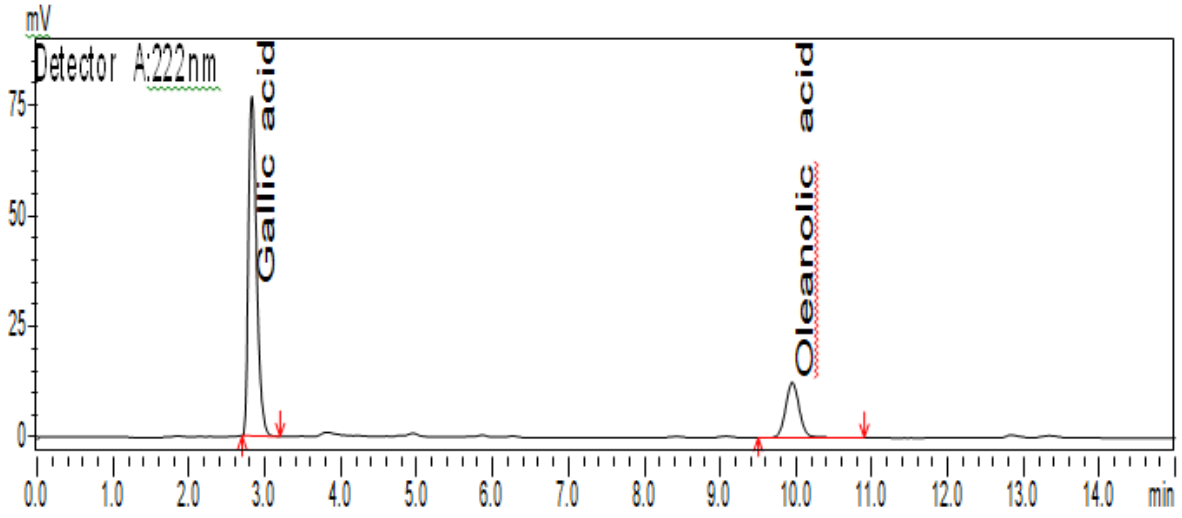


Figure 5.5 HPLC Chromatogram of Simultaneous estimation of Gallic acid and Oleanolic acid

Table 5.18Peak symmetry for Gallic acid and Oleanolic acid.

Name	RT	Peak start	Peak End	Height	Area	Area %	Tailing factor	Theoretical plate	Resolutionon
Gallic acid	2.844	2.700	3.200	61108	575350	78.65	1.218	3088.996	-
Oleanolic acid	9.949	9.500	10.90	11714	156109	21.34	1.076	14402.220	26.501

5.3.2 HPLC Method Validation:

Linearity parameters:

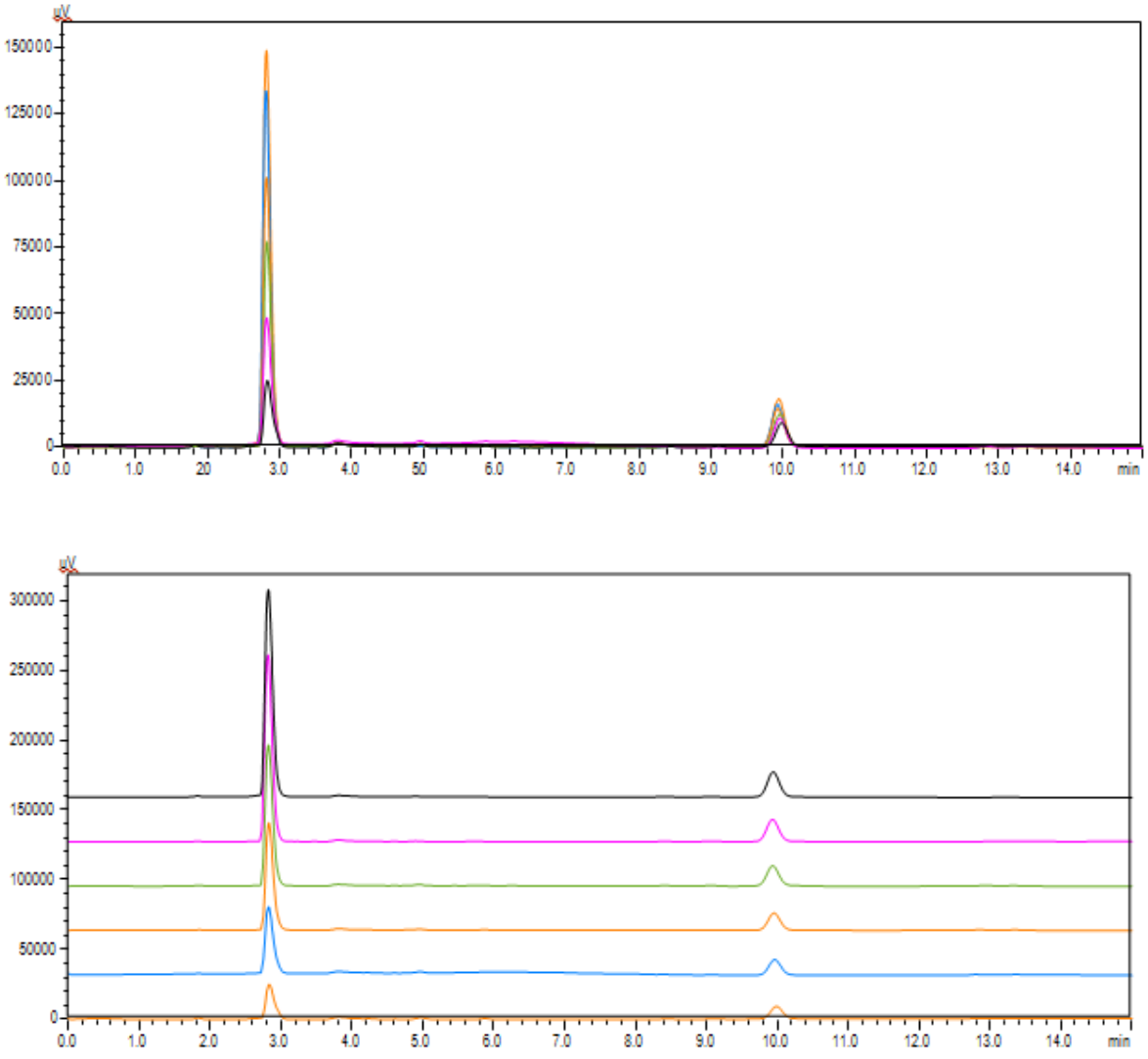


Figure 5.6Overlay HPLC Chromatogram for different linearity concentration for both

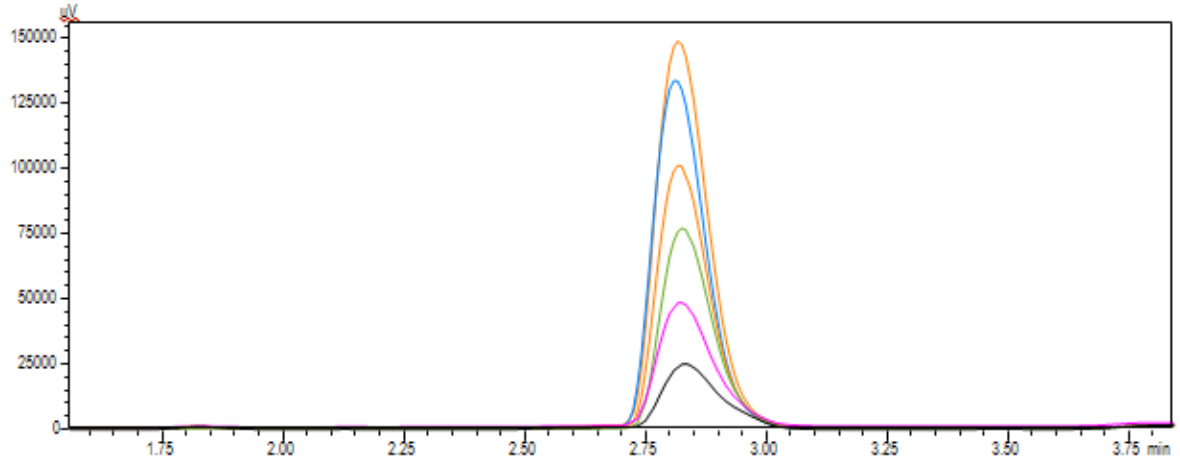


Figure 5.7Overlay HPLC Chromatogram for different linearity concentration forGalic Acid

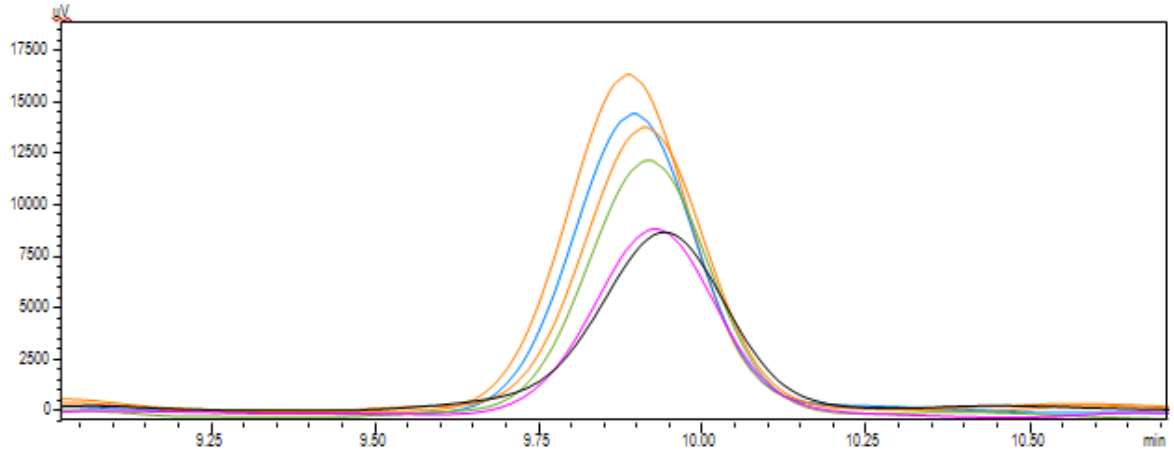


Figure 5.8Overlay HPLC Chromatogram for different linearity concentration for Oleanolic acid

Concentration of GA in µg/ml	Avg. Area of Gallic acid
1	204339
2	379961
3	597321
4	775443
5	984878
6	1128298

Concentration of OA in µg/ml	Avg. Area of Oleanolic acid
50	118299
60	140246
70	163724
80	189978
90	209210
100	229411

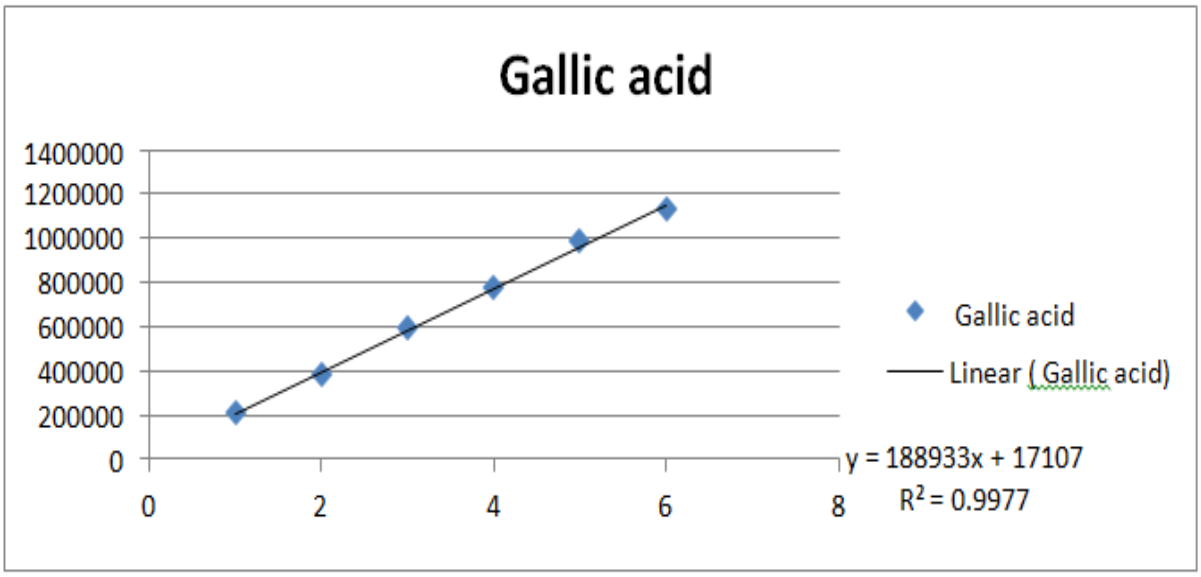


Figure 5.9Calibration curve between Area of peak GA verses its Concentration

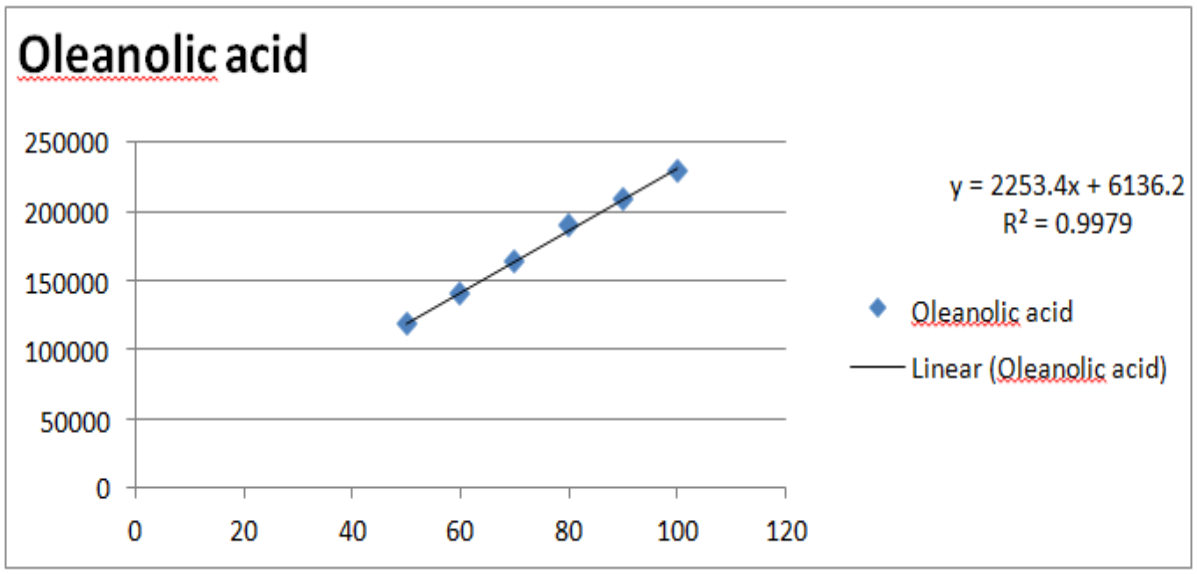


Figure 5.10Calibration curve between Area of peak OA verses Concentration

Precision data:

Table 5.19Interday and Intraday precision data

Marker	Conc. (µg/ml)	Intraday (n=3)			Interday (n=3)		
		Area ± SD	**% RSD of Area**	**%RSD of RT**	**Area ± SD**	**% RSD of Area**	**%RSD ofRT**
Gallic acid	**1**	252159 ± 3911.02	1.5510	0.248	272457 ± 5289.85	1.9415	0.582
	4	717412.7 ±7355.19	1.025	0.102	723515 ± 7016.34	0.9698	1.388
	6	1239953 ±27936.93	2.2530	0.529	1082755 ± 41290	3.8134	0.600
Oleanolic acid	**50**	104470 ± 2265.33	1.2174	0.768	122503 ± 1728.42	1.4109	1.0217
	80	155964± 8882.91	1.5507	0.566	195600 ± 2910.17	1.4878	0.181
	100	245101.3 ± 16488.8	0.7220	0.256	228147 ± 5850.26	2.564	0.117

Limit: % RSD of RT should be less than 2.0 and for area NMT 5.0. Here both the markers in combination mixture at lower, middle and higher concentration range showed %RSD of Retention time and Peak area in limit specified in ICH guideline.

Accuracy: Accuracy was performed by recovery study where known concentration of markers was to be added and calculated the amount to be recovered which shown in following table

Table 5.20Recovery study of HPLC method

Markers	Initial Amount (A)	Addition of known quantity (B)		A+B	Amount recovered (mg)	% Recovery	Acceptedd % Limit for Recovery
Gallic acid	0.031	80%	0.025	0.0558	0.0561	100.54	98-102%
		100%	0.031	0.062	0.0619	99.84	
		120%	0.0372	0.0682	0.0689	101.03	
Oleanolic acid	0.01	80%	0.008	0.018	0.0182	101.1	
		100%	0.01	0.02	0.0198	99	
		120%	0.012	0.022	0.0219	99.54	

Robustness data:

Here different parameters like flow rate, detection wavelength and mobile phase composition were taken and found out whether the method was robust or not.

Table 5.21Robustness data for method validation

Parameters	Changes	Conc.in µg/ml		RT in minute		RSD of RT		Area Under Peak		RSD of Area	
		GA	OA	GA	OA	GA	OA	GA	OA	GA	OA
Flow rate	0.9 ml	4	80	3.136	10.987	0.08	0.05	1007007	234933	0.15	1.40
	1 ml			2.827	9.93	0.10	0.41	769777	191644	1.11	0.62
	1.1 ml			2.56	9.020	0.11	0.07	828029	193256	0.23	0.6
Detection	221 nm			2.827	9.805	0.089	0.76	839239	230555	2.285	0.76
	222 nm			2.835	9.687	0.058	0.26	775443	189978	1.154	0.12
	223 nm			2.817	9.751	0.23	0.45	725557	156890	1.852	0.25
Mobile phase compositionn	90: 10			2.82	19.3	0.35	0.21	243330	1377	2.012	1.8
	98 : 2			2.804	7.5	0.21	0.14	238514	129056	1.478	2.45
	97 : 3			2.826	7.916	0.41	0.45	256412	156256	0.75	1.89

For changes in mobile phase combination, flow rate and detection wavelength, the results showed that the % Relative Standard Deviation of RT and Peak area passed the specified limit as per ICH Guideline. Therefore, method should be robust.

LOD and LOQ:

Table 5.22LOD & LOQ data

Parameters	Gallic acid	Oleanolic acid
LOD	0.012	1.2116
LOQ	0.039	3.6723

5.3.3 Quantification of Markers in developed polyherbal tablet

Table 5.23HPLC Chromatogram for developed polyherbal tablet

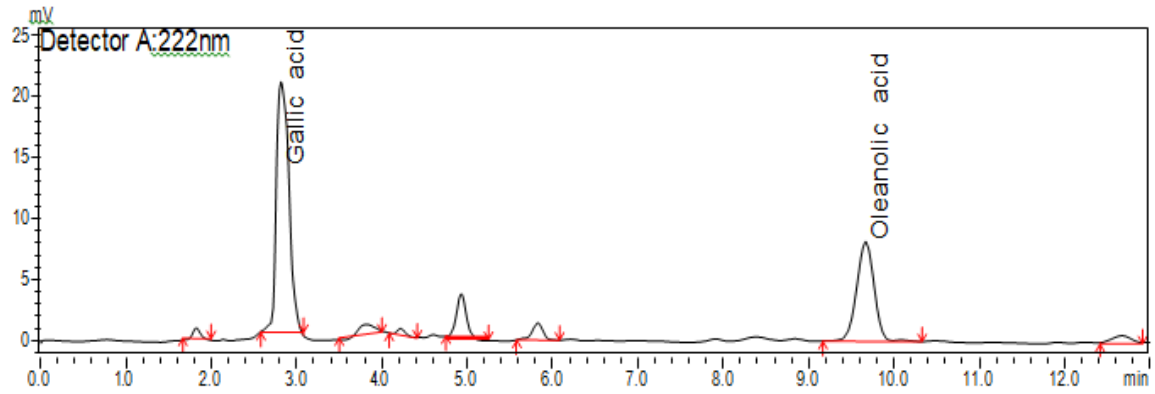


Table 5.24Quantification of markers in laboratory formulated tablet.

Sample	Amount	
	Gallic acid	**Oleanolic acid**
Polyherbal tablet	**20.92%**	**1.58%**

Summary:

Parameters	**Gallic acid**	**Oleanolic acid**
Retention time	3.2±0.005	9.9 ±0.045
Defection wavelength	222 nm	
Linearity(Correlation coefficient)	0.9978	0.9979
Beer's range(µg/ml)	1-6	50-100
Regression equation	Y=188933x + 17107	Y=2253.4x + 6136.2
Precision	0.72-1.59	0.98-1.41
LOD	0.012	1.2116
LOQ	0.039	3.6723
Accuracy	99.84-100.82	99.14-101.2
Robustness	Robust	Robust

5.4 HPTLC Analytical method development and validation:

5.4.1 Method development:

Table 5.25Optimized HPTLC condition

HPTLC Plate	**Aluminium plates pre coated with silica gel 60 F 254 (10 x 10 cm).The plates were activated at 110 ^{0} C for 30 minutes Prior to chromatography.**
Development chamber	Camag twin through glass chamber (20x20 cm). The optimized chamber saturation time for mobile phase was 45 Min at room temp.
Mobile Phase	Toluene : Ethyl Acetate : Formic acid (7:6:1)
Band width	6 mm
Length of chromatogram run	80 mm
Injection volume	20 µl
Detection wavelength	UV at 270 nm. 368 nm after spraying with Methanolic Sulphuric acid.

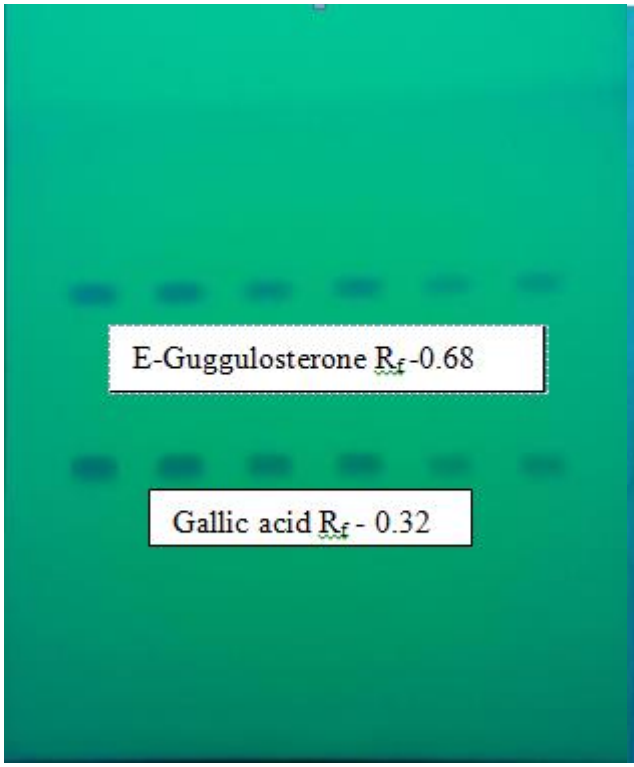


Figure 5.11TLC plate at UV scan

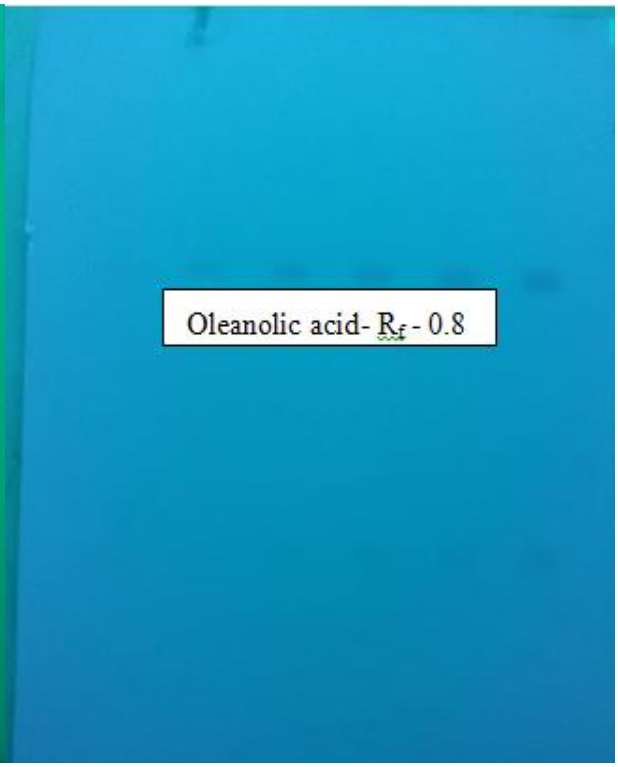


Figure 5.12TLC Plate after spraying with reagent methanolic sulphuric acid

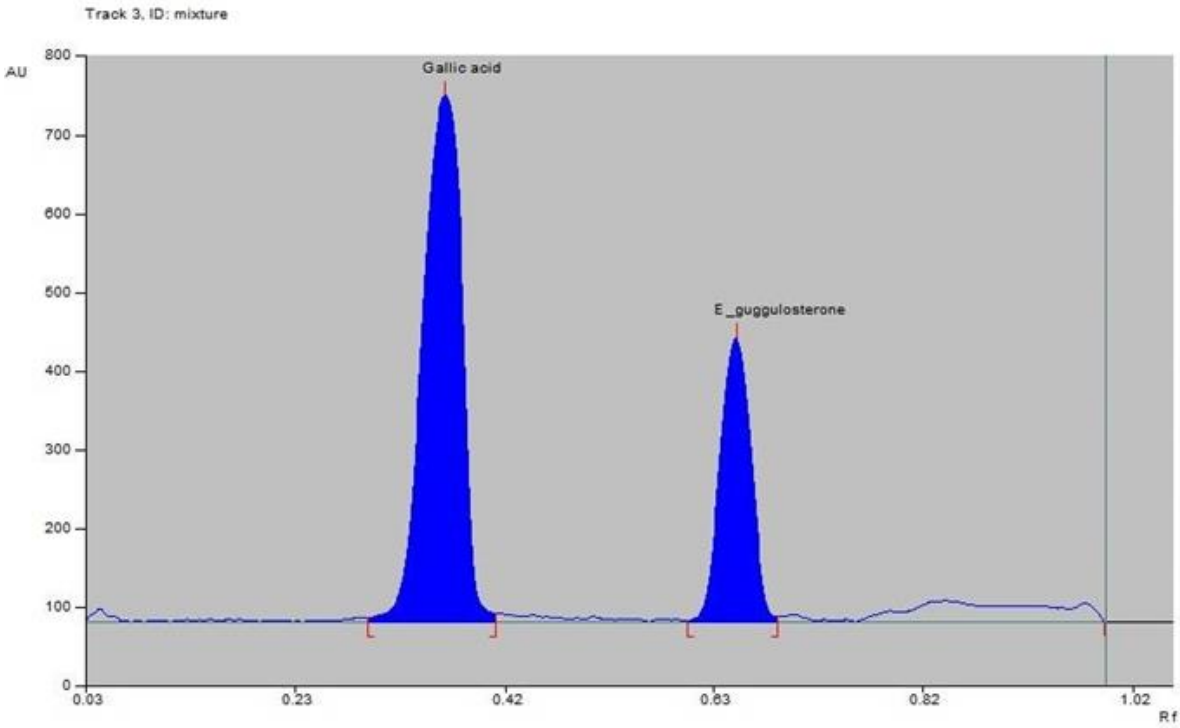


Figure 5.13HPTLC Chromatogram of Gallic acid and E-Guggulosterone at 270 nm

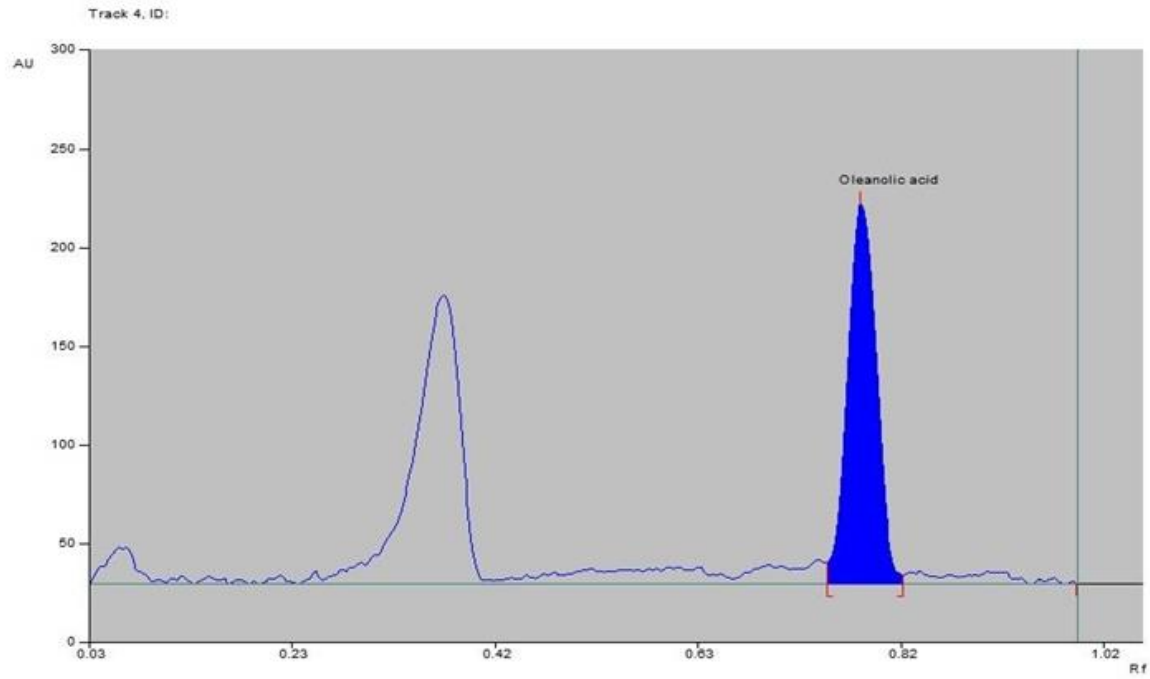


Figure 5.14HPTLC Chromatogram of Oleanolic acid after spraying with reagent scanning at 368 nm

5.4.2 HPTLC Method Validation:

Linearity

The linearity of proposed method was evaluated by analyzing series of six different concentrations of each marker. The standard solutions were analyzed in triplicate for the establishment of calibration curve. The calibration curve was plotted by using the value of peak area v/s concentration of compound.(x, ug/ml).

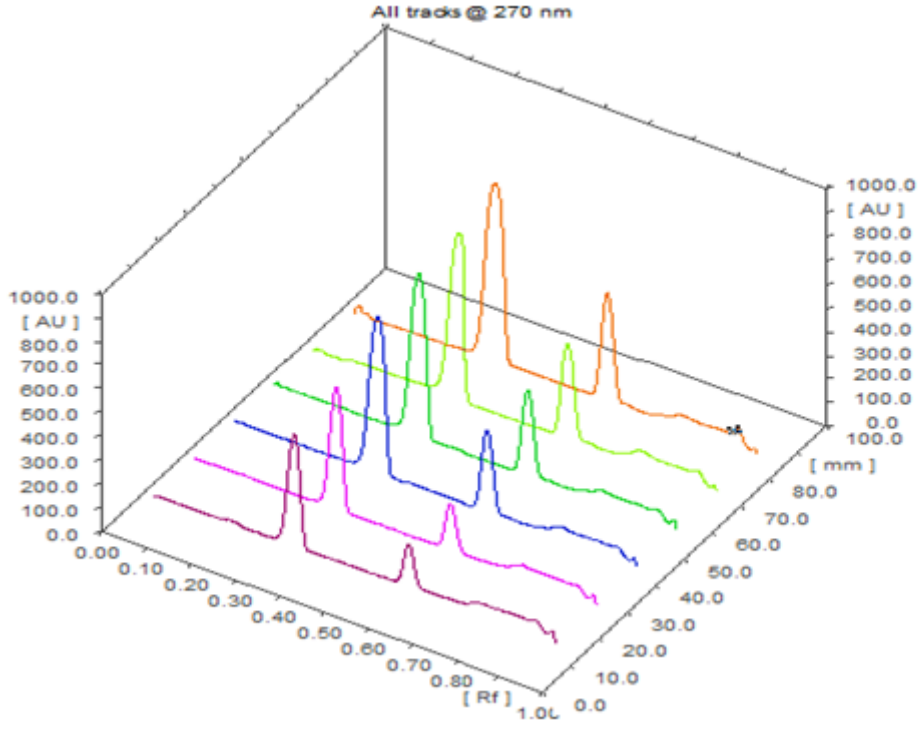


Figure 5.15HPTLC graph for linearity of Gallic acid and E-Guggulosterone scannd at 270 nm

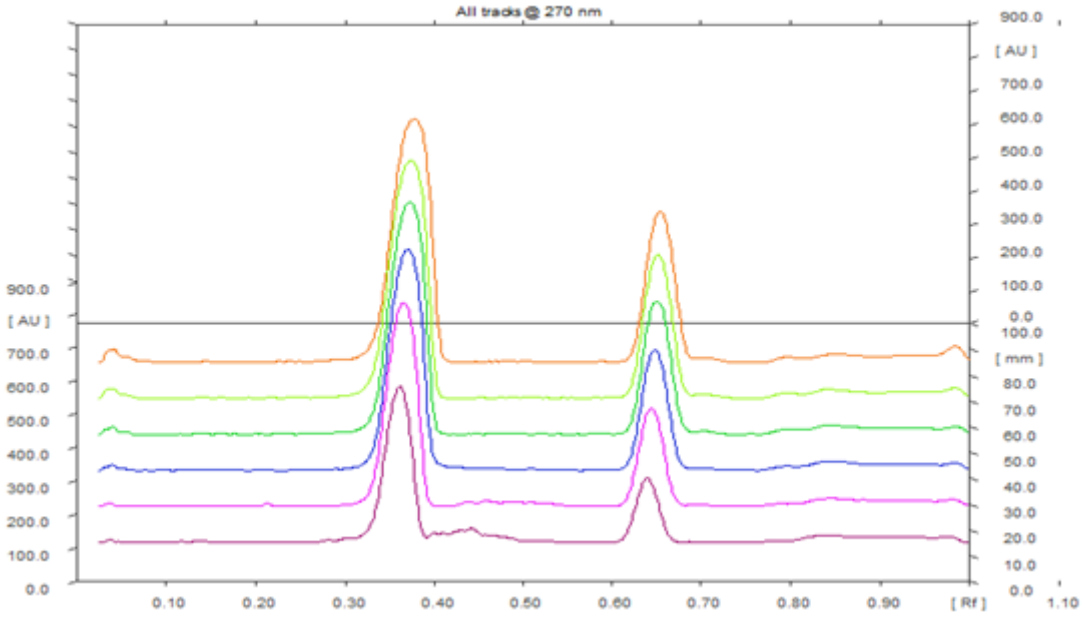


Figure 5.16Overlay HPTLC chromatogram for Linearity of Gallic acid and E-Guggulosterone scanned at 270 nm

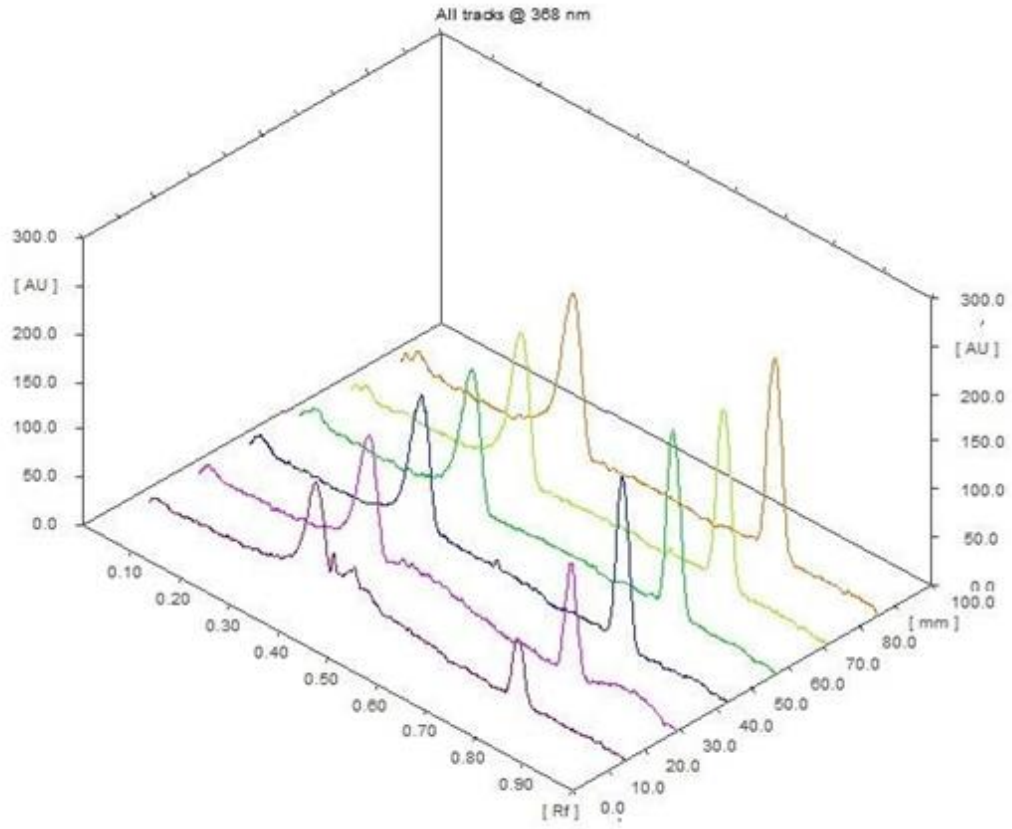


Figure 5.17HPTLC chromatogram for Linearity of Oleanolic acid scanned at 368 nm.

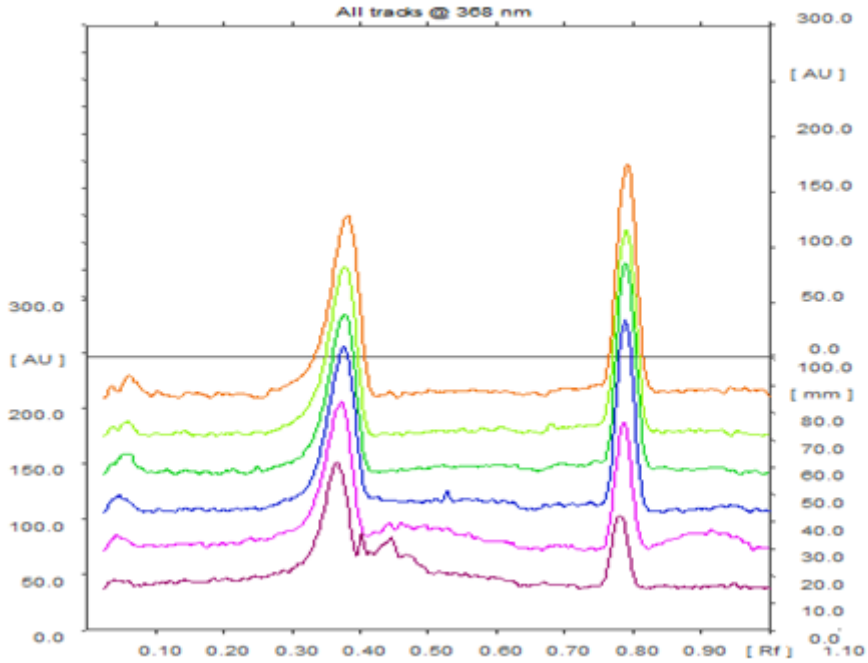


Figure 5.18Overlay HPTLC chromatogram for linearity of Oleanolic acid scanned at 368 nm

Table 5.26HPTLC Peak area for three marker

Markers	Concentration Injection volume in µl	HPTLC Peak Area		
		Gallic acid	E-guggulsterone	Oleanolic acid
Linearity	5	12165	4078	1423
	10	15526	5530	2214
	15	18845	7646	3108
	20	22514	9120	3990
	25	25651	10862	4640
	30	28494	12773	5474

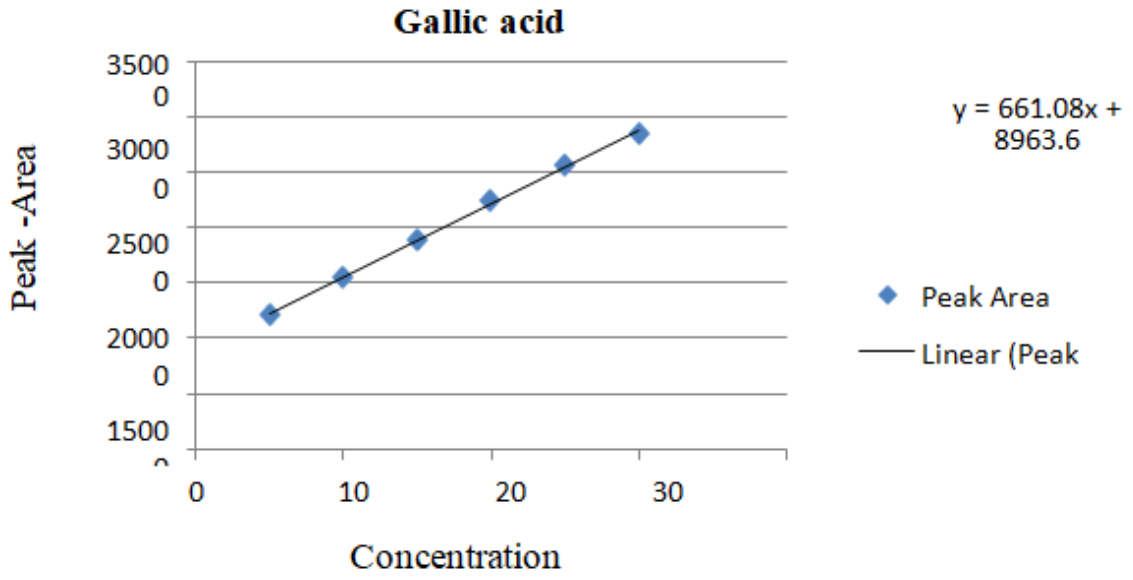


Figure 5.19Calibration curve of Gallic acid.

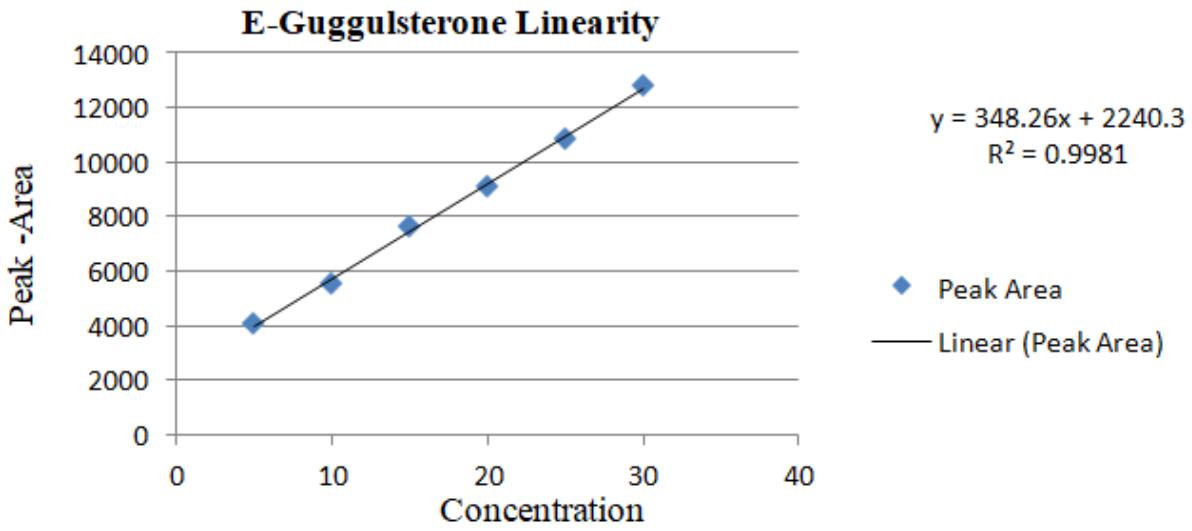


Figure 5.20Calibration Curve of E-Guggulsterone

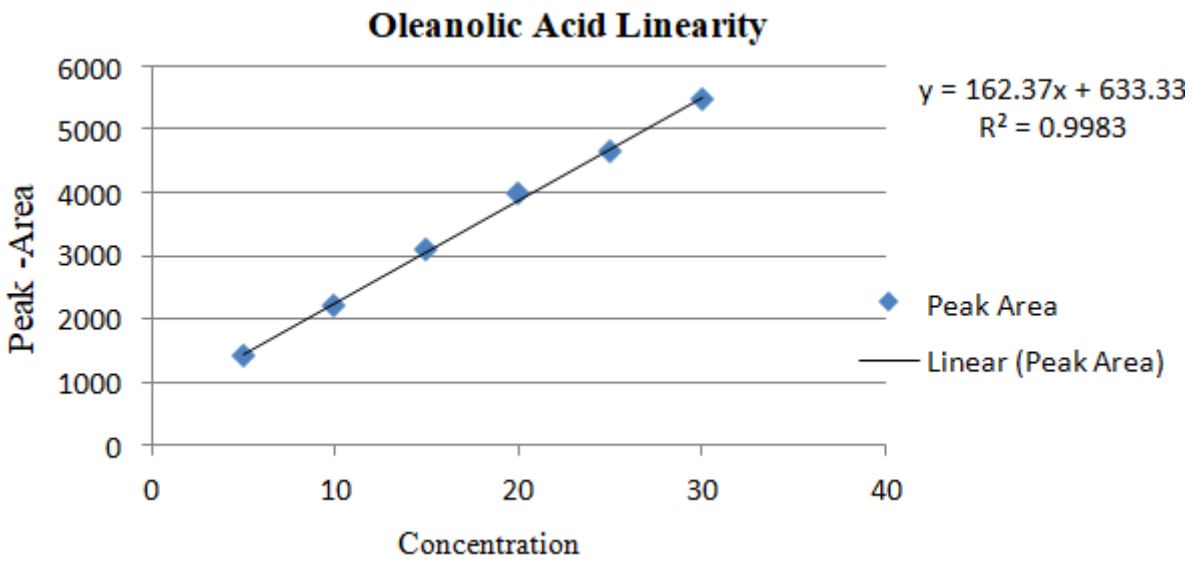


Figure 5.21Calibration Curve of Oleanolic acid.

Precision data:

Intra-day and inter-day precision were performed using mixture of 2 standard solutions at 3 concentrations in order to evaluate intermediate precision.

The intraday test was determined by injection of the same standard solutions thrice a day. And interday was determined by analysing same standards thrice each day for 3 days.

Precision was expressed as relative standard deviation (RSD). Generally, the values of RSD within 2% are acceptable. The results of precision test for each analyte are summarized below.

Table 5.27 Interday and Intraday precision data

Marker	Conc. (ug/spot)	Intraday (n=3)		Interday (n=3)	
		Area ± SD	% RSD of Area	Area ± SD	% RSD of Area
Gallic acid	5	12440 ± 197.41	1.586	12597 ± 220.95	1.753
	20	22516 ± 29.09	0.129	22383 ± 235.045	1.050
	30	28431 ±56.72	0.199	28341 ±199.95	0.705
E-Guggulsterone	5	4085 ± 65.31	1.598	4107 ± 72.59	1.767
	20	9126 ± 16.07	0.176	9193 ± 68.98	0.750
	30	12674 ± 105.04	0.828	12741 ± 209.844	1.646
Oleanolic acid	5	1451± 25.86	1.782	1447 ± 31.89	2.203
	20	3978 ± 17.87	0.444	3958 ± 1.320	1.320
	30	5463 ± 13.63	0.249	5496 ± 67.71	1.231

Table 5.28HPTLC Robustness data

Factor	Gallic acid		E-Guggulosterone		Oleanolic acid	
	Area±SD	%RSD	Area±SD	%RSD	Area±SD	%RSD
Saturation time						
30	21435 ± 427.59	1.879	8929 ± 27.59	0.304	917 ± 0.715	0.715
60	24402 ± 412.59	1.752	9150 ± 36.04	0.393	1226 ± 15.23	1.210
Wavelength						
256	21402 ± 427.59	1.997	9070 ± 38.97	0.429	-	-
280	25435 ± 402.78	1.583	9209 ± 51.159	0.555	-	-
320	-	-	-	-	970 ± 140.01	1.444
380	-	-	-	-	1123 ± 20.075	1.787

LOD and LOQ:

Table 5.29LOD and LOQ for all three markers

Marker	Rf Value	Regression equation	R^2	Linear range	LOD (ug/ml)	LOQ ug/ml)
Gallic acid	0.32	y = 661.08x + 8963.6	0.9987	5-30 ug/spot	0.243	0.737
E-Guggulosterone	0.68	y = 348.26x + 2240.3	0.9981		0.512	1.554
Oleanolic acid	0.80	y = 162.37x + 633.33	0.9983		0.846	2.563

Accuracy:

It was determined by adding 3 concentrations of each standard solution into sample solution. Recovery (%) was evaluated.

Table 5.30HPTLC Recovery data of all markers

Markers	Initial Amount(A) (mg)	Addition of known quantity (B)		A+B	Amount recovered (mg)	% Recovery	Accepted % Limit for Recovery
Gallic acid	0.028	80%	0.0224	0.0504	0.05035	99.90	98-102%
		100%	0.028	0.056	0.0558	99.64	
		120%	0.0336	0.0616	0.062	100.65	
E-Guggulsterone	0.014	80%	0.0112	0.0252	0.0258	101.38	
		100%	0.014	0.028	0.0279	99.64	
		120%	0.0168	0.0308	0.0311	100.97	
Oleanolicacid	0.011	80%	0.0088	0.0198	0.0199	100.51	
		100%	0.011	0.022	0.0218	99.09	
		120%	0.0132	0.0242	0.0246	101.47	

5.4.3 Quantification of markers by HPTLC method

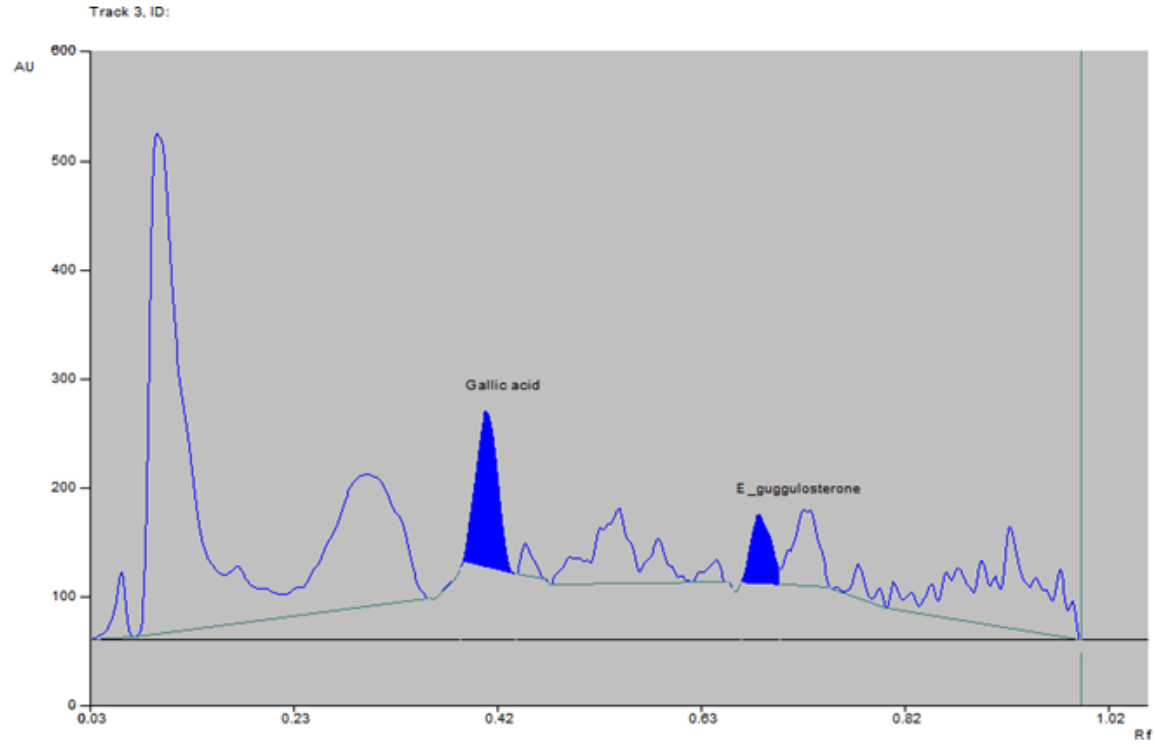


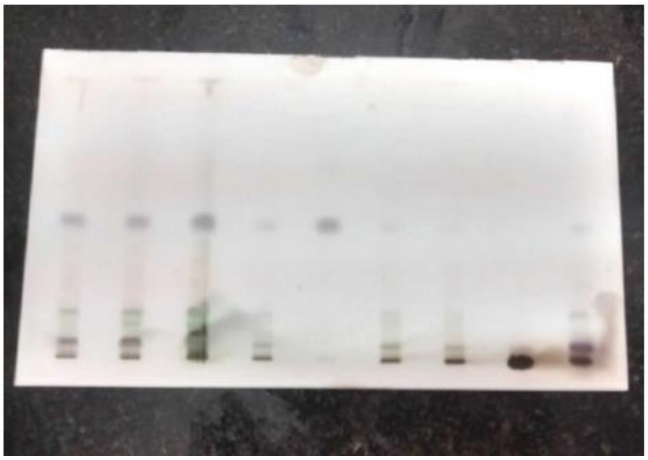
Figure 5.22HPTLC chromatogram of laboratory prepared polyherbal tablet

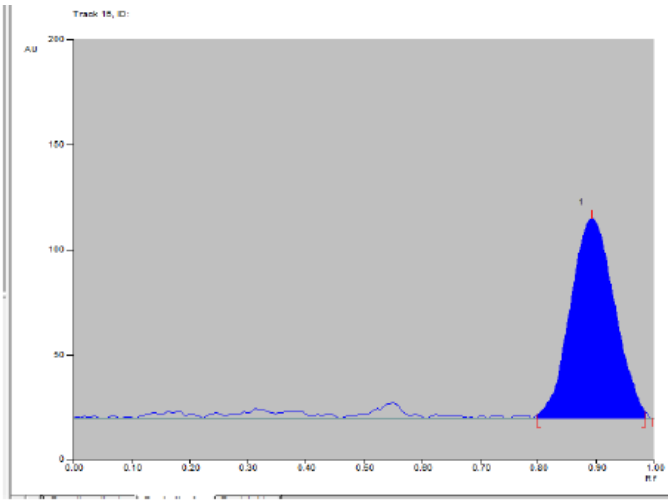
Table 5.31Quantification of markers by HPTLC method

Sample	Amount	
	Gallic acid (%)	E-Guggulsterone (%)
Polyherbal tablet	21.62%	1.32%

HPTLC Method for Mahanimbine in *Morraya koinigi* ext.

Stationary phase Aluminum oxide 150 F254, neutral
Mobile phase n- hexane: ethyl acetate (9:1)
Calibration range 2- 15 µL
Detection Scanned under UV at 254 nm
Derivatization 5 or 10% H2SO4 : violet purple spots





5.5 In silico evaluation results:

5.5.1 Pancreatic α-Amylase (Inhibitory activity):

Molecular Docking Study:

The PDB file (6GXV) of the Crystal Structure of the AliC GH13 alpha-amylase was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 1 gives the binding energies of all active constituents of natural products and standard drugs with 6GXV (binding energies ranged from –9.0 to -4.7 kcal/mol). In addition to, visual examination of the computationally docked optimal binding poses of all NPs on alpha-amylase revealed the important role of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions in the stability of NPs. Among all NPs, oleanolic acid showed highest docking score compared to standard drugs against alpha-amylase. The docking score of all other NPs is given in Table.

Table 5.32Molecular docking results of NPs and some standard drugs with alpha-amylase (6GXV)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	**Oleanolic acid**	**-9.0**
2	Garcinia	-4.7
3	Guggulsterone	-7.6
4	Mahanine	-8.1
5	Quercetin	-7.6
6	**Acarbose**	**-7.6**
7	Orlistat	-5.3
8	Clofibrate	-5.1
9	Rimonabant	-7.9
10	Atorvastatin	-8.5

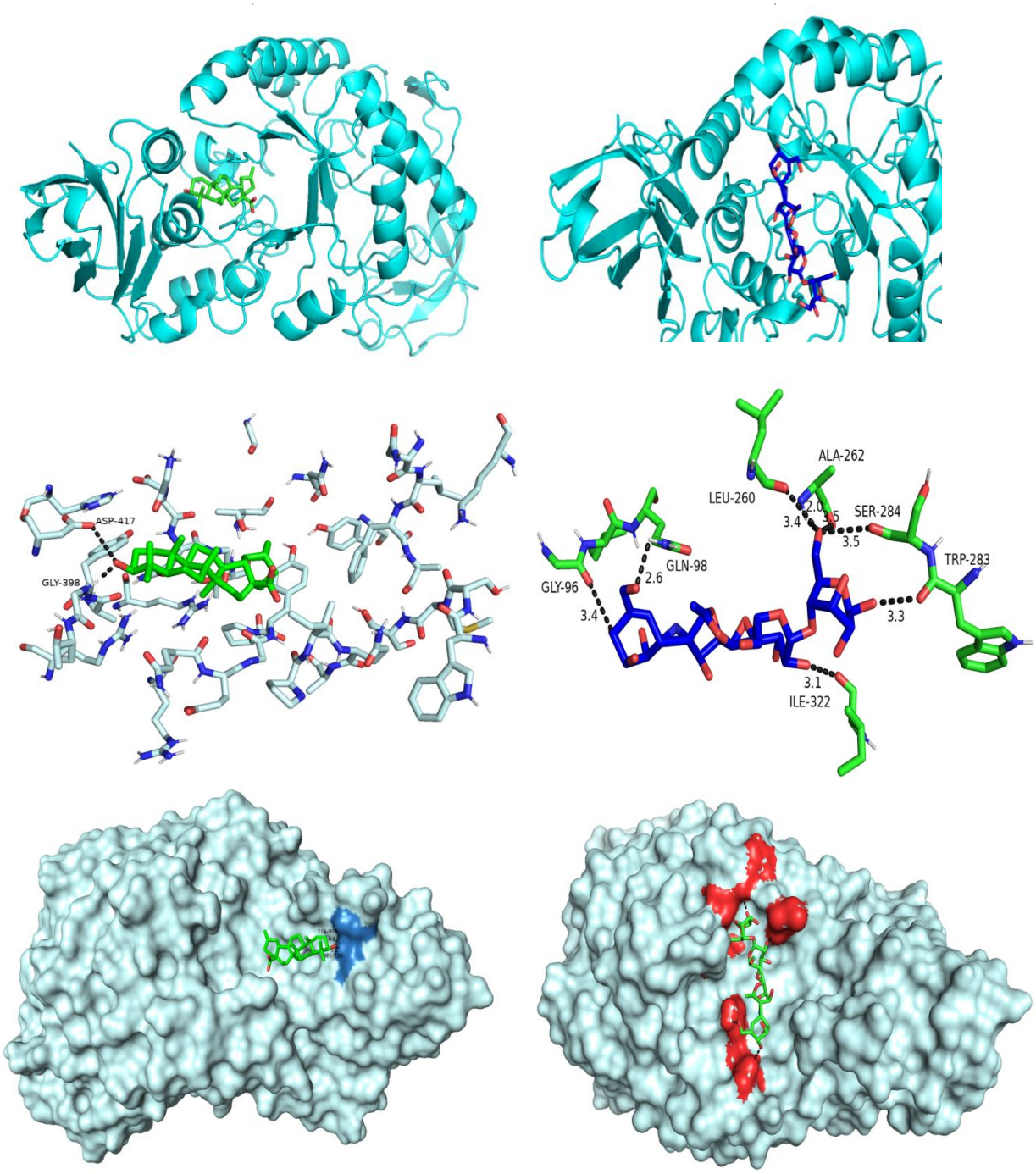


Figure 5.23Docking interactions of (A) Oleanolic acid and (B) Acarbose in the active sites of alpha-amylase (PDB id: 6GXV)

Oleanolic acid bound efficiently to the active site of alpha-amylase with good complementarity, and the docking score is -9.0 kcal/mol. which is far more compared with standard drug acarbose (docking score -7.6 kcal/mol). The Oleanolic acid binds to alpha-amylase by two hydrogen bond with GLY-398 and ASP-417 other nonbonding hydrophobic interactions. Moreover, mahanine has also showed comparative binding with alpha-amylase (Figure 5.23). Among the all standard drugs used for docking, atorvastatin showed comparative binding to alpha-amylase protein. However, identifying the ligand binding site

(composed of amino acids) for each specific protein molecule is crucially important when trying to find a suitable drug molecule for the target. It is also essential to understand the function of the protein. These binding interactions (Figure 5.23) present a clear view that Oleanolic acid can have good interactions with alpha-amylase.

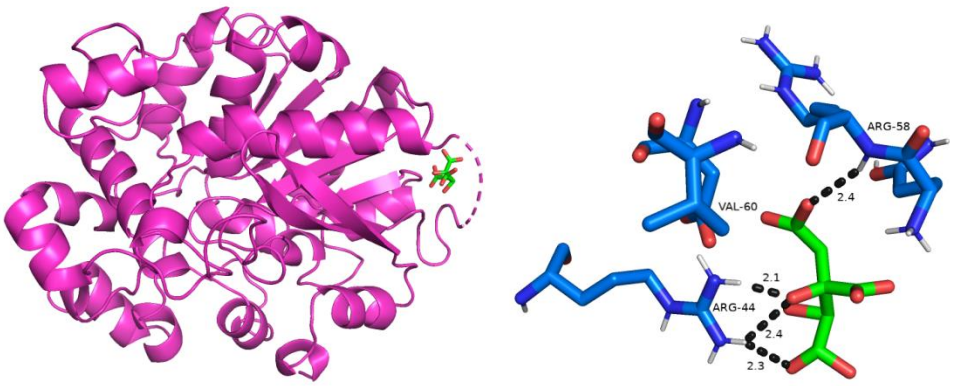
5.5.2 Pancreatic Lipase:

Molecular Docking Study

The PDB file (1HLG) of recombinant human gastric lipase was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 2 gives the binding energies of all active constituents of natural products and standard drugs with 1HLG (binding energies ranged from –8.4 to -4.7 kcal/mol). In addition to, visual examination of the computationally docked optimal binding poses of all NPs on human gastric lipase revealed the important role of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including π–π stacking, π–cation, and π–σ interactions in the stability of NPs. Among all compounds, Oleanolic acid and Guggulsterone have shown the best docking score against human gastric lipase. The docking score of all other NPs is given in Table.

Table 5.33Molecular docking results of NPs and some standard drugs with human gastric lipase

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.4
2	**Garcinia**	**-4.7**
3	Guggulsterone	-7.6
4	Mahanine	-6.4
5	Quercetin_meletin	-7.2
6	Acarbose	-7.2
7	**Orlistat**	**-4.8**
8	Clofibrate	-5.1
9	Rimonabant	-7.1
10	Atorvastatin	-7.1



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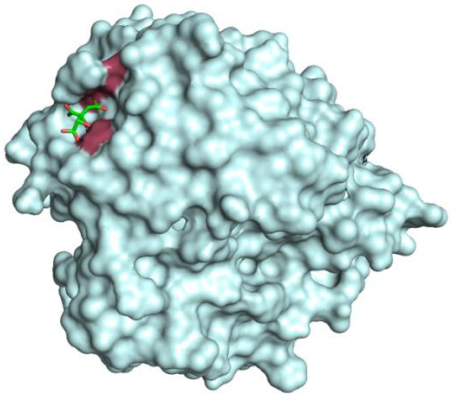

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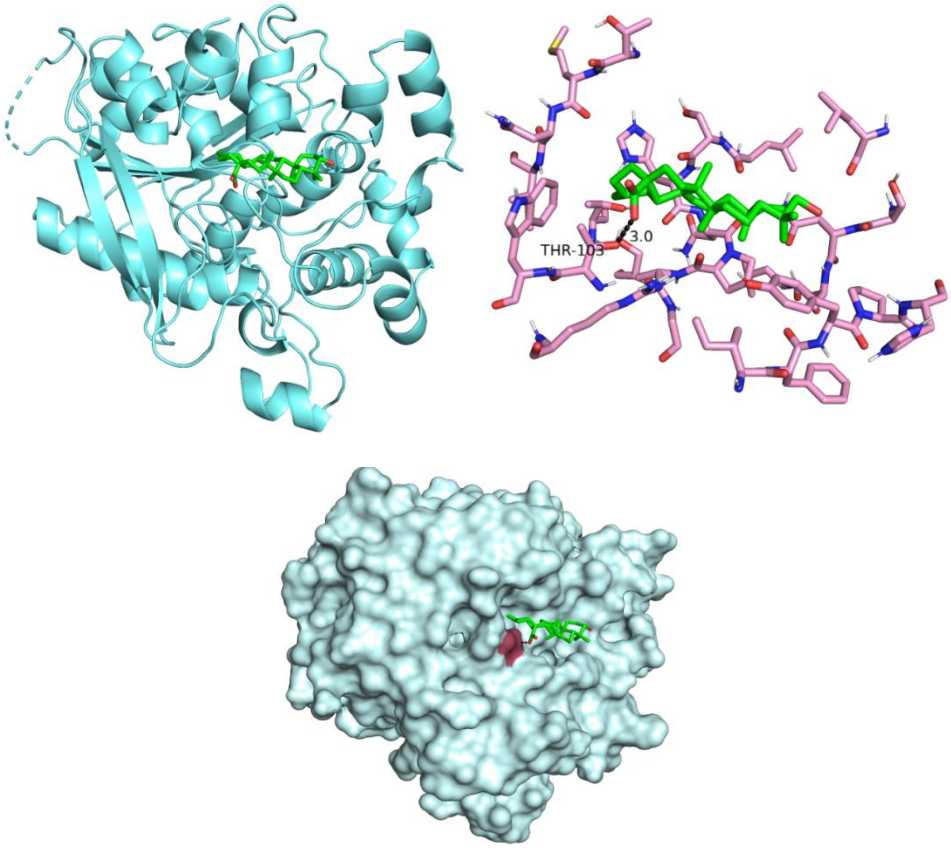

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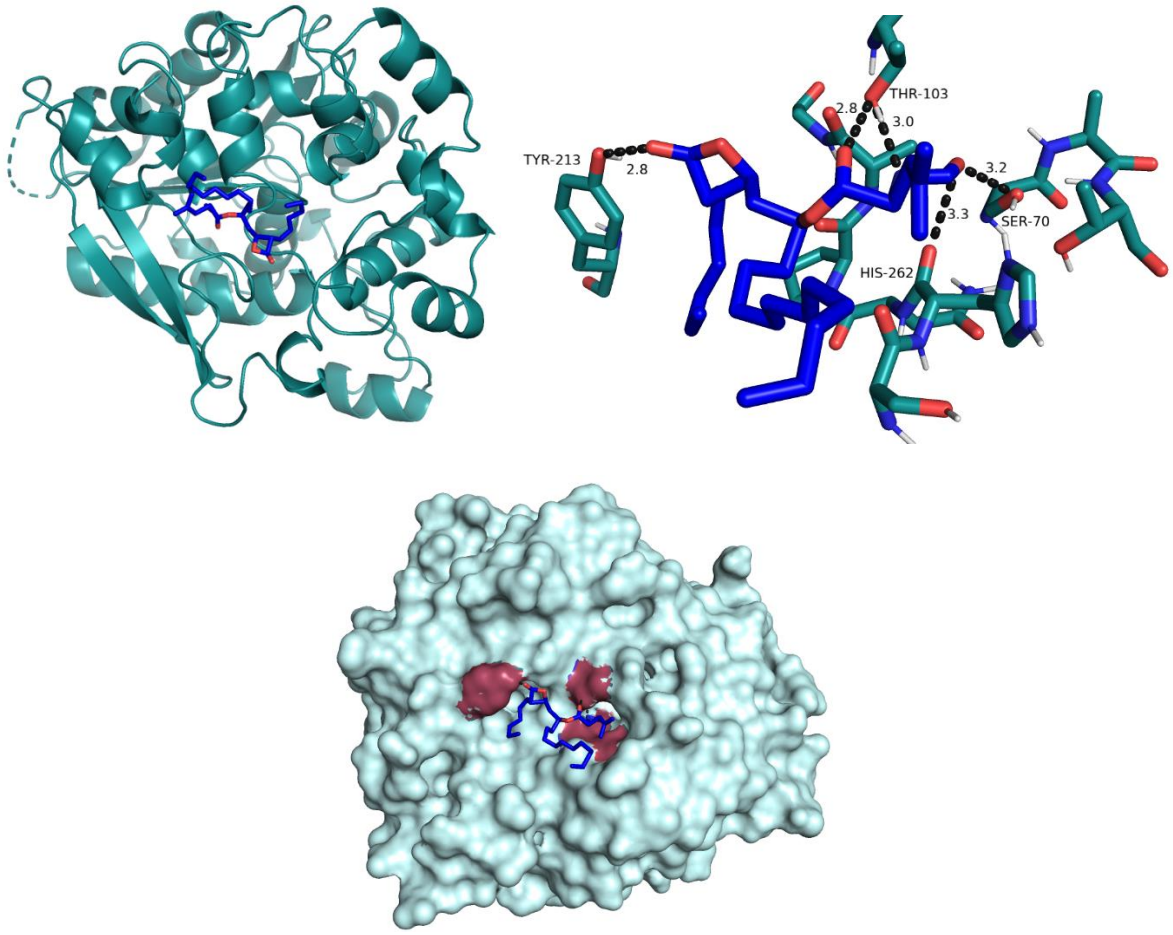


Figure 5.24Docking interactions of (A) Garcinia (B) Oleanolic acid and (C) Orlistat in the active sites of human gastric lipase (PDB id: 1HLG)

Garcinia showed some interactions with human gastric lipase protein through hydrogen bonding and other nonbonding interactions but the interactions are not strong enough as the docking score is -4.7 kcal/mol while Oleanolic acid shoed good binding interection with docking score of -8.4 kcal/mol (Table 2). Garcinia has formed four hydrogen bonding with the active site of human gastric lipase amino acid- ARG 44, and ARG 58 along with hydrophobic interaction (Figure 3), while Oleanolic acid has formed one hydrogen bonding with THR 103 and hydrophobic interaction (Figure 5.24). However, the standard selected drug Orlistat doesn't showed any good interections (docking score is -4.8 kcal/mol) compared to Oleanolic acid. These binding interactions (Figure 5.24) present a clear view that Oleanolic acid interact with human gastric lipase stronger then Garcinia and Orlistat.

5.5.3 PPARs (peroxisome proliferator activated receptor) (PPARalpha):

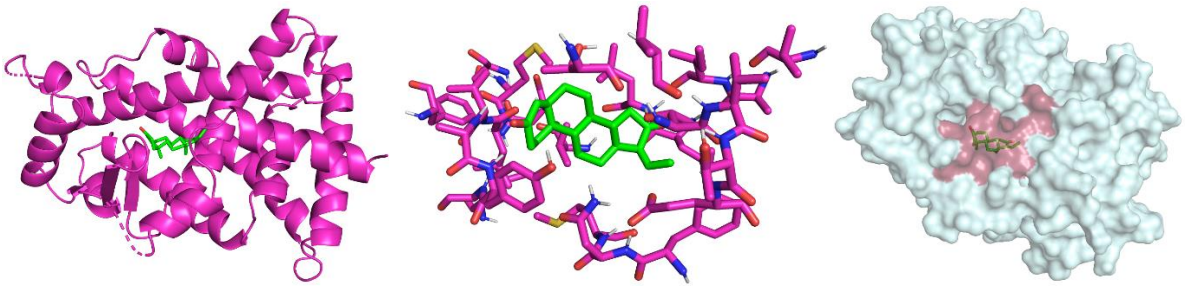
Molecular Docking Study:

The PDB file (3VI8) of human PPAR alpha was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 3 gives the binding energies of all active constituents of natural products and standard drugs with 3VI8 (binding energies ranged from –9.9 to -4.3 kcal/mol). In addition to, visual examination of the computationally docked optimal binding poses of all NPs on human PPAR alpha revealed the important role of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions in the stability of NPs. Among all compounds, Oleanolic acid, Guggulsterone, Mahanine, and Quercetin have shown the best docking score against human PPAR alpha. The docking score of all other NPs is given in Table.

Table 5.34Molecular docking results of NPs and some standard drugs with human PPAR alpha (3VI8)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.0
2	Garcinia	-5.9
3	**Guggulsterone**	**-7.9**
4	Mahanine	-8.5
5	Quercetin	-9.5
6	Acarbose	-5.7
7	Orlistat	-4.3
8	**Clofibrate**	**-6.2**
9	Rimonabant	-9.9
10	Atorvastatin	-6.3

A



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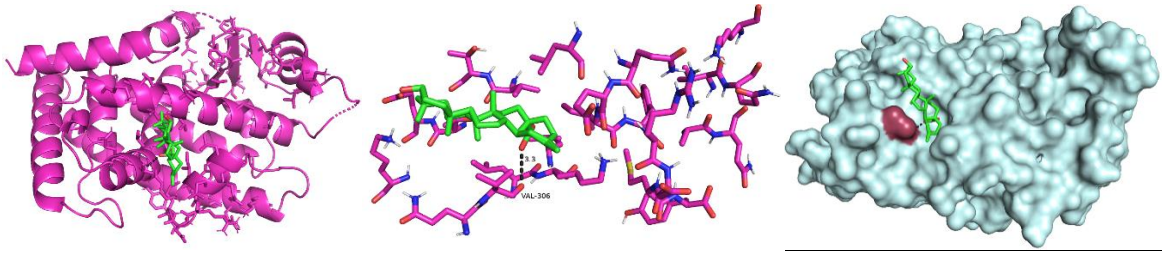

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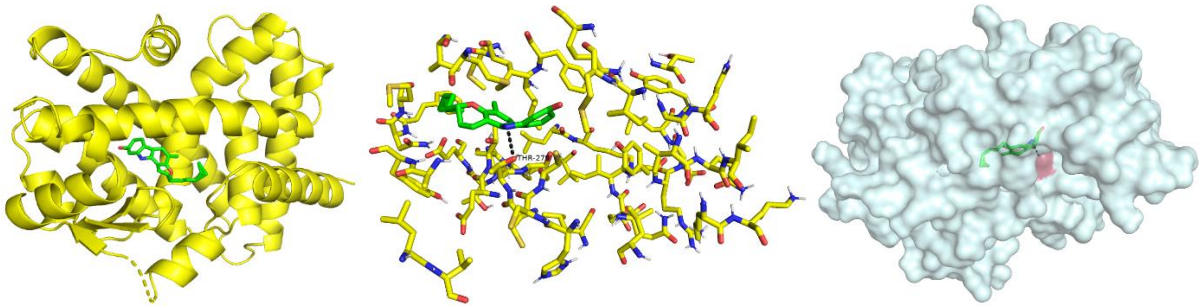

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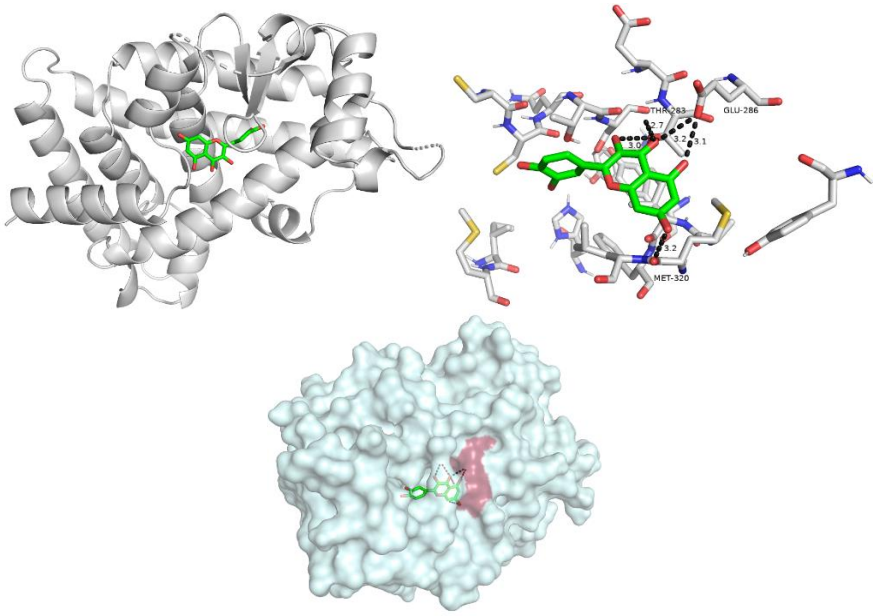

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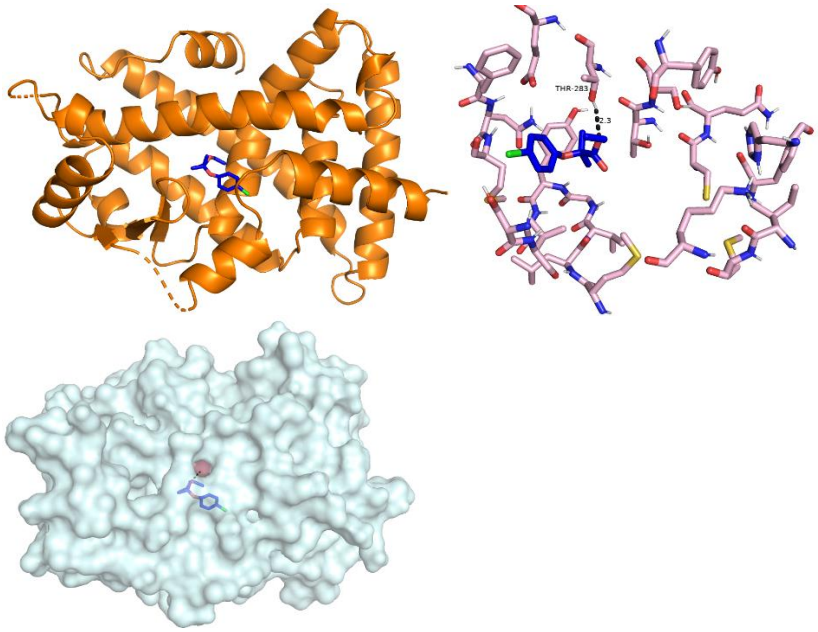


Figure 5.25Docking interactions of (A) Guggulsterone (B) Oleanolic acid (C) Mahanine (D) Quercetin and (E) Clofibrate in the active sites of human PPAR alpha (PDB id: 3VI8)

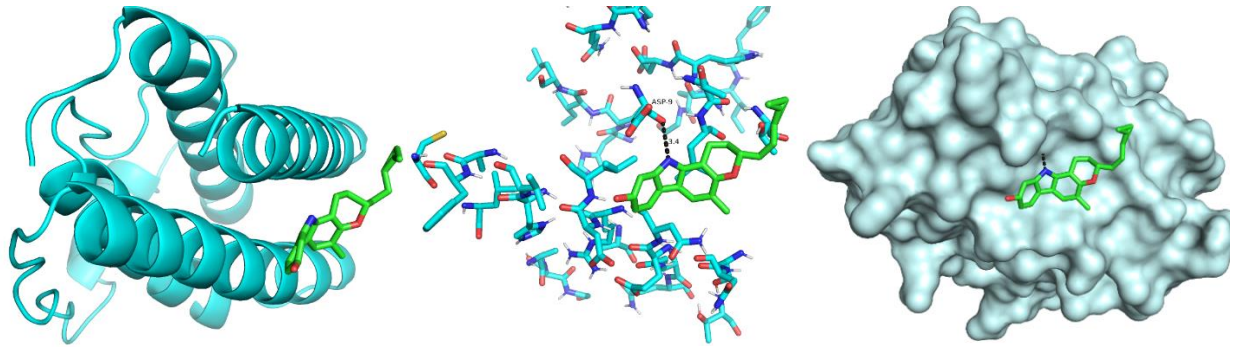
Guggulsterone, Oleanolic acid, Mahanine, and Quercetin showed good binding interactions with the human PPAR alpha efficiently as the docking score is -7.9, -8.0, -8.5, and -9.5 kcal/mol, respectively (Table 3). Moreover, all the natural compounds have formed many hydrogen bonding with the active site of human PPAR alpha amino acids (Figure 4) along with hydrophobic interactions. To find a suitable drug molecule for the target, the binding site was identified in proper way to eliminate false results. The binding interactions (Figure 4) present a clear view that the NPs like Guggulsterone, Oleanolic acid, Mahanine, and Quercetin can irreversibly interact with human PPAR alpha. Bond distance and binding interaction of Guggulsterone, Oleanolic acid, Mahanine, and Quercetin with an active amino acid of human PPAR alpha are less than 3 Å indicatind stronger interactions. On the other side the selected drugs don't have good binding interactions (docking score ranges from -4.3 to -6.3 kcal/mol) except Rimonabant (docking score -9.9 kcal/mol). The reported drug Clofibrate has showed docking score of only -6.2 kcal/mol indicating poor interactions compared to NPs.

5.5.4 Leptin (LEP-R, LEP-Rb):

Molecular Docking Study

The PDB file (1AX8 and 3V6O) of human obesity protein, leptin and leptin receptor-antibody complex were downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 4 gives the binding energies of all active constituents of natural products and standard drugs with 1AX8 (binding energies ranged from –3.9 to -7.9 kcal/mol) and with 3V6O (binding energies ranged from –4.3 to -7.5 kcal/mol). In addition to, visual examination of the computationally docked optimal binding poses of all NPs on leptin and leptin receptor-antibody complex revealed the important role of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions in the stability of NPs. Among the ten compounds, mahanine, and oleanolic acid have shown the best docking score against leptin and leptin receptor-antibody complex while from the drug molecules only rimonabant showed comparable binding. The docking score of all other NPs and drugs is given in below table.

A



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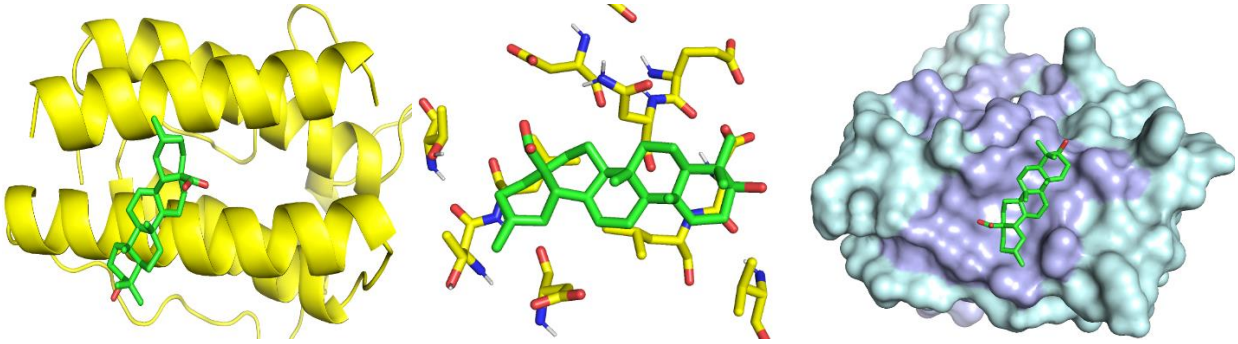

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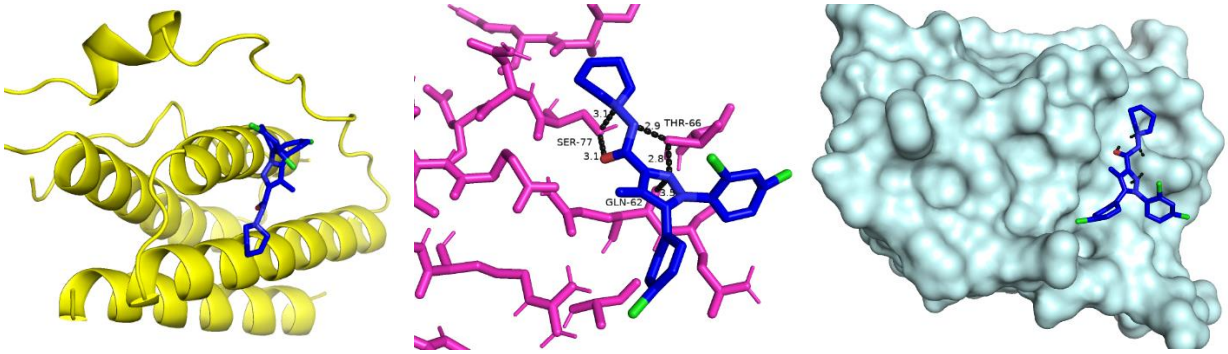
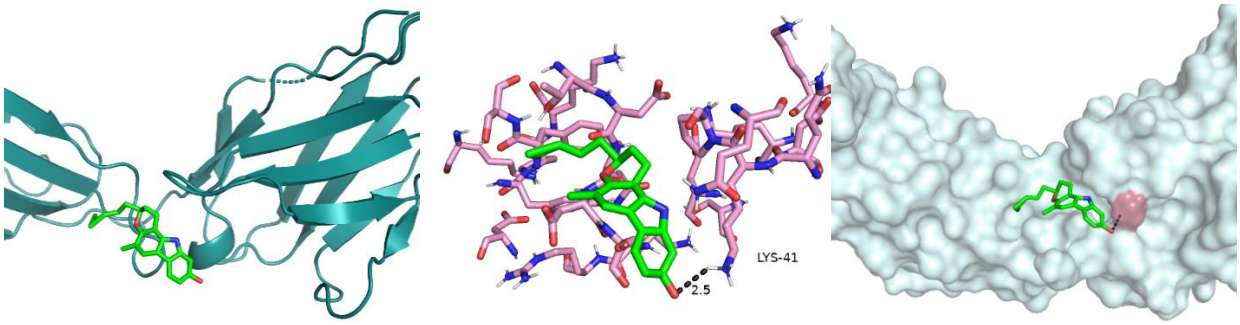


Figure 5.26. Docking interactions of (A) Mahanine (B) Oleanolic acid (C) Rimonabant in the active sites of human obesity protein, leptin (PDB id: 1AX8)

A



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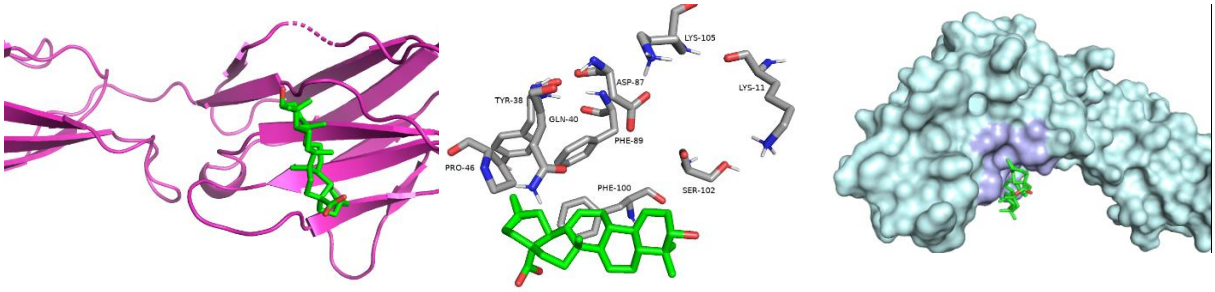

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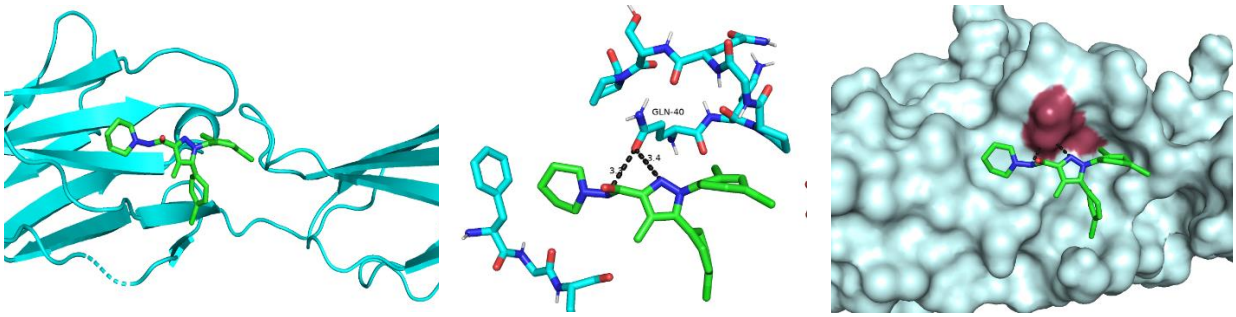


Figure 5.27Docking interactions of (A) Mahanine (B) Oleanolic acid (C) Rimonabant in the active sites of leptin receptor (PDB id: 3V6O)

Amongst all the NPs, Mahanine and Oleanolic acid are showing interactions with the active site of human obesity protein, leptin with good complementarity, and the docking score is -

6.4 and -7.9 kcal/mol, respectively for 1AX8 whereas -6.4 and -7.5 kcal/mol, respectively for 3V6O (Table 4 and Table 5). Moreover, the drug Rimonabant also showing bonding interactions with the active site of leptin with the docking score of -7.1 kcal/mol for 1AX8 (Figure 5) whereas -6.6 kcal/mol, for 3V6O (Figure 6). The pose and interaction suggested that the oleanolic acid binds strongly with the leptin in all the protein structures. The binding interaction of mahanine and oleanolic acid with leptin (PDB id: 1AX8 and 3V6O) are outlined in below table.

Table 5.35Molecular docking results of NPs and some standard drugs with leptin (1AX8)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-7.5
2	Garcinia	-4.3
3	Guggulsterone	-6.4
4	**Mahanine**	**-6.4**
5	Quercetin	-6.4
6	Acarbose	-6.3
7	Orlistat	-4.4
8	Clofibrate	-4.3
9	Rimonabant	-6.6
10	Atorvastatin	-6.6

Table 5.36Molecular docking results of NPs and some standard drugs with leptin (3V6O)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-7.9
2	Garcinia	-4.2
3	Guggulsterone	-6.9
4	**Mahanine**	**-6.4**
5	Quercetin	-7.0
6	Acarbose	-6.8
7	Orlistat	-3.9
8	Clofibrate	-4.4
9	Rimonabant	-7.1
10	Atorvastatin	-6.9

5.5.5 Cannabinoid receptor type 1(CB1):

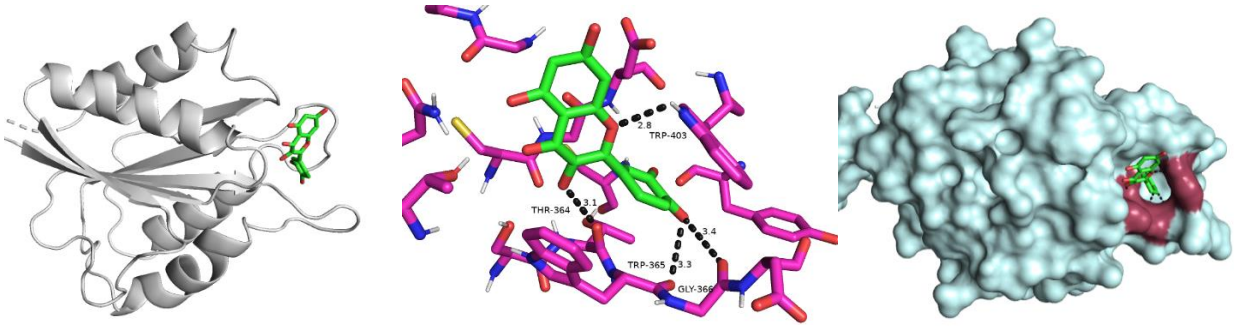
Molecular Docking Study

The PDB file (7V3Z) of the cannabinoid receptor type 1 was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 6 gives the binding energies of all active constituents of natural products and standard drugs with 7V3Z (binding energies ranged from –4.4 to -8.2 kcal/mol). In addition to, visual examination of the computationally docked optimal binding poses of all NPs on alpha-amylase revealed the important role of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions in the stability of NPs. Among all compounds, quercetin, and oleanolic acid have shown the best docking score against cannabinoid receptor type 1 which was more than the standard drug rimonabant. The docking score of all other NPs is given in below table.

Table 5.37. Molecular docking results of NPs and some standard drugs with cannabinoid receptor type 1 (7V3Z)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.2
2	Garcinia	-4.8
3	Guggulsterone	-7.8
4	Mahanine	-7.9
5	**Quercetin**	**-8.1**
6	Acarbose	-6.3
7	Orlistat	-4.4
8	Clofibrate	-6.2
9	**Rimonabant**	**-7.1**
10	Atorvastatin	-7.0

A



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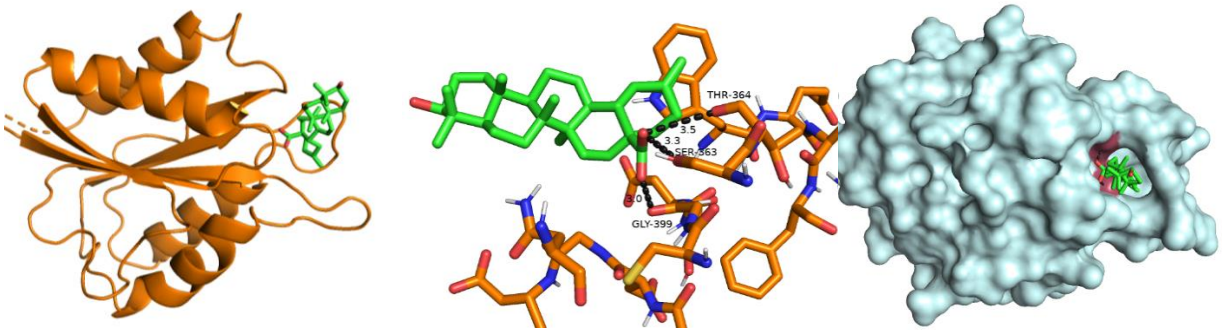

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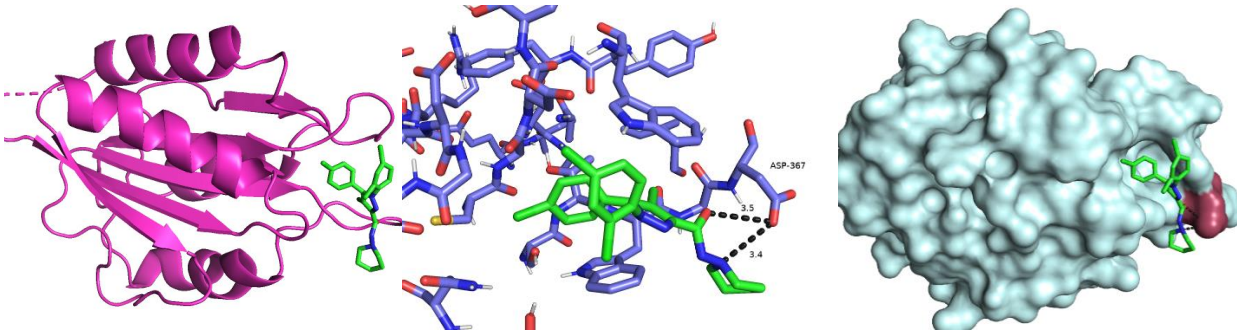

Figure 5.28**Docking interactions of (A) Quercetin (B) Oleanolic acid (C) Rimonabant in the active sites of cannabinoid receptor type 1 (PDB id: 7V3Z)**

Quercetin, and oleanolic acid are bound efficiently to the active site of cannabinoid receptor type 1 with good complementarity, and the docking score is -8.1 and -8.2 kcal/mol, respectively (Table 2). All the selected drug molecules showed comparatively low docking score than quercetin, and oleanolic acid. Moreover, the reported drug rimonabant also shows less binding interactions with docking score of -7.1 kcal/mol. Quercetin, and oleanolic acid showed four and three hydrogen bond with the amino acid THR 364, TRP 365, GLY 366, TRP 403 and THR 364, SER 363, GLY 399 respectively from cannabinoid receptor. In comparison to this the drug rimonabant only have two hydrogen bond with the amino acid

ASP 367 of cannabinoid receptor. These binding interactions (Figure 7) present a clear view that quercetin, and oleanolic acid can irreversibly interact cannabinoid receptor type 1. Binding interaction of quercetin, and oleanolic acid can irreversibly interact cannabinoid receptor type 1 are outlined in above given table.

5.5.6 HMG CoA Reductase:
.Molecular Docking Study

The PDB file (1DQA) of the catalytic portion of human HMG-CoA reductase with HMG, CoA, and NADP+ was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 7 gives the binding energies of all active constituents of natural products and standard drugs with 1DQA (binding energies ranged from –8.9 to -5.2 kcal/mol). In addition to, visual examination of the computationally docked optimal binding poses of all NPs on human HMG-CoA reductase revealed the important role of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions in the stability of NPs. Among all compounds, oleanolic acid, guggulsterone, mahanine, and quercetin have shown the best docking score against human HMG-CoA reductase. The docking score of all other NPs and drugs is given in below table.

Table 5.38Molecular docking results of NPs and some standard drugs with catalytic portion of human HMG-CoA reductase (1DQA)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.9
2	Garcinia	-5.9
3	Guggulsterone	-8.1
4	Mahanine	-8.3
5	**Quercetin**	**-8.2**
6	Acarbose	-8.3
7	Orlistat	-5.4
8	Clofibrate	-5.2
9	Rimonabant	-8.9
10	**Atorvastatin**	**-8.2**

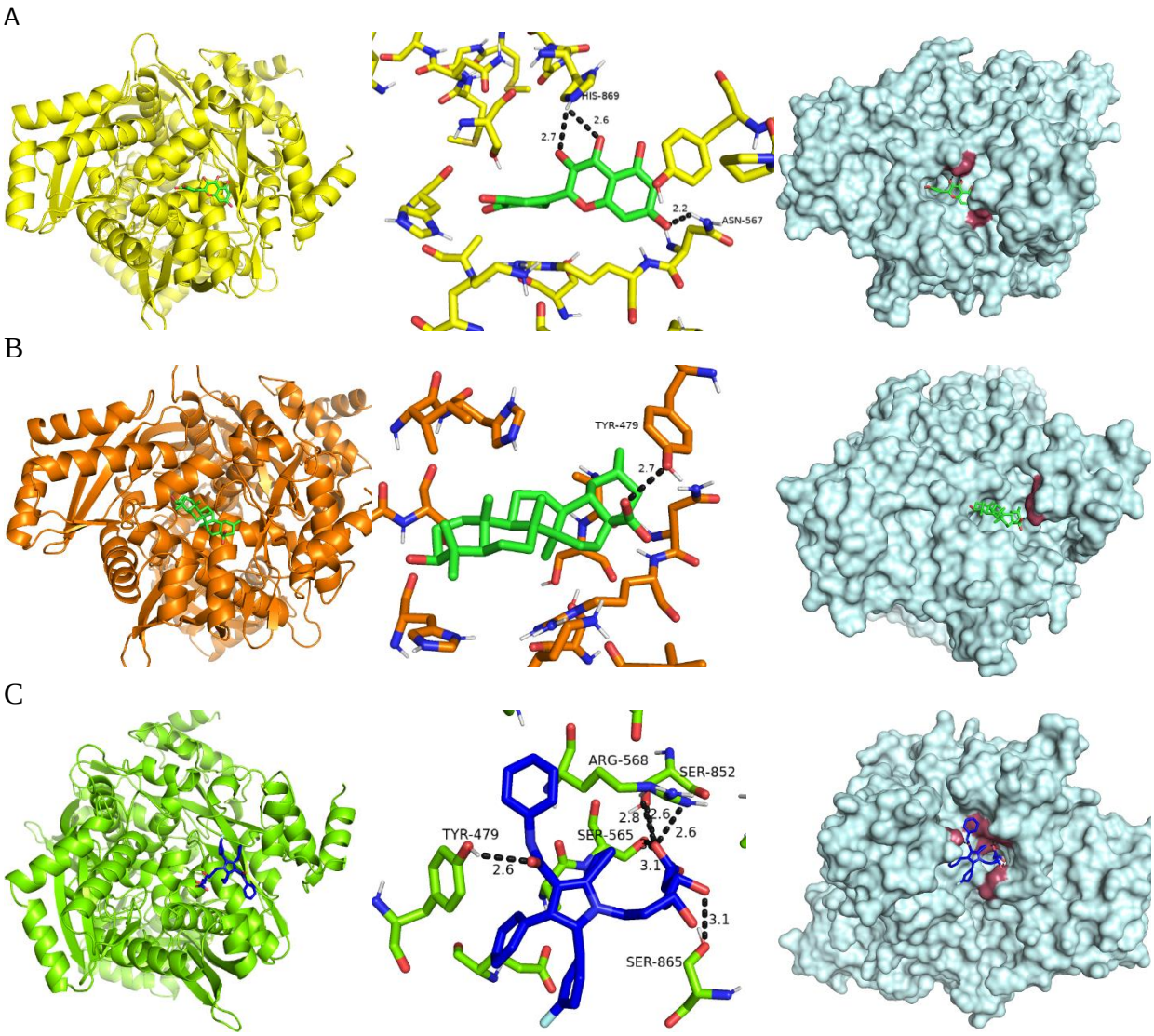

Figure 5.29Docking interactions of (A) Quercetin (B) Oleanolic acid (C) Atorvastatin in the active sites of human HMG-CoA reductase (PDB id: 1DQA)

Quercetin, oleanolic acid, guggulsterone, and mahanine, are bound efficiently to the active site of human HMG-CoA reductase with good complementarity, and the docking score is -8.2, -8.9, -8.1 and -8.3 kcal/mol, respectively. Moreover, oleanolic acid has highest interactions with the active site of human HMG-CoA reductase, while other NPs also showed significant docking score for the binding with protein. In the selected drugs, Rimonabant, and Atorvastatin have shown good binding interactions with the docking score of -8.9 and -8.2 kcal/mol, respectively (Table 7). Quercetin is forming three hydrogen bond with ASN 567 and HIS 869 while oleanolic acid formed one H-bond with TYR 479. Also quercetin, and oleanolic acid formed many nonbonding interaction with protein. The standard drug atorvastatin formed many H-bonds but has less nonbonding interactions. All the interactions can be visualized from the figure 5.29.