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Estimation of Bioactive Compounds by Molecular Docking and GC–MS analysis of Polyherbal Extract

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ABSTRACT

This study focuses on the antioxidant potential of the bioactive compounds present in a polyherbal ethanol extract prepared from *Phyllanthus emblica*, (Amla), *Psidium guajava* (Guava leaves), *Phyllanthus niruri* (Gale of wind) (Keezhanelli) and *Silybum marianum* (Milk Thistle). The plant materials were collected, shade-dried, powdered and extracted for 48 hrs using 90% ethanol in a Soxhlet apparatus. The polyherbal extract was subjected to estimation of total phenol, total flavonoid, *invitro* antioxidant, molecular docking and GC–MS analysis. The total phenol content (TPC) was estimated by Folin-Ciocalteu method, Gallic acid was used as standard. The TPC in the Polyherbal extract was 40.12 mg/g and 43.65 mg/g was found in the standard. The total flavonoid content (TFC) in the polyherbal extract was estimated by AlCl3 calorimetry method, the absorbance was measured 510nm using UV-Visible spectrophotometer, the quercetin was used as standard. *Invitro* antioxidant scavenging activity was performed by ABTS assay showing the highest percentage inhibition. In GC-MS analysis around 57 volatile and semi-volatile mixture of compounds were identified.

Keywords: Soxhlet apparatus, TPC, TFC, GC-MS analysis, *invitro* antioxidant, Molecular docking, UV-Visible spectrophotometer.

1. INTRODUCTION

The exploration of herbs for medicinal purposes is as vast as it is ancient around in the world. The history of human beings has turned to the natural world for healing and wellness. Herbs have been central to various traditional medicinal systems, begin with Traditional Chinese system of Medicine integrates herbal remedies, acupuncture and energy flow derived from natural principles. Similarly, the Indian system of medicine Ayurveda and Siddha dates back over 3,500 years and emphasizes harmony between body, mind and nature using herbs, diet and lifestyle. Plants and plant-derived formulations have been used by mankind from the ancient period of time across history, humans have consistently looked to the natural world for healing, rooted from plants, animals, minerals and marine sources.

Herbal treatments involve bioactive compounds such as flavonoids, alkaloids, and saponins, which have been found effective against infections and chronic diseases. Polyphenols and Flavonoids are the major classes of naturally occurring compounds present in many plants, vegetables, fruits, nuts and green leaves with potential health benefits. In recent years, dietary polyphenols have gained significant interest among researchers due to their potential chemo protective functions and prevent the chronic disorders to maintain the human health. It is believed that dietary polyphenols and flavonoids exert powerful antioxidant potential [1,2].

Based on the evidence the present selected plants *Phyllanthus emblica* (Amla) [3], *Psidium guajava* (Guava leaves)[4], *Phyllanthus niruri* (Keezhanelli) and *Silybum marianum* (Milk Thistle) were selected based on the flavonoids and phenolic content.

2. MATERIALS AND METHODS

2.1. Preparation of Polyherbal Extract

50gm each powdered materials of the selected plants Amla, Guava leaves, Keezhanelli and Milk

Thistle were mixed in equal proportion. The respective parts of the selected plant materials were dried under shade for a week and coarsely powdered for extraction. The Soxhlet apparatus was used to make an extract using 90% ethanol at 60–70 °C for 48 hrs. The extract was filtered through Whatman filter paper under reduced pressure in a rotary evaporator, the extract was dried and concentrated. The dried ethanolic polyherbal extract was stored in an airtight bottle in desiccator at room temperature and used for the present study. [5]

Table – 1: Life of the Plants used in the present study

Common Name	Botanical Name	Parts Used
Indian Goose Berry - Amla	*Phyllanthus emblica*	Fruit
Guava	*Psidium guajava*	Leaves
Gale of wind - Keezhanelli	*Phyllanthus niruri*	Entire Plant
Milk Thistle	*Silybum marianum*	Aerial parts

2.2. Determination of Total Phenol Content (TPC) and Total Flavonoid Content (TFC)

2.2.1. Estimation of Total Phenol Content (TPC)

The total phenolic content (TPC) of the polyherbal ethanol extract (PHE) was determined using the Folin-Ciocalteu Reagent (FCR) method, by colorimetric assay for quantifying phenolic compounds. Phenolic compounds reduce the FCR under alkaline conditions, producing a blue coloured chromophore. The intensity of the colour was measured by spectrophotometric method, is proportional to the concentration of phenol in the sample. The protocol involves preparing a reaction mixture containing 500 µl of polyherbal extract and 500 µl of Folin-Ciocalteu Reagent followed by incubation for 5 minutes at room temperature. Subsequently, 5 ml of 7% sodium carbonate (Na_2CO_3) and 6.5 ml of distilled water were added to the mixture, then incubated for 90 minutes at room temperature. The absorbance of the resulting solution was measured at 765 nm using a spectrophotometer. A standard curve was prepared using gallic acid as standard (ranging from 0 to 500 µg/ml) and the TPC is expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.[6]

2.2.2. Estimation of Total Flavonoid Content (TFC)

The total flavonoid content of PHE was estimated using the aluminium chloride ($AlCl_3$) by colorimetric method. Flavonoids form a stable complex with $AlCl_3$, which can be measured spectrophotometrically. The procedure involves preparing a reaction mixture containing 1 ml of polyherbal extract, 4 ml of distilled water, 0.3 ml of 10% sodium nitrite and 0.3 ml of 5% aluminium chloride. The mixture was incubated for 5 minutes at room temperature, then 2 ml of 1M sodium hydroxide (NaOH) was added. In addition, the mixture was incubated for an additional 30 minutes at room temperature and the absorbance was measured at 510 nm. A standard curve was prepared using quercetin as standard (ranging from 0 to 500 µg/ml) and the TFC is expressed as milligrams of quercetin equivalents (QE) per gram of extract. [7]

2.3. *Invitro* Antioxidant activity

2.3.1. Scavenging activity on ABTS˙+

ABTS˙+ scavenging activity was assessed according to the method described by Re et al. (1999) [8]. A mixture of ABTS (7.0 mmol/L) and potassium persulfate (2.45 mmol/L) in water was prepared and stored at room temperature for 12 h in a dark room to produce ABTS˙+. The ABTS˙+ solution in water was diluted to the level of absorbance of 1.50 at 414 nm for the analysis. Different concentrations of the extract solution (1 mL) were added to the diluted ABTS˙+ solution (2 mL). The absorbance was recorded at 734 nm using an UV-Visible Spectrophotometer.

Scavenging activity (%) = A blank – A sample / A blank x 100

2.4. Molecular Docking

The molecular docking was carried out to evaluate the interaction of bioactive compounds present in the selected plants of Amla, Guava, Keezhanelli and Milk thistle react with target proteins against diabetes. The present phytoconstituents were identified from ethanolic polyherbal extract of the selected plants using ligands. The three-dimensional structures of the ligands were obtained from the PubChem database and the target protein structure was retrieved from the Protein Data Bank. The protein structure was prepared by removing water molecules, adding hydrogen atoms and optimizing the structure. The ligands were energy-minimized and converted into suitable docking format. Molecular docking was then performed using AutoDock Vina to predict the binding affinity and interaction between the ligands and the target protein. The docking results were analyzed based on binding energy values and hydrogen bond interactions. Finally, the ligand-protein interactions were visualized using PyMOL and Discovery Studio to identify the most stable binding poses and potential bioactive compounds responsible for the biological activity.

2.5. GC-MS Analysis

The ethanolic Polyherbal extract was subjected to GC-MS analysis to screen the present Phytoconstituents. The dried polyherbal extract

was dissolved in analytical grade methanol and filtered through a 0.22 µm membrane filter. About 1 µL of the sample was injected into the Gas Chromatography–Mass Spectrometry instrument equipped with a capillary column. The carrier gas helium, was utilized at a steady flow rate of 1 ml/min. The injector temperature was maintained at 250 °C and the oven temperature was programmed from 60 °C to 280 °C at a gradual rate. The mass spectrometer was operated in electron ionization mode at 70 eV with a scan range of 40–600 m/z. The compounds present in the extract was identified by comparing their mass spectra with those available in the NIST library database. The obtained chromatogram provided peaks corresponding to various phytochemical compounds present in the polyherbal extract. [15]

3.RESULT AND DISCUSSION

3.1. Extractive Value

The extractive value of the selected plants in the ethanol polyherbal extract was calculated as follows.

Weight obtained / Weight taken x 100

108/800 * 100 = 13.5% w/w

The polyherbal extract value = 13.5% w/w

3.2. Total Phenolic content (TPC)

The Folin-Ciocalteu assay is a common and simple method to determine total phenolic content in the polyherbal extract, relying on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline media.

The TPC value was obtained from the calibration curve y= 0.544x = 0.3129 with R2 = 0.9858 absorbance at 765 nm.

The total Phenol content in the sample was found to be 40.12 & 43.65 mg/g. Calculated as standard graph of Gallic acid.

S.No	Concentration (µg/ml)	Standard (Gallic acid)	Polyherbal Extract (PHE)
1.	10	0.53±0.00318	0.563±0.0316
2.	20	0.326±0.0026***	0.337±0.0401**
3.	40	0.368±0.00693***	0.342±0.0132**
4.	60	0.237±0.00606***	0.210.0816**
5.	80	0.136±0.00354***	0.127±0.0412**
6.	100	0.0733±0.0026**	0.081±0.00289*
Ic 50 values		43.65 (µg/ml)	40.12 (µg/ml)

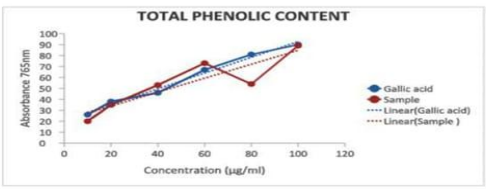


Figure 1: Total Phenol Content determination of standard and sample

Total phenolic compound and flavonoid content of the ethanol polyherbal extract was listed in Figures 1, 2

3.3. Total Flavonoid Content (TFC)

The TFC of the Polyherbal extract was estimated against Quercetin as standard at the absorbance of 510 nm by spectrophotometer.

The TFC in the sample was found to be 63.26 & 50.12 mg/g calculated against graph of Quercetin.

Table 4 : Concentration and absorbance of standard Quercetin and sample

S.No	Concentration (µg/ml)	Standard (Quercetin)	Sample (PHF)
1.	10	0.0822±0.0203	0.123±0.0182
2.	20	0.090±0.0404***	0.086±0.0202**
3.	40	0.086±0.0216***	0.091±0.0215***
4.	60	0.0443±.0982***	0.0723±0.0206**
5.	80	0.0968±0.0702***	0.065±0.0173*
6.	100	0.028±0.0216*	0.023±0.0216**
Ic 50 values		63.26 (µg/ml)	50.12 (µg/ml)

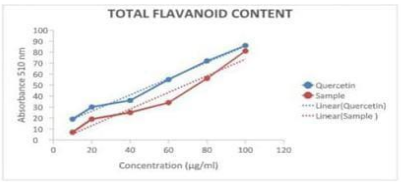


Figure No. 02 Total Flavonoid Content determination of standard and sample

3.4. Molecular Docking

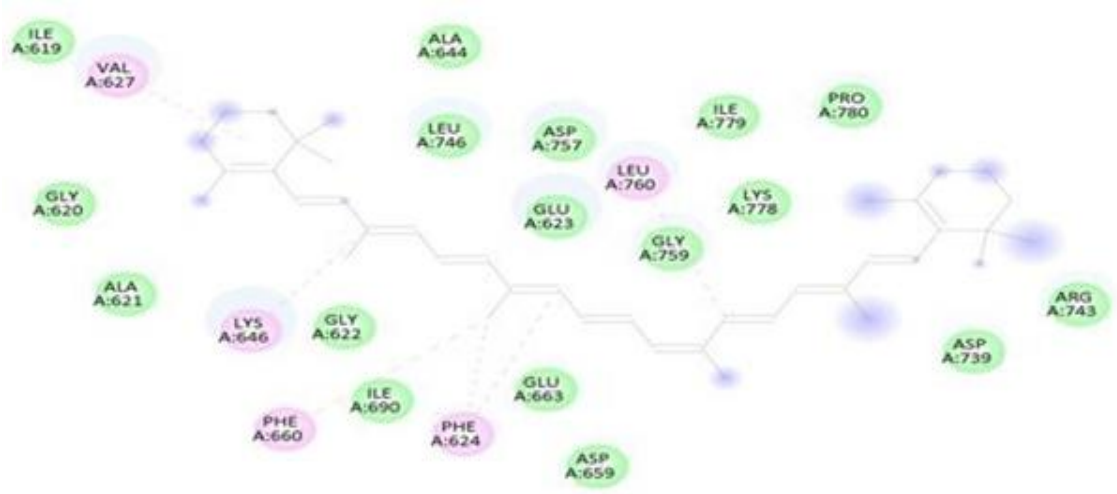
Molecular docking has been increasingly important tool for drug discovery.

The studies were carried out to evaluate the interaction of bioactive compounds present in the selected plants Amla, Guava, Keezhanelli and Milk Thistle screened against targeted proteins using respective ligands obtained from PubChem database and the targeted protein structure was received from Protein Data Bank.

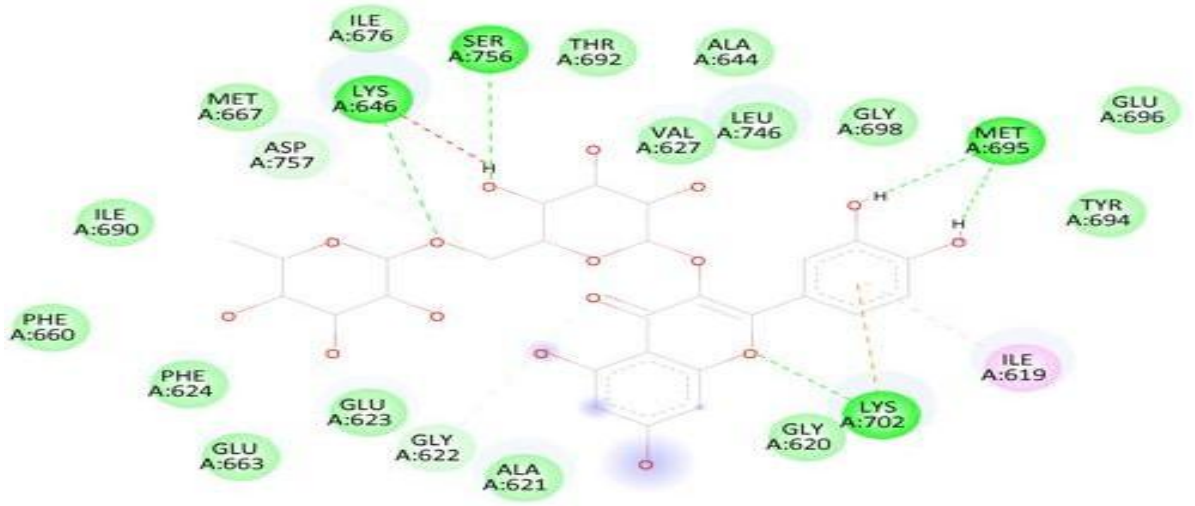
EphA2 & TNF-alpha receptors binding affinity against polyherbal extracts were screened, the Silymarin was used as standard.

The selected plants in the polyherbal ethanol consists of several phytoconstituents among that around 9 constituents binding affinity it's close to the standard value of Silymarin, which reported in table no.6.

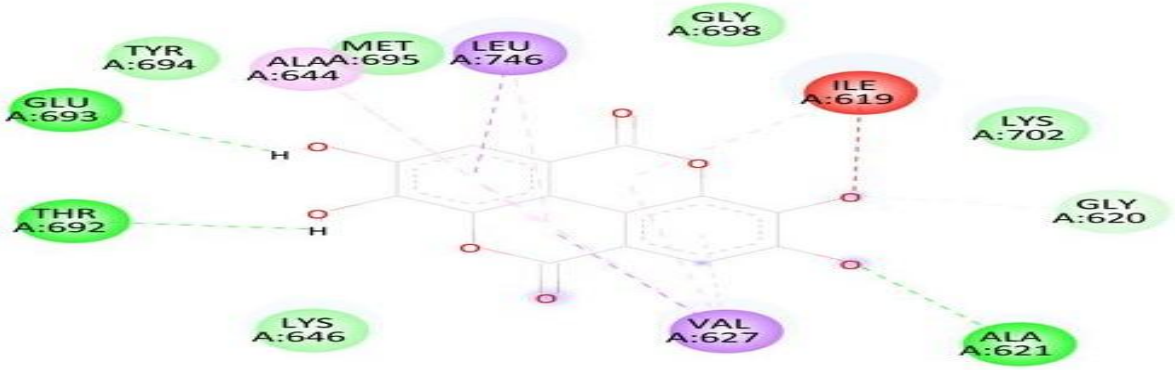
The docking results show that Silymarin (-9.5) has the highest affinity for the EphA2 receptor, while Guaijaverin (-9.7) shows the strongest binding to the TNF-α receptor. Compounds like Rutin, β-carotene, and Ellagic acid also exhibit good binding affinities. Overall, the phytoconstituents present in the polyherbal extract demonstrate promising hepatoprotective potential by effectively targeting both receptors.



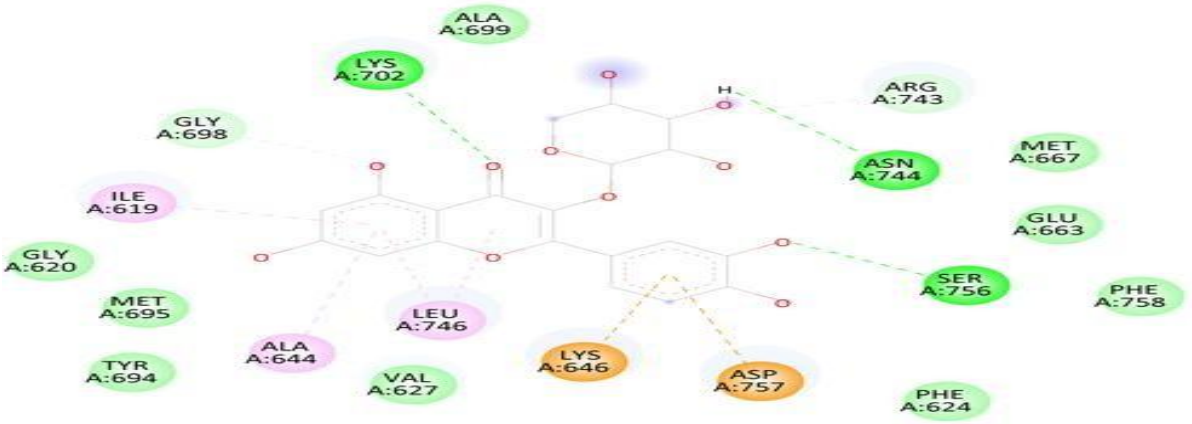
Beta Carotene



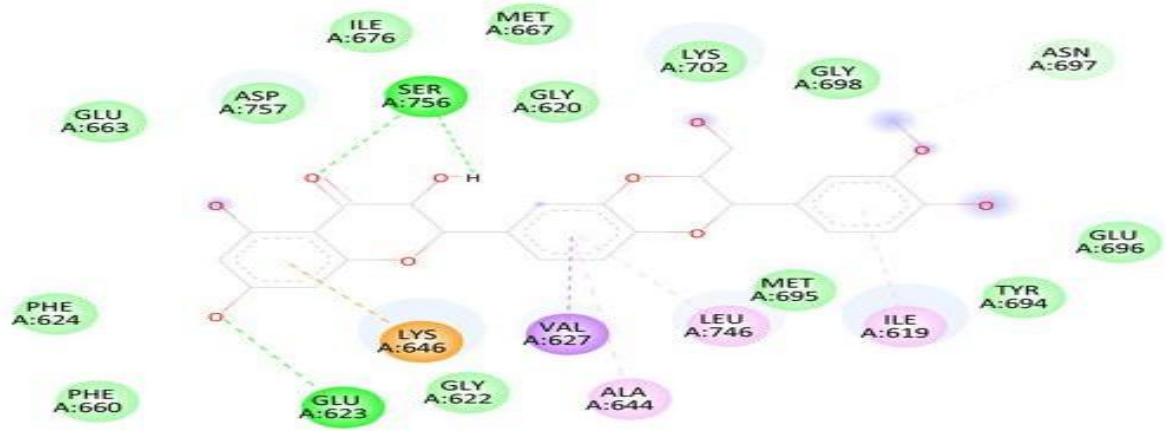
Rutin



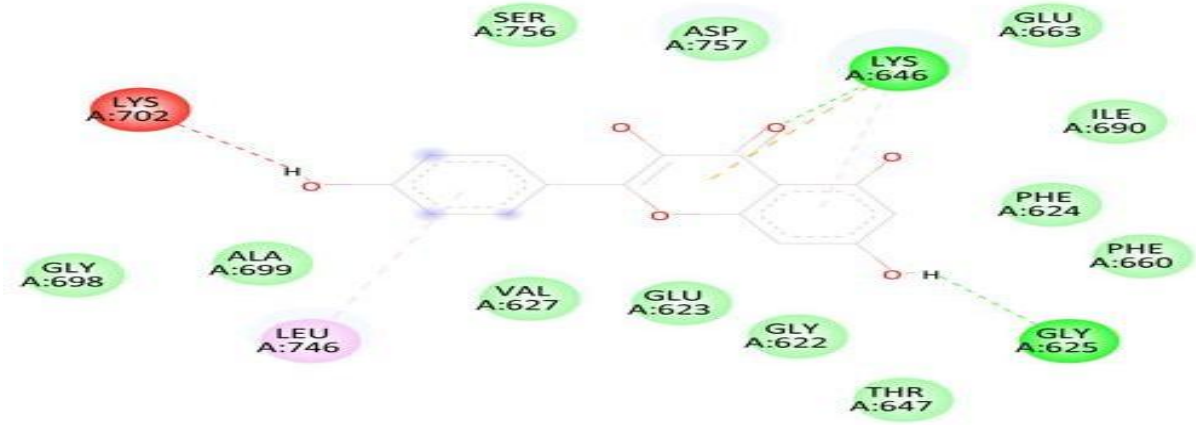
Ellagic acid



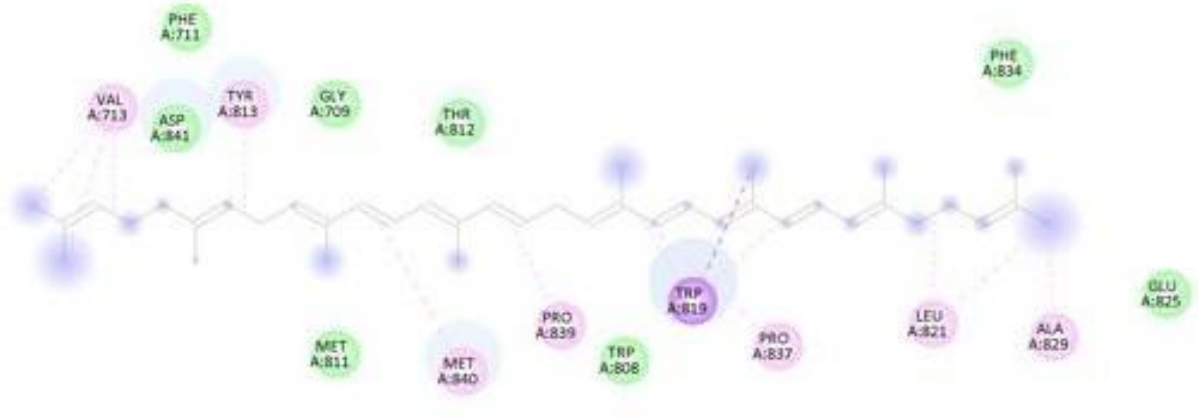
Guaijaverin



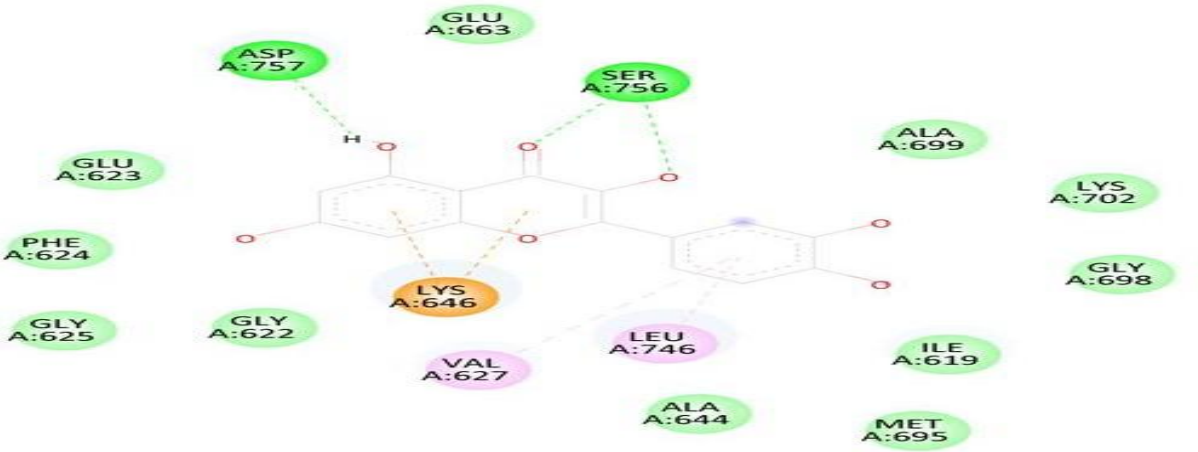
Isosilybin



Kaempferol

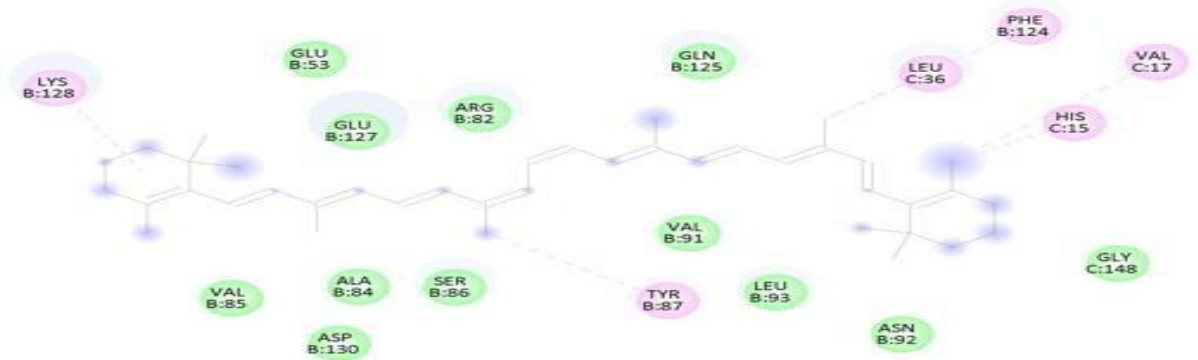


Lycopene

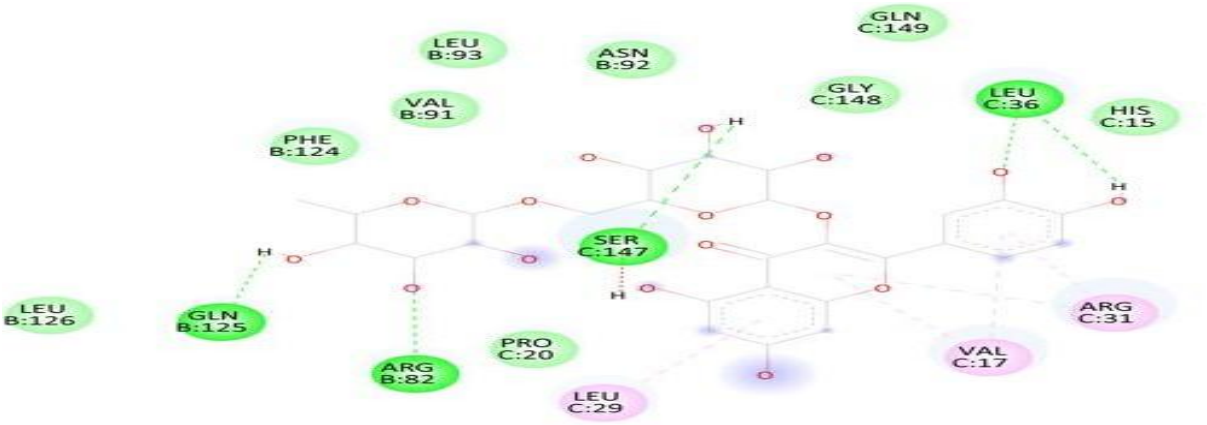


Quercetin

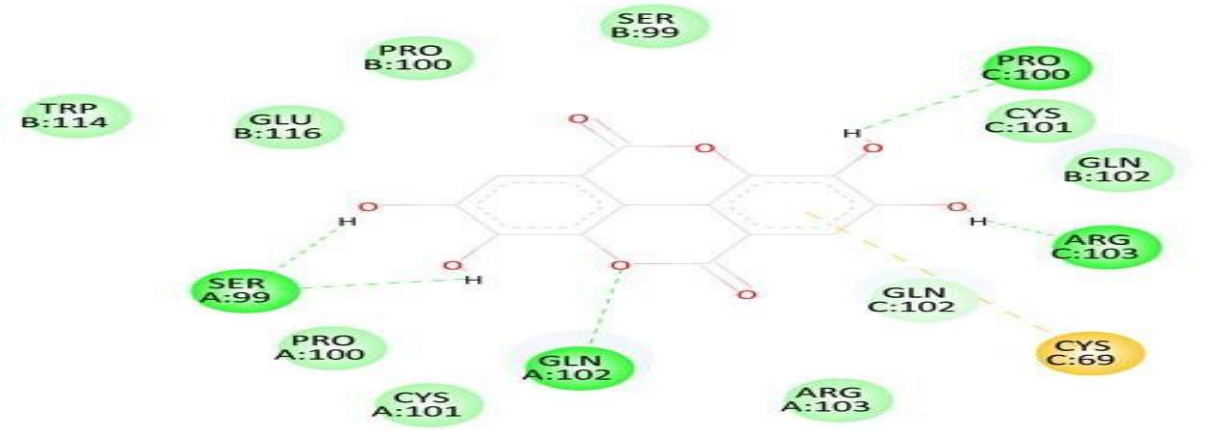
2)TNF-Alpha Receptor Protein



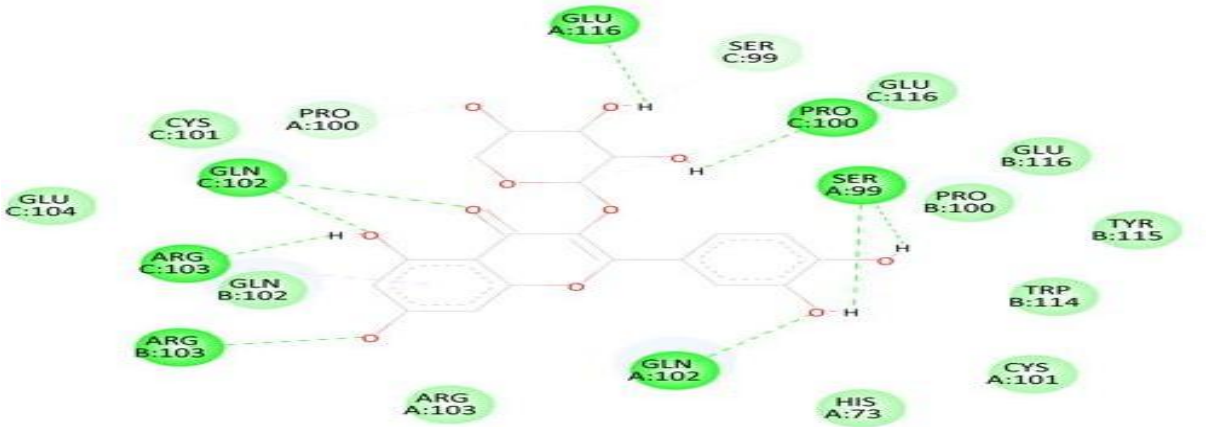
Beta carotene



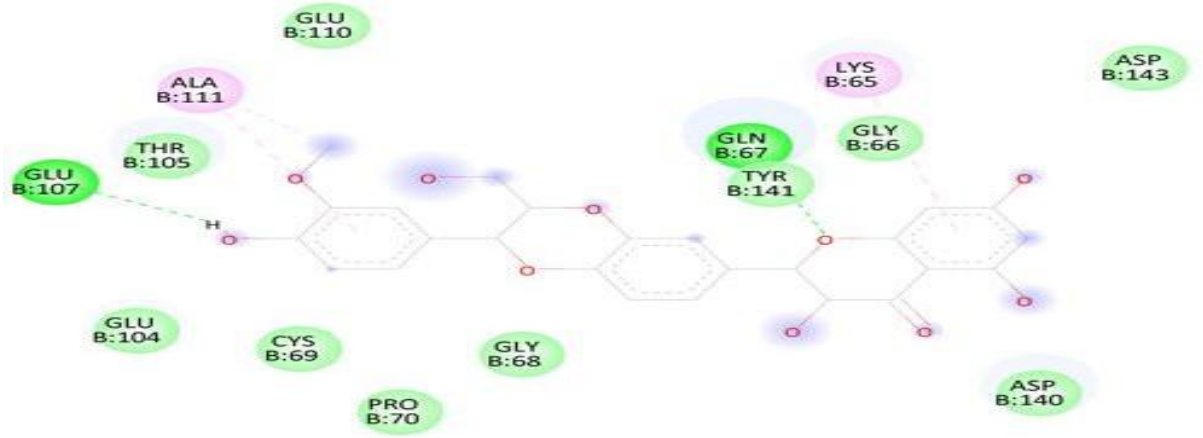
Rutin



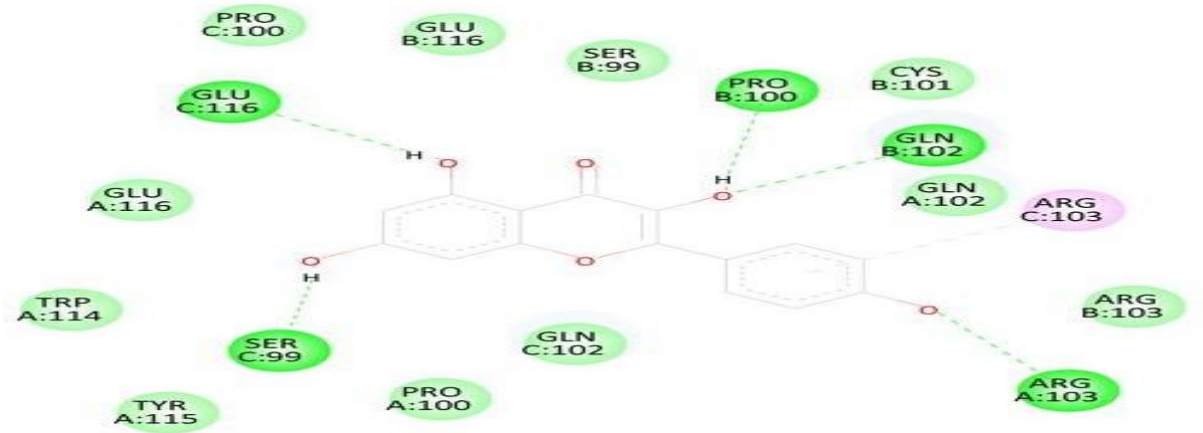
Ellagic acid



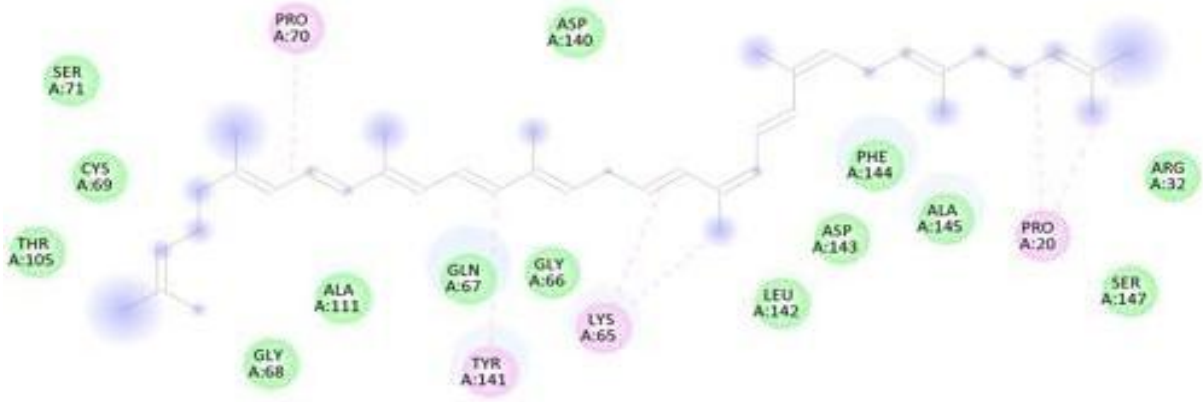
Guaijaverin



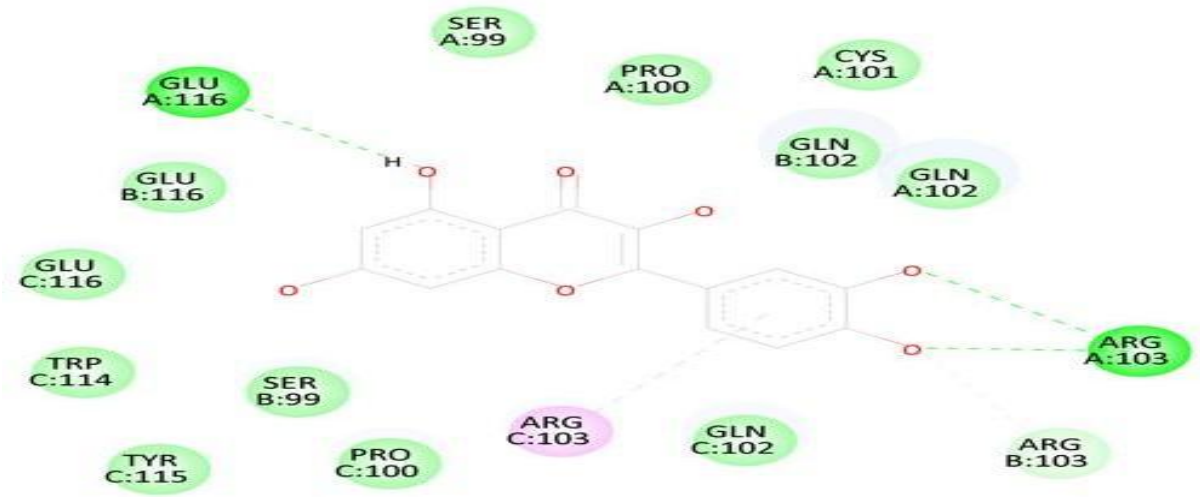
Isosilybin



Kaempferol



Lycopene



Quercetin

Table 6: Table shows the binding affinity of the Polyherbal Extract and Standard

Table - 6: Table shows the binding affinity of the Polyherbal Extract and Standard

Chemical Constituents	EphA2receptor binding affinity	TNF- alpha receptor binding affinity
Rutin	-9.1	-7.7
Beta carotene	-9.0	-7.5
Ellagic acid	-8.5	-9.1
Gallic acid	-5.9	-6.8
Guaijaverin	-8.4	-9.7
Isosilybin	-8.8	-7.5
Kaempferol	-7.6	-8.8
Lycopene	-7.6	-6.6
Quercetin	-7.8	-8.8
Standard (Silymarin)	-9.5	-7.6

It was observed that hydrogen bond interactions against EphA2 & TNF-alpha receptor, the binding affinity of the Rutin= -9.1, Ellagic acid= -9.1, Beta carotene= -9.0, Kaempferol and Quercetin= -8.8 and the standard value of Silymarin was found to be -9.5.

In the present study revealed that the binding affinity of the few phytoconstituents present in the polyherbal extract near to the standard Silymarin value. So, it's recommended for the hepatoprotective activity of the polyherbal extract containing herbs Amla, Guava, Keezhanelli and Milk Thistle.

3.5. GC-MS analysis

GC-MS analysis revealed present some volatile and semi-volatile compounds in the Polyherbal extract as complex mixtures. It provides a fingerprint of the chemical composition, identifying bioactive components like alkaloids, flavonoids, glycosides, terpenoids, esters etc.

In this analysis estimated around 57 compounds in the polyherbal extract reported at different peak area. Around 12 important compounds like 1,2,3-Benzenetriol (Pyrogallol), 5-Hydroxymethylfurfural (HMF), Catechol, Neophytadiene, n-Hexadecanoic Acid (Palmitic acid), Phenolic antioxidants, Anti-inflammatory / antimicrobial compounds were present in the extract.

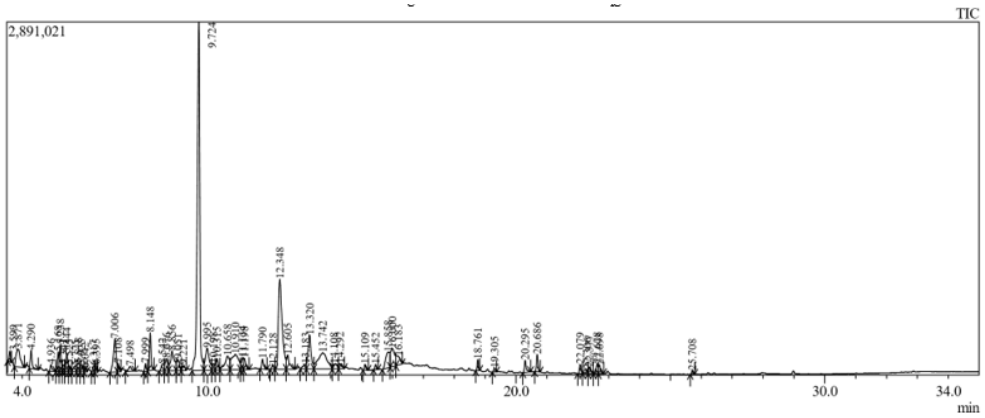
Malic acid, D-Gluconic acid lactone and Succinic acid derivatives these compounds collectively reduce oxidative stress, lipid peroxidation, and inflammation and hepatoprotective properties has been reported.

a) 1,2,3-Benzenetriol (Pyrogallol)

One of the major peaks (Area: 6,440,325) in your GC-MS data. A phenolic compound with strong free-radical scavenging ability. Phenolics are known to prevent lipid peroxidation in liver cells. This compound possess Strong antioxidant, Antimicrobial & Anticancer. These properties help protect hepatocytes from oxidative damage. [16]

b) 5-Hydroxymethylfurfural (HMF)

Largest peak in your chromatogram (Area: 16,321,897). This compound possess Antioxidant, Anticancer & Antimicrobial. Literature reports that HMF can reduce oxidative stress and protect tissues, including liver cells. This compound is a key contributor to hepatoprotective potential. [17]



Peak Report TIC

PeaK#	R.Time	Area	Height	Name	Biological activity
1	3.599	280308	88225	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N	Antimicrobial potential
2	3.871	1330551	145355	(S)-(+)-2-Amino-3-methyl-1-butanol	Antibacterial
3	4.29	566883	149243	1,2-Cyclopentanedione	Antioxidant potential
4	4.936	242692	54919	2-Furancarboxaldehyde, 5-methyl-	Antioxidant
5	5.168	518528	161850	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	Antioxidant
6	5.238	768064	219042	Phenol	Strong antimicrobial
7	5.341	233506	54465	Hexanoic acid, 3-ethyl-, methyl ester	Antimicrobial
8	5.444	623327	147760	2H-Pyran-2,6(3H)-dione	Antioxidant
9	5.525	225919	50631	7,8-Dioxabicyclo[3.2.1]oct-2-ene	Antimicrobial potential
10	5.725	357417	49246	4-Hexen-3-one, 5-methyl-	Antioxidant
11	5.818	396928	67633	1-Heptene, 2-methyl-	Antimicrobial
12	5.925	363118	54298	2-Butanone, 4-hydroxy-3-methyl-	Antioxidant
13	6.042	135196	28581	2-Ethyl-5-propylcyclopentanone	Antimicrobial
14	6.317	200066	49510	Benzeneacetaldehyde	Antimicrobial
15	6.395	226869	58567	2-Thiazolamine, 4,5-dihydro-	Antibacterial, Antifungal
16	7.006	1681318	270259	Cyclohexanamine, N-3-butenyl-N-methyl-	Antimicrobial
17	7.108	315135	67967	Oxalic acid, cyclobutyl hexyl ester	Antioxidant
18	7.498	313364	39910	Cyclohexasiloxane, dodecamethyl-	Antimicrobial
19	7.999	215826	63390	Ethanamine, N-ethyl-N-nitroso-	Antimicrobial
20	8.148	1132508	308160	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	Antioxidant
21	8.542	376550	56368	Cyclobutane-1,1-dicarboxamide, N,N'-di-benzo	Antimicrobial
22	8.676	606174	100418	Ethyl hydrogen succinate	Antioxidant
23	8.856	1367314	162552	2,2'-Bi-2H-pyran, octahydro-	Antioxidant, Antimicrobial
24	9.051	574744	91604	2-Methylbutanoic anhydride	Antimicrobial
25	9.221	265843	43307	Catechol	Strong antioxidant, Antimicrobial
26	9.724	16321897	2851323	5-Hydroxymethylfurfural	Antioxidant, Anticancer, Antimicrobial
27	9.995	1516198	179719	Octanoic acid, 3-hydroxy-, methyl ester	Antimicrobial
28	10.198	323506	52762	D-Gluconic acid, gamma.-lactone	Antioxidant
29	10.315	453746	94443	Butanedioic acid, hydroxy-, diethyl	Antioxidant

				ester, (+/-)	
30	10.658	1171424	113150	Malic Acid	Antioxidant
31	10.91	2002694	126502	4-Ethyl-1-[2-(piperidin-4-yl)ethyl]piperidine	Antimicrobial
32	11.134	436438	98304	2-Methoxy-4-vinylphenol	Antioxidant, Antimicrobial
33	11.198	427310	92302	Methylene asparagine	Antioxidant
34	11.79	354014	80327	d-Mannitol, 1-O-(22-hydroxydocosyl)-	Antioxidant, Diuretic
35	12.128	172077	36219	3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trim	Antioxidant potential
36	12.348	6440325	735890	1,2,3-Benzenetriol	Strong antioxidant, Antimicrobial, Anticancer
37	12.605	697587	115514	Cyclohexanol, 3,3,5-trimethyl-, acetate, cis-	Antimicrobial, Antiinflammatory
38	13.183	295995	39796	Succinic acid, hept-2-yl non-3-en-1-yl ester	Antioxidant
39	13.32	1870230	276889	DL-Proline, 5-oxo-, ethyl ester	Antioxidant, Antimicrobial, Cytoprotective
40	13.742	2784682	131494	Melezitose	Antioxidant, Antimicrobial
41	14.108	412766	37775	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.	Antioxidant
42	14.292	278254	41888	Propanoic acid, nonyl ester	Antimicrobial
43	15.109	169114	45603	Phenol, 4-ethenyl-2,6-dimethoxy-	Strong antioxidant, Antimicrobial, Anti-inflammatory
44	15.452	132376	41220	Dodecanoic acid, ethyl ester	Antimicrobial, Antifungal
45	15.858	1256872	128044	Melezitose	Antioxidant, Antimicrobial
46	16	1297608	147713	Ethyl .alpha.-d-glucopyranoside	Antioxidant, Antimicrobial
47	16.183	692459	72540	.alpha.-D-Galactopyranoside, methyl	Antioxidant, Antimicrobial
48	18.761	281760	89150	Neophytadiene	Antioxidant, Antimicrobial, anti-inflammatory
49	19.305	109925	30889	Neophytadiene	Antioxidant, Anti-inflammatory
50	20.295	424978	98825	n-Hexadecanoic acid	Antioxidant, Antimicrobial, Anti-inflammatory
51	20.686	482401	140554	Hexadecanoic acid, ethyl ester	Antioxidant, Antimicrobial
52	22.079	274551	75147	3,7,11,15-Tetramethyl-1-2-hexadecen-1-ol	Antimicrobial
53	22.306	166758	36182	Cyclooctene, 3-methyl-	Antioxidant, Antimicrobial
54	22.377	259358	53259	cis,cis,cis-7,10,13-Hexadecatrienal	Antimicrobial
55	22.628	334484	81359	9,12-Octadecadienoic acid (Z,Z)-	Antioxidant, Anti-inflammatory
56	22.698	299141	74011	9,12,15-Octadecatrienoic acid, 2,3-dihydroxy	Antioxidant, Antimicrobial, Anticancer
57	25.708	98409	29977	Cyclohexane, 1,3,5-triphenyl-	Antioxidant, Anti-inflammatory

c) Catechol

A powerful phenolic antioxidant. This compound possess Strong antioxidant & Antimicrobial Catechol derivatives are known to neutralize ROS, protecting liver tissues from toxin-induced damage. Important liver-protective antioxidant compound.[18]

d) Neophytadiene

Identified twice in your sample. This compound possess Anti-inflammatory, Antioxidant & Antimicrobial. Anti-inflammatory compounds are crucial in preventing liver inflammation. Supports hepatoprotection through anti-inflammatory action. [19]

e) n-Hexadecanoic Acid (Palmitic acid)

1)A bioactive fatty acid. This compound possess Antioxidant, Anti-inflammatory & Antimicrobial Fatty acids like this can stabilize cell membranes

and reduce inflammation, aiding liver protection. [20]

Other Compounds Contributing to Hepatoprotection

Several additional compounds indirectly support hepatoprotective activity:

I. Phenolic antioxidants

2-Methoxy-4-vinylphenol, Phenol derivatives & 1,2-Cyclopentanedione

II. Anti-inflammatory / antimicrobial compounds

Neophytadiene, 9,12-Octadecadienoic acid & 9,12,15-Octadecatrienoic acid

III. Organic acids with antioxidant roles

Malic acid, D-Gluconic acid lactone & Succinic acid derivatives

These compounds collectively reduce oxidative stress, lipid peroxidation, and inflammation, which are the main mechanisms of hepatoprotection. [21]

4. CONCLUSION

The present study concluded that the relationship between the total phenol content, total flavonoid content co-related with ABTS assay was screened for the ethanolic polyherbal extract consists of selected plant constituents possess significant activity.

The role of antioxidant potential present in the phytoconstituents of the selected plants like Rutin, Quercetin, etc., were further confirmed by molecular docking study.

The binding affinity of these compounds possesses significant interaction with selected receptors EphA2 & TNF-alpha receptor in the molecular docking study.

The GC-MS analysis reported the present volatile and semi-volatile compound in the crude polyherbal extract around 57 compounds were reported.

In further investigation needed to find out the antidiabetic activity and its mechanisms of plants in the polyherbal extracts by *in vivo* models.

5. REFERENCE

1. A Review on Hepatoprotective Activity of Medicinal Plants" by C. Hari Kumar, A. Ramesh, J. N. Suresh Kumar, and B. Mohammed Ishaq, published in IJPSR, 2011, Vol. 2(3): 501-515.https://ijpsr.com/bft-article/a-review-on-hepatoprotective-activity-of-medicinal-plants/
2. Adewusi EA, Afolayan AJ. A review of natural products with hepatoprotective activity. J Med Plants Res, 2010; 4(13): 1318-1334. https://www.researchgate.net/publication/228473528_A_review_of_natural_products_with_hepatoprotective_activity.
3. Borah, N., & Anagha, A. G. (2022). *Phyllanthus emblica* (Amla): A review of nutritional and medicinal properties. International Journal of Current Microbiology and Applied Sciences, 11(1), 375–389. https://doi.org/10.20546/ijcmas.2022.1101.045
4. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn. leaf extract. Indian J Exp Biol, 2006; 44(4): 305-311. https://pubmed.ncbi.nlm.nih.gov/16629373/
5. Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
6. Genwali GR, Acharya PP, Rajbhandari M. Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. Nepal J Sci Technol, 2013; 14(1): 95-102.DOI: https://doi.org/10.3126/njst.v14i1.8928
7. Pandey B, Rajbhandari M. Estimation of total phenolic and flavonoid contents in some medicinal plants and their antioxidant activities. Nepal J Sci Technol, 2014; 15(1): 53-60. https://www.nepjol.info/index.php/NJST/article/view/12010
8. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3
9. Wu, M.; Liu, M.; Wang, F.; Cai, J.; Luo, Q.; Li, S.; Zhu, J.; Tang, Z.; Fang, Z.; Wang, C.; Chen, H. The inhibition mechanism of polyphenols from *Phyllanthus emblica* Linn. fruit on acetylcholinesterase: A interaction, kinetic, spectroscopic, and molecular simulation study. Food Res. Int. 2022, 158, No. 111497.https://doi.org/10.1016/j.foodres.2022.111497
10. Cao T., Kim J.A., Woo M.H., Min B.S. SARS-CoV-2 main protease inhibition by compounds isolated from Lyffa yunnanica using molecular docking. Bioorg. Med. Chem. Lett. 2021, 40,

127972.https://doi.org/10.1016/j.bmcl.2021.127972

11. Fortunato, M. & Colina, C. M. (2017). SoftwareX, 6, 7–12. https://doi.org/10.1016/j.softx.2016.12.002
12. Hsin K.Y., Ghosh S., Kitano H. (2013). Combining machine learning systems and multiple docking simulation packages to improve docking prediction reliability for network pharmacology. PLoS ONE, 8:e822.https://doi.org/10.1371/journal.pone.0083922
13. Gregory Sliwoski, Sandeepkumar Kothiwale, Jens Meiler and Edward W. Lowe Jr., "Computational Methods in Drug Discovery," Pharmacological Reviews, January 2014, 66(1), 334-395. https://doi.org/10.1124/pr.112.007336
14. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10(1):1523.https://doi.org/10.1038/s41467-019-09234-6/
15. Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., & Uma, C. (2013). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. Journal of Food Science and Technology, 52(2), 1212–1217. https://doi.org/10.1007/s13197-013-1105-9
16. Bahramikia, S., Shirzadi, N., & Akbari, V. (2025). Protective effects of Pyrogallol and Caffeic acid against Fe2+ -Ascorbate-induced oxidative stress in the wistar rats liver: An in vitro study. Heliyon, 11(3), e42518. https://doi.org/10.1016/j.heliyon.2025.e42518
17. Li, W., Qu, X., Han, Y., Zheng, S., Wang, J., & Wang, Y. (2015). Ameliorative Effects of 5-Hydroxymethyl-2-furfural (5-HMF) from Schisandra chinensis on Alcoholic Liver Oxidative Injury in Mice. International Journal of Molecular Sciences, 16(2), 2446–2457. https://doi.org/10.3390/ijms16022446
18. Boots, A. W., Haenen, G. R., Hartog, G. J. D., & Bast, A. (2002). Oxidative damage shifts from lipid peroxidation to thiol arylation by catechol-containing antioxidants. Biochimica Et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1583(3), 279–284. https://doi.org/10.1016/s1388-1981(02)00247-0
19. Bhardwaj, M., Sali, V. K., Mani, S., & Vasanthi, H. R. (2020). Neophytadiene from Turbinaria ornata Suppresses LPS-Induced Inflammatory Response in RAW 264.7 Macrophages and Sprague Dawley Rats. Inflammation, 43(3), 937–950. https://doi.org/10.1007/s10753-020-01179-z
20. Liu, L., Xie, B., Fan, M., Candas-Green, D., Jiang, J. X., Wei, R., Wang, Y., Chen, H., Hu, Y., & Li, J. J. (2019). Low-Level saturated fatty acid palmitate benefits liver cells by boosting mitochondrial metabolism via CDK1-SIRT3-CPT2 cascade. Developmental Cell, 52(2), 196-209.e9. https://doi.org/10.1016/j.devcel.2019.11.012
21. Saha, P., Talukdar, A. D., Nath, R., Sarker, S. D., Nahar, L., Sahu, J., & Choudhury, M. D. (2019). Role of natural phenolics in hepatoprotection: A mechanistic review and analysis of regulatory network of associated genes. Frontiers in Pharmacology, 10, 509. https://doi.org/10.3389/fphar.2019.00509