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RESEARCH ARTICLE

Phytochemical Profiling of Various Extracts of *Glycine max* (L.) Seeds and *In-silico* Approach for Hepato-Protective Activity

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Abstract

Cirrhosis and fibrosis are mainly characterized by the frequent and repeated inflammation of the renal cells. These renal disorders may also lead to hepatocarcinoma and even death, so we are in need of complementary and alternative medicine to treat renal diseases. The Soybean (*Glycine max* (L.) Merr.) seed is reported to have medicinal properties and pharmacological activities like anti-inflammatory and anti-oxidant. Here, we have evaluated various extracts (Water, Ethanol, Methanol, Hexane and Benzene) of *Glycine max* (L.) to predict their phytoconstituents and found that methanolic extract has more phytoconstituents. The finest chemicals in the methanolic extract, such as 3-Methoxy-hexane-1,6-diol, Choline, 9,12,15-Octadecatrien-1-ol, and tetradecane, were docked against Transforming Growth Factor-(TGF-β) a cytokine, transcription factors such as Peroxisome Proliferator-Activated Receptor (PPAR) and Pregnene X Receptor (PXR), and Nuclear Factor kappa-b (NF-KB) a protein complex. Comparatively, Choline shows higher negative binding energies against all the receptors and possesses hepato-protective activity.

Keywords: Choline, *Glycine max* (L.), NF-KB, PPARα, PXR, TGF-β

1. Introduction

Nowadays, the prevalence of liver diseases and dysfunctions has increased, and they can only be detected through diagnosis, leading to Hepatitis, Fibrosis (Scarring), Cirrhosis (Severe Scarring), and cancer[1]. The severe conditions of injury may also lead to renal failure, misfunctions, hepatocarcinoma, and ultimately death. Drug metabolism via CYP450 enzymes, bile juice excretion, blood clotting factor production and xenobiotic metabolism are all functions of the liver[2]. The outcomes of these events result in chronic inflammation, hepatic cell replication, scar formation, and connective tissue formation in the liver. Toxins, long-term alcohol addiction, autoimmune illnesses, infection, genetic and metabolic abnormalities are among the causes of chronic liver disease[3]. Cirrhosis is considered to be the end stage of chronic liver disease that is characterised by asterixis, disruption of hepatic architecture, the formation of widespread nodules, and vascular reorganization[4,5]. Mainly, it recruits quiescent fibroblasts while hepatic stem cells are responsible for parenchymal regeneration. The focus is on the common etiologies, clinical symptoms and treatment options for chronic liver disease, which is a very common clinical ailment[6]. For the treatment of renal diseases, we are in need of developing complementary and alternative medicine from natural sources like plants and marine[7,8]. Many plant-based chemical constituents are reported to have versatile medicinal properties like anticancer, antimicrobial, anti-obesity, hepato-protective activity, anti-inflammatory and wound healing properties[9,10].

Soybean seeds were considered a rich source of protein, fibers, and carbohydrates, and were regarded as the most important part of Indian cuisine[11]. Due to its rich oil content, soybean edible oil has separate market value

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and uses for cooking and other medicinal practices[12,13]. Many studies have reported that soybeans have various phytoconstituents such as alkaloids, flavonoids, phenols, and terpenes that are able to offer various pharmacological activities such as antimicrobial, antioxidant, anticancer, anti-inflammatory, antiviral, and antiproliferative activities[10,14–17].

In this study, we used both qualitative and quantitative analysis, as well as GCMS, to profile the phytoconstituents in several extracts of *Glycine max* seeds. The best chemicals in the extract were docked against the receptors Transforming Growth Factor- (TGF-β) a cytokine, transcription factors such as Peroxisome Proliferator-Activated Receptor (PPAR) and Pregnene X Receptor (PXR), and Nuclear Factor kappa-b (NF-KB), a protein complex to determine their binding affinities as prescribed by previous studies[18,19].

2. Materials and Methods

2.1 Preparation of *Glycine max* (L.)

Soybeans were acquired in a sterile package from the Thanjavur local market. The soybeans were washed properly and steeped in water for 10 hours before being drained. A 50-gram sample was obtained and roasted for 25 minutes in a rolling metal cylindrical basket inside a 230°C oven. Different temperatures (ranging from 180 to 230°C) and durations (10-40 minutes) were used to test the roasting conditions.

2.2 Extraction

Glycine max seeds (L.) were weighed and extracted using distilled water, methanol, ethanol, benzene, and hexane on Soxhlet's equipment[20]. Then the extracts were filtered with Whatman filter paper, and the remaining solvents were extracted using a rotary vacuum evaporator before being stored in a desiccator for later use.

2.3 Qualitative Screening of Phytochemicals

All the qualitative screening of phytoconstituents of the *Glycine max* seed extracts was performed as per the described procedure[21].

2.3.1 Wagner's Test

To 4 mL of extract, 3 drops of Wagner's reagent were added and left undisturbed for 5 minutes. The reddish-brown precipitate is an indicator of alkaloid presence.

2.3.2 Sodium Hydroxide Test

To dissolve 0.2 g of the extract, a cold dilute solution of sodium hydroxide and diluted hydrogen chloride were utilized. The absence of the yellow color is an indicator of flavonoid presence.

2.3.3 Copper Acetate Test

To 5 mL of extract, 12 drops of $Cu(OAc)_2$ solution were carefully added and incubated. The formation of a beryl green color is an indicator of terpene presence.

2.3.4 Salkowski Test

To 5 mL of extract, 2.5 mL of $CHCl_3$ and 2.5 mL of concentrated H_2SO_4 were added and carefully mixed. The red fluorescence of the chloroform layer and the greenish yellow fluorescence of the acid layer demonstrate the steroid availability in the sample.

2.3.5 Foam Test

To 3 mL of the extract, 2 mL of water was added and agitated rapidly for roughly 10 minutes. A stable foam appearance is the indicator of saponin presence.

2.3.6 Ferric Chloride Test

For 10 minutes, a mixture of 0.5 mL of plant extract and 5 mL of distilled water was cooked. To the 2 ml of collected filtrate, 3 drops of a 10% ferric chloride solution were added. A greenish blue or violet color is an indicator of phenolics' presence.

2.3.7 Lead Acetate Test

4 mL of $Pb(C_2H_3O_2)_2$ solution were thoroughly mixed with 4 mL of extract. The presence of white precipitate is an indicator of tannins and phenols.

2.3.8 Borntrager's Test

2 mL of extracts were boiled and filtered with mild sulphuric acid. The filtrate was then completely combined with chloroform and shaken. Ammonia was gradually added after the organic layer was separated.

The ammoniacal layer changes color from pink to red when anthraquinone glycosides are present.

2.3.9 Fluorescence Test

A 1N sodium hydroxide solution was mixed with 2 mL of extract. The fluorescence of bluish green is the indicator of coumarin glycosides presence.

2.3.10 Kellar Killani's Test

A mixture of glacial acetic acid, ferric chloride and strong sulphuric acid was used to dissolve a few mL of extracts in water. The formation of a brown ring at the junction[9] indicates the presence of cardiac glycosides.

2.3.11 Spot Test

2 mL of extract was sandwiched by Whatman paper and squeezed for around 2-3 minutes. The residual oil on paper is an indicator of fixed oil's presence.

2.4 Total Phytoconstituents Quantification

The total alkaloid content, total flavonoid content, total phenolic content, total saponin content and total tannin content of phytoconstituents of the *Glycine max* seed extracts were performed as per the described procedure[21].

2.4.1 Total Alkaloids Content

2 mg/mL equivalent of extract was dissolved in DMSO, followed by the addition of 1 mL of 1N HCl, and then filtered. In addition to this, 4 mL of bromo-cresol green dye solution and 4 mL phosphate buffer were also added to it. The reaction mixture is then violently shaken with 1-5 mL of chloroform before being collected and the volume made up with $CHCL_3$. Atropine was used as a standard with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 470 nm.

2.4.2 Total Flavonoid Content

The $AlCl_3$ assay was performed to quantify the total flavonoid content. After mixing 2 mL of the extract with 8 mL of distilled water and 0.6 mL of a 5% $NaNO_2$ solution the mixture was kept undisturbed. Approximately after 8 min, 0.6 mL of 10% $AlCl_3$ and 4 mL of 1.5M NaOH solutions were added and made up to 10 mL of total volume. Quercetin was used as a standard, with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 510 nm.

2.4.3 Total Saponin Content

To 20g of extract, 150 mL of 15% ethanol was added and heated for 3 hr at 58°-60° C with stirring. The filtered sample was extracted with 350 mL of 15% ethanol, and the mixture was heated to evaporate the excess solvent. To the 50 mL of extracted sample, 25 mL of $(C_2H_5)_2O$ was added, and only the aqueous layer was collected and fractioned by n-butanol. Then the whole fraction was washed thrice with 25 mL of 4% NaOH. Diosgenin was used as a standard with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 550 nm.

2.4.4 Total Tannins Content

Folin-Ciocalteu reagent was used to quantify the total tannin content of the extracts. To 3 mL of extract, 5 mL of water, 0.4 mL of Folin-Ciocalteu, and 1.5 mL of 25% Na_2CO_3 solution were added, and the total volume was made up to 10 mL and kept indiverted for 30 min. Gallic acid was used as a standard, with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 725 nm.

2.4.5 Total Phenolics Content

Folin-Ciocalteu reagent was used to quantify the total phenolic content of the extracts. To 2 mL of extract, 7 mL of water and 1.5 mL of Folin-Ciocalteu were added and kept undisturbed for some time. After 8 min, 6.5 mL of 10% sodium carbonate was added and then made up to the 30 mL of the total column and again incubate for 1 hr at 37°C. Gallic acid was used as a standard, with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 550 nm.

2.5 Quantification of Phytochemicals through GC-MS

The GCMS-QP2010 SE was used to quantify the phytoconstituents present in the *Glycine max* seed extracts. The RTX-5 MS capillary column of 0.25m in diameter and 25m in length was used to identify and quantify chemical components. Working conditions for the GC were kept between 42 and 285°C, with a rise of 6°C every minute. The oven and injection port

temperatures were set to 110°C and 290°C, respectively. He was used as a carrier gas (mobile phase) with a 2 mL/min flow rate. The ioniser temperature was set at 210°C and the interface temperature 270°C. The detector voltage was set to 0.10 kV and the solvent cut-off period was set to 4 minutes. The mass range of 20-300 m/z was set and the compounds were compared with Wiley library[20].

2.6 Molecular Docking

PDB and PubChem were used to find the three-dimensional structures of the hepato-protective receptors and drugs. External ligands and surplus water molecules attached to the protein were removed after downloading the protein and ligand files. Then the files were converted into PDBQT files for molecular modelling in AutoDock 4.2. The grid box was set and all the parameters were kept as default. Finally, the binding affinities of ligands against receptors along with their binding energies were predicted[22].

3. Results

3.1 Qualitative Screening of Phytochemicals

The qualitative screening of Ethanolic Extract (EE), Aqueous Extract (AE), Methanol Extract (ME), Hexane Extract (HE), and Benzene Extract (BE) of *Glycine max* (L.) were determined. The results revealed that protein is present, steroids and carbohydrates are in all the extracts. Flavonoids were only absent in benzene extract. Both, saponin and tannins were absent in benzene and in methanolic extract. Amino acids, Cardiac glycosides and tannins were absent in methanolic extract. Protein

Table 1. Qualitative screening of *Glycine max* seed extracts

S.No	Chemical constituents	Test Name	EE	AE	ME	HE	BE
1.	Alkaloids	Wagner's test	+	+	++	-	-
2.	Flavonoids	Sodium hydroxide test	+	+	++	+	-
3.	Terpenoids	Copper acetate test	++	+	+	-	-
4.	Carbohydrates	Molisch's test	-	-	+	-	+
5.	Proteins	Millon's test	+	+	+	+	+
6.	Amino acids	Ninhydrin test	+	+	-	+	-
7.	Fats and oils (Fixed)	Soponification	+	-	+	-	+
8.	Steroids	Salkowski Tests	-	+	+	-	-
9.	Cardiac glycosides	KellarKillani's test	+	+	-	+	-
10.	Phenolics	Ferric chloride test	+	+	++	-	-
11.	Saponins	Foam test	+	++	+	+	-
12.	Tannins	Ferric chloride test	+	+	-	+	-

Note: ++ is highly present, + is Present, - is Not detected.

Table 2. Total phytoconstituent content of *Glycine max* seed extracts

S.No	Extracts	Alkaloids / Atropine equivalent	Flavonoids / Quercetin equivalent	Saponins / Diosgenin equivalent	Tannins / Tannic acid equivalent	Phenols / Gallic acid equivalent
1.	Ethanol extract	16.80±0.20	12.05±0.5	2.20±0.5	1.55±0.005	10.60±0.5
2.	Water extract	18.20±0.05	10.40±0.25	2.50±0.05	3.50±0.05	8.50±0.05
3.	Methanol extract	26.10±1.5	21.60±0.05	2.65±0.05	1.30±0.05	18.20±0.05
4.	Hexane extract	15.05±0.05	18.10±0.05	1.25±0.25	2.25±0.05	5.50±0.5
5.	Benzene extract	10.25±0.05	6.15±0.05	1.95±0.05	3.60±0.05	7.20±0.05

content was present in all the extract. From the above results, it showed that the methanolic extract shows high amount of phytoconstituents compared to the others as shown in the Table 1.

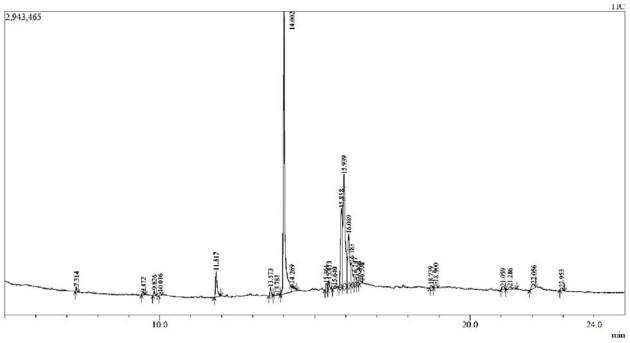


Figure 1. Chromatogram Peak of Methanolic Extract of *Glycine max (L.)*.

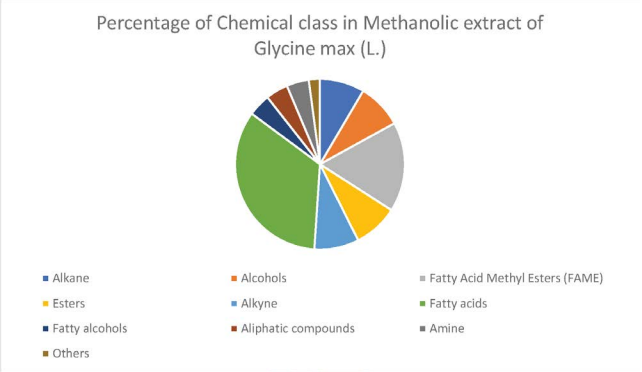


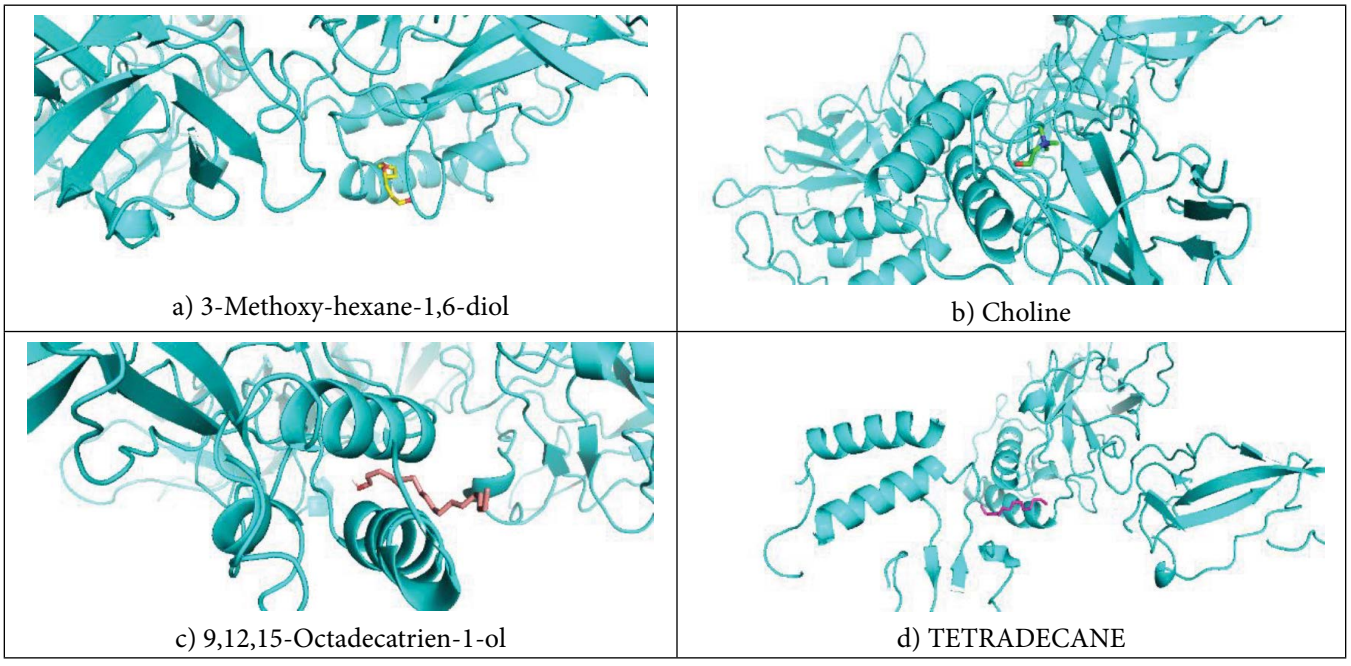
Figure 2. Percentage of chemical classes in Methanolic extract of *Glycine max* (L.).

Table 3. GCMS peak report

Peak No.	RT	Area%	Height%	Compound name
1	7.314	0.45	0.78	PENTADECANE
2	9.472	0.36	0.35	1-Pentanol, 2,3-dimethyl-
3	9.826	0.41	0.79	Nonane, 3,7-dimethyl-
4	10.016	0.32	0.50	Phthalic acid, di-(1-hexen-5-yl) ester
5	11.817	3.68	3.99	PENTADECANOIC ACID
6	13.573	0.84	1.27	HEXADECANOIC ACID, METHYL ESTER
7	13.783	0.30	0.27	Cyclopentanol, 2-(2-propynyloxy)-, trans-
8	14.002	37.77	42.89	Choline
9	14.269	0.32	0.48	ETHYL PENTADECANOATE
10	15.366	0.84	1.00	11,14-Eicosadienoic acid, methyl ester
11	15.453	0.98	1.41	9,12,15-Octadecatrienoic acid, methyl ester, (Z
12	15.640	0.32	0.44	HEXANOIC ACID, 2-METHYL-
13	15.858	12.25	12.00	9,12-OCTADECADIENOIC ACID (Z,Z)-
14	15.939	21.56	17.06	TETRADECANE
15	16.089	9.14	7.92	9-OCTADECENOIC ACID (Z)-
16	16.183	3.50	3.43	ETHYL (9Z,12Z)-9,12-OCTADECADIENOA
17	16.297	1.28	1.27	BUTYL STEARATE
18	16.408	0.72	0.62	DECANOIC ACID
19	16.508	0.57	0.31	3-Methoxy-hexane-1,6-diol
20	18.739	0.38	0.42	7-Hexadecyne
21	18.900	0.86	0.93	cis,cis,cis-7,10,13-Hexadecatrienal
22	21.059	0.62	0.44	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
23	21.286	0.81	0.35	9,12,15-OCTADECATRIEN-1-OL
24	22.056	1.24	0.68	2,2,3,3,4,4 HEXADEUTERO OCTADECAN
25	22.953	0.48	0.40	1,2-BENZENEDICARBOXYLIC ACID, DIN

Table 4. Binding energy of Ligands and receptors

Receptor name	Ligand name	Mean Binding energy (Kcal/mol)
Nuclear factor kappa-b (NF-KB) (1NFK)	3-Methoxy-hexane-1,6-diol	-4.4
	Choline	-5.2
	9,12,15-Octadecatrien-1-ol	-4.9
	TETRADECANE	-4.2
Peroxisome proliferator-activated receptor α (PPARα) (5HYK)	3-Methoxy-hexane-1,6-diol	-4.0
	Choline	-5.9
	9,12,15-Octadecatrien-1-ol	-5.8
	TETRADECANE	-4.7
Pregnene X Receptor (2O9I)	3-Methoxy-hexane-1,6-diol	-4.2
	Choline	-5.8
	9,12,15-Octadecatrien-1-ol	-5.5
	TETRADECANE	-4.8
TGF-β (Transforming growth factor-β) (1VJY)	3-Methoxy-hexane-1,6-diol	-3.8
	Choline	-5.1
	9,12,15-Octadecatrien-1-ol	-4.6
	TETRADECANE	-3.9



a) 3-Methoxy-hexane-1,6-diol

b) Choline

c) 9,12,15-Octadecatrien-1-ol

d) TETRADECANE

Figure 3. Ligand interaction with 1NFK.

3.2 Quantitative Phytochemical Determination

The quantitative determination of all the seed extracts of *Glycine max* (L.) was determined and all the standard gram equivalent values of tannins, alkaloids, saponins, steroids and glycosides were shown as mean value ± SD in triplicates as provided in Table 2. Total alkaloid content was higher (26.10±1.5) in methanolic extract, flavonoid content was less in the benzene extract and

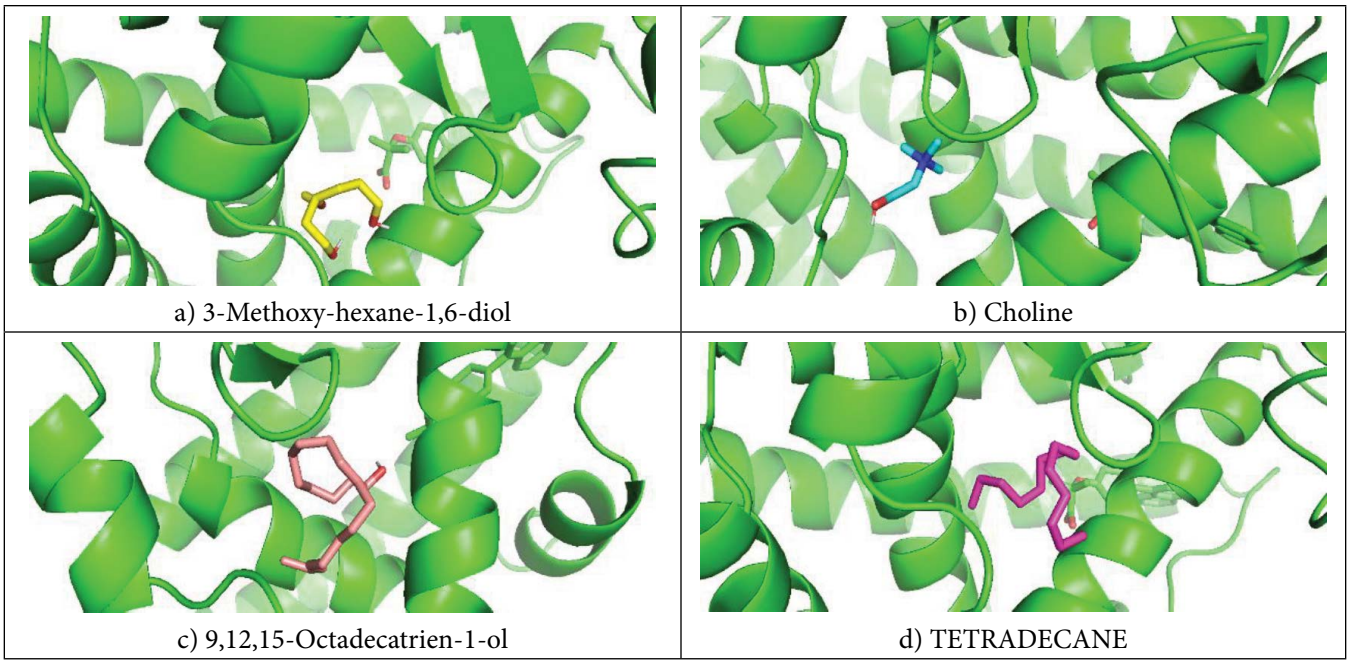


Figure 4. Ligand interaction with 5HYK.

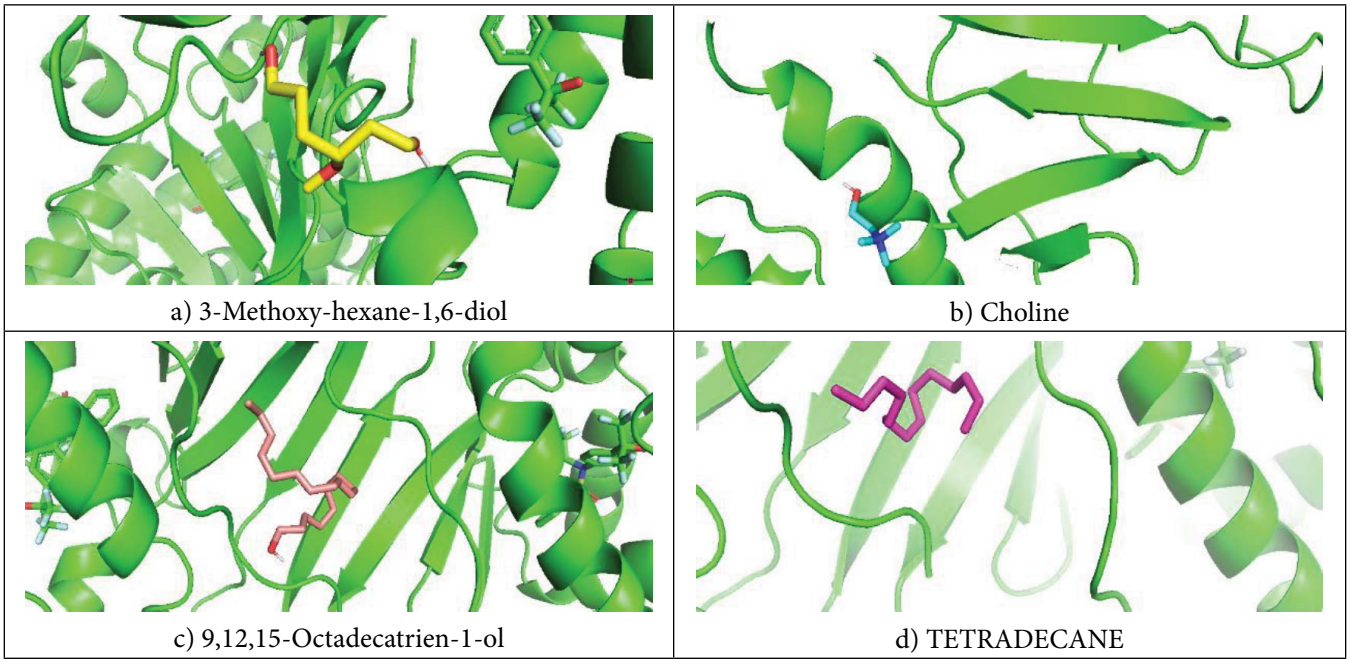


Figure 5. Ligand interaction with 2O9I.

saponin was less in benzene and hexane extracts and higher in methanolic extract (2.65±0.05). Also, the tannin content was very low in the methanolic extract when compared to all other extracts. Compared to the other extracts, methanolic extract showed high alkaloid, phenolic and flavonoid content and low tannin content compared to the other extracts. Therefore this methanolic extract was taken into further study.

3.3 GCMS Analysis

The GCMS analysis of the methanolic extract of *Glycine max* (L.) soybean seeds reported nearly 25

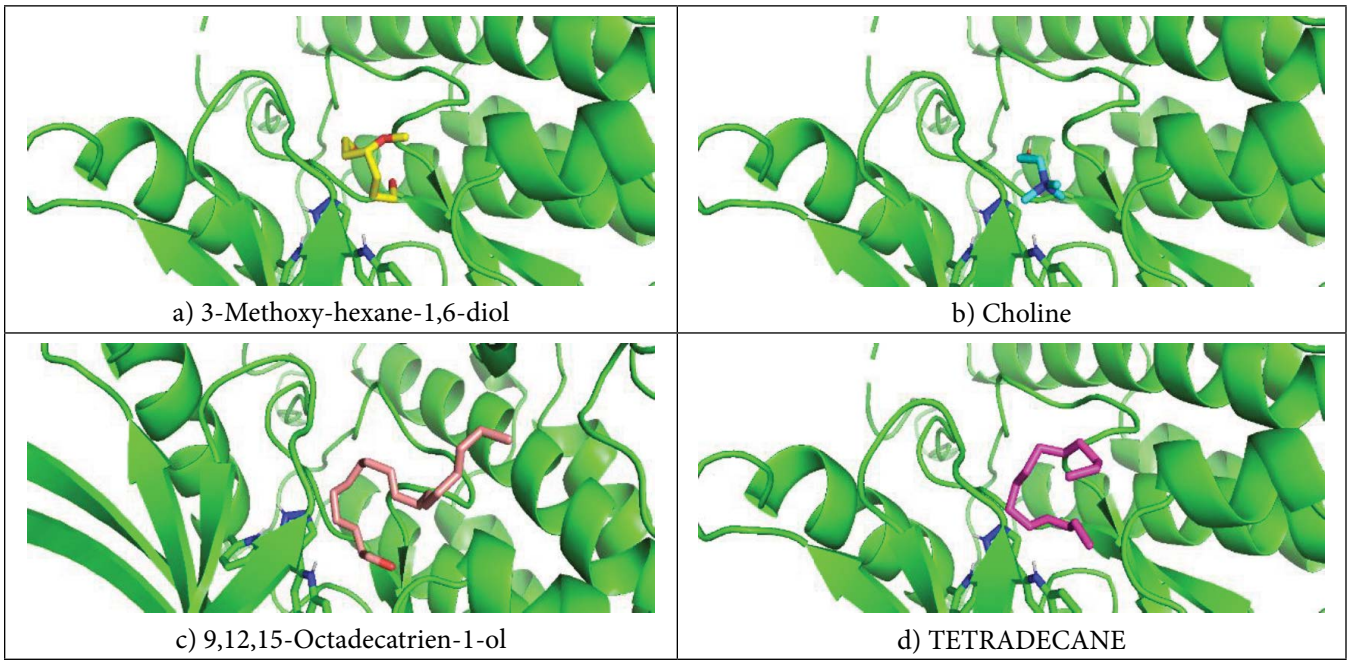


Figure 6. Ligand interaction with 1VJYs.

compounds based on their different Retention Time (RT) and peak area%. The first hit was identified at Retention Time (RT) of 7.314 min was pentadecane, whereas 1,2-benzenedicarboxylic acid, din was the last hit with 22.953 min RT as given in the Table 3. Choline, a dietary amine was identified at 14.002 RT and 42.89 area% which have the highest composition in the methanolic extract of *Glycine max* (L.) seeds (Figure 1).

The phytoconstituents predicted from the GCMS analysis of the methanolic extract of *Glycine max* (L.) were classified based on their functional groups as shown in Figure 2. The compounds were briefly categorised into alkane (2), alcohols (2), Fatty Acid Methyl Esters (FAME) (4), esters (2), alkyne (2), fatty acids (8), fatty alcohols (1), aliphatic compounds (1), amine (1), and other compounds (2).

3.4 Molecular Docking

From the GCMS analysis, the best four phytoconstituents were taken for the molecular modelling studies. These compounds were docked against four hepato-protective receptors namely, Transforming Growth Factor- (TGF-β) a cytokine, transcription factors such as Peroxisome Proliferator-Activated Receptor (PPAR) and Pregnene X Receptor (PXR), and Nuclear Factor kappa-b (NF-KB) a protein complex and their PDB IDs were retrieved as 1VJY, 5HYK, 2O9I and 1NFK respectively (Figures 3, 4, 5 and 6). The binding affinities of these four compounds against these four receptors were evaluated and their binding energies (Kcal/mol) values are given in Table 4. In comparison, choline has high binding affinities for all four receptors.

4. Discussion

Today soybeans were considered as the significant source of proteins (70%) and oils (30%) and have been widely cultivated worldwide[12, 23–25]. The soybean was reported to have various pharmacological activities such as antimicrobial, antioxidants and anti-inflammatory properties[26, 27]. The phytoconstituents present in the soybeans were also reported to have anti-diabetic, hepato-protective, anti-cancer, anticarcinogenic, anti-bacterial, and anti-viral and plays an significant role in human health[12, 13]. The phytoconstituents profiling of the various extracts of *Glycine max* (L.) revealed that the methanolic extract is known to have the highest amounts of alkaloids, flavonoids, phenolics and lower amount of tannins and steroid compounds. Choline is a water-soluble component that is found in large levels in eggs, beef, and soy. Phosphatidylcholine is the most common type of choline in the diet (PC). It helps

the liver's health by blocking the receptors that cause chronic liver disease[28–30].

5. Conclusion

The presence of a high amount of phytoconstituents in the methanolic extract of Glycine max (L.) seeds suggestes that methanol could be a suitable solvent for extracting these phytoconstituents.The molecular modelling of four compounds from the GCMS analysis against the receptors showed these compounds have good binding affinities. In comparison, choline had higher negative binding energies, indicating that it may have hepatoprotective activity, which will need to be confirmed in the near future using an *in vitro* approach.

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