Indian Journal of Experimental Biology
Vol. 62, January 2024, pp. 17-27
DOI: 10.56042/ijeb.v62i01.4628

Potential drug candidates against Hepatitis C virus: *In silico* screening of phytoconstituents from *Phyllanthus fraternus* G.L. Webster

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Received 03 December 2022; *revised 07 August 2023*

Hepatitis C is a liver inflammatory disease caused by the Hepatitis C virus. *Phyllanthus fraternus* G.L. Webster, commonly called Gulf leaf-flower, is an important medicinal plant and possesses hepatoprotective activity against hepatitis. Here, we have determined and characterized the active ingredients from the leaf extract of *P. fraternus* by gas chromatography-mass spectrometry (GC-MS) analysis. It resulted in the identification of fifteen compounds, which are the first to be reported from *P. fraternus*. For finding the compounds responsible for HCV, identified compounds were docked against important enzymes of HCV virus metabolism viz; HCVNS3 enzyme, HCVNS5, and hepatic damage indicator enzymes AST and ALT. To ensure drug-likeness, the pharmacokinetic, physiochemical, and toxicity properties of the compounds were also evaluated. The study has demonstrated the presence of seven hepatoprotective compounds alpha-cadinol, elemol, 1,6-germacradien-5-ol, phytol, carissanol dimethyl ether, phyltetralin and 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl- in the leaves extract of *P. fraternus* against the hepatitis C virus.

Keywords: Blood brain absorption, $CaCo_2$ permeability, Gulf leaf-flower, Human intestinal absorption, Liver

Hepatitis C is a disease of the liver with both acute and chronic infection[1]. In more than 50% of cases, the disease becomes chronic and if left untreated, it leads to severe liver diseases like liver cirrhosis and hepatocellular carcinoma. In most cases, the disease is not diagnosed due to no clear symptoms and leads to serious conditions. The major sources of the infection are unsafe blood transfusion, drug abuse, and poorly handled healthcare facilities. Patients with human deficiency virus (HIV) infection, receiving hemodialysis, and engaging in unsafe sexual behaviour are at the highest risk with the prevalence of infection ranging from 3.5 to 44.7%[2]. The NS3 and NS5 are the important non-structural proteins of HCV required for viral metabolism[3]. There are no effective medications or vaccines against HCV to date. Therefore, there is a need to search for new sources of medicines and new drug formulations. Moreover, the HCV virus mutates very rapidly and multiple resistances have already been reported against new treatments[4].

Plants of the genus *Phyllanthus* are reported to possess antiviral activity against the hepatitis B virus and related hepadnaviruses[5,6]. There are reports that suggest the hepatitis-protecting ability of the Gulf leaf-flower, *P. fraternus* G.L. Webster[7]. It was reported that[8] methanolic extracts of *P. amarus* leaf (PAL) and root (PAR) inhibited HCV DNA replication. The PAR inhibited the HCV-NS3 protease enzyme, whereas PAL inhibited the HCV-NS5B RNA-dependent RNA polymerase.

GC-MS analysis is one of the fastest and most accurate techniques for the detection of bioactive compounds and requires a very small amount of extract[9,10]. Computer-aided drug design has emerged as an advanced tool for drug discovery that minimizes the time, money, and energy required in the drug discovery process[11]. In the present study, we tried to determine the active ingredient responsible for the hepatoprotective activity of *Phyllanthus fraternus*. We extracted the leaves of *P. fraternus* in an aqueous solution and subjected to GC-MS analysis for identification of important bioactive compounds. The

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Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GC-MS, Gas chromatography-mass spectrometry; HCV, Hepatitis C Virus; HCVNS3, Hepatitis C virus non-structural protein 3, HCVNS5B, Hepatitis C virus non-structural protein 5B; PF, *Phyllanthus fraternus*

identified compounds were docked against HCV-NS3 protease, HCV-NS5B polymerase, and two important liver enzymes AST and ALT, to determine their activity against the hepatitis C virus.

Materials and Methods

Plant collection and extract preparation

Leaves of *P. fraternus* were collected from the campus of Banaras Hindu University, Varanasi (25.2677^{0}N, 82.9913^{0}E) from the months of August to September 2021. The voucher specimen was deposited in the herbarium of the Drvyaguna Department, Institute of Medical Sciences, Banaras Hindu University, Varanasi, with accession number DG/21-22/354. For the preparation of the extract, leaves were washed thoroughly under running tap water, oven-dried at 50-60ºC for two days and then powdered in a mechanical grinder. The previous protocol[12] was followed for aqueous extract preparation. The extract was made by boiling 100 g of powdered leaf in 250 mL of water for 40 min and then drying in a rotatory evaporator at 45ºC. The % yield (w/w) was calculated with the original amount of coarse powder used for extraction. It was about 35%.

GC-MS analysis

A modified protocol[9] was used for the GC-MS analysis of the leaf extract of PF. The leaf extract of PF was subjected to GC-MS analysis on a GC-MS-QP 2010 Plus (Shimadzu, Kyoto, Japan) system with a headspace sampler (AOC-20s) and auto-injector (AOC-20i), equipped with the mass selective detector, with an iron source temperature of 230ºC, interface temperature of 270ºC, a solvent cut time of 3.50 min threshold of 1,000 eV and mass range of 40 to 650 m/z. Compounds were separated using an Rtx 5 MS capillary column (Restek Company, Bellefonte, USA) with a cross-bonding of 5% diphenyl and 95% dimethyl polysiloxane and dimensions of 30 m (length)×0.25 mm (diameter) × 0.25 µm (film thickness). The split mode at a ratio of 10:1 was used. The temperature of the injector was initialized to 260ºC, having a split injection mode. The temperature was programmed from 80ºC (2 min), then further increased to 250ºC at a ramp rate of 10ºC/min (5 min hold) and finely increased to 280ºC at a ramp rate of 15ºC/min (24 min hold). Helium (>99.999%) was used as the carrier gas at a linear flow velocity of 40.5 cm/s. The debit of the gas (helium) vector with a total flow of 1.21 mL/min was fixed to 16.3 mL/min. The volume of the injected sample was 2 µL of methanol extract. The total MS running time was 46 min.

Ligands and proteins for *in silico* docking

All the compounds identified through GC-MS analysis were used as ligands for molecular docking studies. Ribavirin and simeprevir were used as standards against HCV NS3 and HCV NS5B enzymes, while, silibinin was used against AST and ALT enzymes. The three-dimensional (3D) structure of the compounds was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in an SDF file and converted into a PDB file through Open Babel Gui[13]. The protein structure (3D) of the enzymes, viz. HCV NS3, HCV NS5B, ALT and AST with PDB ids 1W3C[14], 3PHE[15], 3IHJ[16] and 3WZF[17], respectively, was downloaded from the protein data bank (https://www.rcsb.org/) in PDB format. Grid dimensions for the active site were set at 96×72×66 XYZ points for ALT with 0.375 spacing and 62×84×114 XYZ points for AST with 0.5 spacing 90×90×90 for NS3 and NS5B with 0.5 grid spacing for each atom type.

Molecular docking analysis

The molecular docking studies of all compounds and proteins were performed with Auto doc 4[18]. The interaction between ligand and protein was assessed based on binding energies. Ligands were prepared by incorporating gastegier charges and allowing for the greatest number of torsions possible. For the conformation search, a Lamarckian genetic algorithm was used. The following docking parameters were used: 30-140 GA runs, a population size of 150, maximum energy evaluations in the 25,000 range, a maximum number of generations of 27,000, a mutation rate of 0.02, and a cross-over rate of 0.8.

Admet screening of the compounds

ADMETlab2[19], swiss adme[20], and protox-2[21] online web servers were used to measure the physiochemical, pharmacokinetic, and toxicity properties of the compounds. Different parameters like blood-brain absorption (BBB), human intestinal absorption (HIA), CaCo2 permeability, virtual decoy sets (VDS), and clearance of a drug (CL) were used for scoring ADMET properties. Swiss adme was used for the determination of the physiochemical parameters like molecular weight (MW), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), lipophilicity, log (log P), molar refractivity (MR), topological polar surface area (TPSA), number of

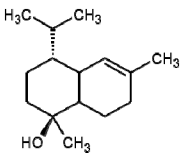
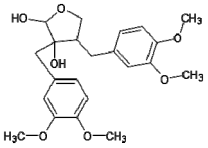
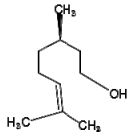
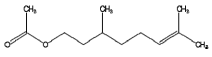
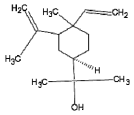
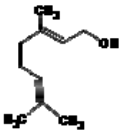
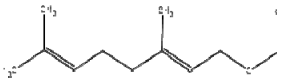
rotatable bonds (nRot) and drug-likeness. Toxicity parameters like human ether-a-go-go related gene (hERG Blockers), human hepatotoxicity (H-HT), drug-induced liver injury (DILI), and AMES toxicity were measured through ADMET Lab2 and protox-2.

Results and Discussion

Identification of compounds through GC-MS analysis

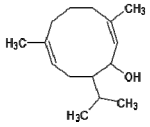
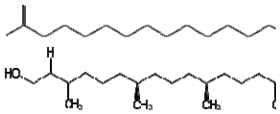
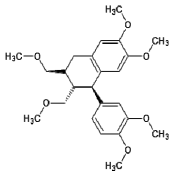
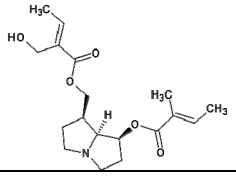
Fifteen compounds were tentatively identified by GC-MS analysis of the extract (Table 1 & Fig. 1). The components were identified by comparison of their retention indices (RI) to homologous alkane series (purchased from Sigma, St. Louis, USA) and by comparison of their mass spectral fragmentation patterns with the data provided in WILEY8.LIB and NIST11.LIB. Identification was achieved by a good match between the mass spectrum and RI. Peaks 1-8 of Fig. 1 correspond to the terpenoid compounds phytol, geraniol, citronellol, geranyl acetate, citronellyl acetate, elemol, 1,6-germacradien-5-ol, and α-cadinol, which are identified for the first time in *P. fraternus*. The retention time at which these compounds were identified and their % peak areas are given in Table 1. Peak (9) indicates an alkaloid molecule 2,6,10-trimethyl,14-ethylene-14-pentacene, and peak (10) indicates the presence of pyrrolidine-2-carboxylic acid, methyl-phenyl-amide, a compound with unknown activity. Peak 11 indicates a fatty acid hexadecenoic acid. Peaks 12 and 13 indicate two compounds carissanol dimethyl ether and 3-furan-methanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl- respectively with unknown activity and class.

Table 1 — List of bioactive compounds identified through GC-MS

Compound name	Retention time	Peak Area %	Molecular formula	Class of compound	Structure
α-Cadinol	21.443	0.53	$C_{15}H_{26}O$	sesquiterpenoid	
Benzene, 4-ethyl-1,2-dimethoxy	45.156	24.42	$C_{10}H_{14}O_2$	unknown	-
Carissanol dimethyl ether	42.749	1.48	$C_{22}H_{28}O_7$	unknown	
Citronellol	10.582	1.01	$C_{10}H_{20}O$	Terpenoid	
Citronellyl acetate	13.823	0.41	$C_{12}H_{22}O_2$	Monoterpene	
Elemol	18.922	1.50	$C_{15}H_{26}O$	Sesquiterpenoid	
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl-	42.857	49.66	$C_{22}H_{28}O_7$	Unknown	-
Geraniol	11.23	2.38	$C_{10}H_{18}O$	Terpenoid	
Geranyl acetate	14.57	0.51	$C_{12}H_{20}O_2$	Oxygenated monoterpene	

(*Contd.*)

Table 1 — List of bioactive compounds identified through GC-MS (*Contd*.)

Compound name	Retention time	Peak Area %	Molecular formula	Class of compound	Structure
1,6-Germacradien-5-ol	19.61	0.39	$C_{15}H_{26}O$	Monocyclicsesquiterpene	
Hexadecanoic acid	27.83	1.62	$C_{16}H_{32}O_2$	Fatty acid	
Phytol	9.310	0.29	$C_{20}H_{40}O$	Terpenoid	
Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide	25.998	2.64	$C_{12}H_{16}N_2O$	unknown	-
Phyltetralin	43.069	1.48	$C_{24}H_{32}O_6$	Lignan	
2,6,10-trimethyl,14-ethylene-14-pentadecne	25.262	0.66	$C_{20}H_{38}$	alkaloid	

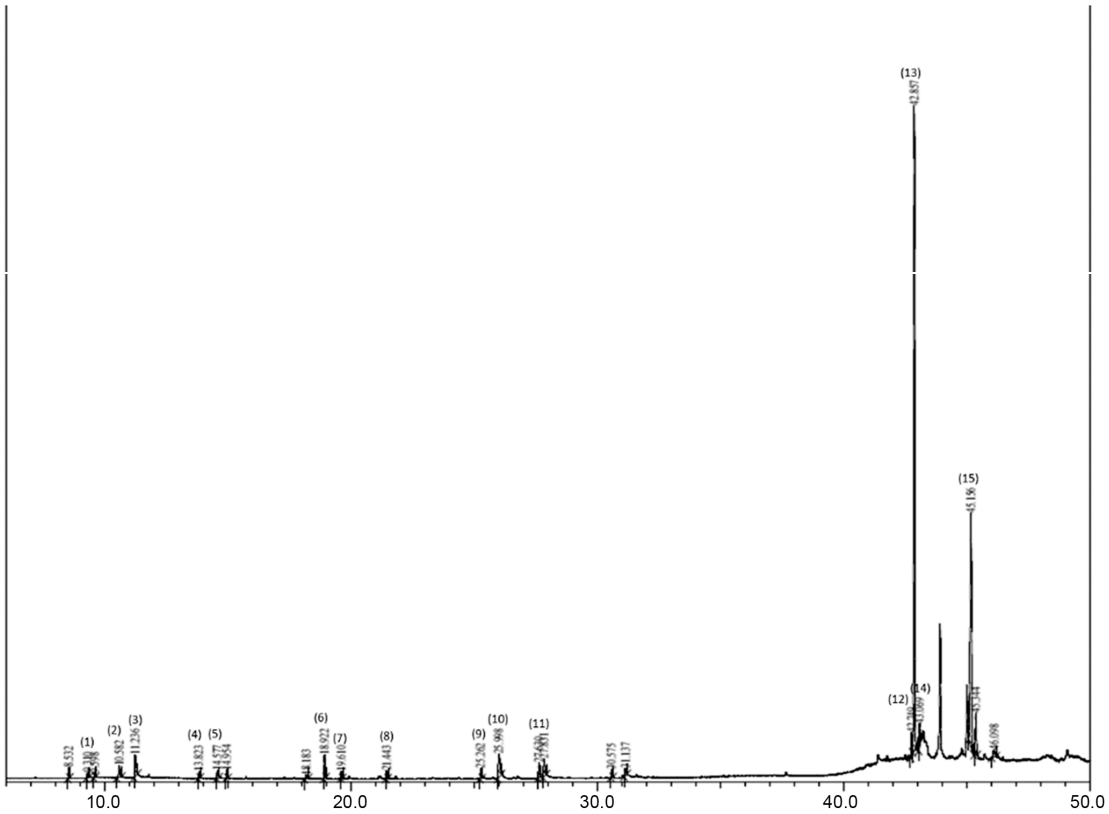


Fig. 1 — GC-MS total ion chromatogram showing the presence of bioactive compounds, peak (1) phytol; peak (2) citronellol; peak (3) geraniol; peak (4) citronellyl acetate; peak (5) geranyl acetate; peak (6) elemol; peak (7) 1,6-germacradien-5-ol; peak (8) α-cadinol; peak (9) 2,6,10-trimethyl,14-ethylene-14-pentadecne; peak (10) pyrrolidine-2-carboxylic acid, methyl-phenyl-amide; peak (11) hexadecenoic acid; peak (12) carissanol dimethyl ether; peak (13) 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl; peak (14) phyltetralin; and peak (15) Benzene, 4-ethyl-1,2-dimethoxy

Table 2 — Molecular docking of hepatitis C NS3 enzyme with identified compounds

Name of the compound	Compound ID	Binding energy (Kcal/mol)	No. of hydrogen bonds involved in binding	Amino acids involved in bonding
Ribavirin	CID37542	–5.30	5	Thr10, Asp30, Asp30, Gln 34, Val35,
Simeprevir	CID24873435	–4.47	Nil	Nil
Alpha cadinol	CID6431302	–7.26	1	GLU32
Benzene, 4-ethyl-1,2-dimethoxy	CID79990	–4.51	Nil	Nil
Carissanol dimethyl ether	CID 592113	–7.10	5	Asp30, Gln8, Thr10, Tyr6
Citronellol	CID8842	–4.53	3	Asp30, Asp30, Gln34
Citronellyl acetate	CID8842	–4.44	1	Thr10
Elemol	CID9017	–6.91	1	Gln8
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	CID92138	–7.25	1	Arg11
Geraniol	CID592103	–4.75	3	Asp30, Asp30, Gln34
Geranyl acetate	CID637566	–4.44	1	Tyr6
1,6-Germacradien-5-ol	CID1549026	–7.42	2	Ala65, Thr63
Hexadecanoic acid	CID 91748908	–4.75	2	Asp30, Gln34
Phytol	CID985	–6.67	2	Thr10
Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide	CID5280435	–6.12	2	Gln8
Phyltetralin	CID550989	–6.98	1	Asp30

Table 3 — Molecular docking of hepatitis C NS5 enzyme with identified compounds

Name of the compound	Compound ID	Binding energy (Kcal/mol)	No. of hydrogen bonds involved in binding	Amino acids involved in bonding
Ribavirin	CID37542	–4.60	3	Gly449, Asp559
Simeprevir	CID24873435	–5.99	Nil	Nil
Alpha cadinol	CID6431302	–6.65	1	Asp458
Benzene, 4-ethyl-1,2-dimethoxy	CID79990	–4.61	2	Arg337
Carissanol dimethyl ether	CID 592113	–4.27	4	Lys 90, Lys90
Citronellol	CID8842	–4.69	2	Arg109, Ser563, Ser218, Thr364
Citronellyl acetate	CID8842	–4.84	2	Ser218, Thr364
Elemol	CID9017	–6.20	1	Gln8
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	CID92138	–5.65	2	Lys141
Geraniol	CID592103	–4.88	2	His402, Thr403
Geranyl acetate	CID637566	–4.95	1	Leu384
1,6-germacradien-5-ol	CID1549026	–6.08	1	Asn142
Hexadecanoic acid	CID 91748908	–3.98	2	Arg32, Arg32
Phytol	CID985	–4.54	1	His467
Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide	CID5280435	–5.64	Nil	Nil
Phyltetralin	CID550989	–6.37	1	His467

Molecular docking analysis

All the compounds identified through GC-MS analysis were docked against HCV NS3 and HCV NS5, AST, and ALT except 2,6,10-trimethyl,14-ethylene-14-pentadecane for which a sdf file was not available. Standard drugs like ribavirin and simeprevir were used as a control against HCV NS3 and HCV NS5 enzymes while silibinin was used against AST and ALT. Molecular docking reveals that three compounds alpha-cadinol, elemol and 1,6-germacradien-5-ol showed the best binding affinity against all enzymes (Tables 2-5). The binding energies of the alpha-cadinol against HCV NS3, HCV NS5 AST, and ALT enzyme were −7.26, −6.65, −7.06 and −6.33, respectively. Elemol showed binding energies of −6.91, −6.20, −6.02 and −6.12 against HCV NS3, HCV NS5 AST and ALT enzymes, respectively. Whereas 1,6-germacradien-5-ol had shown binding energies of about −7.41, −6.08, −6.02 and −5.54 against HCV NS3, HCV NS5 AST and ALT enzymes, respectively. All of these compounds showed greater binding affinities than standard drugs

Table 4 — Molecular docking of alanine aminotransferase enzyme with identified compounds

Name of the compound	Compound IDs	Binding energy (Kcal/mol)	No. of hydrogen bonds involved in binding	Amino acids involved in bonding
Silibinin	CID31553	–5.63	3	Glu 407, Asp 304, Ser 308
Alpha cadinol	CID6431302	–6.33	2	Tyr 302, Gly342
Benzene, 4-ethyl-1,2-dimethoxy	CID79990	–3.83	1	Arg92
Carissanol dimethyl ether	CID 592113	–5.15	2	Tyr302, Asn305
Citronellol	CID8842	–5.24	1	Ile68
Citronellyl acetate	CID9017	–4.05	1	Phe313
Elemol	CID92138	–6.12	1	Tyr 302, Gly342
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	CID592103	–4.35	2	Arg92, Val64
Geraniol	CID637566	–4.86	2	Glu332, Asn330
Geranyl acetate	CID1549026	–5.71	1	Arg92
1,6-Germacradien-5-ol	CID 91748908	–5.54	1	Ser340
Hexadecanoic acid	CID985	–3.25	2	Asn412, Lys416
Phytol	CID5280435	–3.57	1	Arg 169
Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide	CID550989	–5.26	1	Phe313
Phyltetralin	CID 11223782	–5.20	1	Phe 313

Table 5 — Molecular docking of aspartate aminotransferase with compounds

Name of the compound	Compound ID	Binding energy (Kcal/mol)	No. of hydrogen bonds involved in binding	Amino acids involved in bonding
Silibinin	CID31553	–6.86	3	Lys165, Leu168, Pro200
Alpha cadinol	CID6431302	–7.06	2	Arg235 Asp236
Benzene, 4-ethyl-1,2-dimethoxy	CID79990	–4.64	1	Lys258
Carissanol dimethyl ether	CID 592113	–5.56	2	His68, Ala300
Citronellol	CID8842	–5.30	4	Ser 230, Arg235, Arg235, Asp236
Citronellyl acetate	CID9017	–5.81	3	Ser230, Arg235
Elemol	CID92138	–6.02	2	Tyr302, Gly342
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	CID592103	–5.10	4	Glu141, Glu141, Arg386, Lys258
geraniol	CID637566	–4.84	2	Arg235, Ala239
geranyl acetate	CID1549026	–5.10	1	Asp199
1,6-Germacradien-5-ol	CID 91748908	–5.90	1	Pro200
Hexadecanoic acid	CID985	–4.67	3	Ser 230, Arg235, Arg235
Phytol	CID5280435	–4.82	2	Arg235, Ala239
Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide	CID550989	–5.57	1	Glu69
Phyltetralin	CID 11223782	–4.86	1	Asp199

ribavirin, simeprevir and silibinin used in the analysis (Table 2-5). Besides this, four compounds phytol, carissanol dimethyl ether, phyltetralin and 3-furan-methanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl- showed high inhibitory potential against HCV NS3 enzyme with their binding energies of –6.67, –7.10, –6.98 and –7.25, respectively. Phyltetralin was also found to be inhibitory against the HCV NS5 enzyme with a binding energy of –6.37.

ADMET properties

All the compounds subjected to docking showed good physiochemical properties (Table 6). All the compounds were showing excellent intestinal absorption, distribution, and drug clearance as evident from the values of VDS (virtual decoy sets) and CL (drug clearance) (Table 7). Most compounds showed poor blood-brain barrier distribution except 1,6-Germacradien-5-ol, phytol, hexadecenoic acid, carissanol dimethyl ether, and phyltetralin (Table 7). All the compounds were good substrates for the P-glycoprotein substrate (PGS) except carissanol dimethyl ether and phyltetralin. All the compounds were non- hERG I blockers, non-hepatotoxic, and did not induce liver injury while the synthetic drugs ribavirin and simeprevir, and phyltetralin were found to induce liver injury (Table 8). Simeprevir was found to show hepatotoxicity as well. They were non-toxic in the Ames mutagenicity test as well.

HCV infection is a worldwide problem. Although modern treatment strategies have provided some relief, they have limited access due to their high cost and associated side effects[22]. Thus, there is a need for

Table 6 — Physiochemical properties of bioactive compounds from *Phyllanthus fraternus*

Name of compound	HBD	HBA	MLOGP	MR	TPSA	Drug Likeness
Alpha-Cadinol	1	1	3.67	70.72	20.23	Yes (0)
Carissanol dimethyl ether	2	7	1.21	107.23	86.61	Yes (0)
Elemol	1	1	3.56	72.10	20.230	Yes (0)
1,6-Germacradien-5-ol	1	1	3.56	72.32	20.23	Yes (0)
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl-	2	7	2.37	107.23	86.61	Yes (0)
Phytol	1	1	5.25	98.84	20.23	Yes (1)
Phyltetralin	0	6	2.03	115.73	55.38	Yes (0)
Ribavirin	5	8	–2.94	51.06	143.72	Yes (0)
Simeprevir	2	9	1.48	208.52	193.51	No (2)
Silibinin	5	10	–0.40	120.55		Yes (0)

[HBA: Hydrogen bond acceptor; HBD: Hydrogen bond doner; MLOGP: Moriguchioctanol-water partition coefficient; MR: Molar refractivity; TPSA: Topological polar surface area; DL: Drug likeliness]

Table 7 — Important pharmacokinetic properties of the best-docked compounds

Compound Name	$CaCo_2$	HIA	PGS	VDS	CL	BBB
Alpha cadinol	–4.368	E	Yes	2.281	14.24	Av.
Carissanol dimethyl ether	–4.817	E	No	0.945	7.231	Av.
Elemol	–4.369	E	Yes	1.359	5.137	Poor
1,6-Germacradien-5-ol	–4.315	E	Yes	3.231	9.338	Av.
3Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	–4.810	E	Yes	1.192	7.432	Av.
Phytol	–4.338	E	Yes	3.722	8.161	Good
Phyltetralin	–4.775	E	No	0.947	7.719	E
Ribavirin	–5.582	E	Yes	0.875	4.138	Poor
Simeprevir	–5.207	E	Yes	0.632	6.054	E

[$CaCo_2$ Human colorectal carcinoma cell line; HIA: Human intestinal absorption; PGS: P-glycoprotein substrate; VDS: Virtual decoy sets, CL: clearance of a drug; BBB: Blood-brain barrier; E: Excellent; Av.: Average]

Table 8 — Toxicity profiling of the best-docked compounds

Compounds	hERG I	H-HT	DILI	AMES toxicity	Toxicity Class
Alpha cardinol	No	No	No	No	Fourth
Carissanol dimethyl ether	No	No	No	No	Fourth
Elemol	No	No	No	No	Fourth
1,6-Germacradien-5-ol	No	No	No	No	Fourth
3-Furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	No	No	No	No	Fourth
Phytol	No	No	No	No	Fourth
Phyltetralin	No	No	Yes	No	Fourth
Ribavirin	No	No	Yes	No	Fourth
Simeprevir	No	Yes	Yes	No	Fourth
Silibinin	No	No	No	No	Fourth

[hERG: human Ether-a-go-go related Gene; H-HT: human hepatotoxicity; DILI: Drug-induced liver injury]

some alternative sources of drugs or drug formulations. *P. fraternus* Webster is an important hepatoprotective plant known for its activity against hepatitis viruses. Currently, GC-MS analysis is widely used for the identification of bioactive compounds from medicinal plants to establish their biological properties[23]. In the present study, the aqueous leaves extract of *P. fraternus* was characterized by GC-MS analysis to determine the active ingredients. The analysis has resulted in the identification of fifteen bioactive compounds (Table 1). Among these compounds, two compounds 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl and benzene, 4-ethyl-1,2-dimethoxy were the most abundant compounds as evident from their peak areas 49.66 and 24.42 respectively (Table 1 & Fig. 1). Eleven of these compounds belong to the terpenoid, alkaloid, lignan, and fatty acid classes and four belong to the unknown class. These 15 compounds have been identified for the first time in *P. fraternus*. Among eight terpenoid compounds viz. phytol, geraniol, citronellol, geranyl acetate, citronellyl acetate, elemol, 1,6-germacradien-5-ol and α-cadinol, the four compounds, namely phytol, geraniol, citronellol and α-cadinol have been reported from some other species of the genus *Phyllanthus*. Phytol, citronellol and geraniol were identified by GC-MS from *P. salviaefolius*[24]. Geraniol and citronellol was also identified from *P. muellerianus*[25]. α-cadinol was identified from *P. nivosus*[26]. One lignan, named phyltetralin has been identified, which is already reported from some species of the genus *Phyllanthus* viz. *P. niruri*[27], *P. amarus*[28] and *P. urinaria*[29]. Among four unknown compounds, pyrrolidine-2-carboxylic acid, methyl-phenyl-amide and 3-furanmethanol, alpha-(3,4-dimethoxyphenyl)

tetrahydro-3-hydroxy-4-veratryl- and benzene, 4-ethyl-1,2-dimethoxy have not been identified in any species of the genus *Phyllanthus* and their biological activity is also not established, while carissanol dimethyl ether[30] has been identified in *P. amarus* but its activity is unknown.

Computational tools have enabled the selection of methodologies for pharmaceutical and technological research[31]. Molecular docking is an effective and inexpensive method for designing and testing drugs as it provides information about drug-receptor interactions through their binding potential[32]. It is the most commonly used tool for virtual screening and structure-based drug design. It analyses all non-covalent interactions (hydrogen bonding and hydrophobic interactions) of the ligand with the macromolecule. For docking, AutoDock is the most suited program because it calculates the grid automatically for the required atom. A more improved version, AutoDock 4 equipped with a Python molecular viewer and a visual programming environment brings the accuracy of the binding pose prediction to a faster magnitude[33-35]. All the compounds were subjected to *in silico* docking analysis against four important enzymes HCV NS3, HCV NS5B, ALT and AST, to determine the active ingredient against the hepatitis C virus. The *in silico* analysis has resulted in the identification of seven hepatoprotective lead compounds alpha-cadinol, elemol, 1,6-germacradien-5-ol, phytol, carissanol dimethyl ether, phyltetralin and 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl- which were showing maximum binding energy against tested enzymes (Tables 2-5). The different kinds of bonding interactions viz. electrostatic, van der Waals, covalent and hydrogen bonds have been formed by these compounds with enzymes. The number of hydrogen bonds formed by these compounds and amino acids involved in the bonding is shown in Tables 2-5 and Fig. 2. It is clearly evident from the data of molecular docking that bioactive compounds are showing high binding affinity against important enzymes of HCV virus metabolism HCVNS3 and HCVNS5 as compared to synthetic drugs (ribavirin and simeprevir) used as controls (Tables 2 and 3). Many drugs are rejected due to their poor pharmacological, pharmacokinetic, and toxicological potential[34]. Therefore, assessing the physiochemical, and pharmacokinetic properties of the drugs and toxicity parameters is a prerequisite. Analysis of the ADMET properties and drug-likeness prediction helped in the discovery of new drug targets and anticipation of the biological activities of any compounds[35]. In the present analysis, all the docked compounds complied with the desired ADMET properties and showed good physiochemical properties required for drug ability (Table 6). They showed excellent intestinal absorption, distribution, and drug clearance (Table 7). A good drug should be distributed to the entire body for efficient metabolism and action. VDS in the range of 0.04-20 indicates good distribution. Drug clearance (CL) is an important pharmacokinetic parameter that defines the half-life, and thus, the frequency of a drug dose. The CL value greater than 5 m/min/kg indicates good clearance[36]. All the compounds were showing better clearance properties than synthetic drugs. All compounds showed poor blood-brain barrier (BBB) penetration except phytol, and phyltetralin. But for non-CNS (central nervous system) drugs, good BBB penetration is not required rather these are associated with side effects[37]. The human colon adenocarcinoma cell lines (CaCo-2) are used as an alternative for the human intestinal epithelium, to estimate the *in vivo* drug permeability of the drug due to their morphological and functional similarities. The CaCo-2 permeability value higher than −5.15 log cm/s indicates a good permeability parameter. All the identified compounds showed good permeability values as compared to synthetic drugs ribavirin and simeprevir (Table 7). Toxicity is the most important attribute among all ADMET properties. Many drugs are rejected after clinical trials due to their toxic nature[38]. All the compounds were nonhERG I blockers, non-hepatotoxic and did not induce liver damage, while synthetic drugs were found to induce hepatic damage. They were non-toxic in the Ames mutagenicity test as well. The Ames toxicity test is an indicator of both mutagenicity[39] and carcinogenicity[40]. Besides, the synthetic drugs available on the market are associated with several side effects such as tiredness, headache, nausea, fever, muscle pains, mood swings, rash, itching and nausea[41]. Hence, the present analysis validates the presence of seven hepatoprotective compounds in the aqueous leaf extract of *P. fraternus* that can be used in the drug formulations of the hepatitis C virus after clinical trials.

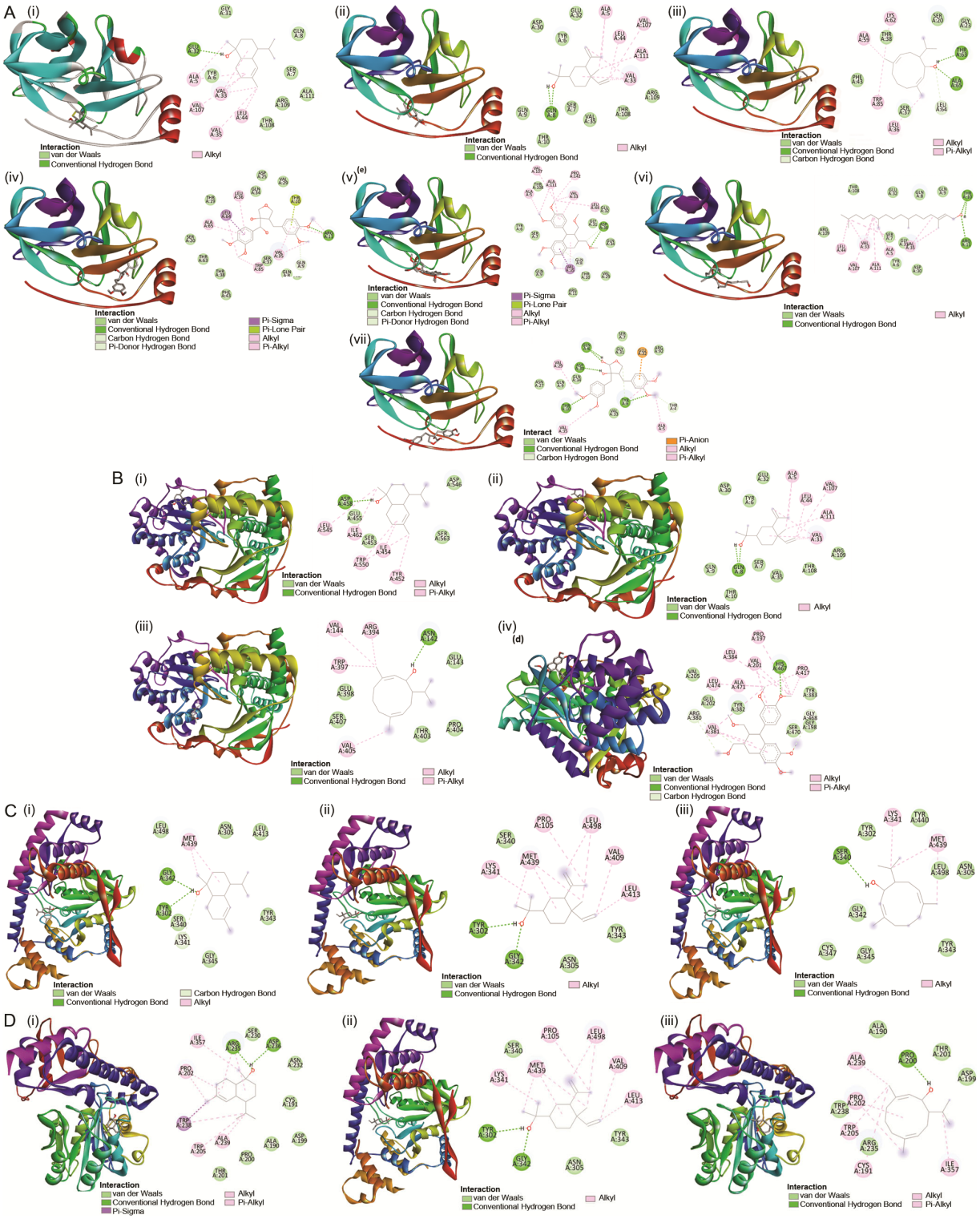


Fig. 2 — Molecular docking and 2D level interaction of (A) HCV NS3 enzyme against (i) alpha- cadinol, (ii) elemol, (iii) 1,6-germacradien-5-ol, (iv) 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl-, (v) phyltetralin, (vi) phytol, and (vii) carissanol dimethyl ether; (B) HCV NS5B enzyme against (i) alpha- cadinol, (ii) elemol, (iii) 1,6-germacradien-5-ol, and (iv) phyltetralin; (C) alanine aminotransferase enzyme against (i) alpha- cadinol, (ii) elemol, and (iii) 1,6-germacradien-5-ol; and (D) aspartate aminotransferase enzyme against (i) alpha cadinol, (ii) elemol, and (iii) 1,6-germacradien-5-ol

Conclusion

The present investigation gives us useful insight into the preclinical identification of hepatoprotective lead compounds from *Phyllanthus fraternus* aqueous leaves extract against the hepatitis C virus. The fifteen compounds have been identified from the leaf extract of *P. fraternus* by GC-MS analysis. All the compounds were subjected to *in silico* docking analysis against HCVNS3, HCVNS5B and two liver enzymes ALT and AST, which resulted in the identification of seven lead compounds viz; alpha-cadinol, elemol, 1,6-germacradien-5-ol, phytol, carissanol dimethyl ether, phyltetralin and 3-furanmethanol, alpha-(3,4-dimethoxy-phenyl) tetrahydro-3-hydroxy-4-veratryl- against the HCV. There is no report of any biological activity of 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetra-hydro-3-hydroxy-4-veratryl- and carissanol dimethyl ether till date. The present analysis first time reports the hepatoprotective activity of 3-furanmethanol, alpha-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl- and carissanol dimethyl ether by *in silico* approach. All seven compounds showing activity against the hepatitis C virus could be subjected to *in vivo* and clinical trials for their use in hepato-protective formulations.

Acknowledgment

Author KNT acknowledge BHU Varanasi for providing financial assistance through incentive grant under the IoE scheme (6031) for this research work

Conflict of Interest

Authors declare no competing interests.

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