Trends Phytochem. Res. 8(3) 2024 188-202

Trends in Phytochemical Research (TPR)

Journal Homepage: https://sanad.iau.ir/journal/tpr

Original Research Article

In vitro experiments, LC-MS profiling, and *in silico* studies reveal anti-hepatocellular carcinoma properties of *Canna indica* L.

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ABSTRACT

Hepatocellular carcinoma is one of the leading causes of cancer-related deaths worldwide, highlighting the urgent need for early detection and effective treatment. Consequently, there is a pressing demand to develop alternative drugs from natural sources. In our study, we investigated the rhizome of *Canna indica* as a potential resource for drug development. We assessed the anti-proliferative efficacy of the methanolic extract of the rhizome against Hep G2 liver cancer cell line and normal cells. Our observations indicated that the extract exhibited specific anti-proliferative effects against the liver cancer cells. These results prompted us to conduct LC-MS-based metabolomic profiling of the extract, which revealed a predominance of alkaloids and phenolic compounds. Furthermore, *in silico* docking studies demonstrated that cadabicine methyl ether, mahanimbinol, ganoderic acid F, and cassythine exhibited strong binding affinities with key proteins involved in hepatocellular carcinoma. Therefore, we conclude that the rhizome of *Canna indica* is a promising source for the development of liver cancer therapeutics.

ARTICLE HISTORY

Received: 17 June 2024
Revised: 25 September 2024
Accepted: 26 September 2024
ePublished: 30 September 2024

KEYWORDS

Canna indica
Hepatocellular carcinoma
Hep G2
In-silico
LC-MS
Liver cancer
Metabolomic profiling

1. Introduction

According to the World Cancer Research Fund International website (https://www.wcrf.org), liver cancer is the sixth most common cancer worldwide. As per the statistical estimates of the American Cancer Society more than 800 thousand people are diagnosed with this cancer each year throughout the world (https://www.cancer.org; Mohadjerani and Asadollahi, 2019; Dekdouk et al., 2024). Liver cancer is also a major cause of cancer mortality worldwide, accounting for more than 700 thousand deaths per year. In 2024, about 29 thousand deaths have been estimated resulting from liver cancer. It is estimated that between 2020 and 2040, there will be a 55.0% increase in new cases and 56.4% mortality annually, potentially leading to 1400 thousand diagnoses and 1300thousand deaths by 2040 (Rumgay et al., 2022).

Reports of NIH-National Cancer Institute say that for the treatment of hepatocellular carcinoma, many synthetic drugs have been approved, like sorafenib, lenvatinib, durvalumab, and tremelimumab. These synthetic drugs act in a non-specific manner, affecting normal cells in the process and leading to side effects like diarrhoea, hypertension, fatigue, bilirubin elevation, thrombocytopenia, decreased appetite, and weight loss (Robinson et al., 2017; Pang et al., 2022). Apart from the side effects, drug resistance, cancer recurrence and secondary malignancies are major setbacks of conventional drug treatment.

However, as compared to conventional synthetic medicines, plant-derived drugs are safer with fewer side effects, less expensive, and less harmful. Plant-derived drugs fall under the category of traditional, complementary and alternative medicine (TCAM) (Iqbal et al., 2017).

Indian shot, also known as *Canna indica* of the Cannaceae family, is a well-known decorative plant with both

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medicinal and industrial uses (Al-Snafi, 2015). *Canna indica* leaves and rootstocks are used to treat wound healing, AIDS, dropsy fever, diarrhoea, diaphoretics, malaria, and wound healing, according to ethnomedical research (Chigurupati et al., 2021). Amenorrhea and gonorrhoea are treated with roots, and dermatosis is treated with a powdered combination of leaves and seeds (Odugbemi et al., 2007; Thepouyporn et al., 2012). Crude extract of the plant showed anti-proliferative potential against colon cancer (Widyarini et al., 2020). According to reports, the plant demonstrated good antioxidant benefits due to the presence of specific anthocyanins like cyanidin, quercetin, and lycopene (Srivastava et al., 2010). However, there is no report on the plant's potential in the treatment of hepatocellular cancer. Nonetheless, research has demonstrated that *Canna indica* extract inhibits the HCV virus's ability to replicate (Huang et al., 2009).

Therefore, in our study, we evaluated the antiproliferative efficacy of the methanolic extract of *Canna indica* against Hep G2 cancer cell lines through MTT assay and Trypan Blue Exclusion assay. Since LC-MS is a comparatively preferred way for distinguishing phytoconstituents (Vignesh et al., 2022), therefore we carried out the LC-MS analysis of the methanolic extract of the rhizome of *Canna indica*. Further, we carried out molecular docking of the compounds available from the metabolomic profile of the extracts against c-met, VEGFR, EGFR, FGF4, and IGFR, which are hallmark proteins involved in hepatocellular carcinoma. *In silico* molecular docking is believed to be themost effective, affordable, and sophisticated method to ascertain how a biologically active molecule behaves inside an organism based on druggability and ADMET qualities and for predicting the best candidates for drug development (Durán et al., 2020; Flores et al., 2021; Kiran et al., 2023).

2. Experimental

2.1 Chemicals

All the chemicals that were used for experiments were molecular grade. The chemicals used were methanol, hexane (Sisco Research Laboratories), DMEM (Dulbecco's Modified Eagle Medium), L-glutamine, FBS (Foetal Serum Albumin), penicillin, streptomycin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Thermo Fisher), DMSO(dimethyl sulfoxide), PBS (phosphate buffer saline), trypan blue (Sigma-Alrich).

2.2. Collection of plant material

The rhizomes of *C. indica* plants were collected from a field in Kolkata near Dhakuria railway station during September-October 2023. The rhizomes were chopped and air-dried in shade for nearly 20 days.

2.3. Preparation of rhizome extract

Dried samples were mechanically ground into fine powder and stored in a vacuum desiccator. 50-g portions of rhizome powder soaked in methanol (95.0%). The extract was filtered through filter paper and phase separation was done using hexane and methanol. The methanolic fraction was separated and dried in a rotary evaporated (Eyela), under reduced pressure.

2.4. Detection of antiproliferative potential using MTT assay and trypan blue exclusion assay

2.4.1. Cell lines and culture

Hep G2 (human liver hepatocellular carcinoma), HEK293 (Human embryonic kidney cells) cell lines cultured and maintained in DMEM supplemented with L-glutamine (2 mM), FBS (heat inactivated, 10 *v/v*%), penicillin (10 µg/mL) and streptomycin (10 µg/mL), and incubated at 37 °C in a humidified atmosphere of CO_2 incubator (5%; HF90) (Ray et al., 2022).

2.4.2. MTT assay

An MTT assay is based on the ability of metabolically active cells to convert water-soluble3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into an insoluble formazan. This assay was performed to evaluate the effect of methanolic extract on cell viability. Nearly, 1×10^4 cells were seeded in 96 well plates in presence of different concentrations of the extract (100-900 µg/mL), except the control sets and incubated for 24 hours. SDS lysis buffer (5.0%) was used to prepare the 100% lysed cell. After the incubation period cell culture medium was replaced with MTT solution (0.5 mg/mL) in phenol red-free DMEM (5.0%) and incubated for 4 hours in a humidified incubator containing CO_2 (5.0%) at 37 °C. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 560 nm. Absorbance was proportional to the percentage of viable cells and the percentages of the viable cells were calculated using the formula:

Cell viability (%) = (O.D. sample - O.D. 100%lysis)/(O.D. 0%lysis - O.D. 100%lysis) × 100 (Eqn. 1)

To check the specificity of the extract towards cancer cells, healthy HEK293 cells were selected, and the assay was repeated using the same concentration in the same process as mentioned above (Ray et al., 2022).

2.4.3. Trypan blue exclusion method

The trypan blue exclusion assay is a technique which classifies viable and non-viable cells based on membrane integrity. A viable cell with an intact membrane will not take trypan blue, whereas a non-viable cell will take the dye and havetrypan blue positive cytoplasm. Post treatment, cells were resuspended in 1 × PBS and mixed with trypan blue solution (0.4%) in 1:1 dilution for 4 minutes and counted with a hemocytometer (Parida et al., 2018).

2.5. LC-MS-based identification of plant constituent:

Metabolomic profiling produces an outline of possible bioactive molecules present in any extract. The methanolic extract of *C. indica* was subjected to metabolomic profiling to identify the probable list

of compounds by QTOF-HRLC-MS. HRLC-MS-QTOF Agilent Technologies, USA instrument was used, and Agilent Mass Hunter software was employed for data acquisition. Agilent Mass Hunter Qualitative Analysis B.06 data processing software was accessed. ZORBaX Eclipse Plus-C18 150*2.1 mm, 5 µm (Agilent) column was used. The binary solvent system used for LCMS was formic acid (0.1%) in Milli-Q water and acetonitrile. A binary pump, dual (positive and negative) AJS ESI ion source and synchronized stroke mode were used. The source parameters were maintained as a gas temperature of 250 °C and 13 L/min gas flow rate. The freshly prepared extract was dissolved in HPLC-grade methanol to prepare 1 mg/mL concentration and filtered using a 0.22 µm syringe filter for LCMS analysis.

2.6. *In silico* molecular docking study

In hepatocellular carcinoma (HCC), the initiation and progression of tumors are significantly affected by many growth factors, including VEGF (vascular endothelial growth factor), HGF (hepatocyte growth factor), EGF (epidermal growth factor), and IGF (insulin-like growth factor) and the crosstalk with their respective receptors (Dimri et al., 2020).

VEGF is a key mediator for angiogenesis and promotes angiogenesis by interacting with its receptors. The chemicals interacting with the two receptors may serve as negative regulators of angiogenesis (Huang et al., 2009; Berretta et al., 2016; Parida et al., 2018). The EGF-EGFR signaling pathway plays a crucial role in development, proliferation, and metastatis of hepatocellular carcinoma cells. Activation of this pathway induces cell proliferation and movement, thereby promoting cancer progression. EGF activates downstream pathways such as PI3K and ERK. Additionally, EGF mediates tumor cell to produce inflammatory factors like CXCL8 and CXCL5. The EGF-EGFR pathway facilitate DNA synthesis, regeneration, and tumor growth in HCC cells, underscoring significance in cancer progression (Huang et al., 2014). Hepatocyte growth factor (HGF) and its receptor, mesenchymal-epithelial transition factor (c-Met), are related to the onset, progression, and metastasis of tumours. The HGF/c-Met axis has a role in normal liver growth, regeneration, and protection (Giordano et al., 2014). Inappropriate Met activation promotes the onset, proliferation, invasion, and metastasis of HCC (Maroun et al., 2014; Wang et al., 2020). Insulin-like growth factors (IGFs) bind to the IGF1 receptor on the cell surface and activate receptor tyrosine kinase activity. Activated receptor phosphorylates several substrates, like Src homology collagen (SHC). Certain Src homology 2 (SH2) domain-containing signalling molecules recognize phosphotyrosine residues in these substrates, like growth factor receptor-bound 2 (GRB2), and SH2-containing protein tyrosine phosphatase 2 (SHP2/Syp). These bindings activate downstream signaling pathways like the PI3-kinase pathway, and (MAP kinase) pathway leading to cell proliferation, cell differentiation, and cell survival (Alexia et al., 2004). FGFR4 selectively binds FGF19 ligand. This binding activation leads to forming FGF receptor substrate 2 (FRS2) and growth factor receptor-bound protein 2 (GRB2) complex, activating Ras-Raf-ERK, MAPK and PI3K-Akt pathways. These pathways are involved in tumor proliferation and anti-apoptosis. Therefore, FGFR4 can be a potential target for HCC treatment (Raja et al., 2019).

Therefore, our work uses the proteins FGFR4, IGF1R, c-MET, EGFR, and VEGFR as target proteins. We performed molecular docking between these 5 receptor proteins and eleven metabolites selected from the LC-MS analysis of the methanolic extract of *Canna indica*. The eleven compounds are 11-amino-undecanoic acid, mahanimbinol, cassythine, isovalerylsarcosine, 2-hexylbenzothiazole, cadabicine methyl ether, S-decyl GSH, ganoderic acid f, *N*-Isovalerylglycine methyl ester, sphinganine, 3-hydroxy-4-methoxy mandelate. These compounds were chosen as they have not been much exploited. The docking results of these compounds were compared with the docking score of a standard drug, Lenvatinib (Hatanaka et al., 2021).

2.6.1. Receptor preparation

The x-ray crystallography structures of EGFR (PDB ID: 3IKA), C-MET (PDB ID: 4XMC), FGF-4 (PDB ID: 1IJT), VEGFR (PDB ID:4CKV), and IGF1R (PDB ID:1IGR) were downloaded from the protein data bank RCSB PDB (hzzzttps://www.rcsb.org) in .pdb format. Receptor proteins were prepared for docking by withdrawing the extraneous water molecules and co-crystallizing ligands and then adding polar hydrogens and Gasteiger charges in AutoDockTools-1.5.7 software and saved in. pdbqt format.

2.6.2. Ligand preparation

For the *in-silico* assessment, the structure of the compounds obtained from the extracts of the plants were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) (Kim et al., 2016) in .sdf format and later changed to .pdb format in Open Babel GUI. The ligands were prepared by adding Gasteiger charges and non-polar hydrogen and rotational interactions were determined and changed in AutoDockTools-1.5.7.

2.6.3. Molecular docking and visualisation

The possible active sites were estimated using BIOVIA Discovery Studio (Prasanth et al., 2021). For docking, the SBD site sphere was placed on a co-crystalized ligand and measurements were recorded in the config file in Biovia Discovery Studio. The config file is represented in Table 1. The 3D structures of the proteins are shown in Fig. 1. The 2-D and 3-D images of the protein-ligand interaction were visualisedin BIOVIA Discovery Studio (Kiran et al., 2023).

2.7. Study of drug-likeliness properties

To be an effective drug, a molecule must reach its target in the body and stay there for a longer period. Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME), which is a pre-requisite in the discovery process.

Table 1
The grid box dimensions for the five receptor proteins.

TARGETPROTEINS	CENTREX	CENTERY	CENTERZ	SIZEX	SIZEY	SIZEZ	EXHAUSTIVENESS
C-MET	-9.008	18.213	-28.408	126	126	126	8
EGFR	-10.616	18.576	31.551	126	126	126	8
VEGFR	-17.062	-33.058	1.313	126	126	126	8
IGF-1R	6.993	54.172	-15.889	126	126	126	8
FGF4	-19.146	8.27	2.318	126	126	126	8

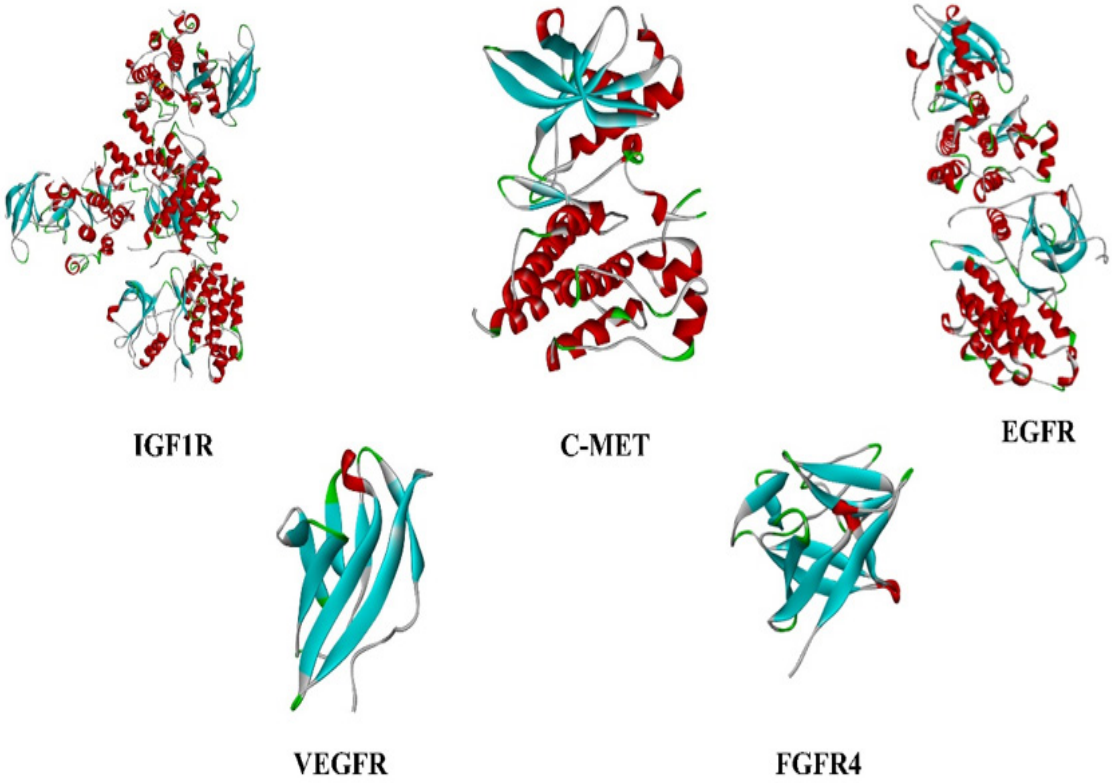

Fig. 1. The 3-D structure of IGF1R, C-MET, EGFR, VEGFR, FGFR4 visualized in Biovia Discovery Studio client 2021.

In this work, we used SwissADME web tool (http://www.swissadme.ch) and pKCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) to predict the ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness (Pires et al., 2015; Daina et al., 2017). The toxicity of the compounds were determined by ProTox-II(https://comptox.charite.de/protox3) (Banerjee et al., 2018).

3. Results and Discussion

Studies have shown that cancer is decreasing the expectancy of lifespan worldwide. In 91 out of 172 countries cancer is in the first or second position for the cause of death (Smith et al., 2019). Even in the recovery stage, the side effects of cancer are of concern (Bhavana et al., 2023). Cancer treatment is very costly, so it is difficult for unprivileged people to opt for it, especially in developing countries like India (Rajpal et al., 2014). So, there is an urgent need for alternating drug therapy with fewer side effects and more specificity. Studies have shown that many angiospermic plants have anticancer properties (Nataru et al., 2014). Secondary metabolites in the plant kingdom such as polyphenols, flavonoids and brassinosteroids have been studied for their potential use as anticancer agents. Collectively they have been shown to possess anticancer activities which include antioxidant activity; inhibition of cancer cell growth; induction of apoptosis; target specificity; cancer cell cytotoxicity (Azmi et al., 2006; Malíková et al., 2008; Cao at al., 2013; Gupta et al., 2014). *Canna indica* is a plant belonging to the family Cannaceae and has many medicinal properties like HIV inhibition, antibacterial, anticancer, anti-diarrheal, and anti-diabetic (Kanase et al., 2018).

According to global cancer data, liver cancer shares

4.7% of global cancer occurrence and a death rate of 8.3% (Sung et al., 2020). Till now there are no reports on *Canna indica* in the field of HCC. Studies have shown that *Canna indica* extract can stop HCV virus replication (Huang et al., 2009).

3.1. Cell viability assay

3.1.1. MTT assay

The methanolic extract of *Canna indica* showed promising anti-proliferative activity *in vitro* against the Hep G2 cancer cell line after 24 hours of treatment. In contrast, minimal changes were observed in normal HEK 293 cells, determining the specificity of the extract towards the cancer cells over normal cells. The cell viability of the Hep G2 cells decreases in a concentration-dependent manner (Fig. 2a), with half maximum inhibitory concentration (IC_{50} value) of 563.4 µg/mL.

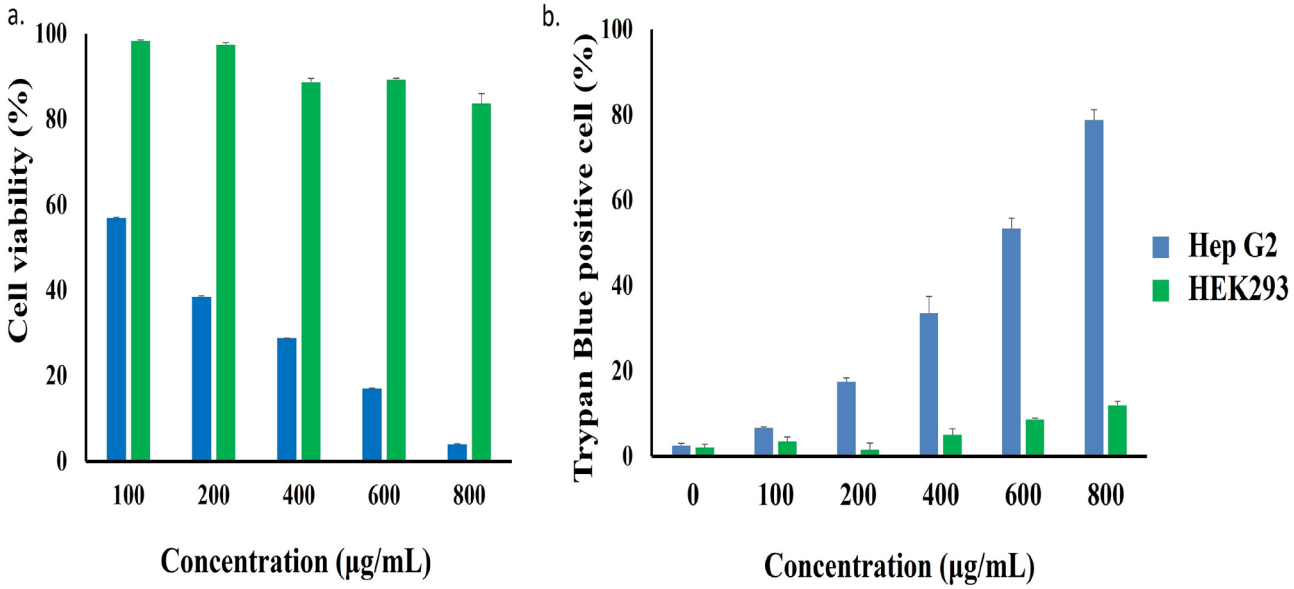

Fig. 2. Anti-proliferative efficacy of the methanolic extract of *Canna indica*. a). Bar graph showing a reduction in cell viability in a concentration-dependent manner detected through MTT assay. b). Bar graph showing an increase in the percentage of non-viable cells with the increasing concentration of the extract as perceived through trypan blue exclusion assay.

3.1.2. The trypan blue exclusion assay

The trypan blue assay showed that with increasing treatment concentration, the number of trypan blue-positive cells increased in the case of the HepG2 cell line. In contrast, in the normal HEK 293 cell line barely any trypan blue positive cells were observed (Fig. 2b). From the MTT and Trypan Blue exclusion assay, it can be inferred that methanolic extract of the rhizome of *Canna indica* can induce selective toxicity to hepatocarcinoma cell line Hep G2. As yet, there are very few reports on the antiproliferative potential of *Canna indica* extract. Dicholoromethane and the ethanol extract of the leaf of red edible *Canna*, shows cytotoxicity against WiDr colon cancer cell line (Widyarini et al., 2020). Another report says two pure compounds stigmasterol and 6-beta-hydroxystigmasta-4, 22-diene-3-one isolated from *Canna indica* exhibits cytotoxicity against P388 leukemic cell line (Sarje et al., 2019).

3.2. LC-MS analysis

Phytochemicals are identified on the basis of predictionsbased on mass fragments obtained on fragmentation at particular mass ion and matched with already available database, METLIN. LC-MS analysis of the methanolic extract of the rhizome of *Canna indica* identified forty-seven natural compounds. The chromatogram is shown in Fig. 3 and the compounds are enlisted in Table 2. The groups that the compounds belong to are enlisted in Table 3.

3.3. Molecular docking analysis

The proteins which are overexpressed during HCC are VEGFR, c-MET receptor, EGFR, IGF receptors, FGFR4. proving to be a good target for our anti-HCC studies. Out of the forty-seven compounds found through LC-MS analysis, only eleven were selected and subjected to molecular docking analysis, as they have not been explored in the field of drug development.The molecular docking scores of the eleven compounds are shown in (Table 4). The docking results suggested that cadabicine methyl ether, ganoderic acid F, mahanimbinol, cassythine has docking scores as good as the standard drug lenvatinib. The 3D docking model

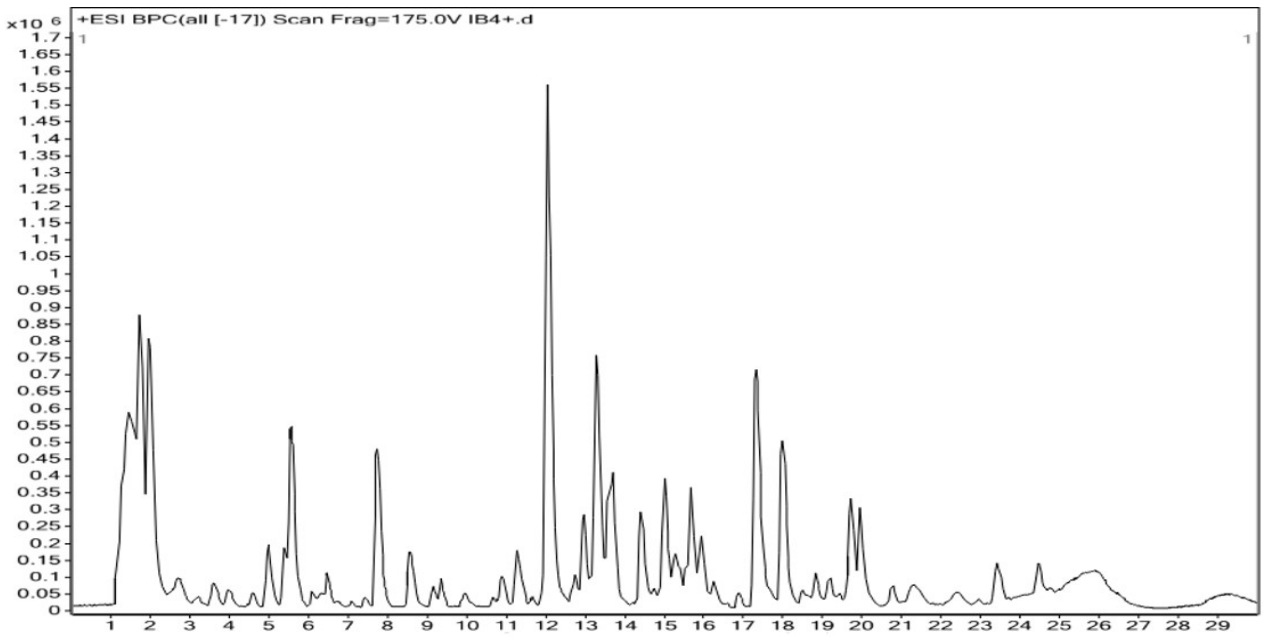

Fig. 3. Chromatogram of the LC-MS analysis of methanolic extract of *Canna indica*.

Table 2
LC-MS analysis of the methanolic extract of *Canna indica* rhizome.

Sl no	Name of phytoconstituent	Molecular formula	Molecular mass	Rt value	%Present
1	Probenecid	$C_{13}H_{19}NO_4S$	285.14	2.54	12.33
2	11-Amino-undecanoicacid	$C_{11}H_{23}NO_2$	201.169	1.89	9.3
3	L-2-Amino-5-hydroxypentanoicacid	$C_5H_{11}NO_3$	133.074	1.61	7.68
4	Mahanimbinol	$C_{23}H_{27}NO$	333.21	5.72	6.28
5	Cassythine	$C_{19}H_{19}NO_5$	341.127	1.56	4.96
6	4-[(2-Furanylmethyl)thio]-2-pentanone	$C_{10}H_{14}O_2S$	198.071	1.62	4.06
7	Isovarylsaconine	$C_8H_{15}NO_3$	173.103	1.42	3.93
8	Lysyl-Valine	$C_{11}H_{23}N_3O_3$	245.174	19.97	3.69
9	2-Hexylbenzothiazole	$C_{13}H_{17}NS$	219.107	1.74	3.61
10	Cadabicinemethylether	$C_{26}H_{31}N_3O_4$	449.235	15.69	3.23
11	S-DecylGSH	$C_{20}H_{37}N_3O_6S$	447.241	7.78	3.23
12	GanodericacidF	$C_{32}H_{42}NO_9$	570.278	25.19	2.65
13	N-Isovalerylglycinemethylester	$C_8H_{15}NO_3$	173.102	1.72	2.59
14	4-Hydroxy-L-threonine	$C_4H_9NO_4$	135.052	2.09	2.49
15	Cyclopiazonicacid	$C_{20}H_{20}N_2O_3$	336.149	1.32	2.42
16	Octanoicacid,7-hydroxy-,(S)-	$C_8H_{16}O_3$	160.11	19.95	2.03
17	Sphinganine	$C_{19}H_{39}NO_2$	301.293	14.46	1.7
18	2-Tetradecylcyclobutanone	$C_{18}H_{34}O$	266.259	11.26	1.69
19	Hydroxyatrazine	$C_8H_{15}N_5O$	197.129	1.29	1.58
20	(±)-(Z)-2-(5-Tetradecenyl)cyclobutanone	$C_{18}H_{32}O$	264.243	11.3	1.32
21	Isoleucyl-glutamate	$C_{11}H_{20}N_2O_5$	260.134	19.96	1.22
22	AntimycinA1	$C_{28}H_{40}N_2O_9$	548.27	23.42	1.15
23	Tolterodine	$C_{22}H_{31}NO$	325.243	6.25	1.06
24	2-Deoxystreptidine	$C_8H_{18}N_6O_3$	246.142	1.83	0.97
25	3-Hydroxyisoheptanoicacid	$C_7H_{14}N_3$	146.094	19.96	0.96
26	23-Acetoxysoladulcidine	$C_{29}H_{47}NO_4$	473.343	21.49	0.92

Table 2
Continued.

Sl no	Name of phytoconstituent	Molecular formula	Molecular mass	Rt value	%Present
27	MG(0:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	$C_{25}H_{38}O_4$	401.273	19.15	0.92
28	Bisoprolol	$C_{13}H_{31}NO_4$	325.22	10.01	0.86
29	6b-Hydroxy-8a-methoxy-7(11)-eremophilen-12,8-olide	$C_{16}H_{24}O_4$	280.17	1.41	0.85
30	L-isoleucyl-L-proline	$C_{11}H_{20}N_2O_3$	228.144	2.32	0.85
31	Carisoprodol	$C_{12}H_{24}N_2O_4$	260.173	19.75	0.81
32	2-Diethylaminoethanol	$C_6H_{15}NO$	117.115	2.77	0.8
33	Tabernamine	$C_{40}H_{48}N_4O_2$	616.384	6.25	0.77
34	(+)-Pronuciferine	$C_{19}H_{21}NO_3$	311.153	1.97	0.75
35	N-(2-Methylpropyl)acetamide	$C_6H_{13}NO$	115.099	5.43	0.75
36	16-Hydroxyhexadecanoicacid	$C_{16}H_{32}O_3$	272.233	19.26	0.74
37	Butyllevulinate	$C_9H_{16}O_3$	172.109	16.26	0.69
38	Columbianetin	$C_{14}H_{14}O_4$	246.088	16.96	0.68
39	3-Oxo-alpha-ionol9-[apiosyl-(1->6)-glucoside	$C_{24}H_{38}O_{11}$	502.241	17.77	0.67
40	2E-Decenedioicacid	$C_{10}H_{14}O_4$	200.104	19.89	0.66
41	MG(22:4(7Z,10Z,13Z,16Z)/0:0/0:0)	$C_{25}H_{42}O_4$	406.304	20.16	0.63
42	Patrinoside	$C_{21}H_{34}O_{11}$	462.209	15.51	0.61
43	Guggulsterone	$C_{22}H_{30}O_3$	342.221	9.31	0.61
44	N-tert-Butyloxycarbonyl-deacetyl-leupeptin	$C_{23}H_{44}N_6O_5$	484.343	4.62	0.62
45	4-Hydroxynornantenine	$C_{19}H_{19}NO_5$	341.127	1.15	0.6
46	4-Oxocyclohexanecarboxylate	$C_7H_{10}NO_3$	142.062	10.77	0.3
47	3-Hydroxy-4-methoxymandelate	$C_9H_{10}O_5$	198.051	7.54	0.23

Table 3
The compounds identified through LC-MS analysis and their chemical categories.

Name	Class
Probenecid	Benzenederivative
11-Amino-undecanoicacid	Aminoacid
L-2-Amino-5-hydroxypentanoicacid	Aminoacid
Mahanimbinol	Alkaloid
Cassythine	Aporphinealkaloid
4-[(2-Furanylmethyl)thio]-2-pentanone	Heteroarene
Isovalerylsarcosine	N-acyl-aminoacid
Lysyl-Valine	Dipeptide
2-Hexylbenzothiazole	Benzothiazoles
Cadabicinemethylether	Azamacrocycle
S-DecylGSH	Peptide
GanodericacidF	Triterpenoid
N-Isovalerylglycinemethylester	N-acylglycine
4-Hydroxy-L-threonine	Hydroxy-aminoacid
Cyclopiazonicacid	Alkaloid
Octanoicacid,7-hydroxy-,(S)-	Carbonylcompound
Sphinganine	Aminoalcohol
2-Tetradecylcyclobutanone	Cyclicketone
Hydroxyatrazine	Triazine

Table 3
Continued.

Name	Class
(±)-(*Z*)-2-(5-Tetradecenyl)cyclobutanone	Cyclicketone
Isoleucyl-glutamate	Dipeptide.
AntimycinA1	Macrolide
Tolterodine	Tertiaryamine
2-Deoxystreptidine	Aminocyclitol
3-Hydroxyisoheptanoicacid	Carbonylcompound
23-Acetoxysoladulcidine	Steroidalalkaloids
MG(0:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	Glycerolipids
Bisoprolol	SecondaryalcoholandaSecondaryamine
6b-Hydroxy-8a-methoxy-7(11)-eremophilen-12,8-olide	Terpenelactone
L-Isoleucyl-L-proline	Dipeptide
Carisoprodol	Carbamateester
2-Diethylaminoethanol	Ethanolamines
Tabernamine	Alkaloid
(+)-Pronuciferine	Isoquinolinealkaloid
N-(2-Methylpropyl)acetamide	Acetamides.
16-Hydroxyhexadecanoicacid	Fattyacid
Butyllevulinate	Oxocarboxylicacid
Columbianetin	Coumarin
3-Oxo-alpha-ionol9-[apiosyl-(1->6)-glucoside]	O-acylcarbohydrate.
2E-Decenedioicacid	Fattyacid
MG(22:4(7Z,10Z,13Z,16Z)/0:0/0:0)	Monoglyceride
Patrinoside	Terpeneglycoside
Guggulsterone	Steroid
N-tert-Butyloxycarbonyl-deacetyl-leupeptin	Isoquinolinealkaloid
4-Hydroxynornantenine	Isoquinolinealkaloid
4-Oxocyclohexanecarboxylate	Monocarboxylicacid
3-Hydroxy-4-methoxymandelate	Phenols

Table 4
Molecular docking showing binding energies (in kcal/mol) of eleven compounds selected from the LC-MS data of the methanolic extract of *Canna indica* and a standard drug.

Chemicals	C-MET	VEGFR	FGFR4	EGFR	IGF-1R
2-Hexylbenzothiazole	-6.8	-4.9	-4.6	-6	-5.4
3-Hydroxy-4-methoxymandelate	-6	-4.4	-4.7	-5.5	-5.3
11-Amino-undecanoicacid	-5.7	-4	-4	-4.5	-4.6
Cadabicinemethylether	-8.2	-7.6	-7.4	-10.2	-9.2
GanodericacidF	-7.7	-6.9	-6.5	-8.9	-8.3
Isovalerylsarcosine	-5.3	-4.1	-3.8	-4.6	-4.6
Mahanimbinol	-10.3	-5.9	-6.3	-8.6	-8.1
N-Isovalerylglycinemethylester	-5.6	-4.1	-4.5	-4.5	-4.5
Cassythine	-6.9	-6.7	-6.1	-8.6	-8.7
S-DecylGSH	-8.7	-6.5	-5.9	-7.3	-6.4
Sphinganine	-8.3	-5.9	-5.8	-5.6	-6
Lenvatinib	-8.9	-6.3	-5.6	-8.6	-8.6

and the 2D interaction of EGFR, C-met, FGFR 4, VEGFR, and IGF-1R with the four ligands mentioned above are represented in Fig. 4, Fig. 5, Fig. 6, Fig. 7, and Fig. 8, respectively.

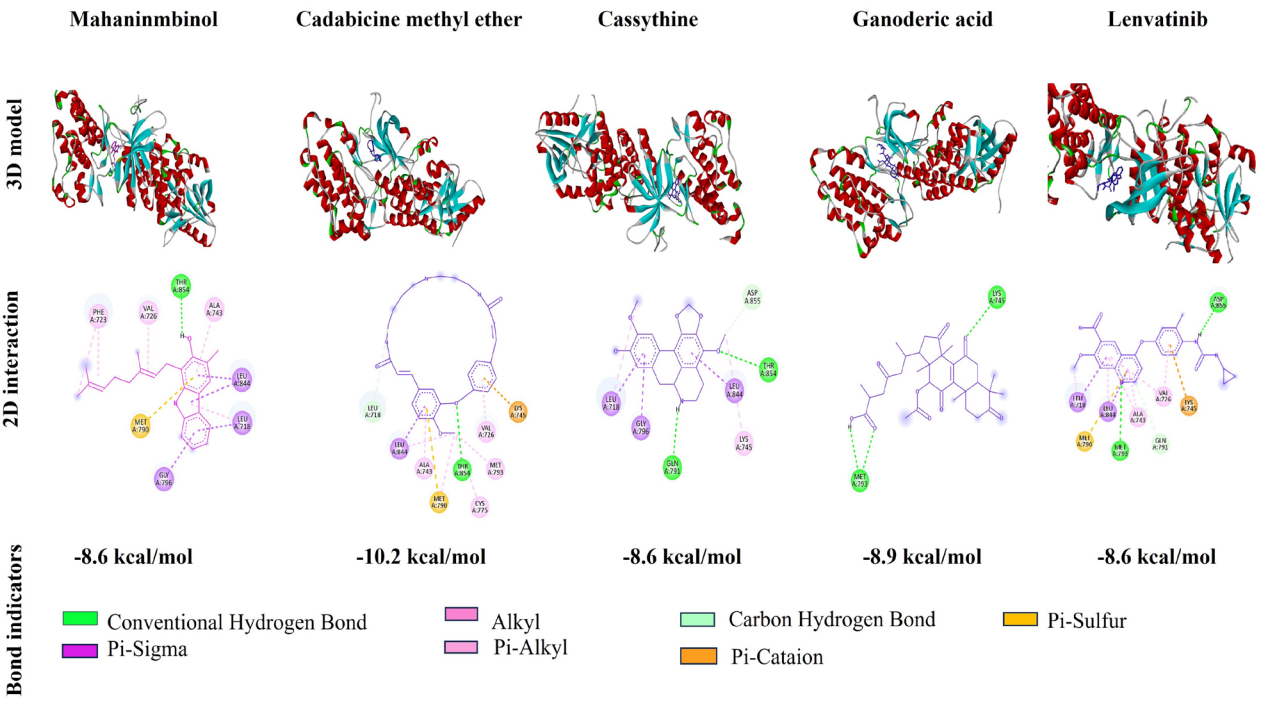

Fig. 4. *In silico* binding of mahanimbinol, cadabicine methyl ether, cassythine, ganoderic acid, and Lenvatinib with EGFR. The 3-D model represents receptor-ligand binding and the 2-D model depicts the interaction between receptor and ligand. Each value represents the binding energy of the docked protein-ligand interaction.

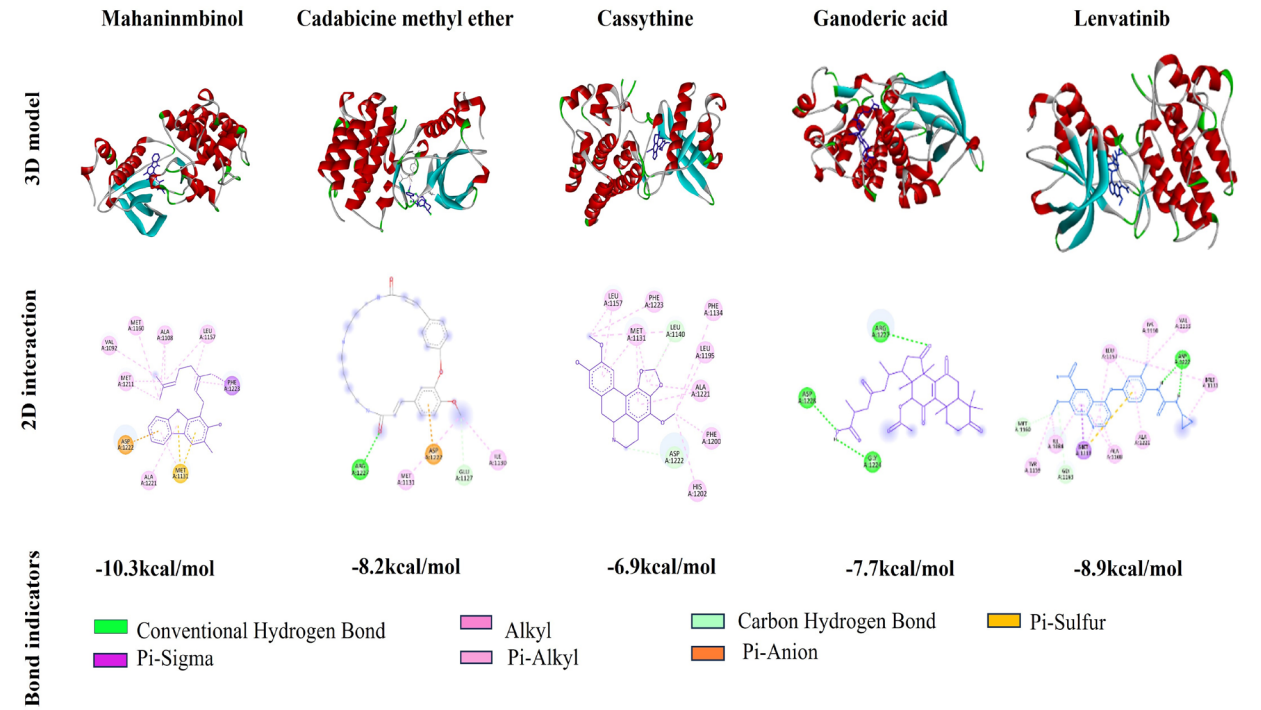

Fig. 5. *In silico* binding of mahanimbinol, cadabicine methyl ether, cassythine, ganoderic acid, and Lenvatinib with C-met. The 3-D model represents receptor-ligand binding and the 2-D model depicts the interaction between receptor and ligand. Each value represents the binding energy of the docked protein-ligand interaction.

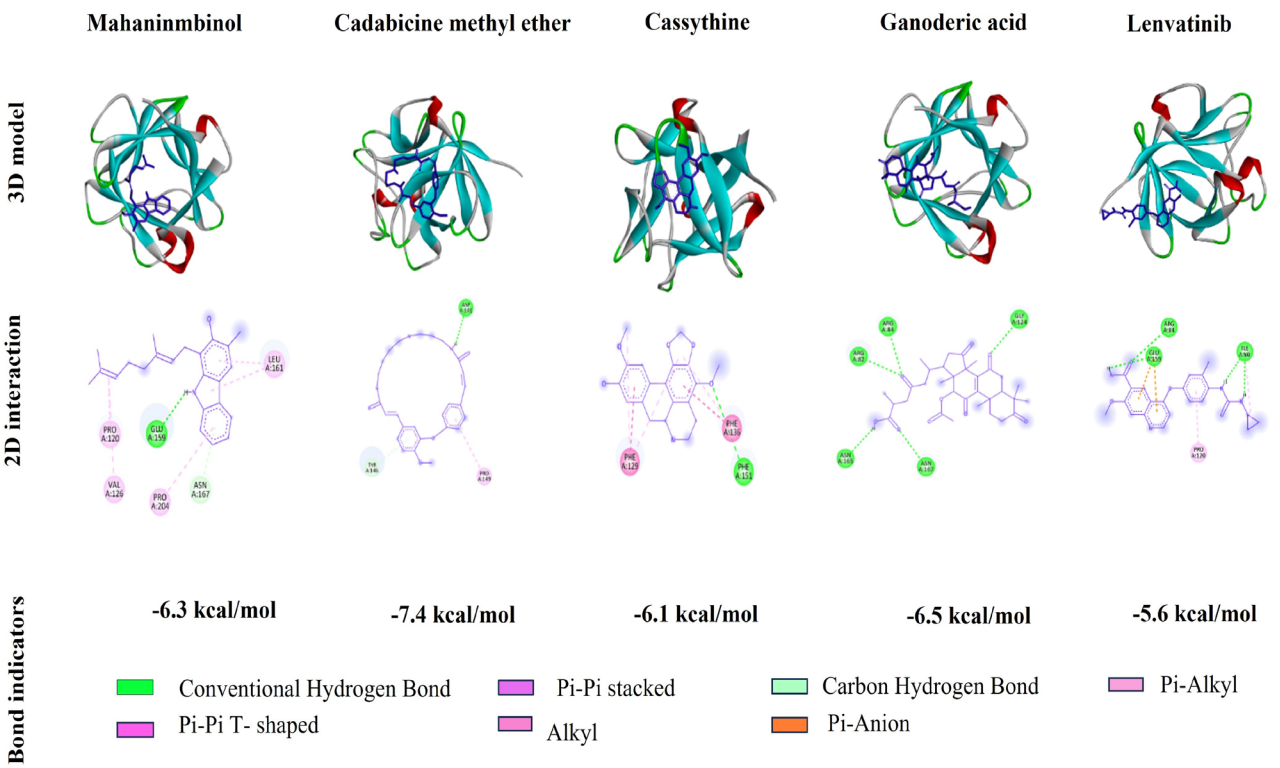

Fig. 6. *In silico* binding of mahanimbinol, cadabicine methyl ether, cassythine, ganoderic acid, and Lenvatinib with FGFR4. The 3-D model represents receptor-ligand binding and the 2-D model depicts the interaction between receptor and ligand. Each value represents the binding energy of the docked protein-ligand interaction.

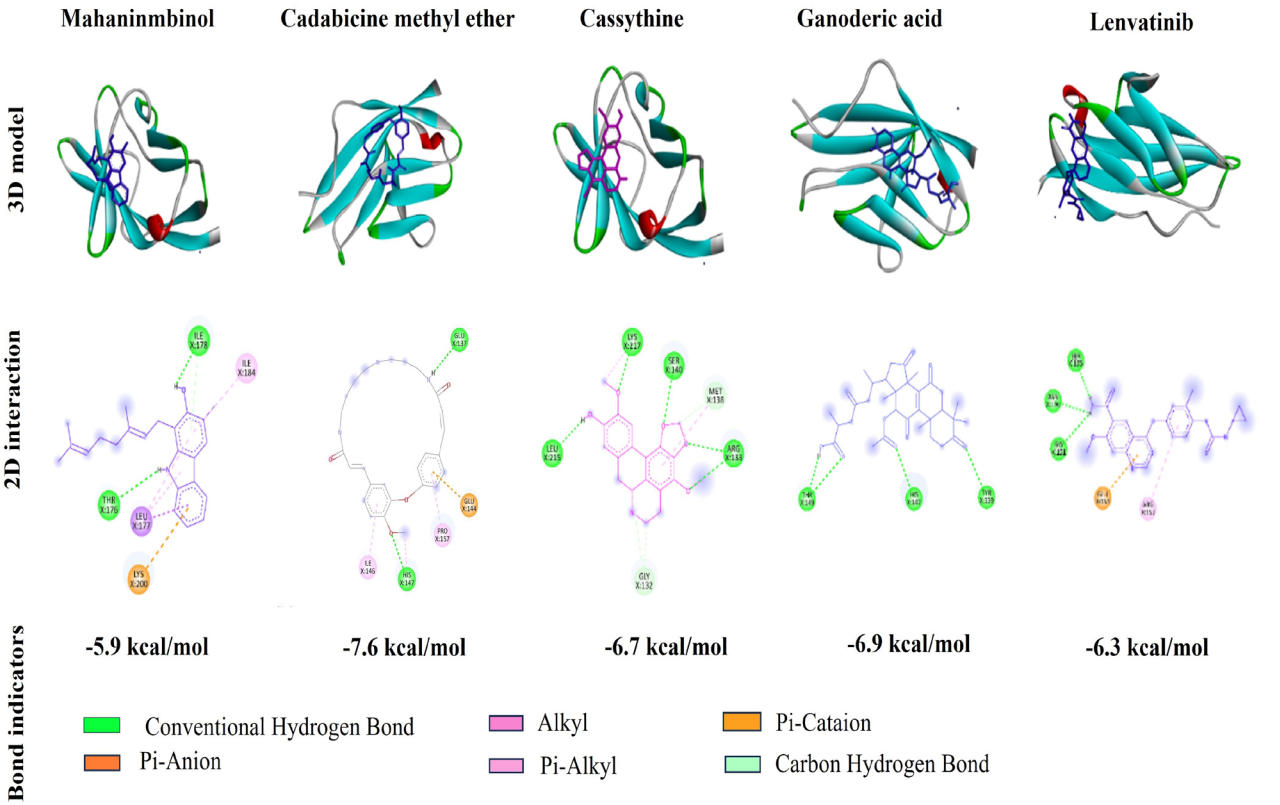

Fig. 7. *In silico* binding of mahanimbinol, cadabicine methyl ether, cassythine, ganoderic acid, and Lenvatinib with VEGFR. The 3-D model represents receptor-ligand binding and the 2-D model depicts the interaction between receptor and ligand. Each value represents the binding energy of the docked protein-ligand interaction.

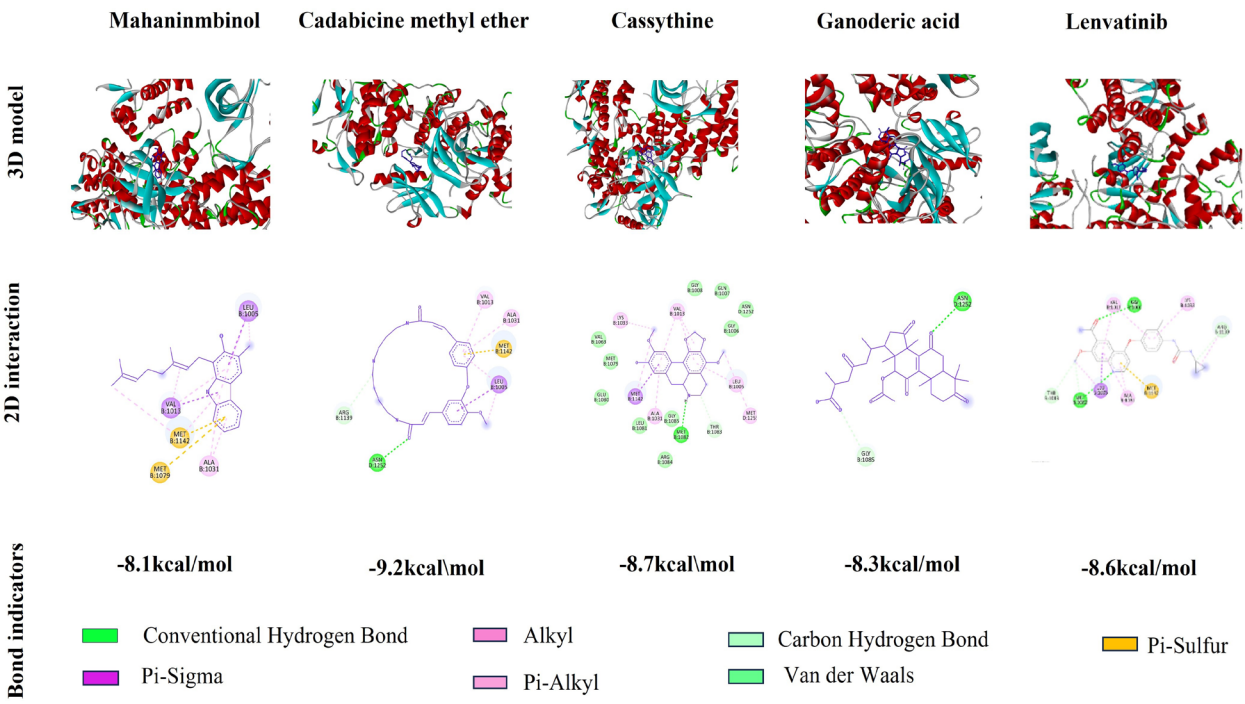

Fig. 8. *In silico* binding of mahanimbinol, cadabicine methyl ether, cassythine, ganoderic acid, andLenvatinib with IGF1R. The 3-D model represents receptor-ligand binding and the 2-D model depicts the interaction between receptor and ligand. Each value represents the binding energy of the docked protein-ligand interaction.

3.4 ADMET analysis of drug candidate

ADMET properties are fundamental for any drug to be acceptable. compounds with higher log p are more lipid permeable and can cross the lipid membrane increasing their absorption in the body. Compounds mahanimbinol and ganoderic acid have shown greater log p values than the standard drug Lenvatinib. Gastro-intestinal (GI) absorption of the compounds mahanimbinol, cadabicine methyl ether, cassythine is fairly good compared to standard drug lenvatinib. The compounds mahanimbinol, cadabicine methyl ether, ganoderic acid have penetration to the blood brain barrier like the standard drug lenvatinib. P-gp is a membrane-bound protein that functions as an efflux transporter. It pumps substance out of the cell. Mahanimbinol is P-gp negative and cadabicine methyl ether,ganoderic acid, cassythine shows positive result for pgp substrate. the standard drug lenvatinib is also positive for Pgp substrate. During drug metabolism, mahanimbinol, cassythine, ganoderic acid gives negative results for CYP1A2 inhibitors. Mahanimbinol was found to be a CYP2C19 inhibitor. Cadabicine methyl ether and cassythine are inhibited by CYP2C9. Cassythine and Mahanimbinol are CYP2D6 inhibitors cadabicine methyl ether, ganoderic acid, and cassythine are CYP3A4 inhibitors. Mahanimbinol and ganoderic acid showed 1 lipinski rule violation. The detailed ADMET analysis of the top 4 compounds with the best docking score is shown in Table 5.

The physiological properties of these compounds are shown in Table 6. The 2-D structures of the compounds and the boiled egg structure of the top 4 compoundsare shown in Fig. 9a and Fig. 9b respectively.

ProTox-II toxicity studies of the compound are shown in Table 7. This study showed that the toxicity range of the four compounds are near the value of toxicity of lenvatinib. All the compounds are found to be moderate to less toxic and thus are safe substitutes for conventional drugs.

4. Concluding remarks

Liver cancer is one of the major reasons for cancer-related death worldwide. Despite the presence of several synthetic drugs, drug resistance and non-specificity play a pivotal role in the field of alternative drug development. Although *Canna indica* has several medicinal properties but it has been very less explored in the field of anti-cancer drug discovery. Our present study summarises, that the methanolic extract of the rhizome of *Canna indica* exhibits significant anti-hepatocellular carcinoma activities. Notable, among several phytochemicals identified in the extract, 4 compounds, namely mahanimbinol, cadabicine methyl ether, cassythine, ganoderic acid are paramount components behind these bioactivities as they have shown strong binding affinity with the hallmark proteins of hepatocellular carcinoma. Future research regarding isolation and evaluation of anti-hepatocellular properties of these compounds may open a new window to liver cancer therapy.

Table 5
ADMET analysis of the top four compounds and comparison with standard drug Lenvatinib.

Pharmacokineticsproperties	Mahanimbinol	Cadabicinemethylether	Ganodericacid	Cassythine	LENVATINIB
LOGP	6.57	3.51	4.13	2.54	4.07
GIABSORPTION	High	High	Low	High	High
BBBPERMEANT	No	No	No	Yes	No
PGPSUBSTRATE	No	Yes	Yes	Yes	Yes
CYP1A2inhibitor	No	No	No	Yes	No
CYP2C19inhibitor	Yes	No	No	No	No
CYP2C9inhibitor	No	Yes	No	Yes	Yes
CYP2D6inhibitor	Yes	No	No	Yes	No
CYP3A4inhibitor	No	Yes	Yes	Yes	Yes
Lipinskiviolation	1	0	1	0	0

Table 6
Physicochemical properties of the top four compounds and standard drug Lenvatinib.

Physiochemical properties	Mahanimbinol	Cadabicinemethylether	Ganodericacid	Cassythine	Lenvatinib
Molecularweight	333.47	449.54	570.67	341.36	426.85
Num. heavy atoms	25	33	41	25	30
Num. atom heavy atoms	13	12	0	12	16
FractionC sp3	0.3	0.31	0.72	0.37	0.19
Num.rotatable bonds	5	1	8	2	8
Num. H-bond acceptor	1	5	9	6	5
Num. H-bond donors	2	3	1	2	3
Molar Refractivity	110.07	141.33	150.02	95.14	112.86
TPSA	36.02	88.69	148.95	69.18	115.57

Table 7
Toxicity profiling of the top four compounds using ProTox-II.

Chemicals	Volume %	Toxicity score	LD_{50} value
Cadabicinemethylether	3.23	4	1180
GanodericacidF	2.65	6	9000
Mahanimbinol	6.25	5	2300
Cassythine	4.96	5	2300
Lenvatinib		5	3000

Author contribution statement

Conceptualization and fund acquisition was done by Santanu Paul. Investigation, data curation, formal analysis, visualization, validation, was performed by Rohit Kumar Dutta and Saparja Saha. Rohit Kumar Dutta prepared the first draft of the manuscript, Saparja Saha and Santanu Paul, critically analysed and gave suggestions to finalize the manuscript.

Lists of abbreviations

DMEM: Dulbecco's Modified Eagle Medium; **DMSO:** Dimethyl sulfoxide; **EGFR:** Epidermal growth factor receptor; **FBS:** Fetal bovine serum; **FGFR 4:** Fibroblast growth factor receptor; **HCC:** Hepatocellular carcinoma;

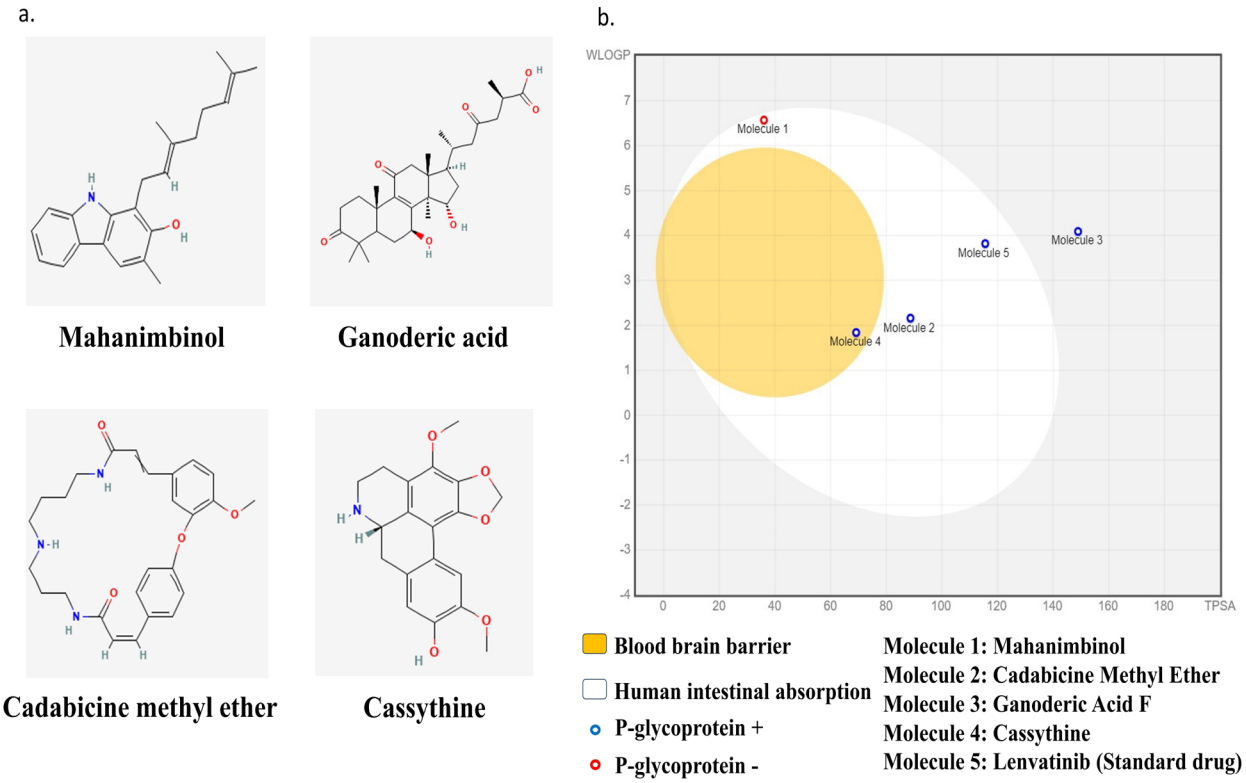

Fig.9. a. Structure of four phyto-compounds showing best molecular docking result. **b.** Evaluation of drug-like properties of ligands, represented by Boiled-Egg structure of the four compounds showing best molecular docking results.

IGF1R: Insulin like growth factor 1 receptor; **LC-MS:** Liquid chromatography-mass spectrometry; **MTT:** (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); **VEGFR:** Vascular endothelial growth factor receptor.

Conflict of interest

The authors declare that there is no conflict of interest.

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