

Effect of Ficus carica against DEN-Induced Hepatocellular Carcinoma: In Vivo and In Silico Analysis

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Abstract

Hepatocellular carcinoma (HCC) is one of the most fatal cancers responsible for mortality worldwide. That makes HCC an important cancer to be studied. A randomized controlled study was conducted (on 32 Balb c albino mice) to evaluate the anticancer potential of acetone based extract of *F.carica* variety from Shandong province of China for the first time. Diethyl amine nitrosamine (DEN) and carbon tetra chloride (CCl₄) were used as inducers of hepatic carcinoma in mice. We conducted an in vivo study on *F.carica* based acetone (FA) extract that has already been proven effective against hepatoblastoma cancer (HepG2) cell lines in our previous experiments. FA extract attenuated the liver functional biomarkers (BUN, ALT, AST, ALP) and the level of alpha fetoprotein (AFP) significantly in the serum of mice at a dose of 60 mg/kg of body weight of mice. The histopathological analysis indicated the regeneration of liver tissues to the normal state of liver upon feeding the mice with the extract for a period of 60 days. The standard hepatoprotective drug silymarin was used as a positive control to assess the efficacy of the used extract. Silymarin (50mg/kg of body weight) also decreased the liver injury associated biomarkers; however, its effect was almost same and even the extract efficiently reduced BUN content and the level of AST enzyme in the blood serum of the studied mice. Our in vivo findings are also reinforced by our in-silico studies. This study leverages molecular docking and ADMET profiling to identify promising FA-based compounds. These compounds, have potentially therapeutic effects and exhibit competitive and even better results than the FDA approved drug i.e. Silymarin. Various phytochemicals from FA extract including sitosterol, quercetin, and luteolin, were tested against the key targets of Hepatocarcinoma e.g., EGFR (Epidermal Growth Factor Receptor), VEGFR (Vascular Endothelial Growth Factor Receptor), and MMPs (Matrix metalloproteinases) via molecular docking stimulation. The findings suggest that sitosterol, quercetin, and luteolin show competitive binding and favorable ADMET properties, proposing them as candidates for further experimental validation. This novel extract and further its isolated compounds could serve as a better and economical alternative to traditional drugs in -future.

1. Introduction

The liver is a very sophisticated and multifunctional organ, which is responsible for metabolism, nutrients dissemination and detoxification of the harmful substances (Mao et al., 2024; Chiang, 2014). It is believed that chronic liver diseases result in persistent inflammation, which can lead to liver fibrosis. This fibrosis can progress to cirrhosis (liver cancer) that significantly regulating long-term morbidity and mortality (Hammerich & Tacke, 2023). Thus, the development of cirrhosis is a continuous process which is complicated by decompensation, liver failure or hepatocellular carcinoma. These modulations also impact the metabolism of endogenous and exogenous substances as well as the production of liver-derived protein (Dietrich et al., 2016).

Diethylnitrosamine (DEN) is a powerful hepatocarcinogen present in various processed foods, is genotoxic and carcinogenic nitrosamine generating reactive oxygen species (ROS), produces oxidative stress and damages liver tissues (Almatrafi, 2024). ROS, interact with and induces damage to different cellular components, such as DNA, proteins, and lipids that causes cellular malfunctioning. Consequently, oxidative stress is a critical factor in hepatocarcinogenesis that influencing both the initiation and progression stages of liver cancer. As diethylnitrosamine (DEN) is a highly toxic organic compound; therefore, it is oftenly used in cancer research to induce liver tumors in experimental animal models (Chang et al., 2024). For example, in a research study, a rat model chronically induced with DEN has effectively replicated human liver fibrosis and cirrhosis, eventually progressing to hepatocellular carcinoma development. Due to its ability to cause degenerative, hyperplastic, and neoplastic liver lesions, DEN is usually employed to induce hepatocarcinogenesis (Peng et al., 2024).

Hepatotoxicity is a significant public health issue worldwide. Although modern medicine has made strides, the disadvantages of synthetic drugs often overshadow their advantages. But the modern medical principles for the treatment of the liver diseases can lead to several adverse side effects. This has prompted growing global interest in medicinal plants for curing liver diseases, due to their promising efficiency and affordability (Venmathi Maran et al., 2022). Throughout human history, people have relied on plants for their survival and treatment of various diseases. Even with major advances in modern medicine, a large number of plants continue to be employed for their medicinal benefits (Shawon et al., 2024). These plants contain several phytochemical compounds such as flavonoids, phytosterols, saponins, terpenes, phenols, anthocyanins, amino acids, fatty acids, steroids, tannins, terpenoids, terpenoids, amino acids, vitamins fatty acids and minerals (Ishnaiwer, 2023). These plant-based compounds exhibit a wide range of biological functions (Putra et al., 2020). Modern medical treatments for liver diseases frequently result in numerous unwanted side effects, underscoring the necessity to delve into alternative therapeutic strategies (Hong et al., 2015). Therefore, the plant-based approaches could present an alternative or supplementary action to conventional treatments that potentially declining the risk of negative impacts and fostering long-term liver health. Nevertheless, further research is required to elucidate the mechanisms of action, optimize dosage and formulation, and conduct clinical trials to validate the proficiency and safety of these hepatoprotective plants in managing liver diseases (Shawon et al., 2024).

The fig (*Ficus carica*) belongs to the *Moraceae* family and has been employed in traditional medicine for the treatment of various diseases (Jagtap & Bapat, 2020). It holds special phytocomponents as a source of primary and secondary metabolites such as fatty acids, vitamins, carbohydrates and minerals and flavonoids, anthocyanins, pectin, furanocoumarins and phytosterols (Alzahrani et al., 2024). They show a broad range of bioactivities including antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, anti-diabetic, renoprotective, anticancer and

hepatoprotective features (Alsenani et al., 2023). Furthermore, it is explored that phytochemicals in figs are served as a powerful anti-inflammatory agent by down-regulating different genetic pathways e.g. NF- κ B and JAK-STAT signaling pathway (Rezagholizadeh et al., 2022).

As *Ficus carica* (Fig) reveals a strong hepatoprotective impact against DEN-induced hepatotoxicity in rats and has shown considerable potential in both preventing and treating the disease (Tawfek et al., 2015). HCC is most often diagnosed at advanced stages with limited therapeutic options available. This presents the necessity of developing novel drugs with better therapeutic approach and fewer to no side effects. Despite the promising pharmacological features of figs, there remains a significant gap in the literature regarding its specific effects on diethylnitrosamine (DEN)-induced liver cancer that need to be researched. Therefore, investigating the effect of *Ficus carica* acetone-based extract on DEN-induced liver cancer is pivotal due to the requirement arise for curing hepatic ailments. Diethylnitrosamine (DEN), a potent carcinogen, stimulates the transition of normal hepatocytes into hepatocellular carcinoma (Tamim et al., 2024). Further, the *Ficus carica* holds remarkable medicinal features and is employed in traditional medicine for curing distinct ailments (El-Attar et al., 2024). This is because it contains vital bioactive compounds that has curative outcomes (Rasool et al., 2023). Therefore, *Ficus carica* acetone-based extraction will separate these therapeutic compounds for enhancing their bioavailability and remedial effects. It is hypothesized that the acetone extract of *Ficus carica* may exhibit protective effects against DEN-induced liver injury due to its bioactive components. The aim of the present research is to assess the hepatoprotective potential of *Ficus carica* acetone-based extract in a DEN-induced liver cancer model as well as to validate the in vivo findings, using in silico docking results against critical HCC-associated proteins and analyzes their ADMET properties to determine their potential as drug candidates.

2. Materials and Methods

2.1. Reagents and Materials:

The formalin, diethyl nitrosamine (DEN) and silymarin were acquired from Alladin (China). ALT, AFP, and ALP analysis kits were derived from Zecen Biotechnology Co. Ltd. (Jiangsu, China). Xylene, anhydrous alcohol and eosin reagents were obtained from the China National Medicines Co. Ltd. (Beijing, China) for the Hematoxylin and Eosin (HE) staining purpose. Further, and neutral balsam (ZLI-9555) and hematoxylin dye solution (ZLI-9609) from Zhongshan Golden Bridge Biotechnology (Beijing, China). An enzyme standard instrument (BIORAD 550) and ELISA kit (MDL MD6596) were utilized for the AFP analysis. In this research, all chemicals used were of analytical standard. Moreover, 35 male Balb/c albino mice, aged 5 to 6 weeks and weight between 15 to 20 grams were bought from Kunming (China). All animal care and procedures adhered to the ethical standards developed by the Shandong University of Technology Animal Care Committee. The apparatus employed during the process were a paraffin slicer (Leica RM2235), heating paraffin embedding system (Leica G1150 H), fully automatic dehydrator (Leica ASP200S), water bath crock (Leica HI1220), microscope (Leica DM3000), baking table (Leica HI1220),

2.2. Extraction of plant material

The samples of the *F. carica* were provided by Prof. Yuanda Song from Shandong University of Technology in May 2019. Then, theses were identified by the Department of Agricultural Engineering and Food Science at Shandong University of Technology in Zibo, China. After that, samples were thoroughly washed, air-dried at room temperature, and then grinded it to a mesh size of less than 5 mm by employing the electric grinder. The extraction process was based on the same procedure that were used in our previous in-vitro experiments (Mustafa et al., 2021a), with minimal alterations in the sample quantity. Particularly, 30 grams of grinded leaves were soaked in 900 mL of acetone and extracted for 24 hours at 40°C in a water bath with constant shaking. The obtained extract was filtered via a nylon mesh filter and then centrifugation was performed at 800 × g for 5 minutes. Resultantly, clear supernatant formed, which was concentrated in a rotary evaporator under low pressure and temperature, and eventually dried in an oven at 30°C. The completely dried extracts were preserved at 4°C until needed for the analysis. The percentage yield of extracts was calculated by the following formula:

$$\frac{\text{Weight Of the Extract}}{\text{Weight Of the Grinded Plant Material}} * 100$$

Consequently, 50 mg/g of FA extract were obtained.

2.3. In vivo hepatoprotective effect of FA extract against DEN-induced damage

The lab-based controlled randomized research investigation was conducted in the animal house of the Department of Life Sciences at Shandong University of Technology from 17 September to 29 December, 2019. The mice were sheltered in cages within the facility and were given unlimited access to food pellet and clean drinking water. The animal house maintained a temperature of 25°C with controlled humidity and a constant dark and light cycle. Before the commencement of the experiment, the animals were acclimatized to the environment of the animal house for one week. Consent for animal experimentation were gained from the Institutional Animal Care and Use Committee at Shandong University of Technology. All principles and procedures were adhered to the pertinent criteria and institutional policies according to the National Regulation of China for the Care and Use of Laboratory Animals, as well as the Regulations of China for the Administration of Affairs Concerning Laboratory Animals. Following the acclimation period, DEN was administered twice through intraperitoneal injection at a dose of 50 mg/kg body

weight, with the next dose given one week after the first. Carbon tetrachloride dissolved in corn oil (0.5 mL/kg), was given orally twice a week for 15 days, with minimum changes from the protocol employed in previous research to induce HCC in mice (Mustafa et al., 2021b). We observed the mice for 30 days for the induction of HCC. After 30 days, the development of HCC was validated by assessing the AFP and LFT levels in the serum of the mice. The animals were categorized into 4 groups with each group contain 8 animals. The group-1 was the normal control group which received 1 mL/kg of oil. The group-2 was the carcinogenic control which was given DEN at a dose of 50 mg/kg body weight twice in 15 days. Further, the group-3 was nourished with the FLA extract at 60 mg/kg body weight. Finally, the group-4 fed with 40 mg/kg body weight of Silymarin.

2.4. Biochemical Analysis

The blood samples were collected from both control and DEN-treated mice groups 30 days after the treatment with DEN and CCl₄ for the measurement of the AFP level and to examine the induction of liver cancer in mice. Then, blood was collected from the mice after 60 days of treatment with the FA extract and silymarin to measure the serum AFP, LFT and bilirubin content in the blood. Subsequently, blood samples were collected from the mice 60 days after treatment with FA extract and silymarin for the measurement of the serum AFP, LFT and bilirubin content in the blood. Blood samples of 2 mL were constantly collected from the retro-orbital plexus of anesthetized mice by employing a capillary tube. The serum was separated from the blood by centrifugation at 3000 rpm for 15 minutes and then stored at - 80°C in a refrigerator for the further analysis of biochemical markers.

2.5. Histopathological study of tissue

Approximately 5 µm thick sections of liver tissues were fixed in 10% neutral formalin solution for one day at 4°C in refrigerator. Following fixation, these sections were embedded in paraffin wax. Subsequently, the paraffin-embedded liver sections were stained with hematoxylin and eosin (H&E) and examined microscopically by a pathologist.

2.6. *In-silico* study

We screened plant-derived compounds using PyRx for binding affinity against selected HCC targets. Followed by energy minimization through ChemDraw 3D, we docked compounds against EGFR, MMPs, recorded their binding energies, and compared them to Silymarin. It involved following steps:

1. **Compound Preparation:** Compounds were prepared by structure optimization and energy minimization using ChemDraw 3D.
2. **Docking Simulations:** Each compound was docked to target proteins using AutoDock Vina, with results analyzed based on binding energy and interaction profiles.
3. **ADMET Analysis:** Shortlisted compounds were assessed for drug-likeness via SWISSADME, examining parameters like Lipinski's Rule, TPSA, LogP, and bioavailability.

2.7. Statistical Analysis

All data were expressed as (Mean ± SD) for the eight mice per group. Statistical analysis was performed for all the results employing one-way ANOVA which was followed by the post-hoc test. P-values < 0.05 were statistically significant.

3. Results

From last experimental work, we found *F. carica* based acetone extract most effective against HepG2 cell lines. After an in-vitro study, we proceeded towards in-vivo study on Balb c albino mice. Following the pathological events associated with liver carcinoma after 30 days of DEN and CCl₄ treatment, we started feeding mice with *F. carica* extract and silymarin. The treatment with extract and drug was continued for a period of 60 days and mice were sacrificed for measuring liver functional markers. Alpha fetoprotein is an embryonic protein in mammals, its elevated level is associated with cancer as previously found in our study in mice. The tumor formation in mice was confirmed by measuring AFP level in normal control group versus DEN + ccl₄ treated group on 30 day of establishing pathophysiology in mice. DEN is an acute hepatotoxin, that establishes hepatocellular carcinoma upon its prolonged use. On 60th day of treatment of mice with FA extract and silymarin, the blood was taken with capillary tubes and serum was separated as mentioned in methodology. Various liver functional serum biomarkers, e.g. ALT, AST, ALP and AFP were found to rise significantly high as compared to untreated (normal) control group. We found FA extract significantly reduced these biochemical markers at a concentration of 60mg/kg of mice body weight that is comparable to silymarin at 50 mg/kg body weight of mice (Table 1). The extract also reduced bilirubin content near to normal level in mice as compared to silymarin. The ALT level in extract treated mice also got reduced significantly as compared to cancerous group of mice, however; the reduction was not much close to the normal level (Table 1). It might need higher dose of extract or prolong duration of treatment with FA extract.

The histopathology of tissue section from normal control mice exhibited normal cellular symmetry with a structural integrity, sinusoid space, and nucleus morphology and distribution (Fig. 1). DEN and CCl₄ treated mice liver had signs of liver cirrhosis and necrosis as depicted by

hemorrhagic cells, lack of cellular integrity and large sinusoidal spaces as well as disrupted nuclear morphology and cellular atrophy (shown in Fig. 1).

Table 1
Effect of F.carica based acetone extract and Silymarin on liver functional biomarkers of Balb-c male albino mice.

Groups	AFP(pg/ml)	BUN(nmol/L)	ALP(U/L)	AST(U/L)	ALT(U/L)
Negative control	328 ± 7.09	4.2 ± 0.4	84.8 ± 6.27	124.7 ± 5.50	45.3 ± 5.69
DEN treatment	408.7 ± 18.04	6.66 ± 2.5	193.7 ± 9.07	265.7 ± 27	171.33 ± 16
F.carica extract	331.33*±3.2	4 ± 1	130*±18.3	140*±5	78.7*±15.8
Silymarin	330*±2.08	5.5 ± 2.17	125*±5.03	155.7*±6	50*±6.8

P < 0.05 was considered as statistically significant value (n = 8) and depicted by * sign. The effect of extract and silymarin drug was compared with DEN cancer control group.

Upon daily treatment of DEN mice over a period of 60 days with FA and silymarin reversed the pathology to normal almost. The FA treatment started vascular proliferation and regeneration of tissue integrity gradually as shown in figure. It is in agreement with previous finding on methanolic extract of a Ficus species from India on the CCl4 induced toxicity in rat's liver. The curative effect of our extract is characterized by amelioration of liver functional enzymes as a result of rebuilding membranal integrity of hepatoma cell lines. This finding also agrees well with our in vitro findings where we have noticed in detail that FA prevented the apoptosis markers and downregulated cyclin dependent kinases (CDK) to inhibit cell death and necrosis. The Fig. 1 shows the infiltration of macrophages to remove cellular debris and regeneration of hepatocytes. The extract has also protected well the membrane integrity as we have seen in HepG2 cells previously, that is in agreement with current observation of membranal and cellular integrity, associated with downregulating the release of enzymatic biomarkers (listed in Table 1) from hepatocytes. The silymarin also downregulated these biomarkers very well by refraining their release into blood serum. The effect of silymarin on liver biomarkers has been studied in ICR male mice and our findings agree with the antioxidant potential of silymarin and FA extract on mice liver. The FA proves to be a potential herbal alternative to available chemotherapeutics, it needs further attention in terms of finding its bioactive compounds responsible for curing liver damage associated with chemical carcinogens e.g., DEN and CCl4.

In-silico Study of Plant Based Compounds and Silymarin

We analyzed the interaction among the active phenolics compounds in FA extract and the protein targets associated with human hepatocarcinoma. Thus, We performed docking simulations of the plant-based compounds with the given targets using PyRx and AutoDock Vina, following energy minimization using ChemDraw 3D. The results are as follows.

Table 2
Molecular docking simulation of active compounds in FA extract against key protein targets involved in hepatocarcinoma

Target Protein	Silymarin (FDA Approved Drug)	Top Plant-Based Compounds	Key Insights
EGFR	-8.6 kcal/mol	- Sitosterol: -8.1 kcal/mol - Rutin: -8.2 kcal/mol - Quercetin: -8.0 kcal/mol	Silymarin shows the strongest affinity, but sitosterol, rutin, and quercetin are competitive, indicating potential for drug development.
MMPs	-9.1 kcal/mol	- Luteolin: -9.9 kcal/mol - Apigenin: -9.2 kcal/mol - Quercetin: -9.5 kcal/mol	Plant-based compounds outperform Silymarin, especially luteolin and quercetin, suggesting stronger alternatives for MMP inhibition.
VEGFR	-6.2 kcal/mol	- Sitosterol: -7.2 kcal/mol	Sitosterol surpasses Silymarin in binding affinity, highlighting its potential for VEGFR-targeted drug development.

Our docking simulation results (Table 2) shows that, except for EGFR, our plant -based compounds outperform Silymarin. In case of EGFR though Silymarin exhibits the strongest binding affinity, but sitosterol, rutin, and quercetin demonstrate competitive binding. Our in-silico study suggests the provided plant based compounds as promising drug candidates and potential alternates for FDA approved drug i.e Silymarin.

Compounds ADMET Analysis

We shortlisted three compounds (Fig. 2), namely quercetin, sitosterol, and luteolin for further ADMET analysis. These compounds were shortlisted because quercetin showed better binding affinity in EGFR and MMP's, sitosterol showed promising binding affinity with VEGFR and EGFR. While luteolin has shown exceptional binding affinity with MMP's. So, we took these three compounds for further analysis. Following are the detailed comparative analysis of the shortlisted compounds with Silymarin.

Table 3
Lipinski's Rule of Five

Compound	Molecular Weight	LogP	H-Bond Donors	H-Bond Acceptors	Lipinski Violations
Quercetin	302.24 g/mol	1.23	5	7	0
Sitosterol	416.72 g/mol	7.09	1	1	3 (LogP > 5, MW > 500, Heteroatoms < 2)
Luteolin	286.24 g/mol	1.97	4	6	0
Silymarin	482.44 g/mol	1.59	4	9	1 (MW > 480)

Key:

Lipinski's Rule of Five (Fig. 3) was used to predict the drug-likeness of a compound. These predictions are based on the molecular properties. A compound is more likely to active orally if:

- Molecular weight ≤ 500 g/mol
- LogP ≤ 5
- Hydrogen bond donors ≤ 5
- Hydrogen bond acceptors ≤ 10
- No more than 1 violation.

Table 4
Physicochemical Properties

Compound	Molecular Weight	TPSA ($\text{\AA}^2$)	H-Bond Donors	H-Bond Acceptors	LogP (Consensus)	Solubility (Log S)
Quercetin	302.24 g/mol	131.38	5	7	1.23	-3.16 (Soluble)
Sitosterol	416.72 g/mol	20.23	1	1	7.09	-7.27 (Poorly soluble)
Luteolin	286.24 g/mol	110.28	4	6	1.97	-3.24 (Soluble)
Silymarin	482.44 g/mol	155.14	4	9	1.59	-4.14 (Moderately soluble)

Key:

Physicochemical properties are shown in Table 4. TPSA (Topological Polar Surface Area): Indicates the ability of a molecule for absorption by the body (lower TPSA improves oral bioavailability).

LogP

Measures lipophilicity; values above 5 suggest poor solubility and permeability issues.

Table 5
Other Key ADMET Properties

Property	Quercetin	Sitosterol	Luteolin	Silymarin (FDA Approved)
GI Absorption	High	Low	High	Low
Blood-Brain Barrier (BBB) Permeation	No	No	No	No
P-glycoprotein (P-gp) Substrate	No	No	No	No
CYP Inhibition	CYP1A2, CYP2C9, CYP2D6, CYP3A4	CYP2C9, CYP3A4	CYP1A2, CYP2C9, CYP2D6, CYP3A4	CYP2C19, CYP2C9, CYP3A4
Skin Permeation (Log Kp)	-7.05 cm/s	-2.93 cm/s	-6.45 cm/s	-7.89 cm/s
Bioavailability Score	0.55	0.55	0.55	0.55
PAINS (Pan Assay Interference Compounds) Alerts	1 (catechol)	0	0	0
Lead-likeness	3.23	5.38	1.91	4.45

Key:

Other Key ADMET Properties are shown in Table 5.

GI Absorption

Higher GI absorption indicates the improved potential for oral administration.

CYP Inhibition

Indicates the likelihood of interactions with metabolic enzymes, impacting drug metabolism.

Skin Permeation (Log Kp)

The more negative a value, the more poor skin permeation. It is relevant for topical drugs.

Bioavailability Score

A score of 0.55 indicates moderate bioavailability.

PAINS Alerts

Shows structural alerts that may cause false positives in biological assays.

ADMET of the Shortlisted Compounds

4. Discussion

Hepatocellular carcinoma (HCC) poses a significant mortality risk, ranking as the third leading cause of cancer-related death. Treatment options such as surgery, radio-frequency ablation, ethanol injection, and chemoembolization are available, but no pharmaceuticals exist to prevent or reduce tumor spread or recurrence, which significantly impacts prognosis and survival (Cao et al., 2020). HCC uniquely prohibits the use of traditional chemotherapeutic agents due to liver damage (Shariff et al., 2009). The limited understanding of HCC's pathogenesis hinders the identification of molecular targets for innovative therapies (Weis & Cheresch, 2011). In hepatocellular carcinoma (HCC), studies have shown that interactions between cancer cells and the surrounding tissue, primarily composed of extracellular matrix (ECM) proteins, growth factors, and proteolytic enzymes like matrix metalloproteases (MMPs), which accumulate due to liver cirrhosis, are crucial in influencing the cancer's various biological behaviors and clinical outcomes (Giannelli & Antonaci, 2006). The metabolic switch from premalignant to neoplastic tumor during metastasis is a complex process controlled by various interreceptors crosstalk between EGFR, MMPs and VEGFR and others (Elena & James, 2015). Tumor angiogenesis is one of the key steps involved in establishment of tumors for a transition from non-malignant to neoplastic stage. In this regards, the angiogenic switch has been regarded as one of hallmarks of cancers (Hanahan & Folkman, 1996). The angiogenic factor VEGF plays a critical role in angiogenesis, imported by MMPs into the tumor microenvironment. In xenograft mice models, the trapping or inhibition of VEGF generated by cancer cells resulted in substantial decline in tumor angiogenesis, associated with reduced tumor growth and ultimate progression to metastasis (Wang, Liu, Ren, Pan, & Zhang, 2008). The protein expression of both VEGF and MMP-2 and MMP-9 were found correlated, and this expressions is certainly linked to angiogenesis and metastasis in gastric cancer patients (Zheng et al., 2006). EGFR,

also referred to as ErbB1/HER1, is a 170 kDa transmembrane glycoprotein that belongs to a family of tyrosine kinase receptors (TKRs), which also includes ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4 [23]. These receptors share a common structure, consisting of an extracellular ligand-binding domain, a transmembrane domain, and a cytoplasmic domain that houses the tyrosine kinase region, followed by a carboxy-terminal tail containing tyrosine autophosphorylation sites (Berasain et al., 2011). EGFR is considered as an important signaling hub, where all proliferation and growth signals converge. EGFR cross talks with various other ligands and receptors in HCC and other cancer types. Its role and mutual interaction with other receptors need to be explored for therapeutic interventions, particularly the use of combined therapies in order to overcome the proliferation of cancer cells and to overcome the resistance to cancer cells (Berasain et al., 2011).

The major objectives of current study were to further explore the *in-vivo* anticancer potential of our *in-vitro* proven *F. carica* acetone-based extract in mice, followed by in-silico study to evaluate the drug likeness and potential of its active compounds against the key liver cancer target marker proteins EGFR, MMPs, and VEGFR. DEN induced carcinogenesis in the liver of mice as reported in previous studies (Sotty et al., 2024(Mustafa et al., 2021)). Previously, we found *F. carica* based acetone extract as the most effective antioxidants against HepG2 cells in lab with IC50 value of 0.157 mg/mL enough for 50% growth inhibition in these cells (Mustafa et al., 2021). We used exactly the same FA extract, enriched with phenolic compounds and flavonoids, to study the potential of this extract against liver injury biomarkers and liver histopathology. Our study is novel in terms of extraction method and further evaluation. we compared the efficacy of our extract with that of silymarin, a standard drug used as a hepatoprotective agent as this study is in line with the previous research where silymarin is also employed a standard hepatoprotective drug (Hira et al., 2021). This research explored the impacts of FA extract on the liver cirrhosis induced by DEN and CCl4, in comparison to the silymarin drug. The injected dose of DEN altered the biochemical markers, raised the level of AFP and liver functional enzymes, e.g., ALT, AST, and ALP and BUN content in the blood as these findings aligns with the previous results where CCl4 treatment causes prominent elevation in the level of the liver biomarker enzymes i.e. ALT, AST and ALP and total bilirubin (Hira et al., 2021). Similarly, according to the research performed by the S *et al.*, 2022 the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were raised in the certain mice intoxicated with CCl4. The biochemical and histological changes in necrotic liver tissues were overcome by FA extract comparable to that of silymarin drug. Silymarin drug has been associated with lipid accumulation within liver in past studies (El-Kot et al., 2023), while FA as a pure, novel herbal extract has proven sufficiently enough against liver injury associated biomarkers in our study. It means application of the FA extract on the treated mice notably lowers the level of biochemical markers associated with liver injury. These results are consistent with the previous in vitro research findings where it has been observed that the biochemical markers associated with liver fibrosis and necrosis were suppressed and cyclin dependent kinases (CDK) were downregulated by FA extract treatment, ultimately led to the restoration of hepatocarcinoma cell to normal physiology (Mustafa et al., 2021). Further, the amelioration of liver cancer biomarkers and tissue histology agrees with the previous studies on rats fed with methanolic extract of Ficus (S et al., 2022).

Moreover, we performed in-silico study to evaluate the effect of FA extract derived active metabolites that we already reported in our in-vitro study via HPLC, against the key target proteins associated with hepatocarcinoma e.g., EGFR, MMPs, and VEGFR. Based on our in-silico study, we come to the conclusion that the suggested plant-based compounds are strong candidates for drug development. Where out of many, five compounds stood out with strong binding affinities relative to Silymarin (FDA approved drug). Based on the Venn diagram (Fig. 1) Quercetin, Sitosterol, and Luteolin were selected for further analysis.

To finally check the status of our shortlisted candidates as promising drug candidates, we analyzed their ADMET using SWISSADME which further confirmed the drug-likeness of the two of the shortlisted plant-based compounds. Sitosterol failed the ADMET because of multiple Lipinski violations and poor solubility. After the complete analyses, Quercetin and Luteolin are the promising candidates for further study and experimentation.

5. Conclusion

We found the hepatoprotective role of *F. carica* based acetone extract against DEN and CCl4 induced hepatocarcinoma in Balb-c albino mice. The reversal changes in liver morphology to almost normal recovery phase was comparable to that of silymarin drug. Silymarin is a standard hepatoprotective drug associated with hepatoblastoma regeneration and membrane integrity. The potential efficacy of *F. carica* based acetone extract at 60mg/kg of b.wt of mice is comparable to that of standard silymarin drug, and even it better reduced the BUN content and AST enzyme level in the blood serum of mice, leading to a strategy, where we can use FA extract or its extracted bioactive components against the hepatocarcinoma in pre-clinical studies. The future studies are directed to focus on the role of fractionated bioactive compounds on liver cancer biomarkers *in-vitro* and *in-vivo* as well. Our in-silico study identifies quercetin, luteolin, and sitosterol as promising compounds for HCC therapy, warranting further dynamic simulation, pharmacophore modelling and other required computational analysis to reinforce this study. The competitive binding affinities and favourable ADMET infer the potential for plant-derived drug development with better therapeutic and fewer to no side effects for treating liver cancer.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Conception and research design: Kiren Mustafa; Experimentation: Kiren Mustafa and Tang qi; Analysis of data, assembly of results, and Data curation: Kiren Mustafa, Sania Zaib and Hassan Khan Nasir; Final drafting of manuscript: Sania Zaib and Karishma; Provision of equipment and laboratory facilities: Prof Yuanda Song and Prof Zhihe Li.

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Figures

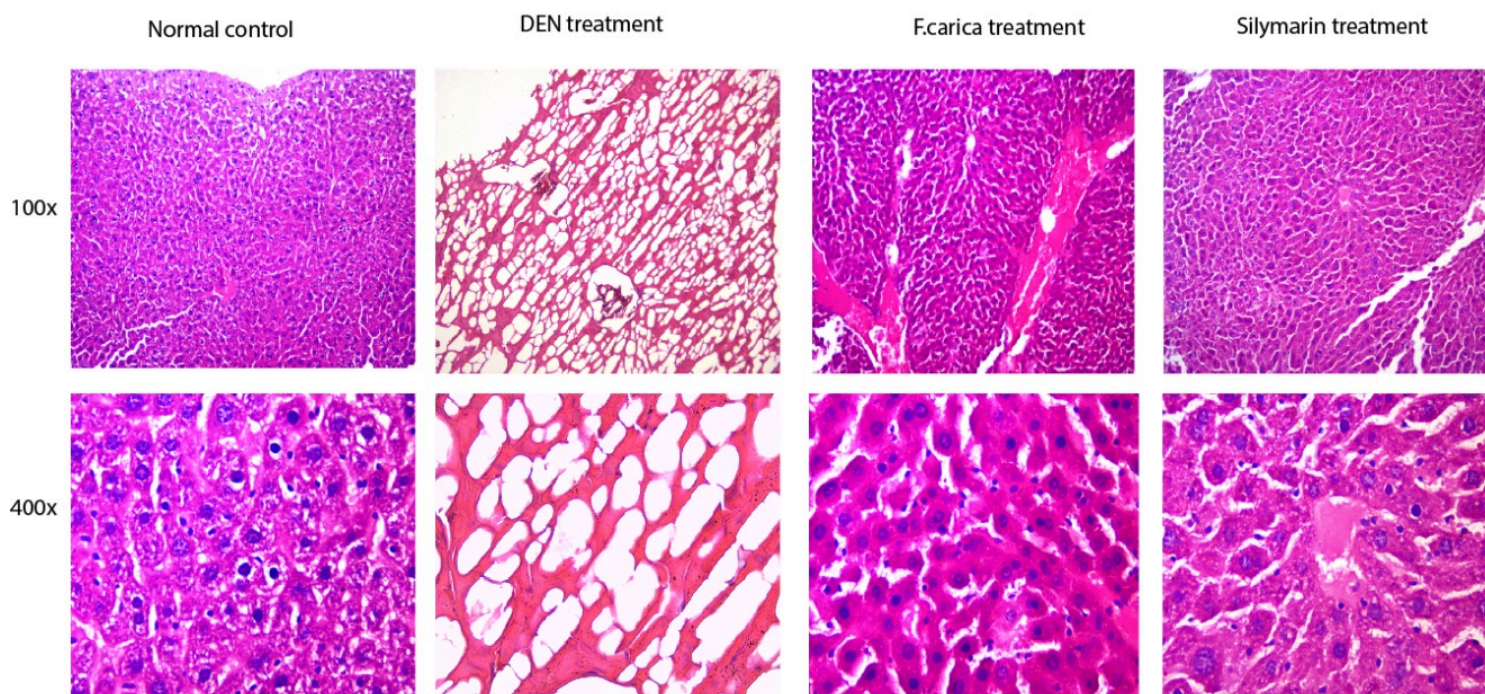


Figure 1

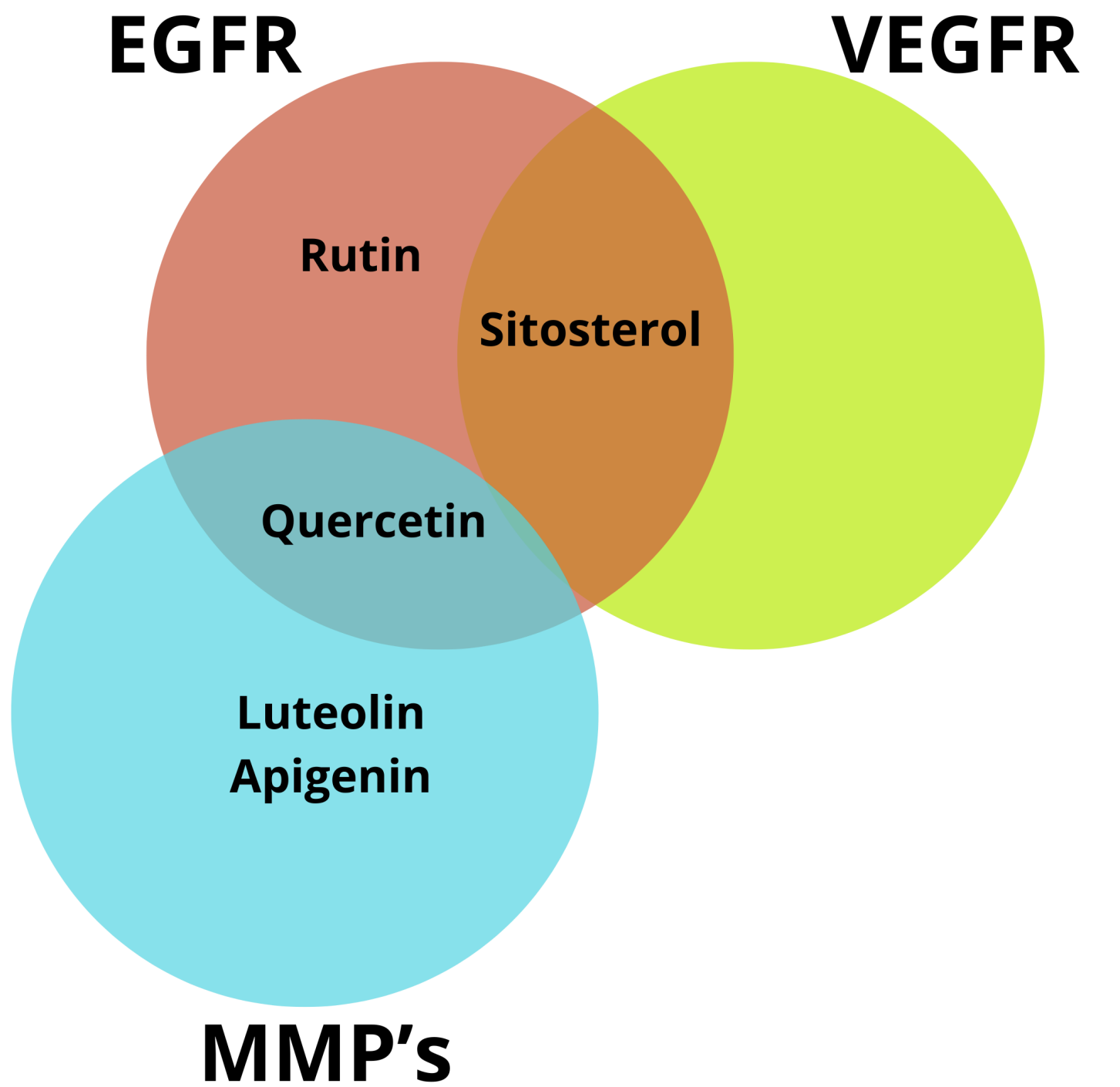


Figure 2
Venn diagram showing shortlisted compounds.

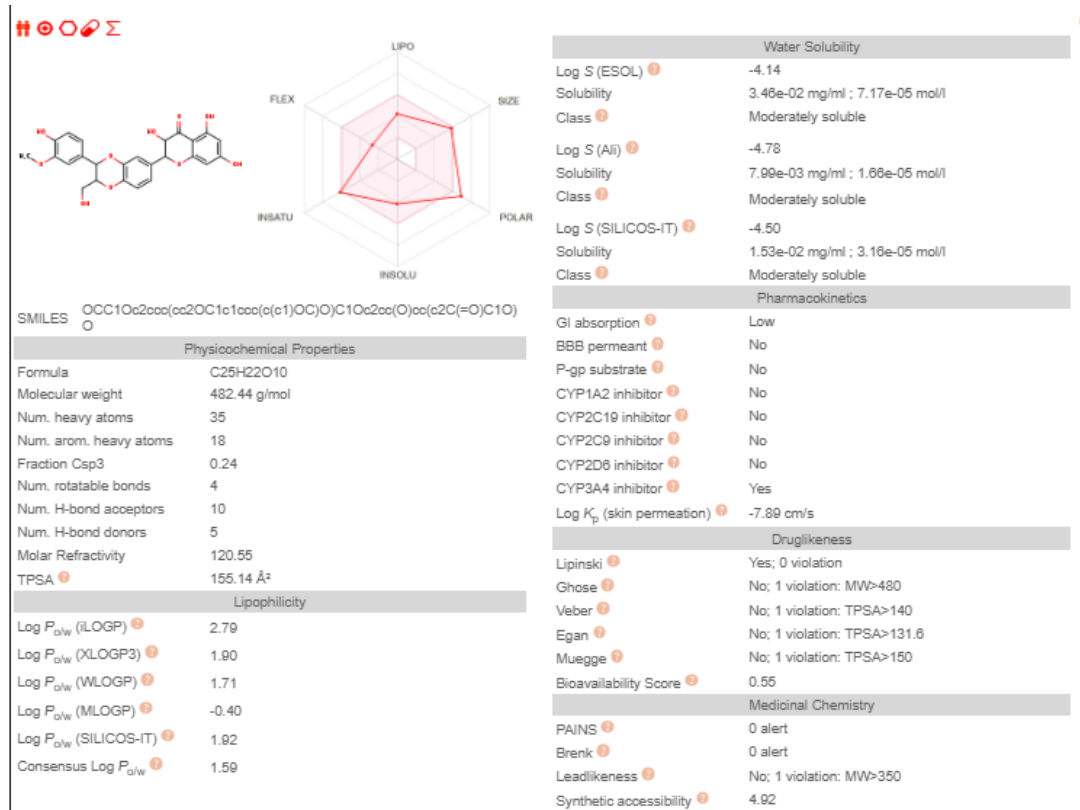


Figure 3

ADMET analysis of Silymarin

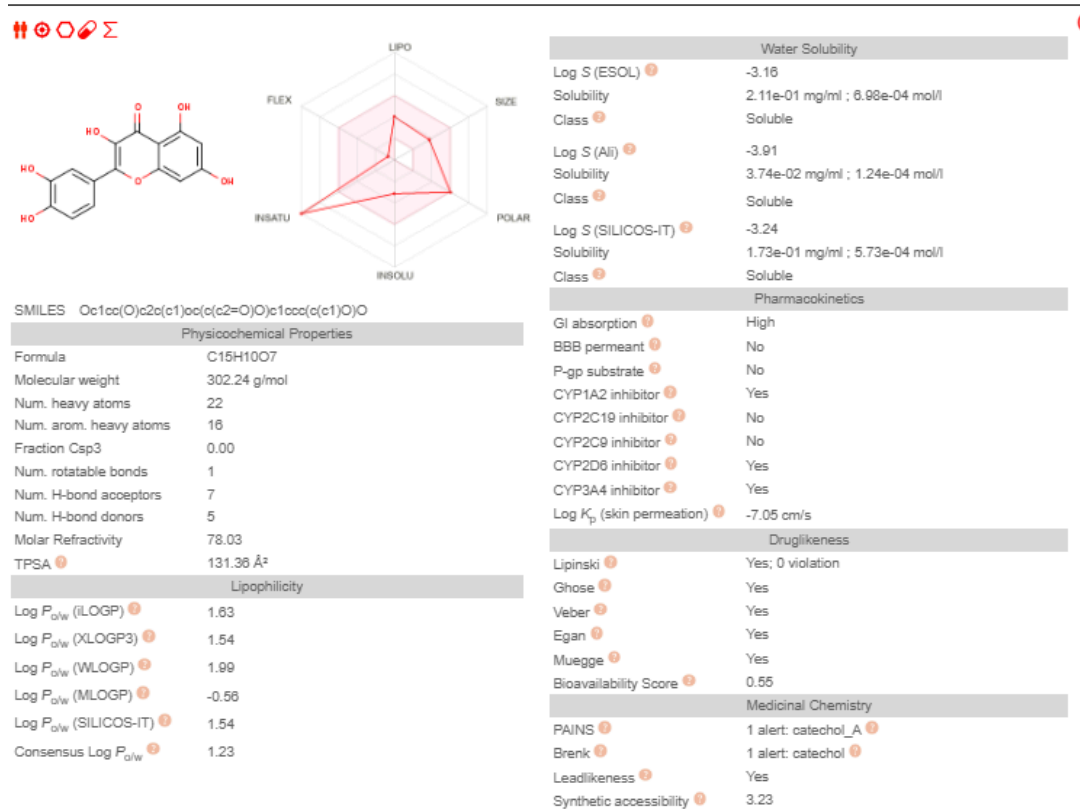


Figure 4

ADMET analysis of Quercetin

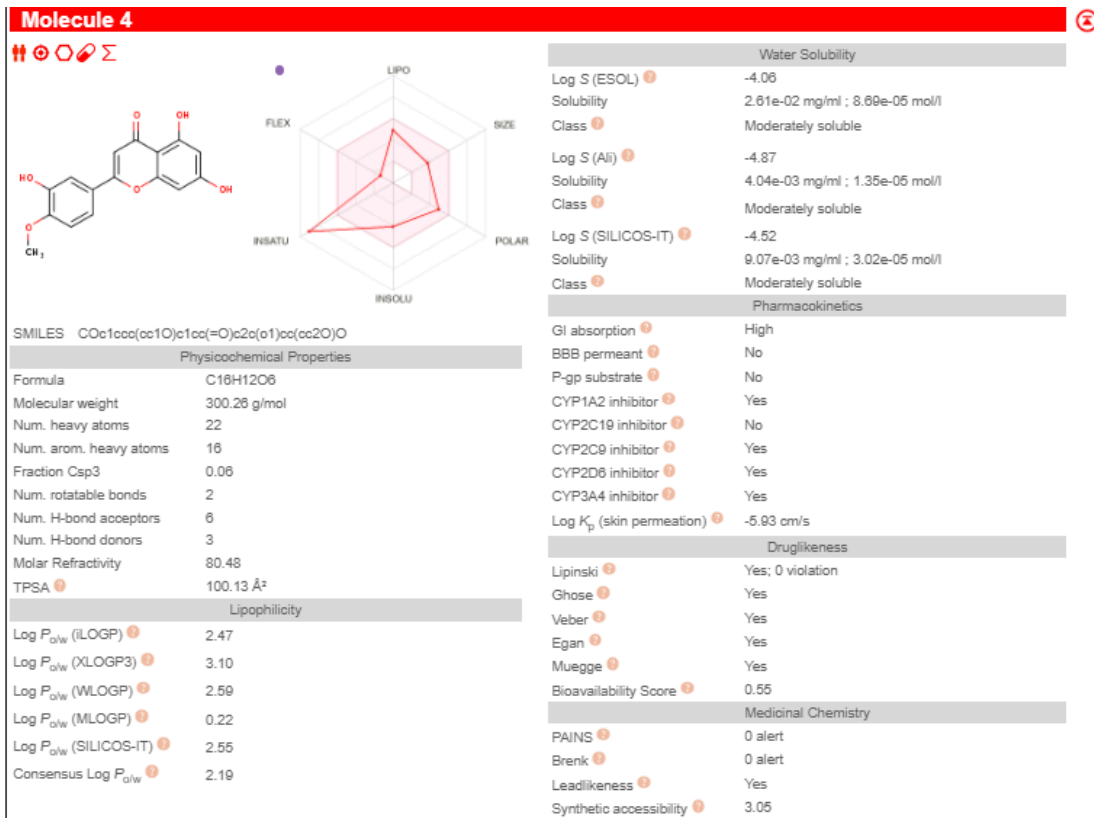


Figure 5

ADMET analysis of Luteolin

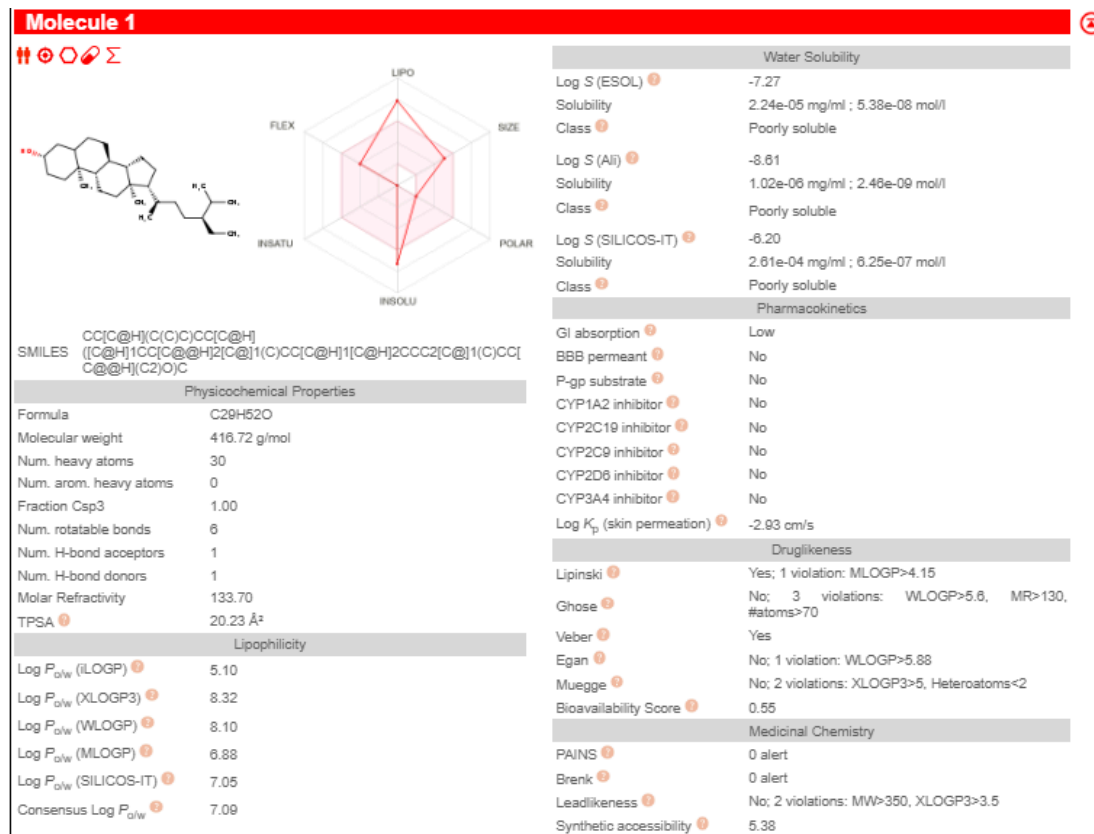


Figure 6

Figure 7

