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Phytochemical, anti-hyperlipidemic and *in silico* studies of extracts of *Ficus carica* seeds

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Abstract: Hyperlipidemia has been considered a disease primarily causing death along with other prevailing diseases such as coronary heart diseases, atherosclerosis and stroke. The present study aims to evaluate the anti-hyperlipidemic potential of *Ficus carica*. Extracts of seeds of *Ficus carica* were investigated for bioactive compounds and screened using *in-vitro* and *in-vivo* anti-hyperlipidemic activities. Fig seeds showed potential *in-vitro* by inhibition of pancreatic lipase while i*n-vivo* study revealed that methanol extract of fig seeds exhibited the anti-hyperlipidemic property by beneficially modifying lipid profile of albino mice comparable to standard drug. GC-MS analysis of methanol extract of seeds of *Ficus carica* exhibited a variety of bioactive compounds. After further evaluation of ligands for their activity by using molecular docking and MM-GBSA study, it is concluded that 1,2,3-benzentriol has the highest binding affinity for pancreatic lipase enzyme. Hence, it is concluded that seeds of *Ficus carica* are medicinally important and have promising anti-hyperlipidemic potential.

Keywords: *Ficus carica*, medicinal plants, anti-hyperlipidemic activity, GC-MS analysis, molecular docking, molecular mechanics-generalized born and surface area solvation.

INTRODUCTION

The medicinal plants being enriched with such ingredients and compounds which can be used in drug development and synthesis Medicinal plants play important role in treatment and cure of many diseases (Rakotoarivelo *et al.,* 2019). Greater than 13,000 species of plants have been extensively used as traditional medicines around the whole world. Since the beginning of twenty first century, trend of new drug discovery has returned to herbal plants. There is an increase in use of herbal remedies due to their cost effectiveness, ease of availability and lesser side effects as compared to synthetic drugs (Yatoo *et al.,* 2018). Roots of traditional systems of medicines such as Chinese, Allopathic and Homeopathic, Unani and Ayurveda emerged from these medicinal plants. Regardless of extensive progress in the synthetic drug discovery, natural plants are still the largest source of medicines. Many plants containing bioactive chemical constituents possess valuable supplemental therapies when used carefully (Suntar, 2020). Current scientific researchers have upgraded their strategies in favor of natural products drug discovery.

Herbal plants, as these owe significant quantity of phytochemicals including proteins, glycosaponins, alkaloids, tannins polyphenols and flavonoids, are used to treat a number of ailments in human (Arshad and Ishtiaq, 2019). The cure of many diseases such as cancer, hypoglycemia, osteoporosis, cardiovascular diseases, central nervous systems disorders and obesity is dependent on such phytochemical constituents present in medicinal plants (Sachdeva *et al*., 2020). In the Essential drug list of 252 drugs enlisted by World Health Organization (WHO), 11% are of specifically originated by plants whereas synthetic drugs obtained from natural precursors occupy a significant number in the list (Barkat *et al.,* 2021).

The severity and prevalence of heart diseases may be increased due to many factors including hyperlipidemia (Powell-willey, 2020). Nowadays, hyperlipidemia has also been considered a disease which is primarily causing death along with other prevailing diseases such as coronary heart diseases, atherosclerosis and stroke (Jain *et al*., 2018). It is a condition characterized by elevated serum cholesterol levels, low density lipoproteins (LDL), very low-density lipoproteins (VLDL) and reduced levels of high-density lipoproteins (HDL). As hyperlipidemia causes lipid disorders due to which it is considered a major cause of atherosclerosis and cardiovascular diseases. The goal of treatment for hyperlipidemia is to reduce the development and occurrence of ischemic heart diseases and cardiovascular diseases respectively. The available anti-hyperlipidemic drugs are associated with many side effects, along with their therapeutic effect, including development of hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver functions (Mazumder *et al.,* 2021). Depending upon the severity of the disease, hyperlipidemia has been classified into two basic types: a primary type and a secondary type. The treatment of primary disease is dependent upon the anti-hyperlipidemic drugs whereas, in the secondary type, the treatment of original diseases like diabetes, hypothyroidism and renal nephritis is mandatory rather

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than treating hyperlipidemia (Stewart *et al*., 2020). Consuming a large amount of fatty foods leads to high levels of VLDL which results in the deposition of large amounts of low density lipoproteins (LDL) to walls of the blood vessels. If the amount of high density lipoproteins (HDL) is insufficient, it may lead to blockage of normal blood flow. Hence, prevention of hyperlipidemia requires improvement in human diet.

Medicinal plants also play a significant role in reducing the serum lipid levels. Minimum of 13000 plants have been evaluated for their pharmacological properties. The treatment for hyperlipidemia and hypercholesterolemia by using herbal and medicinal plants has limited number of side effects, relatively cost effective and can be easily available. Natural lipase inhibitors such as saponins, terpenes, phenols carotenoids and polysaccharides present in medicinal plants can be used against hyperlipidemia (Bejes *et al*., 2022). The lipid lowering action of many medicinal plants is due to the inhibition of hepatic cholesterol biosynthesis, HMG-COA and pancreatic lipase which results into net reduction of lipid absorption in the intestine (Al-Snafi, 2022). Isolated extracts of foodstuffs such as tea, soybean, ginseng, yerba mate, peanut, apple, or grapevine have significant lipase inhibitory activity (Vadivel *et al.*, 2018).

In current study, polar and non-polar extracts of seeds of *Ficus carica* have been analyzed for *in-vitro* and *in-vivo* anti-hyperlipidemic potential. The aim of this study is to identify the compounds in seeds of *Ficus carica* possessing anti-hyperlipidemic activity using extraction, GC-MS analysis, computer aided molecular docking and MM-GBSA technique.

MATERIALS AND METHODS

Materials

HPLC and analytical grade reagents and chemicals including petroleum ether, chloroform, methanol and ethanol were used. Aluminum chloride, Gallic acid, quercetin, atorvastatin (Merck, Germany), Orlistat (Merck, Germany), sodium carbonate, hydrate sodium acetate, Folin-Ciocalteu (FC) reagent, bovine serum albumin (BSA) (Sigma), Sodium cholate (Merck, Germany), cholesterol (Merck, Germany), 4-methyl umbelliferyl oleate (4-MU oleate) (Chem-Imprex International) lipase from procaine pancreas 200-400U/mg (type II) (Sigma Aldrich), sodium cholate and coconut oil were used.

Collection, identification, pulverization of seeds

The fruit of *Ficus carica* was purchased from local market, shah alam, Lahore, Pakistan. It was subjected to authentication from the Department of Botany, Government College University (GCU), Lahore, Pakistan. Voucher number GC.HERB.BOT.3959 was allocated to fruit of *Ficus carica* by GCU herbarium, Lahore. Fruit was soaked in water overnight and seeds were separated from pulp through sieving, washed with water and shade dried. Dried seeds were crushed and pulverized. Powdered seeds were stored in an airtight container at room temperature.

Preparation of seed extracts

Extraction of seeds was performed using sequential hot extraction method in Soxhlet extractor (Pyrex, USA). Solvents of different polarities i.e. petroleum ether, chloroform and methanol were used. The powdered seeds were also subjected to cold extraction using ethanol and water (Nortjie *et al*., 2022). All the extracts were concentrated using rotary vacuum evaporator (HEI – VAP series, Heidolph, Germany). Percentage yield (%) of all extracts was calculated and the extracts were stored at $25^{o}C$ for analysis.

Phytochemical analysis of fig seed extracts

Ficus carica seeds extracts were screened for bioactive compounds. Primary and secondary metabolites quantification was performed by methods as described in the standard protocols. The analysis for primary metabolites including carbohydrates, proteins, lipids (Latif *et al.*, 2020) and secondary metabolites including flavonoids, polyphenols (Abid *et al.*, 2022) and total glycosaponins (Latif *et al.*, 2020) were performed on seeds extracts of *Ficus carica*

Estimation of total protein contents

Protein contents in fig seeds extracts were estimated as described as described by Latif and his coworkers (Latif *et al.*, 2020) with slight modifications. All reagents were freshly prepared. 0.1M sodium hydroxide was mixed with 2% w/v of sodium carbonate to prepare solution-A, while Solution-B was prepared by mixing 1.56 % w/v copper sulfate with 1% potassium sodium tartrate. The analytical reagent named as Solution-C was prepared by mixing 100mL of Solution-A with 2mL of Solution-B. Standard solution of Bovine serum albumin (BSA) was prepared as 1mg/mL. 1.0mg/mL solution of all seeds extracts of *Ficus carica* were prepared using distilled water. Each test and standard solution was vortexed and centrifuged at 2700rpm for 10 minutes. supernatant 0.1mL was collected from each solution and made up to 1mL with distilled water followed by the addition of 3mL of Solution-C in each of the test tubes. All reaction mixtures were incubated for 10 minutes at $25^{o}C$. After incubation, 200µL of folin-ciocalteu's (FC) reagent was added to each test solution. All reaction mixtures were incubated again for 30 minutes at $25^{o}C$. Absorbance of test samples and standard solutions against blank was measured at wavelength 600nm using spectrophotometer. Different concentrations of standard BSA ranging from 25µg/mL to 1000µg/mL were prepared to obtain calibration curve.

Estimation of total Lipids

For the determination of lipid contents in fig seeds extracts, 65g of powdered seeds were macerated and extracted with n-hexane. The extract was dried at room temperature after evaporation of solvent by rotary evaporator (Latif *et al*., 2020).

Total phenolic contents (TPC)

Total polyphenolic contents in seeds extracts of *Ficus carica* were quantified by using spectrophotometric method (Abid *et al*., 2022). Standard stock solution of Gallic acid was prepared as 1mg/mL using methanol as a solvent. Dilutions from stock solution ranging from 10 to 400μg/mL were prepared in methanol. Solutions having concentration of 1mg/mL of different extracts were also prepared in methanol.200μL each of extract, methanol (as blank) and standard solutions were taken in test tubes along with 200μL of folin ciocalteu (FC) reagent followed by addition of 4 mL of distilled water in each reaction tube and incubated at 25^{o}C for 10 minutes. After incubation, 1mL of sodium carbonate (15% w/v) was added. All the reaction mixtures were again incubated at 25^{o}C for 60 minutes. Absorbance of each solution was measured against blank at wavelength 765nm by using spectrophotometer. TPC was expressed as milligram (mg) equivalents of standard Gallic acid per gram of dry matter (mg GA/g). Polyphenolic concentration in different extracts of seeds of *Ficus carica* was determined from standard Gallic acid curve.

Total flavonoid contents (TFC)

Aluminium Chloride colorimetric method (Abid *et al*., 2022) with slight modifications was used for the determination of total flavonoid contents (TFC) in different seeds extracts. Standard reference quercetin was used to prepare calibration curve. Solution of 5mg of quercetin in 1mL of methanol was prepared as stock. Different solutions having concentration of quercetin ranging from 10μg/mL to 120μg/mL were prepared from stock solution. Solution having concentration of 1mg/mL in methanol were prepared from each extract. 200μL of each standard solution and extract solution was mixed with 100μL of 10% aluminum chloride solution.100μL of 1M sodium acetate solution along with 900μL of methanol was added in each solution. The volume was made up to 3mL with distilled water. All reaction mixtures were incubated at 25 C^{o} for 45 minutes after thorough mixing. Absorbance of each reaction solution against methanol as a blank was measured at wavelength 415nm by using spectrophotometer. Total flavonoid content in each extract was determined from the curve of standard quercetin and expressed as equivalent of mg quercetin (QE)/g.

Estimation of total glycosaponins

One gram of each seeds extract was refluxed with methanol 50mL for 30min, repeated twice, pooled, evaporated to volume of 10mL and mixed with acetone 50mL (Latif *et al*., 2020).

Anti-hyperlipidemic activity

Anti-lipase assay

All seeds extracts of *Ficus carica* were investigated for their anti-hyperlipidemic potential using *in-vitro* model of lipase inhibition. The assay was carried out using method described by Zhang (Zhang *et al*., 2010) with slight modifications. Solutions having concentrations of 1mg/mL in methanol were prepared from each extract. Orlistat standard solution was prepared as 1mg/mL in methanol. The reaction mixture was prepared using 25µL sample and standard solution with 25 µL of 16.7 U/mL lipase in Tris-HCl, pH 8.0 buffer solution followed by the addition of 50µL of 0.1M 4-methyl umbelliferyl oleate (4-MU oleate) in Tris-HCl, pH 8.0 buffer solution into reaction mixture. Incubation was carried out at 37^{o}C for 30min. The release rate of 4-methylumbeliferone product formed in control, standard and sample solutions was measured using plate reader (RT-6000) with excitation wavelength of 355nm. Percentage of lipase inhibition was calculated by the formula mentioned below:

$$\%\,\text{inhibition} = 1 - \frac{\text{absorbanceofcontrol - absorbance of sample}}{\text{absorbanceofcontrol}} \text{x100}$$

Anti-hyperlipidemic activity in mice

Male albino mice weighing between 30-40g were acclimatized to laboratory environmental conditions having controlled temperature near to 25°C and controlled humidity. Animals were caged and grouped into four, each group with six (06) mice: Group A: Mice fed with normal diet.

Group B: Control: having high fat diet. Group C: Animals with high fat diet treated with standard drug (Atorvastatin). Group D: Animals with high fat diet treated with methanol fig seed extract as descried in (Rachh *et al.,* 2010).

Hyperlipidemic induction to animals

Animals were induced hyperlipidemia by feeding them high fat diet: prepared by mixing cholesterol 2%, coconut oil 2%, sodium cholate 1% with standard animal food. Animals were fed with diet carefully for seven days.

Administration of standard drug and seed extract

Atorvastatin administered at the dose of 10mg/kg, prepared in 0.5%methylcellulose aqueous solution. The seed extract was dissolved in distilled water and administered at the dose of 200mg/kg once daily, orally along with high fat diet for seven days.

Plasma analysis

After the completion of experiment, blood samples were collected under anesthesia and allowed to clot at room temperature. The samples were centrifuged at 2500 rpm for 30min. Serum was separated and stored in refrigerator until biochemical estimations have been performed.Total cholesterol (TC), triglyceride (TG), high density lipoproteins (HDL) were estimated.

STATISTICAL ANALYSIS

Results were statistically analyzed using one-way ANOVA followed by Tukey's Post-hoc Test with p-value ($p<0.05$) was considered significant. Standard error was calculated. Microsoft Excel Office Professional Plus 2016 was used for statistical analysis conducted on study.

Histological analysis

After sacrifice, the liver of mice was removed and weighed. The samples of liver were fixed with 10% formalin solution. The sections were prepared, stained with hematoxylin and eosin and were microscopically evaluated (Chu *et al.,* 2021).

Gas Chromatography-Mass Spectrophotometer (GC-MS) analysis of Ficus carica seeds extract

Methanol seeds extract of *Ficus carica* was subjected to GC-MS analysis for screening of compounds possessing anti-hyperlipidemic potential. The extract was dissolved in methanol for this purpose. Capillary tube having dimensions of 15m* 0.25 nm with film coating of 0.25µm was used. The flow rate of helium as a carrier gas was 0.25mL/min. The column pressure was 1psi and volume of the injected sample was 4µL. Temperature of oven was maintained at 100^{o}C for 0.5 minutes. Temperature was ramped gradually at rate 10^{o}C for 15min until desired temperature of 200°C was achieved. At the end, temperature was elevated to 320°C for 30min. The GC-MS chromatogram showed various peaks of different compounds present in methanol seeds extract of *Ficus carica*. The interpretation of such peaks was performed by comparing the peaks in the database of National Institute of Standards and Technology NIST20 having minimum quality factor of 80.

In silico studies

Molecular docking

Analysis of methanol extract of fig seeds through GC-MS provided seven ligands for molecular docking studies using Glide with default parameters available in Maestro v12.3 (Glide, Schrodinger, LLC, 2020, NY, USA). The selection of ligands was based on the pharmacological activities and significance of peaks obtained through GC-MS analysis. Structure of the protein pancreatic lipase (1LPB) was retrieved from Protein data bank (PDB: 4JK4) and protein was prepared using the protein preparation wizard available in Maestro v12.3. The missing residues were added and all ligands were removed. Structures of ligands were drawn using Maestro v12.3. Ligand structures were positioned around active site of the protein. Using Glide software in Maestro, molecular docking (XP) calculations were performed. For each ligand, multiple poses were attained after the docking process. (Imtiaz *et al.*, 2022). The above study was helpful regarding understanding the mechanism of interaction of ligand with protein pancreatic lipase.

Prime Molecular Mechanics-Generalized Born and Surface Area Solvation (MM-GBSA)

For each ligand docked complex, Prime MM-GBSA was applied using following equation for calculation of binding energy

$$\Delta Gbind = \Delta EMM + \Delta Gsolv + \Delta GSA$$

Where; ΔEMM is the difference in minimized energy between the 1LPB-inhibitor complex and sum of the energies of unliganded 1LPB and the ligands. ΔGsolv is the difference in MM-GBSA. ΔGSA is the difference in the surface area energies for complex and the sum of the surface area energies for 1LPB and ligand considered as individually. MM-GBSA analysis was performed using OPLS 2005 force field on the molecules that showed maximum docking score using Schrodinger Maestro Suite.

Ethical approval

The Punjab University Institutional Ethics Review Board approved the study for animal experiments and issued the voucher number D/34/FIMS.

RESULTS

Yeilds and phytochemical analysis

The amount of various extracts of seeds of *Ficus carica* was calculated in terms of percentage yield. After extraction process, the extracts obtained were dried and weighed. Calculated yields of petroleum ether, chloroform, methanolic, ethanolic, aqueous extracts were found to be 3.93g, 10.61g, 20.04g, 30.45g and 9.35g respectively.

Seeds extracts of *Ficus carica* were evaluated in detail for phytochemical analysis which revealed the presence of different primary and secondary metabolites. Carbohydrates, lipid and protein contents in powdered seeds were found to be 32.97%, 27.52% and 30.0% with relative standard deviation (RSD) of less than 3%. Aqueous extract contained high amount of proteins and glycosaponins. High amount of flavonoids and phenols were present in methanol seed extract. Evaluation of seed extracts with standard Bovine serum albumin showed maximum concentration of protein with value of 80.25 ± 3.32% in aqueous seed extract. Maximum concentration of TPC was quantified in methanol extract with value of 66.78±5.05mg of Gallic acid equivalent per gram of extract (mg GAE/g). Highest concentration of TFC was found in the methanol extract 114.51±10.46 mg QE/g extract) while the aqueous extract showed highest concentration of glycosaponins 49.35±1.62%.

Table 1: Phytochemical contents of seed extracts of *Ficus carica*

Extracts	Total Protein	Total Polyphenols	Total Flavonoids	Total Glycosaponins
Pet. ether	7.22 ± 4.64	35.60 ± 3.97	43.69 ± 7.49	3.93 ± 1.09
Chloroform	13.81 ± 3.72	42.97 ± 3.88	62.90 ± 7.67	10.61 ± 1.78
Methanol	20.51 ± 6.43	66.78 ± 5.05	114.51 ± 10.46	20.04 ± 1.90
Ethanol	32.79 ± 1.56	50.96 ± 4.47	90.27 ± 8.00	10.45 ± 1.96
Aqueous	80.25 ± 3.32	21.61 ± 5.19	12.86 ± 8.19	49.35 ± 1.62

Table 2: Inhibition of Lipase

Lipase Inhibition of seeds extracts of *Ficus carica*		
Sample	Absorbance	% Lipase Inhibition
Petroleum ether	0.046 ± 0.002	8.28
Chloroform	0.140 ± 0.019	25.77
Methanol	0.335 ± 0.001	61.93
Ethanol	0.194 ± 0.003	35.66
Aqueous	0.224 ± 0.004	41.29

*Control = 0.539 ± 0.002

Table 3: Comparison of lipid profile of groups of mice and liver weight

	Parameters of lipid profile			
Groups	TC (mg/dL)	HDL (mg/dL)	TG (mg/dL)	Liver weight (g)
A	71.7 ± 2.33	38.7 ± 1.71	89.0 ± 1.79	1.81 ± 0.02
B	126.5 ± 2.22	74.3 ± 1.43	106.7 ± 1.80	1.85 ± 0.01
C	104.3 ± 1.90	60.0 ± 1.49	99.8 ± 1.88	1.84 ± 0.02
D	108.0 ± 1.83[c]	65.8 ± 1.46[b]	97.0 ± 1.11[a]	1.85 ± 0.02

* (a $p < 0.05$; b $p < 0.01$; c $p < 0.001$, vs. control) Results were represented as mean ±SEM (n = 6).

Table 4: Important compounds in GC-MS profile of methanol extract of seeds of *Ficus carica*

Sr. No.	RT(min)	Compound Name	Formula	Molecular weight	Area %
1.	6.454	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	0.02
2.	17.552	9,12,15-Octadecatrien-1-ol	$C18H_{32}O_3$	264.40	0.02
3.	19.286	Cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{30}O$	302.45	0.01
4.	21.600	Heneicosapentaenoic acid methyl ester	$C_{22}H_{34}O_2$	333.52	0.03
5.	24.736	Gamma Tocopherol	$C_{28}H_{48}O_2$	416.7	1.18
6.	24.736	Beta Tocopherol	$C_{28}H_{48}O_2$	416.7	1.18
7.	26.530	Gamma Sitosterol	$C_{29}H_{50}O$	414.7	1.24

Table 5: Reported activity of compounds identified in methanol fig seeds extract by GC-MS.

Compound Name	Category	Pharmacological actions
1,2,3-Benzenetriol	Pyrogallol	Antiseptic, antioxidant, antidermatitic, fungicide and insecticide (Mohammad *et al*., 2019).
9,12,15-Octadecatrien-1-ol	Fatty acid alcohol	Anti-oxidant and antibacterial (Olivia and Obinna, 2021).
Cis-5,8,11,14,17-Eicosapentaenoic acid	Fatty acid	Anti-clastogenic, (Sasaki *et al*.,1994). Anti-platelet aggregation (Gryglewski, *et al*., 1979).
Heneicosapentaenoic acid methyl ester	Fatty acid	Anti-microbial (Alsenani, *et al.,* 2020).
Gamma Tocopherol	Methylated phenol	Antidermatitic, anticancer, hepato-protective, antioxidant and antispasmodic (Abraham, *et al*., 2019).
Beta Tocopherol	Methylated phenol	Antioxidant and anti-inflammatory potential (Abraham, *et al*., 2019).
Gamma Sitosterol	phytosterols	Anti-hyperlipidemic and anti-diabetic (Al-Snai, 2019)

Table 6: Molecular docking profile of important biologically active ligands in methanol extract

Ligands	Docking score	XP GScore	Glide ligand efficiency	Glide energy	Glide emodel
Orlistat (Reference)	-5.273	-5.273	-0.151	-52.014	-68.751
1,2,3-Benzenetriol	-6.453	-6.459	-0.717	-20.561	-26.679
Gamma tocopherol	-6.316	-6.316	-0.211	35.141	-49.373
Gamma sitosterol	-6.235	-6.325	-0.208	-30.828	-30.523
Beta tocopherol	-6.014	-6.014	-0.200	-37.770	-46.678
5,8,11,14,17 eicosapentaenoic acid	-5.737	-5.737	-0.249	-39.730	-49.439
Heniecosapentaenoic acid methyl ester	-5.256	-5.256	-0.219	-31.287	-37.920
9,12,15 octadecatrien-1-ol	-4.469	-4.469	-0.235	-25.235	-30.513

Table 7: Prime MM-GBSA and relative binding energies of various ligands (Kcal/mol)

Ligands	MMGBSA-dG-binding energy	MMGBSA-dG-bind (NS)	MMGBSA-dG-Bind coulomb	MMGBSA-dG Bind (NS)-coulomb
1,2,3-Benzenetriol	-21.19	-24.16	-13.45	-16.10
Gamma tocopherol	-76.95	-83.67	-12.93	-12.32
Gamma sitosterol	-72.21	-90.16	-3.79	-3.86
Beta tocopherol	-74.79	-82.05	-9.82	-9.40
5,8,11,14,17 eicosapentaenoic acid	-67.91	-89.62	24.09	-25.50
Heniecosapentaenoic acid methyl ester	-62.84	-88.99	-7.34	-6.60
9,12,15 octadecatrien-1-ol	-58.82	-70.53	-4.59	-4.78

Anti-hyperlipidemic activity

Measurement of lipase inhibition

Seed extracts of *Ficus carica* presented a significant inhibition of enzyme lipase.

The highest inhibition was observed in methanol extract (61.93%) and lowest in petroleum ether extract (8.28%). The order of lipase inhibition potential is methanol> aqueous >ethanol >chloroform >petroleum ether extract.

Anti-hyperlipidemic effect in mice fed with diet containing seed extract.

To evaluate anti-hyperlipidemic potential of methanol seed extract of *Ficus carica*, the serum lipid profile of mice from each group was characterized for total cholesterol (TC), high density lipoproteins (HDL), and triglycerides (TG) after the completion of experiment. Serum lipid profile was significantly modified in the groups fed with high fat diet (table 3). The serum lipid profile of mice fed with standard was comparable with that of the mice fed with methanol extract.

Liver histology

To determine the possible effects of diets on mice liver, each liver was weighed and processed for histological examination. There was no significant difference in the weights of the liver as mentioned in table 2 while changes in liver histology could be noticed (fig. 1). It was observed that mice fed with hyperlipidemic diet had modified hepatic cells filled containing fats (steatosis), while that of the mice fed with diet containing standard drug and extract have comparable illustrations.

Identification of compounds by GC-MS analysis

GC-MS analysis possesses its distinctive ability to identify, determine and authenticate the desired compounds. Hence, the most potent seed extract of *Ficus carica* exhibiting anti-hyperlipidemic potential was subjected to GC-MS analysis. The results have showed that the methanol extract possess variety of compounds including polar and non-polar. National Institute of Standard and Technology (NIST) library was used to identify the compounds present in methanol seed extract. (fig. 2.) Different peaks obtained for a variety of compounds were interpreted through comparison with the database of NIST. This analysis of seed extract of *Ficus carica* revealed the presence of hundred compounds.

Identified compounds having similarity index 80% or more were evaluated including 1,2,3, benzentriol, gamma tocopherol, gamma sitosterol, beta tocopherol, 5,8,11,14,17 eicosapentanoic acid, heiecosaptenoic acid and 9,12,15 octadecatrein-1-ol. GC-MS analysis data of such compounds is mentioned below including retention time (RT), name of identified compound, molecular formula, molecular weight and area percent (%) (table 4).

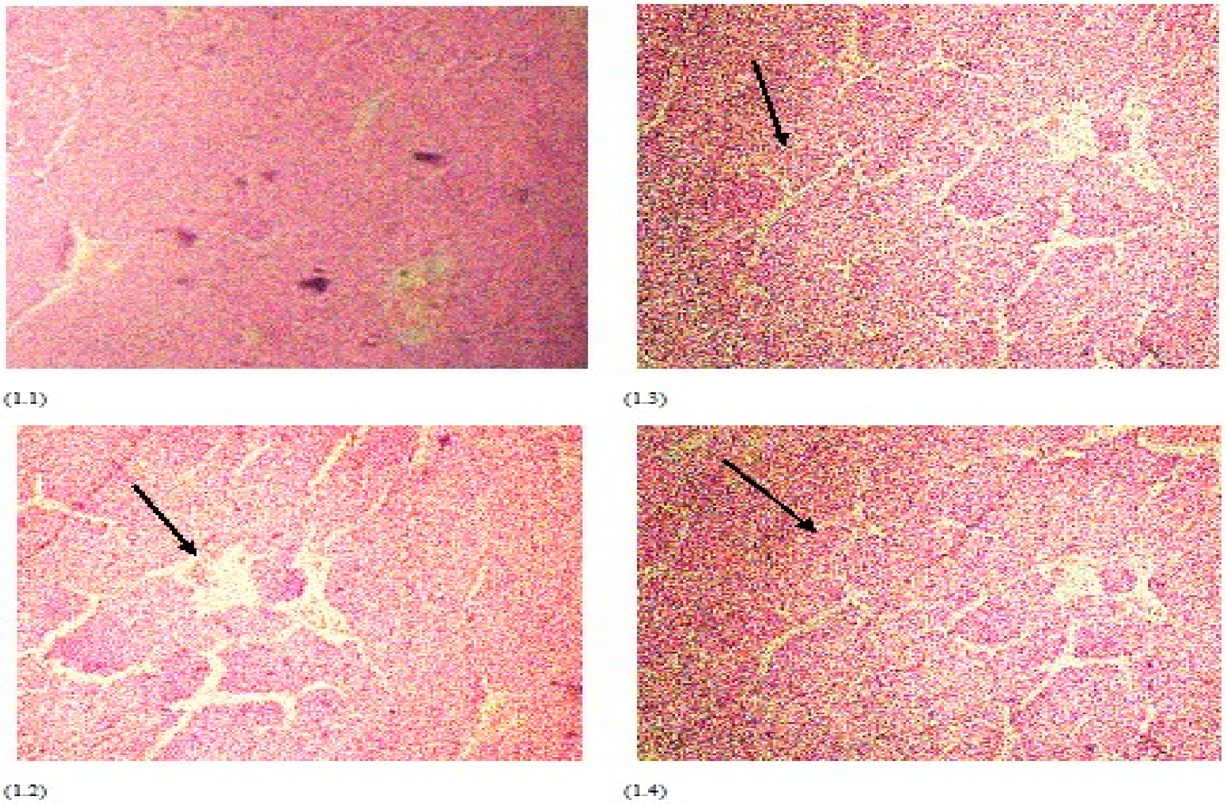

Fig. 1: Histological examination of mice liver under 10x (1.1) Liver cells of mice fed with normal diet. (1.2) Liver cells of mice fed with lipid rich diet indicating that the lipids get deposited into the parenchyma cells of liver (1.3) Mice liver cells treated with methanol extract (1.4) Mice liver cells treated with standard drug. Arrows indicating deposition of fats in the liver tissue.

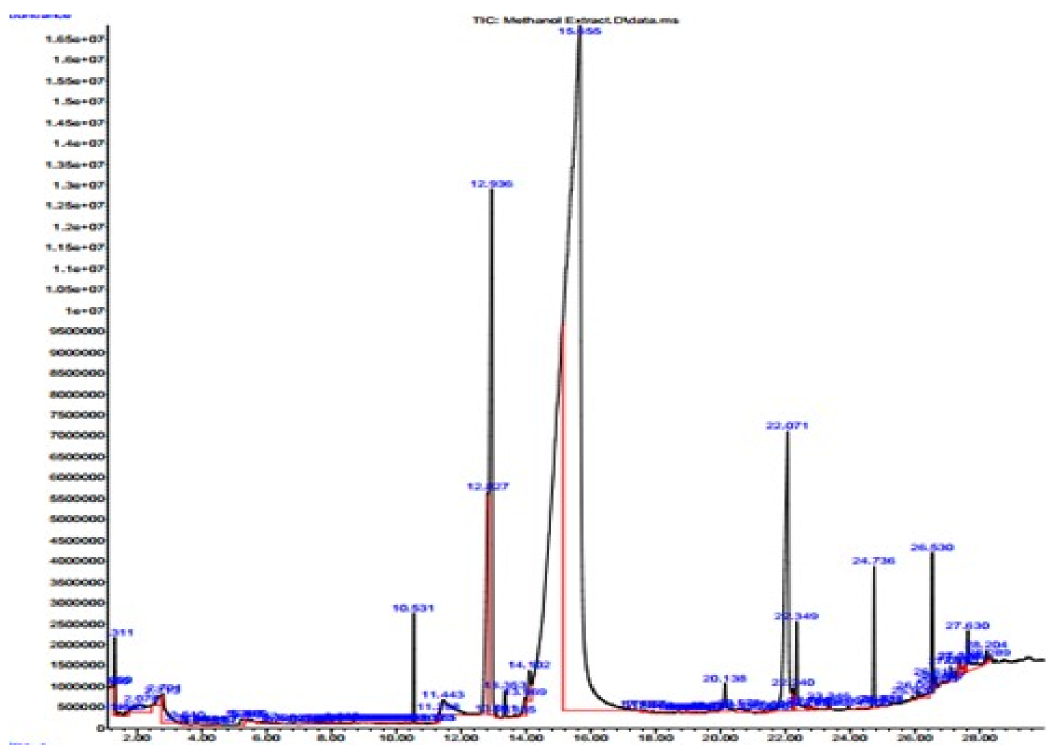

Fig. 2: GC-MS chromatogram of methanol extract of seeds of *Ficus carica*

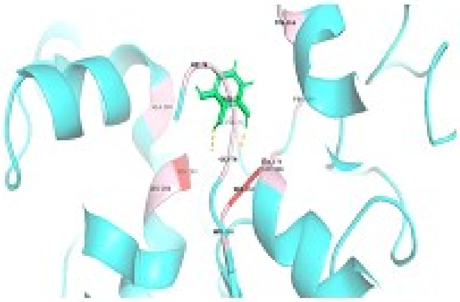

Figure 3.1: 1,2,3-Benzenetriol docked with 1LPB

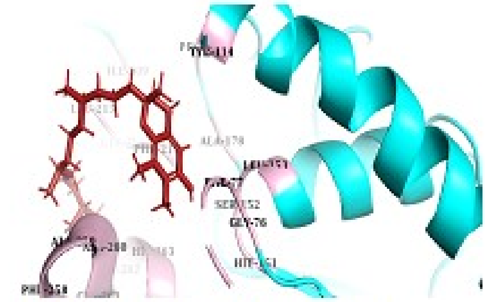

Figure3.2:Gamma tocopherol docked with 1LPB

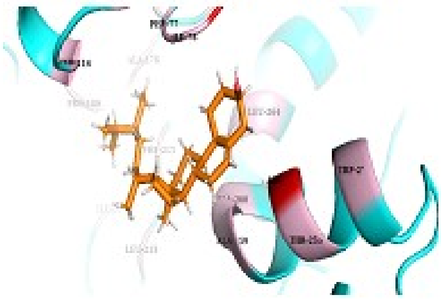

Figure 3.3: Gamma sitosterol docked with 1LPB

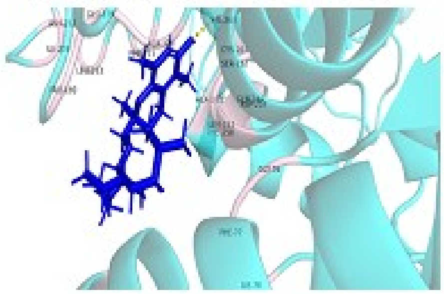

Figure 3.4: Beta tocopherol docked with 1LPB

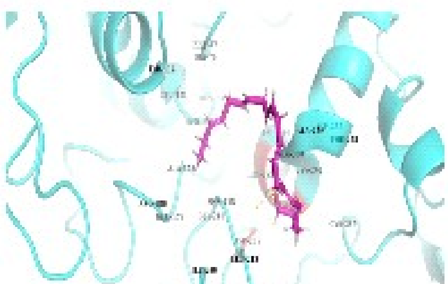

Figure 3.5:5,8,11,14,17-eicosapentaenoic acid docked with 1LPB

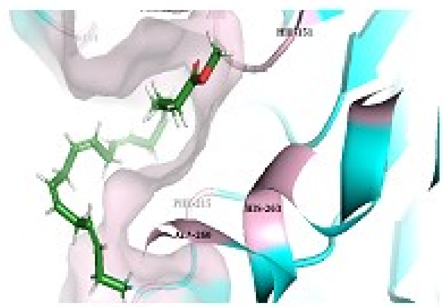

Figure 3.6:Heneicosapentaenoic acid methyl ester docked with 1LPB

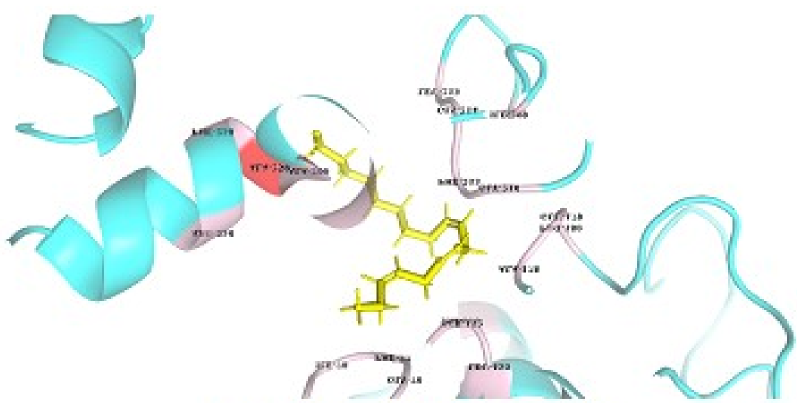

Figure3.7:9,12,15-octadecatrien-1-ol docked with 1LPB

Fig. 3: Attachment of various ligands embedded in the binding site of protein pancreatic lipase whereas; the protein is illustrated in figures by cyan and pink color and various colors including green, brown, orange, blue, magenta dark green and yellow illustrating ligands as mentioned below in each figure.

while table 5. Showed the reported activity of selected compounds present in methanol extract of seeds of *Ficus carica.*

In silico molecular docking

The above mentioned compounds were ranked according to their binding affinities with the protein 1LPB.Orlistat was used as reference ligand. Ligands with a docking score similar to or above that of orlistat were selected for further molecular docking studies, as shown in table 6.

The binding affinity for each of the ligands were calculated which ranged from -4.469 to -6.453 kcal/mol (9,12,15 octadecatrien-1-ol with the lowest binding affinity while that of 1,2,3-Benzenetriol with the highest). These binding affinities were comparable or superior to the reference drug, orlistat, with binding affinity at -5.273Kcal/mol. The docking score of 1,2,3-benzenetriol (6.453- Kcal/mol) represented the high binding affinity (-21.19 Kcal/mol) with 1LPB. Seven docked compounds can be considered for the optimization of drug development as these possess higher and comparable docking score and glide ligand efficency (percentage/potency efficiency index PEI) to that of orlistat.

The ligand 1,2,3-benzenetriol was surrounded by amino acids Ala 260, His 263, Gly 76, Phe 77, Pro 180, Ala 178, Phe 215, Leu 153, Ser 152, Hie 151 through non-polar bonding. Gamma tocopherol formed hydrogen bonding with Ser 152 amino acid of pancreatic lipase. This ligand was surrounded by amino acids Leu 213, Gly 214, Phe 215, Asp 257, Phe 258, Ala 259, Ala 260, Cys 261, Asn 262, His 263, Asp 205, Pro 180, Ala 178, Gly 76, Phe 77, and Ile 78 through non-polar bonding. Gamma sitosterol ligand also formed hydrogen bond with Asp 79 amino acid of pancreatic lipase and was surrounded by amino acids Leu 213, Phe 215, Ser 152, Leu 153, Ala 178, Pro 180, Typ 114, Ile 209, Leu 264, His 263, Ala 260, Ala 259, Arg 256, Trp 252, Phe 77, Ile 78, Asp 79 and Ile 209 through non-polar interactions.

Ligand beta tocopherol was surrounded with amino acids of protein through non-polar bond including Tyr, 114, Phe 77, Ala 178, Glu 179, Pro 180, Leu 153, Ser 152, Asp 257, Phe 258, Ala 259, Ala 260, Gln 244, Cys 261, His 263, Cys 237, Phe 215, Gly 214, Leu 213, Asn 212, Asp 205 and Ile 209. This ligand formed hydrogen bond with Asn 262 amino acid of protein. 5,8,11,14,17 eicosapentaenoic acid ligand formed hydrogen bond with Asp 205 and interacts Asn 262 with oxygen. It was surrounded by His 263, Asn 262, Cys 261, Ala 260, Ala 259, Phe 258, Leu 213, Gly 214, Phe 215, Tyr 114, Ile 209, Pro 180, Glu 179, Ala 178, Ser 152, Leu 153, Phe 77 and Ile 78 amino acid of pancreatic lipase.

Similarly, methyl ester of heniecosapentaenoic acid also formed hydrogen bond with Asp 205 while interacted with Asn 262 through oxygen. This ligand was surrounded by His 263, Asn 262, Cys 261, Ala 260, Ala 259, Phe 258, Leu 213, Gly 214, Phe 215, Tyr 114, Ile 209, Pro 180, Glu 179, Ala 178, Ser 152, Leu 153, Phe 77 and Ile 78 amino acid through non polar bond. 9,12,15 octadecatrien-1-ol was surrounded by Ile 209, Gly 76, Phe 77, Asp 79, His 263, Leu 264, Hie 151, Ser 152, Leu 153, Ala 178, Glu 179, Pro 180, Phe 215, Leu 213, Tyr 114 through non polar bond and with Ala 259 amino acid through hydrogen bond.

Prime MM-GBSA studies were used to calculate relative binding free energy of each ligand, Energy of optimal free ligands, free receptors, complex ligand and receptor (Naseem *et al.,* 2022). 1, 2, 3-benzentriol possessed high binding energy to LPB protein, was -21.19 Kcal/mol. The relative binding energies of molecules are described in table 7.

DISCUSSION

Hyperlipidemia is a disorder characterized by abnormal lipid profile including elevated levels of total serum cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low density lipoproteins. By consuming lipid rich diet directly affects fatty acid consumption and lipid profile which is the major factor modulating the lipid metabolism. Higher intake of fatty acids increases low density lipoproteins (LDL) and cholesterol also it reduces high density lipoproteins (HDL) in blood stream. This condition leads to the development of coronary artery disease because the HDL is inversely related to risk of atherosclerosis, while increased LDL is an important risk factor to cardiovascular events (Nicholls, 2018). Therefore, a balance for the consumption of unsaturated fatty acids is required when selecting food sources and those rich in monounsaturated and polyunsaturated fatty acids. The long chain poly-unsaturated fatty acids must be selected preferentially as these reduce the risk for atherosclerosis (Calder, 2018).

The increased fat diet consumption can also be associated with deposition of triglycerides in the liver and consequently leads to the development of non-alcoholic hepatic steatosis (Lian *et al.,* 2020). Statins are the main class of drugs for the treatment of hyperlipidemia, however, the use of statins is limited due to treatment resistance, intolerance due to adverse events, and a lack of adherence which contribute to poor outcomes. Many patients require adjunctive therapies including niacin, fibric acids, ezetimibe and bile acid sequestrants for adequate control hyperlipidemia (Zodda *et al.*, 2018).

Medicinal plants contain biologically active substances including polyphenols and flavonoids (Asghar *et al.*, 2018). Such plants can be used as medicinal agents due to association of several benefits including antioxidant, anti-inflammatory, antidiabetic, antibacterial, antiviral, anti-allergic and anticancer (Yousaf *et al*., 2022: Imtiaz *et al.,* 2017). During literature studies, it has been concluded that anti-hyperlipidemic potential of the seeds of *Ficus carica* has not discussed so far. The seed extracts of *Ficus carica* were subjected to extensive phytochemical screening. These contain significant levels of secondary metabolites including polyphenols, flavonoids, proteins, carbohydrates, lipids and glycosaponins as determined through phytochemical screening. Phytochemical analysis of dried fig extracts has been documented indicating the presence of poly phenols, flavonoids, anthocyanins and flavanols. These metabolites have been documented as antimicrobial, antidiabetic, cytotoxic, anti-oxidant, antiseizure anti-Parkinson's diseases antiviral, antifungal, cardioprotective, and annalgesic properties (Salehi *et al*., 2021). Therapeutic potential of *Ficus carica* seed extracts may be attributed due to presence of certain phytochemicals.

A robust method for determination of anti-hyperlipidemic activity was selected for assessment of *in-vitro* anti-hyperlipidemic potential of seeds extracts of *Ficus carica*. All the seed extracts showed anti lipase activity indicating the presence of important phytochemicals in the extracts. The results of *in-vivo* study also confirmed that methanol extract of seeds of *Ficus carica* possess significant anti-hyperlipidemic potential by improving abnormal lipid profile in albino mice exposed to a high fat diet and treated with methanol seed extract. Anti-hyperlipidemic activity of methanol extract of fig seeds may be correlated with its flavonoid content (Bencheikh *et al.,* 2021).

The technique GC-MS was used to evaluate and identify the compounds present in methanol extract of *Ficus carica* seeds which revealed the presence of 1,2,3-benzenetriol, gamma tocopherol, gamma sitosterol, beta tocopherol, 5,8,11,14,17 eicosapentaenoic acid, heniecosapentaenoic acid methyl ester and 9,12,15 octadecatrien-1-ol with antiseptic, anti-oxidant, fungicide, insecticide (Muhammad *et al.,* 2019), anti-clastogenic, (Sasaki *et al*., 1994), anti-platelet aggregation (Gryglewski*, et al*., 1979), antidermatitic and antispasmodic (Abraham, *et al*., 2019) anti-hyperlipidemic and anti-diabetic (Al-Snai, 2019) potential.

An *in silico* strategy was employed to gather information about biological activities of the natural products. Molecular docking and MM-GBSA studies predicted ligand-target interactions and provided information on the binding capabilities and potential method of action of substances that are active against proteins (Imtiaz *et al*., 2023). Seven compounds from GC-MS analysis of methanol extract of fig seeds were docked against 1LPB protein. The results provided understanding on the ability of ligand to inhibit protein functions which are correlated to experimental enzyme inhibition. Conclusively, this study confirms our finding of anti-hyperlipidemic potential of seed extract of *Ficus carica* used against hyperlipidemia

CONCLUSION

The present study revealed that *Ficus carica* seed extracts have significant anti-hyperlipidemic potential. Methanol extract of fig seeds possess biologically active compounds including essential oils fatty acids, methylated phenol and phytosterols which showed good anti-hyperlipidemic activity. *Ficus carica* seeds could be a promising source for usage in nutraceuticals, natural and synthetic medicines due to its significant potential for anti-hyperlipidemic activity. A safe and effective anti-hyperlipidemic medicine could be developed by using seeds extract of *Ficus carica*.

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