

Identification and *In-Vitro* Evaluation of using HUH-7 cell line of herbal extract of *Silybum Marianum* and *Carica Papaya* for the treatment of Non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD), now commonly included within the metabolic dysfunction-associated steatotic liver disease spectrum, is characterized by hepatic fat accumulation and may progress to steatohepatitis, fibrosis and cirrhosis. Natural products with antioxidant and hepatoprotective potential are of continuing research interest. Objective: The present work aimed to comparatively investigate ethanolic extracts of *Carica papaya* leaves and *Silybum marianum* seeds using phytochemical screening, infrared (IR/FTIR) spectral interpretation and an in-vitro MTT assay on HUH7 cells. Methods: The materials were defatted with petroleum ether and extracted with ethanol using Soxhlet extraction. Preliminary phytochemical tests and IR spectral analysis were performed. Cytotoxicity was assessed in HUH7 cells using the MTT assay and IC₅₀ was calculated using GraphPad Prism 6. Results: The recorded extraction yield was 12% for *S. marianum* and 15% for *C. papaya*. The phytochemical observations reported the presence of several classes including flavonoids, phenols/tannins and steroids/triterpenoids. Characteristic IR bands were recorded for both extracts. Paclitaxel showed an IC₅₀ of 143.3 ± 0.07 , whereas Sample 1 and Sample 2 were reported as above the maximum dose limit. Conclusion: The thesis data demonstrate that both plant extracts contain detectable phytochemical/functional-group signals and showed low cytotoxicity under the reported HUH7 assay conditions. The available data support further standardized NAFLD-specific pharmacological evaluation, but do not by themselves establish hepatoprotective efficacy in an NAFLD model.

Keywords

NAFLD; *Silybum marianum*; *Carica papaya*; Silymarin; phytochemical screening; FTIR; HUH7; MTT assay; IC₅₀

1. Introduction

NAFLD is characterized by excess fat accumulation in hepatocytes in the absence of excessive alcohol use and is closely associated with obesity, insulin resistance, type 2 diabetes, dyslipidemia and other metabolic abnormalities. The disease can range from simple steatosis to steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. The thesis identifies oxidative stress, inflammation, insulin resistance and dysregulated lipid metabolism as important pathogenic mechanisms. The increasing disease burden and limited disease-specific pharmacological options provide a rationale for investigating natural products.(1)

Silybum marianum (milk thistle) is a member of the Asteraceae family and is a source of silymarin, a mixture of flavonolignans including silybin. The thesis describes antioxidant, anti-inflammatory and hepatoprotective properties associated with milk thistle. *Carica papaya* is a medicinal plant containing flavonoids, phenolics, carotenoids, vitamins and other phytoconstituents. The present study therefore compared the two plant extracts at the preliminary phytochemical, spectral and cell-line level.(2)

2. Materials and Methods

Plant materials: *C. papaya* leaves and *S. marianum* seeds were collected in November 2025 from Sagar, Madhya Pradesh. The thesis reports authentication by Dr. Pradeep Tiwari at Dr. Harisingh Gour University (UTD Sagar), with herbarium/voucher documentation.(3-6)

Extraction: Plant materials were washed, shade-dried, powdered and sieved. *S. marianum* powder (50 g) was defatted with 500 mL petroleum ether at 60–80°C for 4–6 h in a Soxhlet apparatus. Defatted material (25 g) was subsequently extracted with 200 mL ethanol at 60–70°C for 22–24 h. The reported dried extract yield was 12% ($3/25 \times 100$). *C. papaya* powder (40 g) was defatted with 400 mL petroleum ether and the defatted material was extracted with 350 mL ethanol at 60–70°C for 22–24 h. The reported yield was 15% ($6/40 \times 100$). (4-9)

Phytochemical screening: The thesis reports qualitative tests for alkaloids, glycosides, flavonoids, phenols/tannins, steroids/triterpenoids and saponins using standard colour/precipitation or foam reactions.(10)

MTT assay: HUH7 cells obtained from NCCS Pune were seeded at 10,000 cells/well in 96-well plates and cultured for 24 h in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C and 5% CO₂. Samples were prepared in DMSO and tested at different concentrations. After 24 h exposure, MTT solution (5 mg/mL) was added for 2 h. The formazan-containing cell layer was dissolved in 100 µL DMSO and read at 540 nm using an iMark Bio-Rad plate reader. IC₅₀ was calculated with GraphPad Prism 6. Percentage viability was calculated as: % viable cells = (A_{test}/A_{control}) × 100.(11)

Table 1 Chemical Evaluation and analytical Screening

Phytochemical group	Silybum marianum	Carica papaya
Alkaloids	+	+
Glycosides	–	+
Flavonoids	+	+
Phenols/Tannins	+	+
Steroids/Triterpenoids	+	+
Saponins	–	+

3. In Vitro Cytotoxicity Evaluation –MTT Assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide assay is a colorimetric method used for the evaluation of cell viability and cytotoxicity. This assay performed by using Air-jacketed CO₂ incubator (Heal Force HF90), iMark Microplate Reader (Bio-Rad, USA) and Inverted microscope (Olympus EK2), AmScope Digital Camera, 10 MP Aptima CMOS. The assay is based on the reduction of MTT by metabolically active viable cells to form purple-colored formazan crystals. The amount of formazan formed is proportional to the number of viable cells. In the present study, MTT assay was performed to evaluate the in-vitro cytotoxicity of Sample 1 (S1) and Sample 2 (S2) against HUH7 cell line, using Paclitaxel as the reference drug.(12-15). Cell Line HUH7, Culture Medium DMEM (Dulbecco's Modified Eagle Medium, AT149-1L, HiMedia), Fetal Bovine Serum (FBS, RM10432, HiMedia), Penicillin-Streptomycin antibiotic solution (P0781, Sigma-Aldrich). MTT solution (5 mg/mL), Dimethyl Sulfoxide (DMSO, SRL, Cat. No. 67685). Test Sample Ethanolic Extrac .1 (S1 – *Carica papaya*) 2 (S2 – *Silybum marianum*).

Procedure

A. Cell Culture

HUH7 cells were cultured in DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin antibiotic solution. The cells were maintained at 37°C with 5% CO₂.

HUH7 cells were seeded in a 96-well plate at a density of **10,000 cells/well** and incubated for **24 hours** to allow cell attachment.(16)

B. Treatment of Cells

After 24 hours of incubation, the cells were treated with different concentrations of the test samples.

Stock solutions of the samples were prepared in DMSO and subsequently diluted to obtain the required concentrations using incomplete cell culture medium without FBS.

The cells were treated with different concentrations according to the experimental Excel data and incubated for another 24 hours.(17)

C. MTT Treatment

After 24 hours of treatment, MTT solution (5 mg/mL) was added to the cell culture and the cells were further incubated for 2 hours in a CO₂ incubator. Cells without treatment were considered as Control, while cells without MTT were considered as Blank.(18)

D. Formazan Development and Absorbance Measurement

After incubation, the culture supernatant was carefully removed. The cell-layer matrix containing the formed formazan was dissolved in 100 µL DMSO. The absorbance was measured using an iMark Bio-Rad microplate reader at 540 nm. Cell images were captured using an Olympus EK2 inverted microscope with an AmScope digital camera.(19-20)

Calculation

The % of viable cells was calculated using the following formula: % **Viable Cells** = (A **Test** / A **Control**) × 100

Where:

A Test = Absorbance of test sample

A Control = Absorbance of untreated control

Table 2 IC50 Value Determination Treatment

Treatment	IC50 Value
Paclitaxel	143.3 ± 0.07
Sample 1 (S1 – Carica papaya)	Above maximum dose limit
Sample 2 (S2 – Silybum marianum)	Above maximum dose limit

4. Results and Discussion

Table 3 Extraction Yield

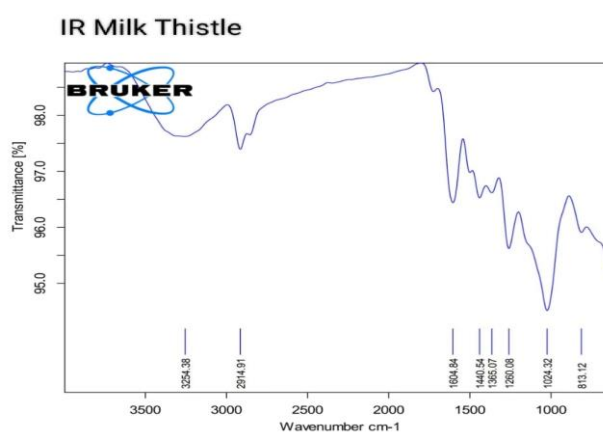
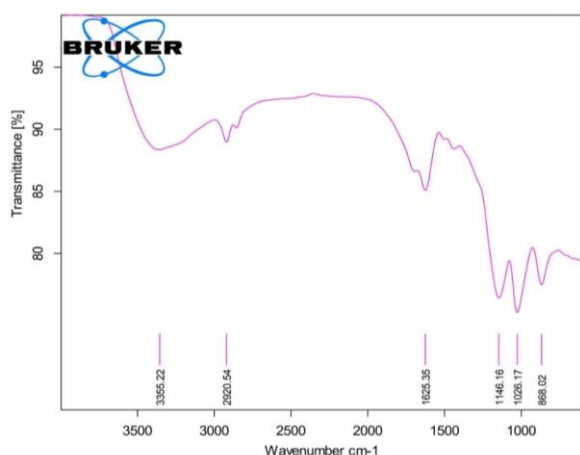
Extract	Starting material	Reported yield
Silybum marianum (ethanolic seed extract)	25 g	12%
Carica papaya (ethanolic leaf extract)	40 g	15%

The recorded observations indicated positive reactions for multiple phytochemical groups in both extracts. The thesis table reports alkaloids, glycosides, flavonoids, phenols/tannins, steroids/triterpenoids and saponins using +/- scoring. Because the original table contains a wording inconsistency referring to “Coriander sativum” in its explanatory sentence, the present article retains only the plant-specific observations and does not reinterpret that inconsistent sentence.(22-25)

Table 4 IR/FTIR Spectral Findings

Plant	Peak (cm ⁻¹)	Interpretation
Carica papaya	3355	O–H stretching; alcohols/phenols/flavonoids
	2920	C–H stretching; alkanes
	1625	C=C stretching / amide I; aromatic/phenolic compounds
	1146	C–O stretching; alcohols/ethers
	1026	C–O stretching; glycosides/polysaccharides
	868	Aromatic C–H bending
Silybum marianum	3254	O–H stretching; phenols/alcohols
	2914	C–H stretching; alkanes
	1604	Aromatic C=C stretching; phenolics/flavonoids
	1440	CH ₂ bending; alkanes
	1365	C–H bending; methyl groups
	1260	C–O stretching; phenols/ethers
	1024	C–O stretching; glycosides
	813	Aromatic C–H bending

Figure No.1 Infrared spectroscopy Carica Papaya extract **Figure No.2 Infrared spectroscopy Milk Thistle Extract**



Phytochemical Screening

The recorded observations indicated positive reactions for multiple phytochemical groups in both extracts. The thesis table reports alkaloids, glycosides, flavonoids, phenols/tannins, steroids/triterpenoids and saponins using +/- scoring. Because the original table contains a wording inconsistency referring to “Coriander sativum” in its explanatory sentence, the present article retains only the plant-specific observations and does not reinterpret that inconsistent sentence.(26)

In-Vitro evaluation analysis

Based on the results obtained from the MTT assay, it was observed that when the cell line HUH7 was exposed to different concentrations of the samples, cytotoxic activity was estimated for samples and 50% inhibitory concentration as mentioned in table 1. All sample were found to be very less cytotoxic. IC₅₀ is the concentration of an inhibitor/sample/ formulation at which the viable cells are reduced by half.(27)

Figure No. 9 Concentration- in HUH7 cells (Standard)

Figure No. 10 Concentration- HUH7 cells (Sample)

MTT Assay

The cytotoxicity of the samples provided on HUH7 cell line (Procured from NCCS Pune) was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L-HIMEDIA) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO₂. Next day cells were treated from different concentrations of the samples (concentration as per mentioned in excel sheet). Stock solution of samples was prepared in DMSO and further diluted to get different concentrations in incomplete Cell culture Medium (Without FBS). After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated (Air-jacketed CO₂ incubator-Heal force-HF90) for 2h. Cells without treatment were considered as Control and cells without MTT were considered as Blank. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO-SRL- Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm. IC₅₀ was calculated by using Graph Pad Prism -6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS). 50% inhibitory concentration (IC₅₀) was presented as Mean ± SEM (Standard Error of Mean).(28)

Treatment concentrations evaluated: 1, 10, 50, 100, 250, 500 and 1000 nM

Control – Magnification = 100× Treated (at 1 nM)

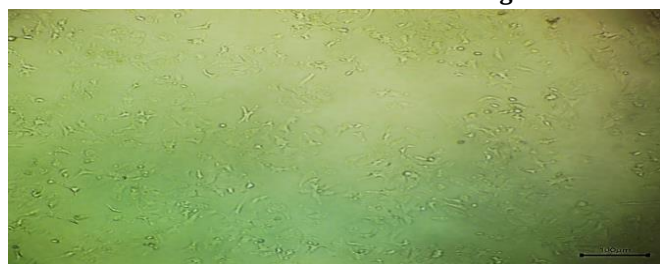


Figure No. 3 Treated (at 10 nM)

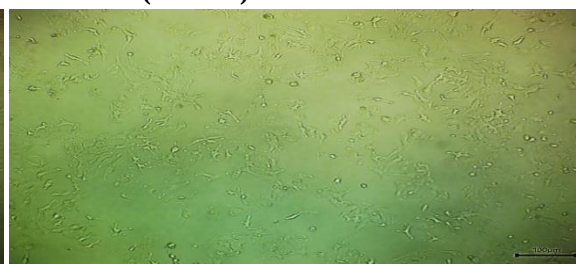


Figure No. 4 Treated (at 50 nM)



Figure No. 5 Treated (500 nM)



Figure No. 6 Treated (1000 nM)



Figure No. 7 Magnification = 100×



Figure No. 8 Magnification = 100×

Table 5 Concentration-wise HUH7 cell viability following MTT assay (mean $\pm$ SD; n=4)

Concentration (nM)	Paclitaxel (% viability)	Carica Papaya (% viability)	Milk Thistle (% viability)
0	100.00 $\pm$ 0.94	100.00 $\pm$ 2.81	100.00 $\pm$ 2.88
1	73.68 $\pm$ 2.19	89.67 $\pm$ 3.71	80.00 $\pm$ 2.95
10	61.92 $\pm$ 2.45	77.17 $\pm$ 1.77	65.71 $\pm$ 2.19
50	59.11 $\pm$ 1.66	76.09 $\pm$ 4.16	64.00 $\pm$ 4.94
100	54.30 $\pm$ 2.20	74.46 $\pm$ 3.71	61.14 $\pm$ 5.07
250	45.03 $\pm$ 2.36	67.39 $\pm$ 6.28	59.43 $\pm$ 4.38
500	43.54 $\pm$ 1.37	64.67 $\pm$ 8.40	53.71 $\pm$ 5.90
1000	38.08 $\pm$ 1.99	63.59 $\pm$ 5.99	52.00 $\pm$ 7.32

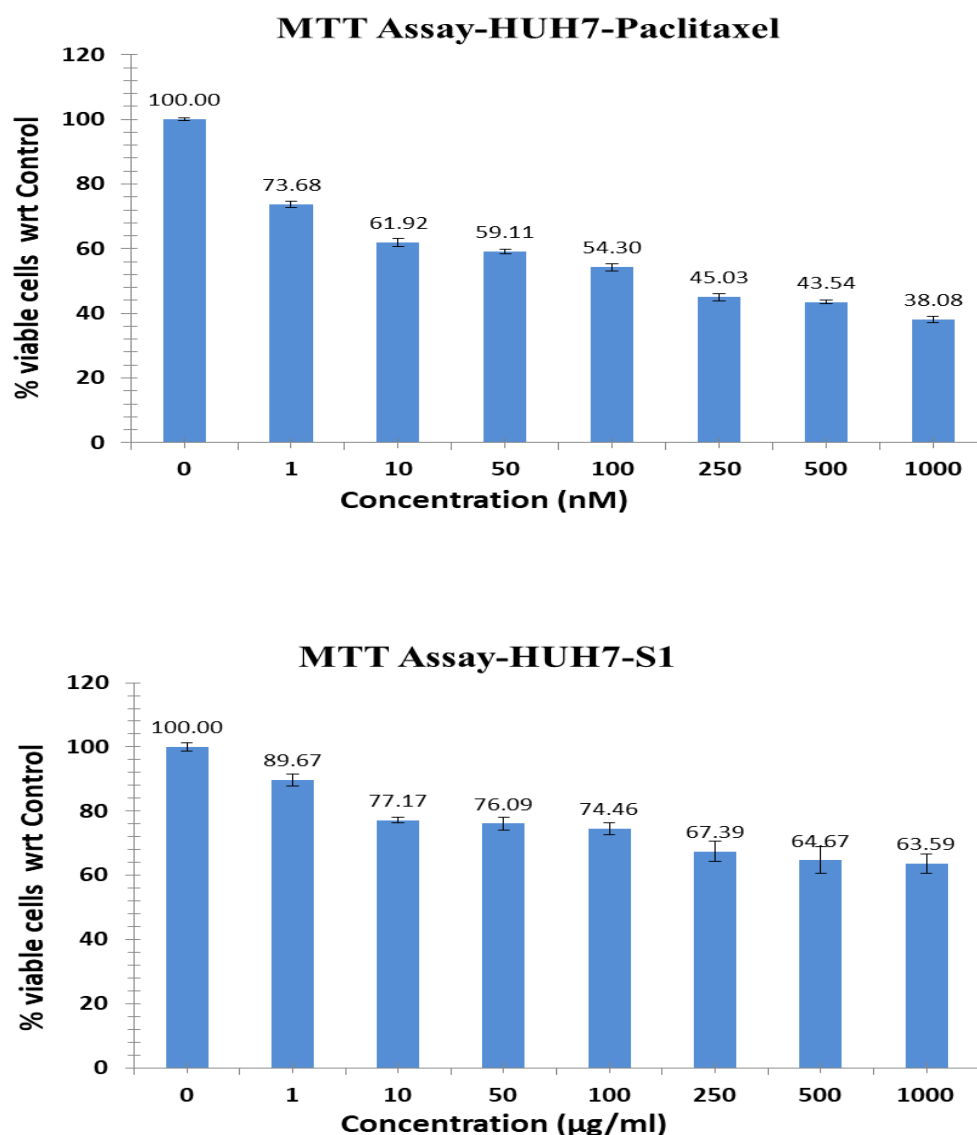


Figure: 4 % Viability of HUH-7 cell line in standard by paclitaxel and with the sample

Calculation

$$\% \text{ Viable cells} = (A_{\text{test}} / A_{\text{Control}}) * 100$$

(A_{test} = Absorbance of test sample)
(A_{Control} = Absorbance of Control)

Inference

Based on the results obtained from the MTT assay, it was observed that when the cell line HUH7 was exposed to different concentrations of the samples, cytotoxic activity was estimated for samples and 50% inhibitory concentration as mentioned in table 2. All sample were found to be very less cytotoxic. IC50 is the concentration of an inhibitor/sample/ formulation at which the viable cells are reduced by half. (29)

Microscopic Images (100×)



Figure No. 11 Control – 0 µg/ml



Figure No. 12 S2 – 1 µg/ml



Figure No. 13 S2 – 10 µg/ml



Figure No. 14 S2 – 50 µg/ml



Figure No. 15 S2 – 100 µg/ml



Figure No. 16 S2 – 250 µg/ml

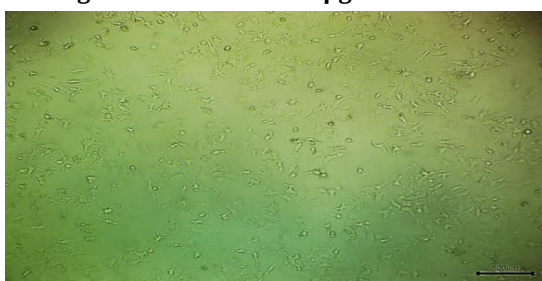


Figure No. 17 S2 – 500 µg/ml

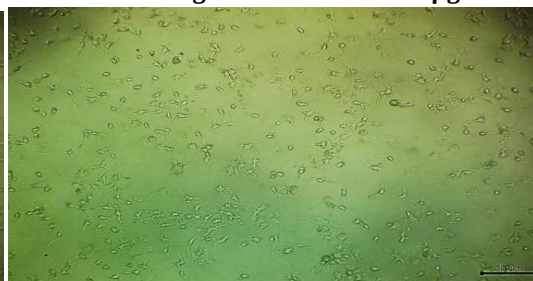


Figure No. 18 S2 – 1000 µg/ml

Table 6 Nonlinear dose-response parameters reported from the MTT dataset.

Treatment	IC ₅₀	Unit	LogIC ₅₀	Hill slope	95% CI for LogIC ₅₀
Paclitaxel	143.3 ± 0.07	nM	2.156	-0.2159	1.952–2.360
S1 (Carica papaya)	11836 ± 0.26	µg/mL	4.073	-0.2042	3.398–4.749
S2 (Silybum marianum)	1505 ± 0.17	µg/mL	3.177	-0.1671	2.733–3.622

One-way ANOVA showed a statistically significant effect of concentration on cell viability for all three treatments: Paclitaxel, $F(7,24)=417.97$, $p=1.74 \times 10^{-23}$; S1, $F(7,24)=25.39$, $p=1.26 \times 10^{-9}$; and S2, $F(7,24)=44.90$, $p=2.89 \times 10^{-12}$. These results confirm significant concentration-related differences in the viability measurements.(30)

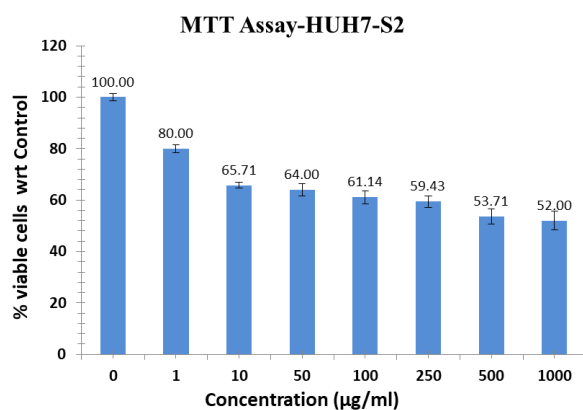


Figure No. 19 Concentration in HUH7 cells (Sample)

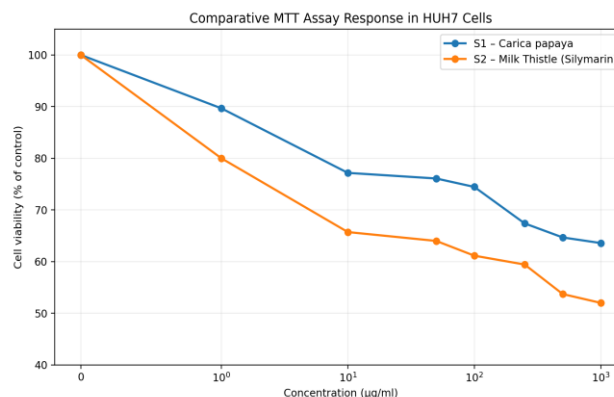


Figure No. 20 Concentration Dose–Response

Note: The IC₅₀ values are reported in the units used in the source dataset. Because Paclitaxel was tested in nM and S1/S2 in µg/mL, direct numerical comparison of IC₅₀ values across different units should be made cautiously.

The supplied presentation contains microscopic images of untreated control HUH7 cells and HUH7 cells treated with Sample S2 at increasing concentrations. The images were reviewed qualitatively for overall cell distribution and apparent morphological changes.(31)

Table 7 One-way ANOVA Within Each Sample *Silybum Marianum* and *Carica Papaya*

Sample	F value	P value in workbook	F critical	Interpretation
Carica papaya	25.393	1.2632	2.423	Concentration effect is significant in supplied ANOVA
Milk Thistle	44.900	2.8860	2.423	Concentration effect is significant in supplied ANOVA

Both samples showed a concentration-related decline in HUH7 cell viability. The S2 curve remained below the S1 curve at all tested concentrations, indicating a greater reduction in cell viability by S2.

S2 > S1 in apparent HUH7 cell viability-reducing activity. Milk Thistle/Silymarin (S2) showed the stronger response across the tested concentrations and the lower fitted IC₅₀. Therefore, S2 demonstrated greater apparent potency than Carica papaya (S1) in the present MTT assay.(32)

Conclusion

Ethanol extracts of *Silybum marianum* seeds and *Carica papaya* leaves were successfully prepared and showed reported extraction yields of 12% and 15%, respectively. Qualitative phytochemical screening and IR spectral analysis indicated multiple bioactive/functional-group signatures in both extracts. In HUH7 cells, the two test samples did not reach 50% inhibition within the reported dose range, indicating low cytotoxicity under the stated experimental conditions. These preliminary findings justify further standardized investigation of the extracts in validated NAFLD models, including lipid-accumulation assays, biochemical markers and other mechanistic endpoints.(33-34)

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References

1. Younossi ZM, et al. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*. 2023;77:1335–1347.

2. Rinella ME, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology*. 2023;78:1966–1986.
3. Rong L, et al. Advancements in the treatment of non-alcoholic fatty liver disease (NAFLD). *Front Endocrinol*. 2023;13:1087260.
4. Huggett ZJ, et al. A comparison of primary human hepatocytes and hepatoma cell lines to model the effects of fatty acids, fructose and glucose on liver cell lipid accumulation. *Nutrients*. 2022;15:40.
5. Morgan DML. Tetrazolium (MTT) is a assay for cellular viability and activity. *Methods Mol Biol*. 1998;79:179–184.
6. van Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol*. 2011;731:237–245.
7. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicol Lett*. 2006;160:171–177.
8. Ahirwar S. Comparative Study of Silybum marianum and Carica papaya on Non-Alcoholic Fatty Liver Disease. M.Pharm Dissertation, RGPV Bhopal/BTIPS Sagar. 2026.
9. Aydin, cansu; özkan-kotiloğlu, selin; yalçın-azarkan, Serap. Comparative cytotoxic and molecular effects of deltamethrin and acetamiprid in normal and cancerous human liver cell lines. *Cell Biochemistry and Biophysics*, 2026, 1-15.
10. Chaudhary, Archana, et al. Arsenic trioxide allosterically inhibits human telomerase, validated by in-silico and in-vitro cancer and non cancer cell lines. *Scientific Reports*, 2026.
11. Kong, Shujia, et al. DDX11/SKP2 Inhibits Cisplatin Sensitivity in Liver Cancer Cells by Regulating DNA Damage Repair and ER Stress. *Applied Biochemistry and Biotechnology*, 2026, 1-18.
12. Zheleznyak, Alexander, et al. Epithelial–Mesenchymal Transition and Stress Adaptations Underlie Yttrium-90 Resistance in Liver Cancer Cell Lines. *Cancer Research Communications*, 2026, 6.1: 178-190.
13. Peyras, Marion, et al. Protective Effects of Neutral Lipids from *Phaeodactylum tricornutum* on Palmitate-Induced Lipid Accumulation in HepG2 Cells: An In Vitro Model of Non-Alcoholic Fatty Liver Disease. *Molecules*, 2026, 31.2: 323.
14. Park, Yongsoo; Ko, Kyung Soo; Rhee, Byoung Doo. Non-alcoholic fatty liver disease (NAFLD) management in the community. *International Journal of Molecular Sciences*, 2025, 26.6: 2758.
15. Feng, Gong, et al. Recompensation in cirrhosis: unravelling the evolving natural history of nonalcoholic fatty liver disease. *Nature Reviews Gastroenterology & Hepatology*, 2024, 21.1: 46-56.
16. Zelber-Sagi, Shira; Moore, J. Bernadette. Practical lifestyle management of nonalcoholic fatty liver disease for busy clinicians. *Diabetes Spectrum*, 2024, 37.1: 39-
17. Abbasi, Amin, et al. A critical review on Akkermansia muciniphila: functional mechanisms, technological challenges, and safety issues. *Probiotics and Antimicrobial Proteins*, 2024, 16.4: 1376-1398.
18. Miao, Lei, et al. Current status and future trends of the global burden of MASLD. *Trends in Endocrinology & Metabolism*, 2024, 35.8: 697-707.
19. Thomas, James A., et al. Hepatocellular and extrahepatic cancer risk in people with non-alcoholic fatty liver disease. *The Lancet Gastroenterology & Hepatology*, 2024, 9.2: 159-169.
20. Ren, Qiannan; Sun, Qiming; Fu, Junfen. Dysfunction of autophagy in high-fat diet-induced non-alcoholic fatty liver disease. *Autophagy*, 2024, 20.2: 221-241.
21. Rafaqat, Saira, et al. Interleukins: pathogenesis in non-alcoholic fatty liver disease. *Metabolites*, 2024, 14.3: 153.
22. Younossi, Zobair M., et al. Clinical profiles and mortality rates are similar for metabolic dysfunction-associated steatotic liver disease and non-alcoholic fatty liver disease. *Journal of Hepatology*, 2024, 80.5: 694-701.
23. Chen, Qingling, et al. Association between triglyceride-glucose related indices and mortality among individuals with non-alcoholic fatty liver disease or metabolic dysfunction-associated steatotic liver disease. *Cardiovascular Diabetology*, 2024, 23.1: 232.
24. Maseko, Tumisan Edward, et al. Comparison of HepaRG and HepG2 cell lines to model mitochondrial respiratory adaptations in non-alcoholic fatty liver disease. *International journal of molecular medicine*, 2023, 53.2: 18.
25. Rong, Li, et al. Advancements in the treatment of non-alcoholic fatty liver disease (NAFLD). *Frontiers in endocrinology*, 2023, 13: 1087260.
26. Rinella, M. E., et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatol (Baltim Md)* 78: 1966–1986 [online]. 2023.
27. Younossi, Zobair M., et al. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*, 2023, 77.4: 1335-1347.
28. Wong, Vincent Wai-Sun, et al. Changing epidemiology, global trends and implications for outcomes of NAFLD. *Journal of hepatology*, 2023, 79.3: 842-852.
29. Cusi, Kenneth, et al. American Association of Clinical Endocrinology clinical practice guideline 2023, 79.3: 842-852.

30. Grander, Christoph; Grabherr, Felix; Tilg, Herbert. Non-alcoholic fatty liver disease: pathophysiological concepts and treatment options. *Cardiovascular research*, 2023, 119.9: 1787-1798.
31. Le, Michael H., et al. Global incidence of non-alcoholic fatty liver disease: a systematic review and meta-analysis of 63 studies and 1,201,807 persons. *Journal of Hepatology*, 2023, 79.2: 287-295.
32. Quek, Jingxuan, et al. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. *The lancet Gastroenterology & hepatology*, 2023, 8.1: 20-30.
33. Grander, Christoph; Grabherr, Felix; Tilg, Herbert. Non-alcoholic fatty liver disease: pathophysiological concepts and treatment options. *Cardiovascular research*, 2023, 119.9: 1787-1798.
34. Tincopa, Monica A.; Loomba, Rohit. Non-invasive diagnosis and monitoring of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *The Lancet Gastroenterology & Hepatology*, 2023, 8.7: 660-670.

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