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Original Article

Analysis of Volatile Compounds in *Caulerpa lentillifera* for Anti-Proliferative Studies in HEPG2 Liver Cancer Cells and *in silico* Comparison

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ABSTRACT

Background: Liver cancer remains a significant cause of mortality despite standard therapies. Recent studies have highlighted the anti-inflammatory properties and cancer prevention potential of certain agents. *Caulerpa lentillifera* (*C. lentillifera*) is known for its anti-inflammatory activity, yet there is limited understanding regarding its impact on liver cancer. **Aim:** Hence, this study aims to identify volatile compounds in *C. lentillifera* and associate them with cytotoxicity and molecular interactions involving the molecular marker SREBP-1A in liver cancer cells. **Materials and Methods:** Compounds present in the extract were identified using gas chromatography MS. Functional analysis involved proliferation and clonogenic assays. The interaction of the fatty acids with the biomarkers was assessed using AutoDock software. **Results:** The most abundant fatty acids identified were palmitic acid and tridecanoic acid. Cellular analysis revealed a dose-response antiproliferative effect on HepG2 cell growth, with a half maximal inhibitory concentration (IC_{50}) of 1.2 mg/mL. Similarly, colony formation was significantly suppressed ($p < 0.05$). *In silico* analysis demonstrated a higher affinity binding of palmitic acid to SREBP-1A compared to alpha-fetoprotein. **Conclusion:** The study suggests that palmitic acid exhibits antiproliferative activity in HepG2 liver cancer cells by binding to SREBP-1A. Further investigations are warranted to determine the regulatory effect of palmitic acid in liver cancer.

Keywords: Hepatocellular carcinoma, Gas chromatography-mass spectrometry, Cytotoxicity, Palmitic acid, SREBP-1A.

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INTRODUCTION

The most common liver cancer is Hepatocellular Carcinoma (HCC), which grows in two different patterns: either as a single tumor or several small nodules throughout the liver.[1] Risk factors associated with primary liver cancer include chronic infection with HBV or HCV, cirrhosis, inherited liver diseases (e.g., hemochromatosis and Wilson's disease), diabetes, Non-Alcoholic Fatty Liver Disease (NAFLD) and exposure to aflatoxins or excessive alcohol consumption.[2] It ranks as the eighth most common cancer in Malaysia, with mortality having risen by 31.5% within three decades.[3]

Treatment options for HCC include ablation techniques, surgery, chemotherapy, radiotherapy, targeted therapy and immunotherapy. Radiofrequency ablation involves tumor removal and is the most common treatment for affected tissues. Targeted therapy using regorafenib (Stivarga) and cabozantinib (Cabometyx) targets specific genes, proteins, or tissue environments that support cancer cell growth and survival. It aims to enhance the immune response against cancer cells.[4]

Intense research has shifted towards natural sources due to their low toxicity.[5-7] One natural source known for its anti-inflammatory function is *C. lentillifera*, a green seaweed found in Southeast Asia and the Asia-Pacific regions. *Caulerpa* species have a high concentration of minerals and iron, serving as nutritional supplements.[8] Its nutritional composition includes carbohydrates, which range from 3.6% to 83.2% of dry matter in the edible species of *Caulerpa*. Additionally, *Caulerpa* species contain a higher abundance of chlorophyll a and chlorophyll b compared to β-carotene, attributes associated with anticancer

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functions. The most prominent secondary metabolite in *C. lentillifera* is *Caulerpin*. *Caulerpin* exhibits antimicrobial effects and inhibits pro-inflammatory cytokines TNF-α and IL-6 production in a dose-dependent manner in RAW 264.7 cells.[9]

According to a previous report, *C. lentillifera* Hexane Extract (CLHE) inhibited growth of A172 human glioblastoma cells. It induced apoptosis by causing cell cycle arrest at G0/G1 phase and inhibition of matrix metalloproteinase activity which lead to accumulation of intracellular ROS levels in the A172.[10] Oligosaccharides derived from *Caulerpa lentillifera* caused apoptosis in MCF-7 breast cancer cells that involved caspase.[11] The *Caulerpa lentillifera* Extract (CLE) reduced the level of IL6 and TNF-α inflammatory cytokines in LPS-activated RAW264.7.[12] These effects were associated with presence of tannic acid, where it suppressed the IL-6 and IL-1β production and reduced the expression of TNF-α.[13]

Alpha-Fetoprotein (AFP) serves as an established liver cancer marker for the diagnosis of Hepatocellular Carcinoma (HCC). Tumour derived AFP interacts with the metabolic regulator Sterol Regulatory Element-Binding Protein-1 (SREBP-1).[14] SREBP1 is necessary to fulfil the increasing demand of lipid by cancer cells.[15] Specifically, Sterol Regulatory Element-Binding Protein-1a (SREBP1a) is a member of the SREBP family of transcription factors that controls homeostasis of lipids.[16] However, anti-proliferative activity of *C. lentillifera* in liver cancer and the molecular interaction of fatty acids in *Caulerpa lentillifera* with liver biomarkers remain unclear.

Therefore, this study aims to identify the volatile compounds present in the methanol extract of *C. lentillifera* and evaluate their cytotoxicity in HepG2 liver cancer cells using cell proliferation and clonogenic assays. These techniques will facilitate the identification of volatile bioactive compounds and their association with antiproliferative activity in liver cancer. Furthermore, *in silico* modeling will aim to demonstrate the molecular interaction between fatty acids and biomarkers for potential therapeutic targeting in liver cancer.

MATERIALS AND METHODS

Preparation of *C. lentillifera* sample

A 500 g sample of *C. lentillifera* was collected from the USM Centre for Marine and Coastal Studies (CEMACS, USM) and identified. The voucher specimen with the code 11909 has been deposited at the USM herbarium.

Upon receipt, the sample underwent a rinsing process with milliQ water repeated five times to remove surface impurities and was subsequently left to dry for 24 hr at room temperature. Following drying, the sample was stored in a round-bottom flask and subjected to a 48 hr lyophilization process. The dried sample was ground into a fine powder using a mechanical blender. The powdered samples were then weighed and placed back in a freezer dryer. The freeze-dried sample was stored at -30°C until further use.

Extraction of *C. lentillifera* sample

Maceration method

A 2 g powdered sample of *C. lentillifera* was placed into a sterile culture tube. The sample was macerated by adding 10 mL of diluted methanol solvent (1:5 ratios) and placed on a shaker at medium speed for 24 hr at room temperature. Following this period, the macerated mixture was filtered using Whatman No. 1 filter paper to collect the filtrate, following a previously established method.[17]

Rotary evaporation of the *C. lentillifera* methanolic extract

The collected filtrate from the extract underwent evaporation in a rotary evaporator. Initially, the chiller was activated and set to cool at 8.1°C. The water bath was filled with distilled water to over half its capacity and heated to 70°C while waiting for the chiller to reach 8.1°C from room temperature. Once the desired temperature was reached, the vacuum source was activated.

Subsequently, the filtrate was transferred into a round-bottom flask and connected to the "bump trap" using a plastic clip. The flask was partially submerged and the rotary evaporator was adjusted to evaporate methanol at 350 mbar with the flask rotating at 50 rpm. Evaporation continued for 2 hr until the crude extract became visible. Upon completion, the extract was collected. To retrieve the extract, 10 mL of milliQ water was introduced into the flask. The extract was then transferred to a tube and subjected to lyophilization for 24 hr to remove water content. The percentage yield of the *C. lentillifera* extract was calculated using the following formula.

$$\text{Percentage of yield of } C.\,lentillifera \text{ extract}(\%) = \frac{\text{Weight of dry } C.\,lentillifera \text{ extract}}{\text{Weight of dry } C.\,lentillifera \text{ sample}} \times 100\%$$

Gas Chromatography-Mass Spectrometry (GC-MS) method

The lyophilized *C. lentillifera* extract (0.7 g) was dissolved in 77 mL of methanol and 1mL of the diluted extract was transferred into a sample vial for subsequent analysis via Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis was conducted using an Agilent Technologies 7890A gas chromatograph coupled with an Agilent Technologies 5975C mass spectrometer. The instrumentation was equipped with a capillary column 250 µm in inner diameter and 0.25 µm in film thickness. The inlet temperature program initially commenced at 50°C initially for 3 min and gradually increased at a rate of 10°C/min until reaching 280°C. Subsequently, the final temperature was elevated to 300°C and held for 10 min. Helium served as the carrier gas at a flow rate of 1.0 mL/min flow rate at 250°C. For the GC-MS spectral detection, an ionization energy of 70

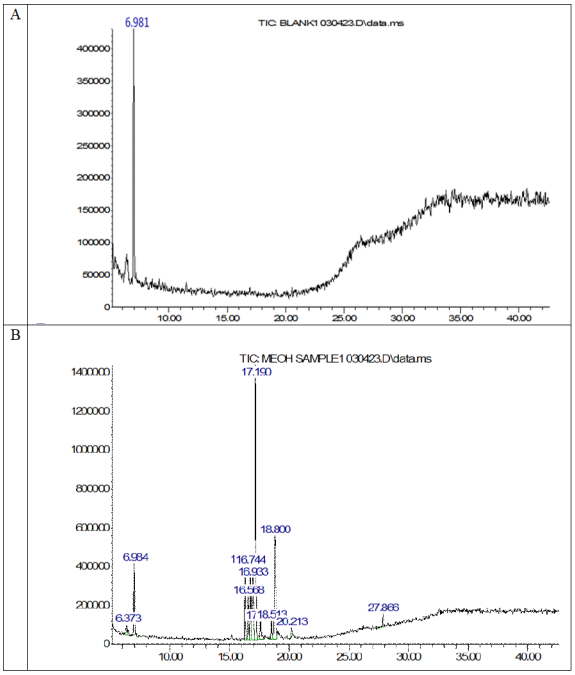

Figure 1: Relative peak values of volatile compounds present in *C. lentillifera* methanol extract (A) methanol only and (B) Methanolic extract of *C. lentillifera*. Analysis was done by Gas Chromatography-Mass Spectrometry (GC-MS). All experiments were performed in triplicates.

eV was employed with a scan time of 0.2 sec and the fragments were detected within the range of 40 m/z to 600 m/z. Based on the retention time, peak area, height of the peak and the mass spectral patterns obtained from the methanolic extracts, the GC-MS results were compared with the standard mass spectra (NIST 2005 MS collection) libraries.[18]

Preparation of complete media

Complete media was prepared from the Roswell Park Memorial Institute (RPMI) medium that was added with 10% foetal bovine serum and 1% penicillin-streptomycin and store at 4°C for cell culture of HepG2 cells.[19]

Cytotoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-Tetrazolium bromide (MTT) assay

HepG2 cells were cultured in a 96-well plate at a density of 1,000 cells/well using EMEM complete media and were incubated at 37°C with 5% CO_2 overnight. The *C. lentillifera* extract was diluted in EMEM complete media for the dose-response study and filtered through a 0.22 µm syringe filter. After the overnight incubation, the medium was replaced with 100 µL of *C. lentillifera* extracts at 10 different concentrations (ranging from 7 x 10^{-9} to 7 mg/mL). The HepG2 cells were treated with the extracts for 48 hr.

For the cytotoxicity assay, a 5 mg/mL MTT solution was prepared by dissolving MTT (Sigma) powder in Phosphate Buffered Saline (PBS, pH 7.4) and filtered using a 0.22 µm syringe filter.[20] After

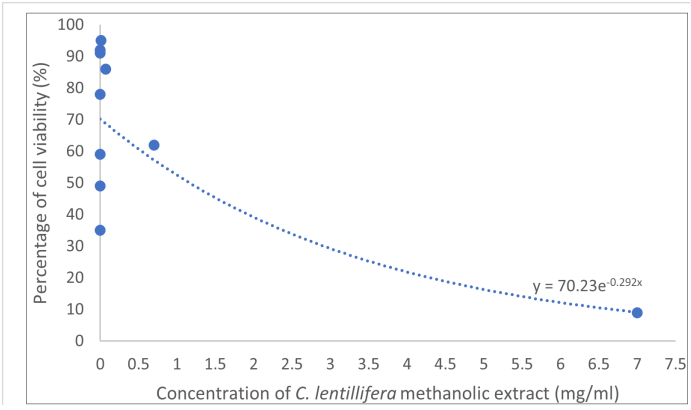

Figure 2: Dose-response curve of HepG2 cell viability treated with the *C. lentillifera* methanolic extract.

the 48 hr treatment of cells, 20 µL of the MTT solution was added to each well and incubated at 37°C for 4 hr. Subsequently, the medium was replaced with 100 µL of DMSO, mixed and the absorbance was measured at 570 nm using an ELISA plate reader. Wells with untreated cells served as negative controls, while wells containing only media were used as blanks. All experiments were conducted in triplicates.[21]

The IC_{50} values were calculated using an exponential graph plotting the percentage of cell viability against the concentration of the methanolic extract of *C. lentillifera*.[22] The percentage of cell viability was determined using the following formula.

$$\text{Percentage of cell viability (\%)} = \frac{\text{Average OD treated} - \text{Average OD blank}}{\text{Average OD untreated} - \text{Average OD blank}} \text{ x } 100\%$$

Cell surviving fraction by clonogenic assay

The HepG2 liver cancer cells were plated in a 6-well plate at 10,000 cells/well density and left overnight in a CO_2 incubator at 37°C for cell attachment. Following attachment, the cells were treated with the IC_{50} concentration of *C. lentillifera* extract (1.2 mg/mL) and incubated at 37°C in a 5% CO_2 atmosphere for 5 days.

Post-incubation, the cells underwent two washes with cold PBS, were fixed using methanol and then stained with 0.5% crystal violet.[23] The colonies with more than 50 cells per field were observed using a phase-contrast microscope and images were captured using the ZEN software. The average count of colony formations and the diameter of these colonies were calculated using the following formula.[24]

$$\text{Average of colony formation or diameter of colony (µm)} = \frac{\text{Number of colonies ordiameter of colonies}}{\textit{Number of replicates}}$$

In silico analysis

Ligand

Palmitic acid was used as a ligand. The Three-Dimensional (3-D) model of the palmitic acid was retrieved from the PubChem database in sdf format.[25]

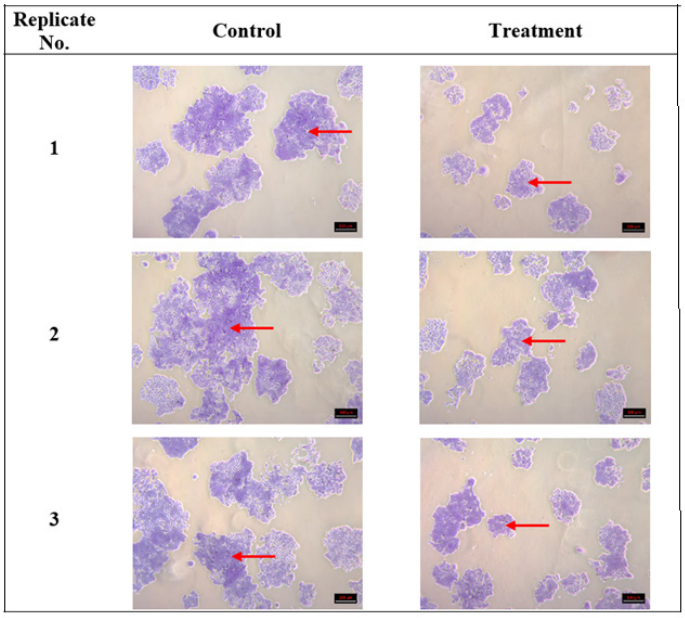

Figure 3: Clonogenic assay shows a morphological difference between control and *C. lentillifera* methanol extract treated HepG2 liver cancer cells. Images were captured with a phase contrast microscope at 500x magnification.

Preparation of ligand

The obtained palmitic acid in sdf format from the PubChem database (PubChem CID: 985) was prepared using Discovery Studio (DS) 4.0. The ligand (palmitic acid) underwent a process involving removal of duplicates, counting of tautomers/isomers, insertion of hydrogen bonds and finally energy minimization using the CHARMM force field.[26]

Identification and Retrieval of Target Proteins

Human Sterol Regulatory Element-Binding Protein-1A (SREBP-1A) and alpha-fetoprotein were selected as the target proteins for the molecular docking analysis. SREBP-1A, a helix-loop-helix transcriptional activator, regulates gene expression for proteins involved in fatty acid and cholesterol biosynthesis. Alpha-fetoprotein acts as a carrier protein for the transportation of fatty acids to cells. The 3-D structures of SREBP-1A (PDB ID: 1AM9) and alpha-fetoprotein (PDB ID: 7YIM) were obtained from the RCSB Protein Data Bank (PDB) in pdb format.[27]

Preparation of Target Proteins and Identification of Active Sites

The 'Prepare protein' method in DS 4.0 was utilized to process the selected target proteins, which involved removal of duplicates, tautomer and isomer counting and addition of hydrogen bonds. DS Visualizer identified the active site of the proteins to facilitate a strong binding affinity with palmitic acid. Furthermore, it analyzed molecular interactions between the target protein's crystal structure and its ligand present in the PDB.[27] A grid box covering the chosen protein-binding site allowed free movement of the ligand while encompassing critical functional residues.

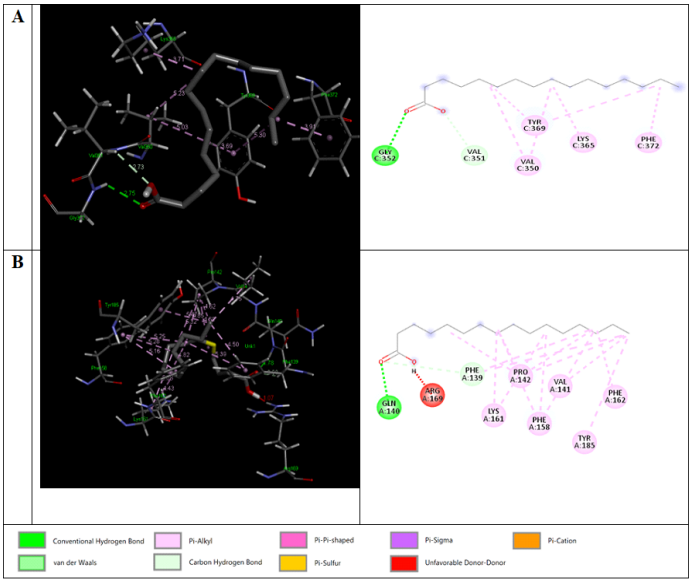

Figure 4: The molecular interaction of palmitic acid with (A) SREBP-1A and (B) alpha-fetoprotein.

Molecular Docking

The target proteins were docked with palmitic acid using Autodock Vina.[28] The resultant model of the target protein-palmitic acid complex was visualized using DS 4.0. Parameters such as binding energy, number of hydrogen bonds and hydrogen bond distances between the target protein and palmitic acid were measured. The most stable and favorable molecular interaction was determined based on the lowest binding energy (kcal/mol) and the most negative value.

Statistical analysis

A t-test was employed to compare differences between treatment and control groups for both colony formation and colony diameter using SPSS software. A mean value less than 0.5 was considered significant and the standard deviation was used as the error value.

RESULTS

Methanol extraction of *C. lentillifera* sample

C. lentillifera extract was obtained from the maceration step with application of an absolute methanol solvent. The percentage yield of methanolic extract was 36.5%.

Identification of volatile compounds in *C. lentillifera* sample

The GC-MS chromatogram displaying the detected compounds of *C. lentillifera* methanolic extract is depicted in Figure 1, while Supplementary 1 provides a comprehensive list of the compounds present. A total of 36 compounds were identified and the highest peak intensity was recorded at 17.2 min, followed by 18.8 min. Supplementary 1 showcases the compounds detected in the analysis. Notably, among these compounds, hexadecanoic

acid and tridecanoic acid were identified as the compounds with the highest peak quality, each demonstrating a purity of 96%. *N*-Hexadecanoic acid has been reported to demonstrate a range of biological properties encompassing anti-inflammatory effects, potential anticancer properties and antioxidant characteristics.[29,30] Moreover, in-line with previous reports, *C. lentillifera* methanolic extract was found to contain several major constituents including palmitic acid, oleic acid, pentadecanoic acid, behenic acid and myristic acid.[31]

Cytotoxicity assay

In this experiment, HepG2 cells were exposed to varying concentrations of *C. lentillifera* extracts in a 96-well plate, followed by incubation with MTT solution and subsequent analysis using an ELISA plate reader. As depicted in Figure 2, an exponential graph was generated, demonstrating a decrease in the percentage of cell viability with increasing extract concentrations. Notably, at the highest concentration of 7 mg/mL, the cell viability was observed to be 8.9%. By fitting the data to the equation y=70.23e-0.292x, the calculated half-maximum concentration of the methanolic extract was determined to be 1.2 mg/mL.

Clonogenic assay

In the clonogenic assay, HepG2 cells treated with a concentration of 1.2 mg/mL *C. lentillifera* extracts in a 6-well plate showed observable colony formation under phase contrast microscopy, as shown in Figure 3, compared to untreated cells. Notably, treated cells significantly reduced the number of colonies compared to untreated cells (p=0.012). Moreover, while the average diameter of treated colonies was smaller (1150 µm) than that of untreated HepG2 cells (1468 µm) as depicted in Figure 3, this difference in diameter was not statistically significant (p=0.081) (Supplementary 2).

Additionally, this study investigated molecular interactions between the target proteins (SREBP-1A and alpha-fetoprotein) and the ligand (palmitic acid) using Autodock Vina. The best and most stable interaction was selected based on the lowest binding energy observed for each target protein-ligand complex. Notably, SREBP-1A and alpha-fetoprotein exhibited the lowest binding affinities with palmitic acid (PubChem ID: 985) at -4.6 and -5.3 kcal/mol, respectively.

SREBP-1A demonstrated interaction with palmitic acid at residue GLY352, forming a hydrogen bond with a length of 2.75 Å (Supplementary 3). On the other hand, Human Alpha-fetoprotein formed a hydrogen bond with palmitic acid at residue GLN140, with a hydrogen bond length of 1.78 Å. Despite the more negative docking energy observed for palmitic acid against SREBP-1A and alpha-fetoprotein, the binding orientation of palmitic acid with SREBP-1A appears more favorable due to the absence of unfavorable interactions, as depicted in the 2-D docking image (Figure 4).

DISCUSSION

In this study, absolute methanol was used for the extraction of volatile compounds from *the C. lentillifera* sample. Methanol can extract both lipophilic and hydrophilic molecules. Furthermore, it has high polarity, allowing better extraction yield from the extract. The identification of volatile compounds contained in the methanolic extract of the *C. lentillifera* sample was performed using a GC-MS. Analysis indicated that the most abundant compound was hexadecenoic acid, followed by tridecanoic acid.

Hexadecanoic acid, also known as Palmitic Acid (PA), is a saturated fatty acid found in various species of seaweeds.[32] It has been associated with antiproliferative activity in prostate cancer.[33] breast cancer.[34] and colon cancer.[35] Similarly, fatty acids extracted from Antarctic macroalgal species *Adenocystis utricularis, Curdiea racovitzae* and *Georgiella confluens* had antitumor activity in breast cancer cell lines MCF-7 and MDA-MB-231 but had no cytotoxicity in the Chinese Hamster Ovary (CHO) cell line.[36] In addition, methanolic extracts of green seaweeds *Ulva intestinalis, Halimeda macroloba* and brown seaweed *Sargassum ilicifolium* also contained n-hexadecanoic acid as one of the major fatty acids. It exhibited toxicity in the breast, colorectal and hepatocellular carcinoma cells.[37] Therefore, PA can be a potent compound that has an inhibitory effect on the growth of cancer cells. Furthermore, tridecanoic acid was identified as the second most abundant compound. It is also known as Tri Decylic Acid (TDA), is an organic saturated fatty acid that can be found in plants like *Peganum harmala* and Bacillus species.[38] A previous study associated the presence of Fatty Acid Methyl Esters (FAMEs) in a commercial seaweed liquid extract product called True Algae Max (TAM). The formulation contained *Ulva lactuca* (green seaweed), *Jania rubens* (red seaweed) and *Pterocladia capillacea* (red seaweed) that stimulated immune response in *Oreochromis niloticus* (Nile tilapia).[39]

Cytotoxicity analysis indicated that the inhibitory effect of the *C. lentillifera* methanolic extract on the growth of HepG2 cells was dose-dependent (Figure 2). The anticancer effect towards cancer cells is directly proportional to the concentration of the *C. lentillifera* methanolic extract, whereby the highest concentration (7 mg/mL) had the highest inhibitory effect towards HepG2 cell growth while the lowest concentration ($7x10^{-9}$ mg/mL) had the lowest inhibition of cancer cell growth. The half-maximal inhibitory dose (IC_{50}) obtained for treatment of HepG2 liver cancer cells was 1.2 mg/mL. This can be associated with the presence of hexadecanoic acid and tridecanoic acid in the *C. lentillifera* methanolic extract. A colony formation assay was performed to determine the role of *C. lentillifera* with anti-metastatic characteristics. There was a significant reduction in the number of colonies after treatment with the extract, with notable diameter reduction, suggesting that the extract contains an anti-metastatic compound which needs to be evaluated further in pre-clinical studies.

In silico modeling demonstrates a potential interaction between hexadecenoic acids and the cancer-associated protein in liver cancer. Activation of Sterol Regulatory Element-Binding Protein 1 (SREBP-1), a master lipogenic transcription factor, is linked with cancer metabolism and metabolic disorders.[40] As a key regulator in lipogenesis, SREBP-1 contributes to the progression of Hepatocellular Carcinoma (HCC) by promoting cancer cell growth and metastasis. Previously, blocking SREBP cleavage-activating protein, which inhibits de novo lipid biosynthesis in the SREBP pathway, suppressed HCC.[41] These findings strongly suggest increased lipogenesis is a significant factor in HCC progression. However, the mechanisms underlying the effect of Palmitic Acid (PA) on human HCC are not fully understood. Further analysis was conducted using *in silico* methods to confirm the binding affinity of PA to SREBP-1 in liver cancer.

Two-Dimensional (2-D) images of docking showed that palmitic acid could exhibit a more stable interaction with SREBP-1A compared to alpha-fetoprotein. However, palmitic acid is involved in unfavorable donor-donor interaction with Arginine 169 residue of chain A for alpha-fetoprotein. Unfavorable interactions observed in the docking data indicate that the ligand and the target exhibit a repelling effect between the molecules. In docking analysis, the emergence of these undesirable interactions may negatively impact the stability of the ligand-target complex.

CONCLUSION

The study suggests that *C. lentillifera* has anticancer potential. The most abundant compound was palmitic acid. It was associated with the antiproliferative activity demonstrated in HepG2 liver cancer cells by binding to SREBP-1A. Further investigations are warranted to determine the regulatory effect of palmitic acid in liver cancer.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Rammiya Rajasegaran-data acquisition, data analysis, manuscript preparation, Asita Elengoe- literature search, data acquisition, data analysis, manuscript preparation. Woo Sau Pinn- design and manuscript review. Ariffuddin Abd Hamid-data acquisition and manuscript review. Noorfatimah Yahaya-manuscript editing and manuscript review. Shahrul Hamid -conception, data analysis, manuscript editing and manuscript review.

DATA AVAILABILITY STATEMENT

Data is provided in the supplementary materials section.

SUMMARY

The current study has identified volatile compounds present in *C. lentillifera* and demonstrated that its methanolic extract potentially suppresses HepG2 cell growth. The metastatic characteristics of this effect could be further explored using scratch assays in the HepG2 cell line. However, this study has not delineated the possible pathways or conducted additional assays to demonstrate suppression or inhibition of cell line growth. Therefore, further research is required to elucidate the expression of downstream molecules in the SREBP1a pathway following treatment with palmitic acid in liver cancer.

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No.	R.T. min	Compounds	Area %	Quality
1.	17.196	Hexadecanoic acid, methyl ester	28.61	96
2.	17.196	Tridecanoic acid, methyl ester	28.61	96
3.	17.196	Hexadecanoic acid, methyl ester	28.61	95
4.	18.804	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)-	20.44	91
5.	16.936	7,10,13-Hecadecatrienoic acid, methyl ester	12.67	90
6.	18.804	7,10,13-Hexadecatrienoic acid, methyl ester	20.44	87
7.	6.979	Undecane	8.75	87
8.	16.321	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, (1. alpha., 2. beta., 5. alpha.)	8.18	80
9.	16.936	Cyclooctane, 3-ethenyl-	12.67	74
10.	6.979	Undecane	8.75	68
11.	6.979	Undecane	8.75	68
12.	18.804	Z, Z, Z-4,6,9- Nonadecatriene	20.44	66
13.	16.321	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-	8.18	64
14.	16.936	3-Tetradecen-5-yne, (Z)-	12.67	64
15.	17.598	Tetrahydropyran 12-tetradecyn-1-ol ether	2.98	59
16.	17.598	2-Methylbutanoic anhydride	2.98	59
17.	16.557	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	4.26	58
18.	16.557	Cyclohexanol, 1-ethynyl-	4.26	53
19.	18.520	Thiazole	2.02	52
20.	18.520	4-Methylpentyl pentanoate	2.02	50
21.	18.520	2H-Tetrahydropyran, 2-[(1-ethenyl-1,5-dimethylhex-4-enyl) oxy]-	2.02	50
22.	16.557	1-Methyl-2-methylenecyclohexane	4.26	47
23.	17.598	Tetrahydrofuran, 2-methyl-5-pentyl	2.98	45
24.	16.321	Tridecanedial	8.18	38
25.	16.747	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.78	38
26.	27.862	3,4,5-Trihydroxybenzoic acid ethyl ester, tris (0-trimethylsilyl0-	1.48	38
27.	16.747	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	7.78	35
28.	16.747	1-Tridecyne	7.78	35
29.	27.862	9H-Imidazo[1,2-a] benzimidazole, 2- (4-chlorophenyl)-9-methyl-	1.48	30
30.	27.862	Phenylhydrazine, 4-nitro-N2- (chloro) (2-trienyl) methylene-	1.48	30
31.	6.364	Propanal, 2,3-dihydroxy-	1.86	28
32.	20.223	DL-Serine, N-[(phenylmethoxy) carbonyl]-	0.98	27
33.	20.223	1,3-Butadiene, -1,4-dichloro, -2,3-dimethyl	0.98	16
34.	6.364	1-Propyne, 1,3,3,3-tetrafluoro-	1.86	9
35.	6.364	1,2,15-Pentadecanetriol	1.86	9
36.	20.223	1H-1,3-Diborole, 1,3,4,5-tetraethy 1-2,3-dihydro-2-methyl-	0.98	9

Supplementary 1: List of volatile compounds present in *C. lentillifera* extract presented by the respective retention time, area percentage and quality measured by GC-MS analysis.

Colony formation	Replicates	Number of colonies formed	Average colony formation ± Standard deviation	P value of colony formation	Diameter (µm) n=30	Average diameter ± Standard deviation	P value of diameter (µm)
Control	1	1066	986 ± 70	0.012	1268	1468 ± 217	0.081
	2	958			1436		
	3	934			1699		
Treatment	1	749	677 ± 101		1095	1150 ± 94	
	2	720			1097		
	3	562			1259		

Supplementary 2: Comparison between control and *C. lentillifera* extract methanolic extract treated HepG2 liver cancer cells based on the number of colonies and their diameters.

Target protein	Ligand	Residues	Distance (Å)	Bond Category	Bond Type
Human SREBP1	Palmitic acid	GLY352	2.75	Hydrogen	Conventional Hydrogen
		VAL351	2.73	Hydrophobic	Carbon Hydrogen
		VAL350	5.03	Hydrophobic	Alkyl
		TYR369	5.30	Hydrophobic	Pi-Alkyl
		LYS365	3.71	Hydrophobic	Alkyl
		PHE372	3.91	Hydrophobic	Alkyl
Human Alpha-fetoprotein	Palmitic acid	GLN140	1.78	Hydrogen	Conventional Hydrogen
		ARG169	1.07	Unfavorable	Unfavorable Donor-Donor
		PHE139	2.63	Hydrogen	Carbon Hydrogen
		PHE139	4.50	Hydrophobic	Pi-Alkyl
		PHE139	5.39	Hydrophobic	Pi-Alkyl
		VAL141	4.75	Hydrophobic	Alkyl
		PRO142	5.32	Hydrophobic	Alkyl
		PRO142	4.62	Hydrophobic	Alkyl
		PRO142	4.59	Hydrophobic	Alkyl
		PRO148	4.85	Hydrophobic	Alkyl
		PHE158	5.25	Hydrophobic	Pi-Alkyl
		PHE158	5.28	Hydrophobic	Pi-Alkyl
		PHE158	5.16	Hydrophobic	Pi-Alkyl
		LYS161	5.08	Hydrophobic	Alkyl
		LYS161	4.43	Hydrophobic	Alkyl
		PHE162	4.82	Hydrophobic	Pi-Alkyl
		TYR185	5.47	Hydrophobic	Pi-Alkyl

Supplementary 3: List of hydrogen bond interactions between target proteins (SREBP1 and human Alpha-fetoprotein) and ligand (Palmitic acid).