Journal of
Materials in Life Sciences

Vol. 3 No. 2 (2024)

Full Paper

In vitro and in Silico Evaluation of *Acalypha Indica* Ethanolic Extract on Lipid Metabolism-Related Protein Expression on Breast Cancer Cells

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Article history
Received
22 September 2024
Revised
13 October 2024
Accepted
23 October 2024
Published online
30 November 2024

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Abstract

Dysregulated lipid metabolism has been recognized as one of the most significant metabolic changes in breast cancer development. *Acalypha indica* is a polyphenol-rich herbal plant with various medicinal applications such as antibacterial, antioxidant, and anticancer activities. However, studies on the inhibitory effect of *A. indica* ethanolic extract (AI) on breast cancer cells and its efficacy as an alternative lipogenesis inhibitor are still less explored. Therefore, this study was designed to investigate the possible inhibition mechanisms of selected phenolic acids and flavonoids from the extract against MCF-7 breast cancer. The antiproliferative effect of AI was determined using MTT assay. Immunocytochemical analysis was used to evaluate the inhibitory effect of AI on the expression of the three lipogenic enzymes; fatty acid synthase (FASN), acetyl-CoA carboxylase (ACCA), and ATP citrate lyase (ACLY). In addition, molecular docking of the phenolic acids (gallic acid, ferulic acid, and caffeic acid) and flavonoids (rutin and quercetin) were performed using AutoDock Tools, and their ADMET properties were determined using SwissADME and pKCSM web servers. AI treatment resulted in inhibition of cell viability of MCF-7 breast cancer cells after 24 and 48 h. In addition, immunocytochemistry revealed a significant decrease in the expression of FASN, ACCA, and ACLY in MCF-7 cells when compared to control. Docking studies demonstrated that AI phytochemical quercetin was considered the most potential lipogenesis inhibitor due to its lowest binding energy to FASN, ACCA, and ACLY compared to other phytochemicals. Nevertheless, results from ADMET analysis suggested that all compounds except rutin possessed acceptable ADME and toxicity properties. Collectively, these findings indicated that the chemoprevention by AI in breast cancer cells is associated with the inhibition of fatty acid synthesis.

Keywords Acalypha indica, fatty acid synthase, acetyl-CoA carboxylase, ATP-citrate lyase, breast cancer

1.0 INTRODUCTION

Breast cancer (BC) is a lipid-dependent cancer that is responsible for 2.1 million new cases among women worldwide. More than 1.5 million women were diagnosed with BC, accounting for 24% of new cancer cases and 15% of cancer deaths in 2018, and incident cases are anticipated to increase by more than 46% by 2040 [1]. Despite advancements in cancer diagnosis and therapy, a significant number of patients relapse after treatment and develop multidrug resistance, resulting in a

poor prognosis, reduced overall survival rates, and lower quality of life. This is a major public health problem, emphasizing the need for effective medicines to prevent or treat BC progression. Due to the high reported cases and mortality rates, BC poses a major public health concern and become one of the targets of the third Sustainable Development Goals (SDGs) which emphasized the need for effective treatment procedures to prevent or treat BC [2]. There is a compelling need to find potential therapeutic strategies with minimum toxicity for the benefit of cancer patients and society.

Dysregulated lipid metabolism in cancer cells has been recognized as a potential target as a new therapeutic target for the development of alternative cancer treatments [3]. Evidence suggests that proteins involved in fatty acid synthesis and oxidation have an important role in the proliferation, migration, and invasion of BC cells [4]. The fact that cancer cells heavily are reliant on fatty acids (FAs) to meet cellular demand is one of their important characteristics. Cancer cells are known to produce a significant portion of their fatty acids by *de novo* synthesis, whereas normal cells get the majority from dietary sources [5]. It is the primary source of lipids in cancer and has been demonstrated to be essential in BC cell survival and development. In contrast, fatty acid demand in normal tissues is mainly met from dietary sources and *de novo* fatty acid synthesis is minimal with the exception of several organs such as the liver, adipose tissue, and brain [6]. Overexpression of key fatty acid synthesis protein levels such as fatty acid synthase (FASN) [7–10], ATP-citrate lyase (ACLY) [11,12] and acetyl-CoA carboxylase (ACCA) [13] was observed in human BC. Besides that, it has been suggested that increased FASN expression is correlated with a more aggressive phenotype and shorter survival in BC patients by promoting multidrug resistance in treatment [14]. Thus, targeting those three enzymes involved in fatty acid synthesis may be a novel approach to the chemoprevention of BC.

Accumulating evidence suggests that plant-derived phytochemicals may be effective for the treatment of various malignancies. *Acalypha indica*, commonly known as “kucing galak” among Malaysians, is a traditional medicinal plant that grows widely throughout Asia, including India, Indonesia, and Sri Lanka [15,16]. *A. indica* is an exceptional source of phenols, flavonoids, tannins, coumarins, alkaloids, glycosides, saponins, volatiles, and fatty acids. *A. indica* has been shown to possess numerous therapeutic properties including anti-diabetic, antioxidant, antibacterial, and anti-inflammatory activities. Notably, the plant extract has also been shown to be safe by causing minimal cytotoxicity and significantly induces the proliferation of human skin fibroblast cells (HSF 1189) [17]. Furthermore, *in vivo* toxicity studies suggested that *A. indica* is safe for human consumption when administered orally [18,19]. *A. indica* flavonoid constituents such as quercetin has also been identified as a potential drug candidate that exhibits anti-obesity activity [20]. Nonetheless, the properties of *A. indica* are not only limited to antibacterial and antioxidant properties. In recent years, the extract of *A. indica* has been shown to have significant anticancer effects on BC cells and to shrink tumours *in vivo* [15,20]. Recent studies showed significant effects of *A. indica* extract in decreasing free fatty acids and reduced fat formation in diabetic rats [21]. Another study found that *A. indica* extract influenced fat formation in rats fed a high-fructose, high-cholesterol diet for four weeks [22,23]. However, it is unknown if *A. indica* ethanolic extract (AI) may regulate fatty acid synthesis in BC and have anticancer effects via interfering with fatty acid synthesis. Therefore, the present study aimed to investigate the potential of AI as a fatty acid synthesis inhibitor in BC cells.

2.0 EXPERIMENTAL

2.1 Preparation of *A. indica* ethanolic (AI) crude extract

Leaves of *A. indica* leaves were extracted using 80% of ethanol (HmbG, Germany) through the ultrasonication-assisted extraction (UAE) method. The extract was then stored in the refrigerator at 4°C for later use. To prepare the stock solution, 10 mg of AI was dissolved in 25 µL of Tween-20 (SYSTERM, Malaysia) and equal volume of 100% Dimethyl sulfoxide (DMSO) correspondingly. Then, 9950 µL of complete medium was added to make up a final volume of 10 mL. The solution was mixed thoroughly with a vortex followed by filtration through 0.22 µM syringe filter.

2.2 Cell culture

The MCF-7 breast cancer cells (ATCC, USA) were maintained as monolayer cultures in Roswell Park Memorial Institute medium (RPMI) 1640 medium (Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). The cells were maintained in a 5% CO_2 incubator (Esco, Singapore) at 37 °C.

2.3 Cell viability assay

MCF-7 cells were seeded in a 96-well plate at a density of 1×10^6 cells/mL in each well before being treated with *A. indica* extract (AI) at different concentrations (7.81 – 500 µg/mL) for 24 and 48 h. Cells of the control group were left untreated. Each well received 100 µL of 0.5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT; Thermo Fisher Scientific, USA) solution after treatment and incubated for 4 h at 37 °C. The generated formazan crystals were dissolved in 100 µL 100% DMSO and incubated for 30 mins. Absorbance was measured at 570 nm using a Spectro Star Nano microplate reader (BMG Labtech, Germany).

2.4 Immunocytochemical analysis

1×10^5 cells/mL MCF-7 cells were plated on a 13 mm glass coverslip in a 24-well plate. Once the cells reached 90% confluency, the cells were treated with three different concentrations of AI (31.25 – 125 µg/mL) for 24 h. Coverslips were fixed in 1% paraformaldehyde (PFA; Sigma-Aldrich, USA) for 20 mins at room temperature and then washed twice with Phosphate-buffered saline (PBS; Oxoid, USA). Following washes with PBS twice, samples were permeabilized for 30 mins with 0.25% Triton X-100 (Sigma-Aldrich, USA) and then washed with PBS twice. The coverslips were incubated overnight at 4 °C with anti-FASN, anti-ACCA or anti-ACLY primary antibodies (Santa Cruz Biotechnology, USA). Samples were washed with PBS and incubated with Alexa Fluor 488 goat anti-mouse IgG (Poly4503; Thermo Fisher Scientific, USA) for 4 h. After repeated washes, the coverslips were counterstained with a nuclear probe (Life Technologies, USA) for 10 mins in the dark at room temperature. After that, the fluorescence-stained cells were visualized under a fluorescence microscope (Nikon Intensilight C-HGFI, Japan). The intensity of the fluorescence was quantified by calculating the corrected total cell fluorescence (CTCF) using ImageJ software.

2.5 Molecular Docking

The docking study was carried out within the FASN (PDB ID: 2PX6), ACCA (PDB ID: 3FF6) and ACLY (PDB ID: 5TDE) active sites, which were retrieved from the Protein Data Bank (PDB) database using AutoDock Tools (ADT) version 1.5.6. The apo-protein structure was prepared by eliminating water and co-crystal ligand molecules followed by merging non-polar hydrogen atoms. A grid box of 40 × 40 × 40 was built and centred on the ligand in the complex with a spacing of 0.375 Å for all proteins. The ligand binding coordinates were determined through control docking procedure. Chemical structure of AI phytochemicals (caffeic acid, ferulic acid, gallic acid, quercetin, and rutin) was retrieved from the PubChem database which was subjected to 150 dependent runs. The best docking conformations were selected based on the free binding energy values and superimposition with the crystal structure. Protein/ligand interactions were determined using Discovery Studio Visualizer 4.0.

2.6 ADME and Toxicity Analysis

ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis of the phytochemicals (caffeic acid, ferulic acid, gallic acid, quercetin, and rutin) in AI was performed using the SwissADME server (Swiss Institute of Bioinformatics Switzerland) and pKCSM web server. The SMILES code of all selected compounds was submitted to both web servers to predict all possible ADMET properties. Parameters such as number of hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), total polar surface area (TPSA) value, lipophilicity, water solubility, P-glycoprotein (P-gp) substrate, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability and cytochrome P450 inhibitors were evaluated.

2.6 Statistical analysis

For statistical analysis, GraphPad Prism software version 8.0.2 (GraphPad Software, USA) was used. The normal distribution of the data was analysed by the Shapiro-Wilk test and the statistical comparisons of the mean between experimental groups were determined by one-way analysis of variance (ANOVA). All values are expressed as mean ± standard deviation (SD). In all comparisons, $P \leq 0.05$ was considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 AI inhibited MCF-7 cell proliferation

To investigate the anti-proliferative potential of AI, MCF-7 was treated with the extract at varying concentrations, ranging from 0 to 1000 µg/mL for 24 and 48 h, after which MTT assay was then performed. After treatment with 15.63 µg/mL of AI at 24 h, there was a significant inhibitory effect on the viability of MCF-7 cells compared to control (Figure 1). A significant drop on cell viability was observed on the MCF-7 cells treated with 62.5 µg/mL of AI at 48 h. After 24 h and 48 h treatment with AI, the IC_{50} values for AI is 138.8 µg/mL and 53.69 µg/mL respectively. This result is in line with a recent study that discovered the AI are rich in polyphenol contents which contribute to inhibition of cell proliferation of cancer cells by inducing apoptosis in a dose and time-dependent way [21]. Another study reported IC_{50} value of 50 µg/mL of AI on MCF-7 cells which is much lower than the result in this study [15]. Thus, the results demonstrated that AI has a cytotoxic effect on MCF-7 cells in a dose-dependent manner.

Table 1. The IC_{50} value of *A. indica* on MCF-7 cells after 24 h and 48 h treatment.

	IC_{50} (µg/mL)	
Time	24	48
Concentration	138.8	53.69

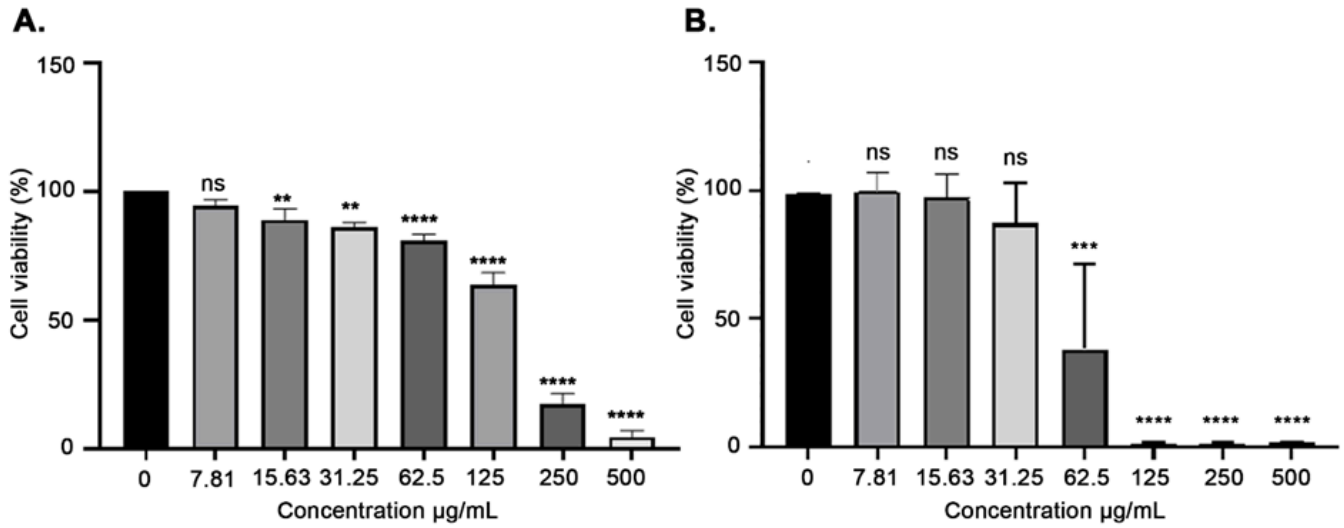

Figure 1 The graph of the cell viability in MCF-7 treated with AI with 24 (A) and 48 (B) hours incubation period. The data is displayed as mean ± standard deviation (SD). The thresholds for statistical significance were * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$, and **** $P < 0.0001$ correspondingly.

3.2 AI reduced lipogenic enzymes expression on MCF-7 breast cancer cells

To investigate whether the cytotoxic effect of AI on BC cells is related to fatty acid synthesis inhibition, immunocytochemistry analysis was performed to determine the effect of AI treatment on the protein levels of FASN (an enzyme complex responsible for the synthesis of saturated fatty acids like palmitic acid from malonyl-CoA), ACCA (an enzyme responsible for conversion of acetyl-CoA to malonyl-CoA), and ACLY (an enzyme responsible for conversion of citrate to acetyl-CoA) [12]. Figure 2 (left) shows representative fluorescence images of FASN (A), ACLY (B) and ACCA (C) proteins in control and AI-treated MCF-7 cells. The protein level of ACLY was significantly reduced by AI treatment in MCF-7 cells in a dose-dependent manner. On top of that, the protein level of ACCA, which is the rate-limiting enzyme in the de novo fatty acid synthesis, decreased significantly upon AI treatment in MCF-7 cell lines. Exposure of BC cells to AI resulted in suppression of FASN protein level. Quantitation of the immunofluorescence using corrected total cell fluorescence (CTCF) confirmed significant downregulation of these proteins in AI-treated cells in comparison with untreated cells.

Phytochemicals such as flavonoid, polyphenol compounds, alkaloids, tannins, and steroids, derived from AI could be the potential compound in inhibiting the expression of fatty acid synthesis enzymes in this study. It was reported in a study that lutein, quercetin, and kaempferol were three naturally occurring polyphenols that can reduce lipogenesis [24]. Furthermore, plant-derived flavonoids, including luteolin, quercetin, taxifolin, galangin, morin, and genistein have been reported to have an inhibitory effect on FASN [25]. On top of that, polyphenols in plants regulate hyperlipidaemia and reduce lipogenesis via acetyl-CoA carboxylases 1 and 2 [26]. Following treatment with flavonoids such as coumarins, diterpenes, triterpenes, lignins, and phenylpropanoid glucosides, the mRNA expression of the FASN, ACCA, and SREBP-1c also decreased [27]. ACCA suppression inhibited palmitic acid production in breast tumour cells, causing cell death, ROS build up, and mitochondrial malfunction. ACLY inhibition reduces the availability of acetyl-CoA, which is required for fatty acid synthesis. Cell death occurs as a result of ACLY inhibition, as does a reduction in energy supplies as a result of the disruption of both fatty acid synthesis and oxidation. The current study's findings established that AI treatment reduced ACLY protein levels. As a result of ACLY inhibition and the resulting loss in energy sources owing to the disruption of both fatty acid synthesis and oxidation, cell death ensues. Inhibiting ACLY also generates a build up of citrate molecules, which has a detrimental impact on glycolysis and the PDH enzyme, as well as catalysing oxidative decarboxylation, which links glycolysis and the Krebs cycle. In various models, inhibiting glycolysis, the tricarboxylic acid cycle, and the insulin-like growth factor 1 receptor pathway inhibits tumour development. In addition, a study reported that ACLY regulates the Akt signalling pathway to support tumour growth and that the PI3K/Akt signalling pathway played a crucial regulatory role in a variety of malignancies [28]. The inhibition of ACLY expression by AI may downregulate the PI3K/Akt signalling pathway, which resulted in a decrease in MCF-7 cell proliferation. As a result, the findings suggested that AI's effect on MCF-7 cells is at least partly related to the suppression of enzymes involved in *de novo* lipogenesis.

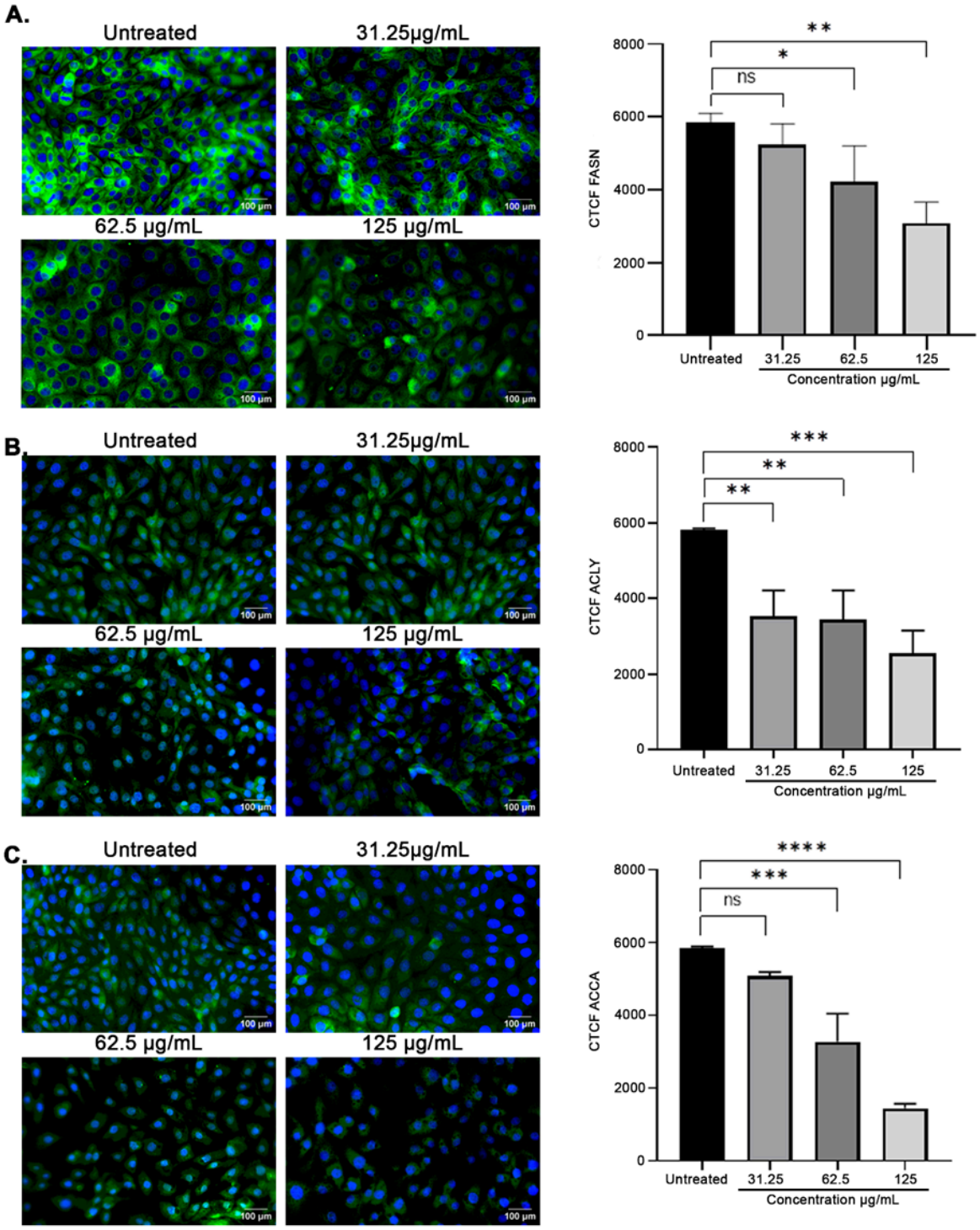

Figure 2 AI treatment decreased the expression levels of lipid metabolism-related enzymes in MCF-7 cells. 1 × 10^6 MCF-7 cells/well were seeded on a glass coverslip in a 24-well plate. 24 hours post-incubation, cells were treated with either AI (31.25 - 125 µg/mL) or left untreated. A day after that, cells were subjected to immunocytochemical staining. (left) Representative confocal images (20 × objective magnification) for (A) FASN, (B) ACLY and (C) ACCA proteins (green fluorescence) in untreated- or AI-treated MCF-7 cells. A nuclear probe (blue fluorescence) was used to stain nuclei. (right) The corrected total cell fluorescence (CTCF) for (A) FASN, (B) ACLY and (C) ACCA protein expression in AI-treated MCF-7 cells is shown in mean ± SEM from three independent experiments (n = 3). *ns*; non-significant, *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control by Students' t-test.

3.3 Molecular docking analysis of lipogenesis enzymes

To gain some more insight into the inhibitory effect of AI in binding to fatty acid synthesis enzymes, molecular docking was performed to predict which phytochemicals can interfere with FASN, ACCA and ACLY. To validate the docking procedures, the co-crystal ligand inside the PDB file of 2PX6, 3FF6 and 5TDE were extracted and re-docked with its target. The RMSD values for these targets were below 2.0 Å. The results showed that among five AI phytochemicals; caffeic acid, ferulic acid, gallic acid, quercetin and rutin identified from published literatures [29,30], only quercetin and rutin demonstrated higher binding to FASN Thioesterase domain than the known FASN inhibitor, Orlistat (-5.47 kcal/mol) [31]. FASN is crucial for fatty acid synthesis, and the TE domain is essential for terminating fatty acid chain elongation and releasing newly generated fatty acids. Orlistat binds specifically to the FASN TE, blocking its active site. This prevents the enzyme from hydrolysing the thioester bond of acyl-ACP, which is necessary for releasing fatty acids. Quercetin (-6.39 kcal/mol) and rutin (-6.22 kcal/mol) demonstrated highest binding affinity followed by caffeic acid (-5.12 kcal/mol), ferulic acid (-4.80 kcal/mol), and gallic acid (-4.39 kcal/mol). The interaction between rutin and quercetin with FASN TE active site revealed that phytochemicals interacted well over the distal pocket and hydrophobic groove by binding to the seven conserved amino acids (Leu2222, Glu2251, Ile2250, Phe2371, Phe2370, Phe2375, and Phe2423) through hydrogen bonding, van der Waals forces, and Π-stacking interaction (Figure 3). In contrast, gallic acid, ferulic acid, and caffeic acid showed less interaction with these conserved amino acids, particularly the Phe2370, probably due to their smaller molecular size. This finding demonstrated that phenolic acids have a lower binding affinity compared to flavonoids which was contributed by the presence of interactions with these conserved amino acid residues of FASN TE domain. By inhibiting the release of fatty acids, AI may reduce the overall synthesis of fatty acids within the cancer cells. The inhibition of FASN leads to decreased levels of fatty acids, potentially lipid metabolism and energy storage causing cancer cells to undergo cell death [31].

Table 2. The binding energy of selected phytochemicals on lipid metabolism-related proteins.

Compounds	Free Binding energy (kcal/mol)		
	FASN	ACCA	ACLY
Caffeic Acid	- 5.12	- 3.54	- 6.85
Ferulic Acid	- 4.80	- 3.25	-7.14
Gallic Acid	- 4.39	- 2.72	-5.42
Quercetin	- 6.39	- 4.79	-7.47
Rutin	- 6.22	- 3.87	-5.07

Compared to other phytochemicals, quercetin showed lower free binding energy value of -4.79 kcal/mol followed by rutin (-3.87 kcal/mol), caffeic acid (-3.54 kcal/mol), ferulic acid (-3.25 kcal/mol) and gallic acid (-2.72 kcal/mol). However, all AI phytochemicals showed weaker binding energy in binding to ACCA compared to control docking ligand, CP-640186 (-7.86 kcal/mol) [14]. Nevertheless, quercetin and rutin exhibited similar interactions to CP-640186 active site amino acids of ACCA namely Gly2162, Arg2158 and Glu2230 (Figure 4). CP-640186 is a selective inhibitor of ACCA. It inhibits ACCA by binding to the active site of the enzyme, which prevents it from catalysing the conversion of acetyl-CoA to malonyl-CoA [32,33]. The binding of AI phytochemicals to ACCA may contribute to the reduction of malonyl-CoA levels, thereby limiting fatty acid synthesis and inhibiting cancer cell growth and proliferation.

On the other hand, quercetin and ferulic acid showed the lowest binding free energy values of -7.47 and -7.14 kcal/mol when docked with ACLY, followed by caffeic acid (-6.85 kcal/mol), gallic acid (-5.42 kcal/mol) and rutin (-5.07 kcal/mol). ACLY is an enzyme that converts citrate from the mitochondria to acetyl-CoA and oxaloacetate in the cytoplasm, which is essential for fatty acid synthesis. Nevertheless, the binding energy of AI phytochemicals was weaker compared to known ACLY inhibitor 2-hydroxycitrate (HCA) (-7.84 kcal/mol) [12,34,35]. HCA is structurally identical to citrate but has a considerably greater affinity for ACLY than citrate, making it a powerful competitive inhibitor of the enzyme ACLY [12]. When HCA occupies the active site of ACLY, it prevents citrate from binding and being converted into acetyl-CoA. This inhibition leads to a decrease in the levels of acetyl-CoA, thereby impacting lipid biosynthesis and other metabolic pathways that depend on acetyl-CoA. Both quercetin and ferulic acid formed hydrogen bonds with Phe347 and Thr348 (Figure 5). Additionally, these ligands were also placed in a predominantly hydrophobic pocket mainly restricted by Phe347 and His760, these forming interactions with all these residues and a hydrogen bond with Ph347 which undoubtedly improved the binding affinity of the phytochemicals on the protein. By preventing the binding and conversion of citrate to acetyl-CoA, these phytochemicals could play a significant role in modulating fatty acid synthesis.

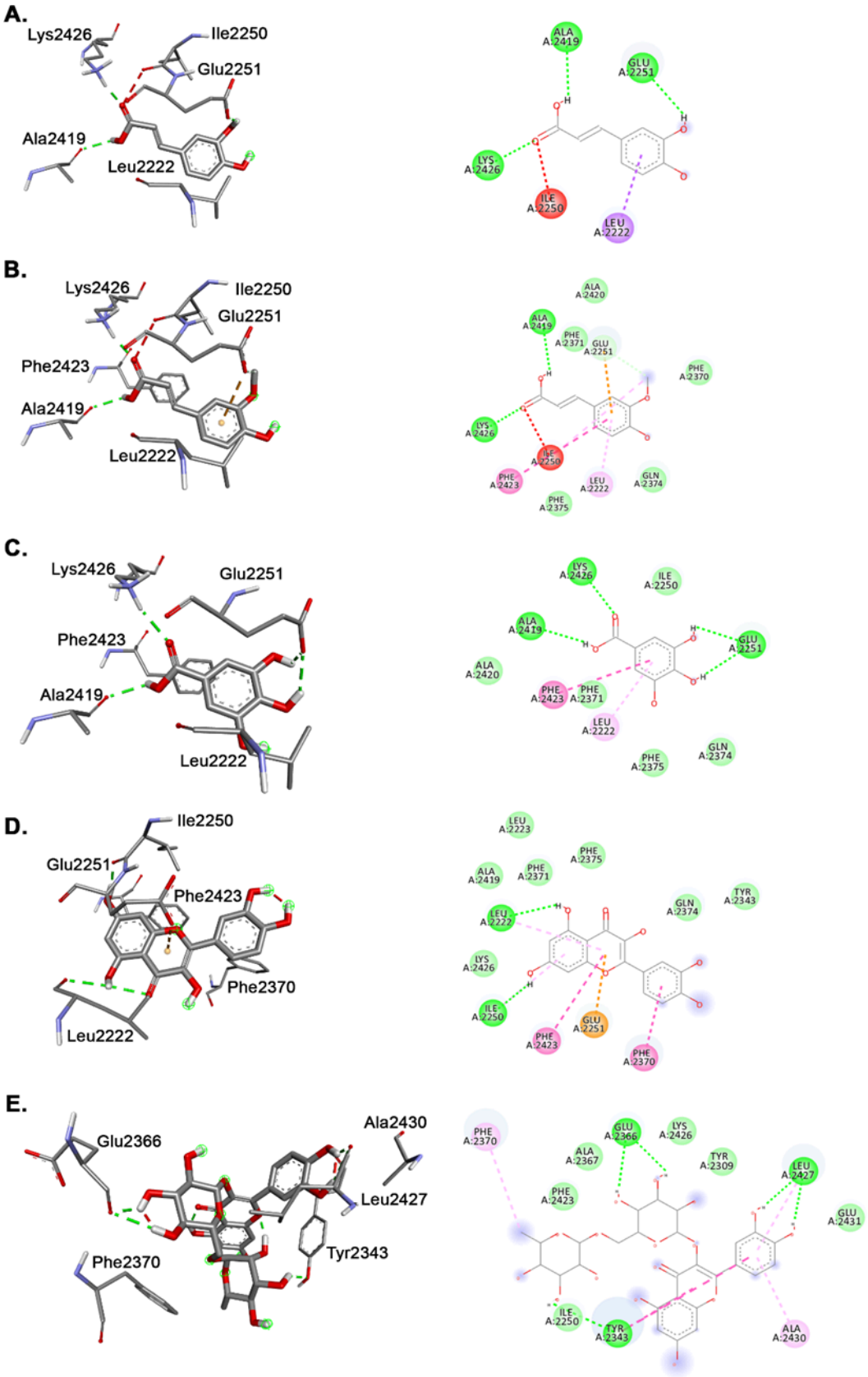

Figure 3 3-D (left) and 2-D (right) representations of FASN with the best docking compounds: (a) Caffeic Acid, (b) Ferulic acid, (c) Gallic acid, (d) Quercetin, and (e) Rutin.

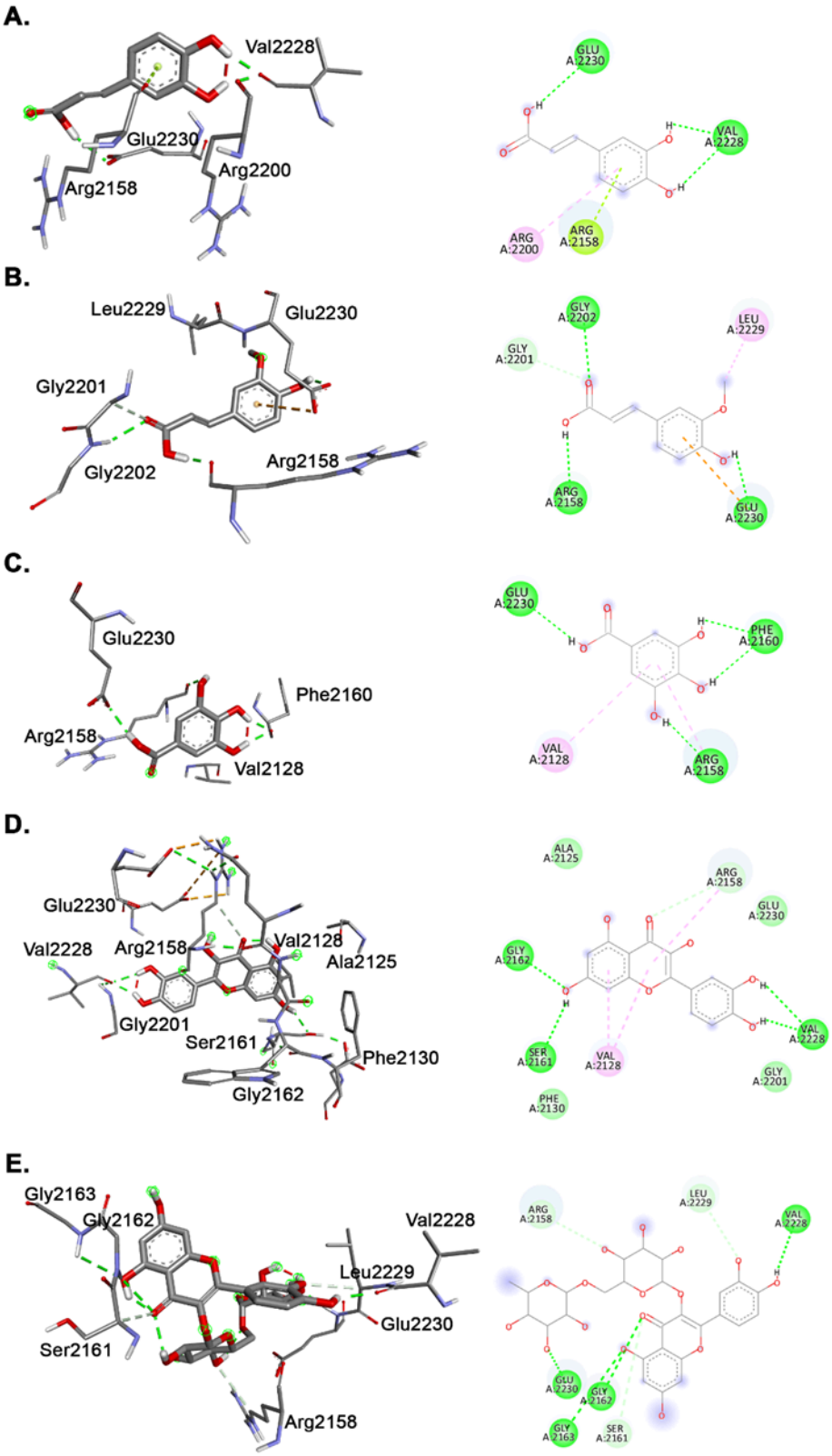

Figure 4 3-D (left) and 2-D (right) representations of ACCA with the best docking compounds: (a) Caffeic Acid, (b) Ferulic acid, (c) Gallic acid, (d) Quercetin, and (e) Rutin

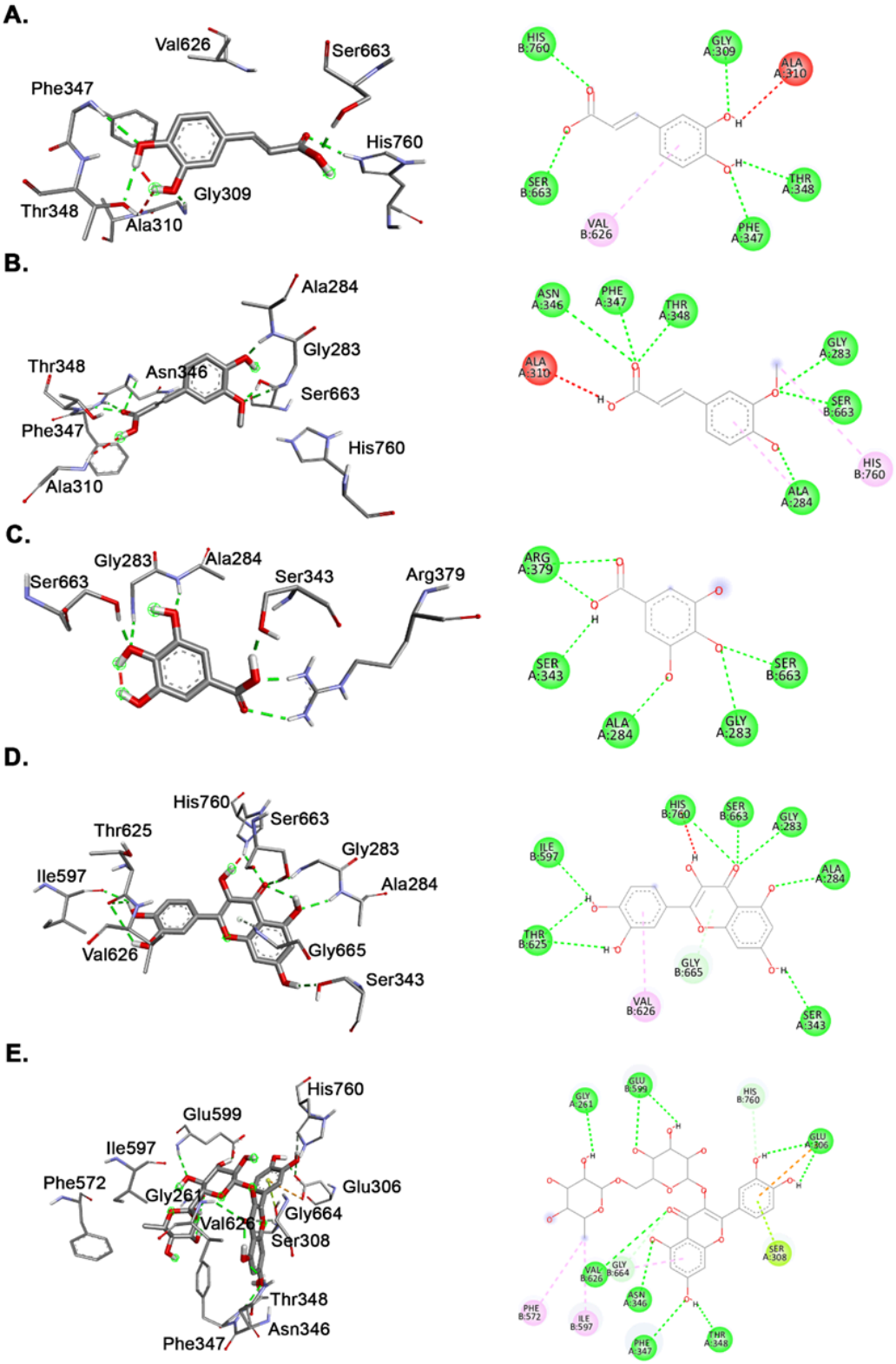

Figure 5 3-D (left) and 2-D (right) representations of ACLY with the best docking compounds: (a) Caffeic Acid, (b) Ferulic acid, (c) Gallic acid, (d) Quercetin, and (e) Rutin

These results suggested that AI phytochemicals may inhibit fatty acid synthesis by interacting with those three lipogenic enzymes, preventing substrate binding and enzyme activity. This could lead to downregulation of ACLY, ACCA and FASN protein expression and activity as shown in Figure 2, resulting in the suppression of MCF-7 cell proliferation (Table 1).

However, further *in vitro* investigations on inhibition of those three lipogenic enzymes by the selected AI phytochemicals should be conducted to confirm the *in silico* results.

3.4 ADME and Toxicity analysis of AI phytochemicals

The pharmacokinetics and drug-likeness profile of the AI phytochemicals were evaluated using SwissADME and pKCSM web servers. Water solubility, gastrointestinal absorption, blood-brain barrier (BBB) penetration, CYP450 inhibition, and skin permeation were among the pharmacokinetic characteristics evaluated. Toxicity parameters determined, on the other hand, included AMES toxicity, hERG blockers, hepatotoxicity, and skin sensitization (Table 3). Compounds having a molecular weight of 500 g/mol, less than 10 hydrogen bond acceptors, less than 5 hydrogen bond donors, and a lipophilicity value (LogP) of less than 5 exhibit strong permeability as well as good absorption and bioavailability [36].

Table 3 The ADMET properties of selected phenolic and flavonoid in *A. indica* by SwissADME and pKCSM

		Compound				
		Caffeic Acid	**Ferulic Acid**	**Gallic Acid**	**Quercetin**	**Rutin**
Physiochemical properties	MW (g/mol)	180.16	194.18	170.12	302.24	610.52
	Fsp^3	0.00	0.10	0.00	0.00	0.44
	RB	2	3	1	1	6
	HBA	4	4	5	7	16
	HBD	3	2	4	5	10
	MR	47.16	51.63	39.47	78.03	141.38
	TPSA ($Å^2$)	77.76	66.76	97.99	131.36	269.43
Lipophilicity	Consensus logP	0.93	1.36	0.21	1.23	-1.29
Drug-likeness	Lipinski	No violation	No violation	No violation	No violation	3 violations: MW>500, NorO>10, NHorOH>5
	Ghose	No violation	No violation	2 violations: MR<40, #atoms<20	No violation	4 violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70
	Veber	No violation	No violation	No violation	No violation	1 violation: TPSA>140
	Egan	No violation	No violation	No violation	No violation	1 violation: TPSA>131.6
	Muegge	1 violation: MW<200	1 violation: MW<200	1 violation: MW<200	No violation	4 violations: MW>600, TPSA>150, H-acc>10, H-don>5
Water Solubility	ESOL	-1.89	-2.11	-1.64	-3.16	-3.30
	LogW	2.32e+00 mg/ml; 1.29e-02 mol/l	1.49e+00 mg/ml; 7.68e-03 mol/l	3.90e+00 mg/ml ; 2.29e-02 mol/l	2.11e-01 mg/ml; 6.98e-04 mol/l	3.08e-01 mg/ml; 5.05e-04 mol/l
	Class	Very soluble	Soluble	Very soluble	Soluble	Soluble
Pharmacokinetics	GI absorption	High	High	High	High	Low
	BBB permeant	No	Yes	No	No	No
	P-gp substrate	No	No	No	No	Yes
	CYP1A2 inhibitor	No	No	No	Yes	No
	CYP2C19 inhibitor	No	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	Yes	No
	CYP3A4 inhibitor	No	No	Yes	Yes	No
	Log K_p (cm/s)	-6.58	-6.41	-6.84	-7.05	-10.26
	Bioavailability	0.56	0.85	0.56	0.55	0.17
Toxicity	Carcinogenicity (AMES Toxicity)	No	No	No	No	No

Cardiovascular toxicity (hERG I Inhibitor)	No	No	No	No	No
Hepatotoxicity	No	No	No	No	No
Skin Sensitization	No	No	No	No	No

Notes: Molecular weight: MW, topological polar surface area: tPSA, Molar Refractivity: MR, fraction of sp3 carbon atoms: Fsp3, HBD: hydrogen bonds donor, HBA: hydrogen bond acceptor, RB: rotatable bonds, LogP values: indicator of Lipophilicity, ESOL: aqueous solubility parameter, Log Kp: skin permeation, F: Bioavailability Score, hERG: human Ether-à-go-go-Related Gene.

Based on the result, caffeic acid, ferulic acid, gallic acid and quercetin were predicted as potential oral drug candidates as they have high possibility of gastrointestinal (GI) absorption and adhering to Lipinski's rule of five. However, rutin was predicted as a poor oral drug candidate due to its low GI absorption and it violated both Lipinski and Veber parameters of the rules. Only rutin have molecular weights greater than 500 g/mol, have more than 10 hydrogen bond acceptors and 5 hydrogen bond donors. Molecular weight and low lipophilicity values suggest that routine might have low membrane permeability and oral bioavailability. In addition, all phytochemicals were predicted to be non-carcinogenic and have satisfactory safety profiles for all major toxicity measures. These results are encouraging but the bioavailability of these phytochemicals and their metabolites after oral administration is yet to be determined. Collectively, the predicted ADMET properties for the selected phytochemicals suggested that almost all compounds except for rutin had orally active drug-likeness properties.

4.0 CONCLUSION

The present study demonstrated that anticancer properties of AI are associated with inhibition of fatty acid synthesis in BC cells. Besides, according to the molecular docking assessment, AI phytochemicals bind to the active sites' amino acids of FASN, ACCA and ACLY through hydrogen bonding and hydrophobic interactions. Additionally, computational studies suggested that quercetin emerged as a lead for inhibition of fatty acid synthesis enzymes, thus providing a new therapeutic approach to BCtreatment. Altogether, AI may represent a potential anticancer therapeutic agent by acting on fatty acid synthesis leading to reduction of MCF-7 cell proliferation.

Acknowledgment

This study was funded by Universiti Teknologi Malaysia (Collaborative Research Grant; R.J130000.7354.4B448 and R.J130000.7354.4B439). The authors thanked the Faculty of Science, Universiti Teknologi Malaysia for providing full support, access, and facilities especially the Cancer Research Laboratory research group until this study was completed.

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