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EVALUATION OF *CARICA PAPAYA* L. EXTRACT AS POTENTIAL THERAPEUTIC AGENTS FOR GASTROINTESTINAL ULCERS: PHYTOCHEMICAL CHARACTERIZATION, BIOCHEMICAL PROFILLING, AND *IN-SILICO* EVALUATION

Adeleye Gbenga Sunday[1], Olalekan Elijah Odesanmi*[2], Blessing Tolulope Owolabi[1], Deborah Odunayo Jesusanmi[1], Wonderful Oluwapamilerin Folayan[1], Oluwafolakemi Oluwayemisi Jeje[1], Oluwanifemi Faith Sunmonu[1], Ebenezer Oluwatobi Ojo[1], Blessing Oluwatobiloba Ajayi[1], Ayooluwa Comfort Shotubo[1], Praise Jesutofunmi Olamakinde[1], Oluwafemi Alexander Kowe[1], Kofoworola Grace Omosanu[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria.

[2]Department of Biochemistry, Faculty of Sciences, Ekiti State University, Ado Ekiti, Nigeria.

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***Corresponding Author: Olalekan Elijah Odesanmi**
Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria.
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ABSTRACT

Introduction: Gastrointestinal ulcers are a significant health concern, and the development of effective and safe treatment options remains a priority. The present study aimed to investigate the potential of *Carica papaya* leaf extract and its bioactive compounds as therapeutic agents for gastrointestinal ulcers by targeting the key proteins FFAR1, FABP4, PPARA, and PPARD that are associated with the pathogenesis of the disease. ***Methods:*** The study began with a comprehensive biochemical analysis of the *C. papaya* L. extract, including assessments of anti-lipoxygenase (ALOX) activity, protein denaturation (PD) activity, membrane stabilizing activity, and in vitro urease inhibition activity. GC-MS analysis was conducted to identify the bioactive compounds present in the extract, and a clustering analysis was performed to assess the physicochemical properties of these compounds. In silico molecular docking studies were then carried out to evaluate the binding interactions of the identified bioactive compounds with the target proteins. ***Results:*** The results demonstrated that the *C. papaya* L. extract exhibited potent ALOX inhibitory activity, high PD inhibitory activity, significant membrane stabilizing effects, and concentration-dependent urease inhibition. The in silico docking studies revealed that several bioactive compounds, including Octadecanoic acid, 2,3-dihydroxypropyl ester, cis-13-Octadecenoic acid, Phytol, Oleic acid, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester, and Diethyl Phthalate, exhibited favorable binding affinities with the target proteins. ***Conclusion:*** The findings of this study provide compelling evidence for the therapeutic potential of *C. papaya* L. extract and its bioactive compounds in the treatment of gastrointestinal ulcers. Further in vivo and clinical investigations are warranted to fully elucidate the mechanisms of action and to establish the efficacy of these natural compounds as novel ulcer-healing agents.

KEYWORDS: Gastroitestinal tract, Ulcer, Carica papaya L., Anti-liopxygenase, In-silico.

1.0 INTRODUCTION

Gastrointestinal (GI) ulcers are a significant public health concern, affecting millions of people worldwide.[1] These painful lesions in the lining of the digestive tract can occur in various parts of the GI system, including the stomach (peptic ulcers) and the duodenum (duodenal ulcers).[2] GI ulcers are primarily caused by an imbalance between the aggressive factors, such as excessive stomach acid, Helicobacter pylori infection, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs), and the protective factors, such as the mucus layer and bicarbonate secretion.[3] The pathogenesis of GI ulcers involves a complex interplay of various signaling pathways and molecular targets. Specific target proteins have been identified as crucial players in the development and progression of GI ulcers which includes free fatty acid receptor 1 (FFAR1), fatty acid-binding protein 4 (FABP4), peroxisome proliferator-activated receptor delta (PPARδ/ PPARD), and peroxisome proliferator-activated receptor alpha (PPARα/ PPARA). FFAR1, also known as G protein-coupled receptor 40 (GPR40), is a receptor for medium and long-chain fatty acids. It plays a role in the regulation of gastric acid secretion, which can contribute to the development of GI ulcers.[4] FABP4, a cytoplasmic protein involved in the intracellular transport of fatty acids, has been implicated in the modulation of inflammatory responses and oxidative stress, both of which are key factors in GI ulcer pathogenesis.[5] PPARδ and PPARα are nuclear receptors that regulate gene expression involved in various cellular processes, including inflammation, cell proliferation, and wound healing.[6] Dysregulation of these PPAR isoforms has been associated with the impaired mucosal defense and increased susceptibility to GI ulcers.[6]

In recent years, there has been growing interest in the exploration of natural compounds as potential therapeutic agents for GI ulcers.[7] *Carica papaya* L., a tropical fruit, has been traditionally used in various cultures for its medicinal properties, including its ability to promote wound healing and exert anti-inflammatory effects.[8] The leaves of *C. papaya* are a rich source of bioactive compounds, such as flavonoids, alkaloids, and terpenoids, which have shown promising pharmacological activities.[9] However, the potential of *C. papaya* L. compounds in targeting these key molecular players involved in GI ulcer pathogenesis. This study aims to explore the therapeutic potential of *C. papaya* L. compounds by investigating their ability to target FFAR1, FABP4, PPARδ, and PPARα through molecular docking studies and in-vitro studies.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The plant *C. papaya* L. were obtained from a private farm in Ado Ekiti, Nigeria, botanically identified and authenticated at the Department of Plant Science, Ekiti State University, Ado- Ekiti, Nigeria.

2.2 Preparation of Plant Sample

The freshly collected *C. papaya* L. were washed and dried under room temperature. After drying, the leaf was grounded using pestle and mortar into smaller particles and then blended to powder using an electric blender. The powdered sample was then stored in containers and kept under room temperature for biochemical analysis.

2.3 Anti-Lipoxygenase Activity Analysis

Anti-Lipoxygenase Activity Anti-LOX assay was carried out using linoleic acid as substrate and lipoxidase as enzyme. *C. papaya* L extract sample (200-800 µg/ml) was dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and 0.25 ml of soybean lipoxidase enzyme solution (final concentration 20,000 U/ml) was added. This mixture was incubated at 25 °C for 5 minutes. Then 1.0 ml of linoleic acid solution (0.6 mM) was added, mixed well and the absorbance was measured

at 234 nm. Indomethacin (60 g/ml) was used as a reference standard. Percent inhibition was calculated using the following equation: (% inhibition = [(AC – AT)/Ac] x 100), where, AC is the absorbance of control; AT is the absorbance of test sample. All tests and analyzes were performed in triplicate and averaged.

2.4 Protein Denaturation Activity Analysis

The protein denaturation assay was carried out according to the method described by Gambhire *et al*. (2009) with some modifications as in Gunathilake *et al.*[10] The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.02 mL of extract. The mixture was mixed and incubated in a water bath (The reaction mixture was then heated to 37 °C for 15 minutes and then heated to 70 °C for 5 minutes. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer (Spectrumlab 752S). Aspirin was used as standard. The percent inhibition of protein denaturation was calculated using the following formula: (% inhibition of denaturation = 100 × (1 − A2/A1)), where, A1 = absorption of the control sample, and A2 = absorption of the test sample.

2.5 Membrane Stabilizing Activity Analysis

The membrane stabilizing activity of the *C. papaya* L. extracts was determined according to the method of Akhtar.[11]

2.5.1 *Effect on Haemolysis*

Erythrocyte suspension: Whole blood was collected from goat under ether anesthesia. NIH was used to prevent clotting. NIH solution was made by adding 5.5 g Trisodium citrate, 2 g citric acid, 6.125 g dextrose in 250 ml distilled water. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 100ml of distilled water: NaH2PO4.2H2O, 0.26 g; Na2HPO4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). The isotonic buffer solution was composed of 154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4).

Hypotonic solution-induced hemolysis: Stock erythrocyte suspension (30 µl.) was mixed with 5 ml of the hypotonic solution containing the *C. papaya* L. extracts at concentrations of 1000 µg/ml, 1500 µg/ml, 2000 µg/ml and 2000 µg/ml, 2500 µg/ml, 3000 µg/ml for locust bean and *C. papaya* L. respectively, while the control sample was mixed with drug free solution. The mixtures were incubated for 10 min at room temperature and centrifuged at 3000 rpm for 10 min. and the absorbance (O.D.) of the supernatant was measured at 560 nm. Acetyl salicylic acid (Aspirin) was used as a reference standard at a concentration 1000 µg/ml, 1500 µg/ml and 2000 µg/ml.

***Calculation*:** The percentage inhibition or acceleration of haemolysis in tests and standard was calculated according to the equation: (% acceleration or inhibition of hemolysis = 100(OD1 – OD2)/OD1), where, OD 1 = Optical density of hypotonic saline solution + blood (control) and OD2 = Optical density of test sample in hypotonic saline solution + blood.

2.6 *In Vitro* Urease Inhibition Activity Analysis

The modified Berthelot method was adopted. Briefly, 0.01g of each sample was dissolved in 10ml of ethanol to obtain a 1mg/ml test solution. Thiourea reference solution used for the comparison was made by dissolving 0.1g of thiourea in 10ml of ethanol to obtain the reference stock solution (10mg/ml). A 1ml solution of the thiourea stock solution was further diluted to 10ml with ethanol to obtain the 1mg/ml thiourea working solution for comparison. The content of the

Blood Urea Nitrogen (BUN) enzyme vial was reconstituted in 10ml of deionized water as specified in the manufacturer's test kit. The assay began by transferring a 1ml aliquot of *C. papaya* L. extracts, reference thiourea solution, ethanol (negative control) into separately labeled test tubes. To each of these test tubes, 1ml of the reconstituted BUN enzyme solution was added, mixed and followed by the addition of 10µl of urea. The mixture was then incubated for 10 min at room temperature. The percentage inhibition of urease activity was then calculated using the formula: (Percentage urease inhibitory activity = 100(A control – A sample) /A control), where, A control = Absorbance of the control solution (containing all reagents except the extracts), and A sample = Absorbance of the plant extracts or thiourea reference solution. For the standard urease inhibitor, the thiourea was employed. Both ureases were purchased as 99.9% pure from Mon Scientific (Lagos).

2.7 Solvent Extraction

The *C. papaya* L. (10 g) were subjected to successive solvent extraction with 95% of ethanol (1:7, m/V) for 48 h by maceration at room temperature and filtered through Whatman No. 4 filter paper. The residues were re-extracted with 95% ethanol (1:5, m/V), as described above, for 24 h. The solvent combined extract was evaporated at 40 °C and stored at 4 °C for further analysis.

2.8 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The *C. papaya* L. powder were subjected to chromatographic analysis using a Varian 3800/4000 gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column VF-5MS fussed silica capillary column (30.0m x 0.25mm x 0.25µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70ev; nitrogen (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 240^{o}C ion-source temperature 200^{o}C. The oven temperature was programmed from 70^{o}C (isothermal for 3 min), with an increase of 10^{o}C/min, to 240^{o}C, ending with a 9 min isothermal at 280^{o}C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 1000Da. Total GC running time is 42 min. The identification of the various components was based on comparison of their mass spectra with those of NIST Library mass Spectra data base and mass spectra from Literature. Only compounds with 90% or greater spectral matching accuracy are reported. No response factors were calculated.

2.9 Computational Drug Designing and Screening

2.9.1 *In-silico Pharmacokinetics*

The in silico ADME (absorption, distribution, metabolism, and excretion) screening of the compounds were done on Swiss ADME server (www.swissadme.ch), which was performed at default parameters using the SMILES format[12], by following the Lipinski's rule of five.

2.9.2 *Clustering Analysis*

The SMILES of the bioactive compounds in *C. papaya* L. identified through GC-MS were gotten from NCBI PubChem Compound database (https://pubchem.ncbi.nlm.nih.gov/) and used to generate clustering analysis of the compounds. The clustering of the chemical compounds was done on the Chemmine R webserver (https://chemminetools.ucr.edu/). The fingerprint of similarity of the compounds based on their physicochemical properties was obtained.

2.9.3 *Ligand Preparation*

The major phytochemical constituents of *C. papaya* L. were identified by G C-MS analysis and their structures were obtained from NCBI PubChem Compound database (https://pubchem.ncbi.nlm.nih.gov/) in sdf formats. LigPrep tool was utilized for ligand preparation which was adorned in Maestro Schrodinger panel (v. 12.8). Using this tool, energy minimization with the OPLS4 force field was performed, which involved applying the standard energy function of molecular mechanics while ensuring that a maximum of one stereoisomer was generated per ligand.

2.9.4 *Target Preparation*

To combat the current situation of gastrointestinal ulcer, protein 3-dimensional structure of receptors were obtained from the Protein Data Bank RCSB PDB: Free fatty acid receptor 1 (PDB ID: 4PHU), Fatty acid-binding protein 4 receptor (PDB ID: 3P6H), Peroxisome proliferator-activated receptor delta receptor (PDB ID: 1Y0S), and Peroxisome proliferator-activated receptor alpha receptor (PDB ID: 3KDU). The protein receptor targets were preprocessed, optimized, minimized and refined using Protein Preparation Wizard. Charges and protonation state were assigned, followed by energy minimization using the OPLS-4 force field available in Maestro 12.8. The active site x, y, and z coordinates of their respective centroid co-crystallized ligands were used to generate docking grid boxes saved in gridbox.zip files.

2.9.5 *Protein-Ligand Docking*

The Glide tool of the maestro Schrodinger package was used to perform the molecular docking using the generated receptor grid file for synthesis and screening [Highthroughput Virtual Screening (HTVS), Standard Precision (SP), and Extra Precision (XP)] setting the ligand sampling to rigid and flexible. Their docking scores were recorded after scrrening. XP method was to weed out false positives and to provide a better correlation between good poses and a good scores, estimate the theoretical interaction of the ligands with the proteins and, to evaluate the interactions between the ligands and amino acids.

2.9.6 *Statistical Analysis*

GraphPad Prism software (version 9.0, GraphPad Software, Inc.) was used for data analysis. All data are reported as mean values with standard deviation (mean ± SD). One-way analysis of variance (ANOVA) followed by Tukey post hoc test was employed for multiple comparisons between groups. Significant difference was set at $P < 0.05$.

3.0 RESULTS

3.1 Anti-Lipoxygenase Activity Analysis

Results for anti-lipoxygenase (ALOX) inhibitory activity and its minimum inhibitory concentration (MIC) of *C. papaya* L. extract as well as those of standard antibiotics (indomethacin) were as shown in Figure 3.1 and Table 3.1. It was observed that the *C. papaya* L. extract showed highest ALOX inhibitory activity of 82.88% when compared with the reference standard at 500 µg/ml concentration, while the MIC of the *C. papaya* L. extract revealed a significant ($p<0.05$) increase when compared with reference standard. Therefore, the *C. papaya* L. extract exhibited potent LOX inhibitory activity, suggesting the *C. papaya* L. may have a potentially high anti-inflammatory effect when compared to the standard used.

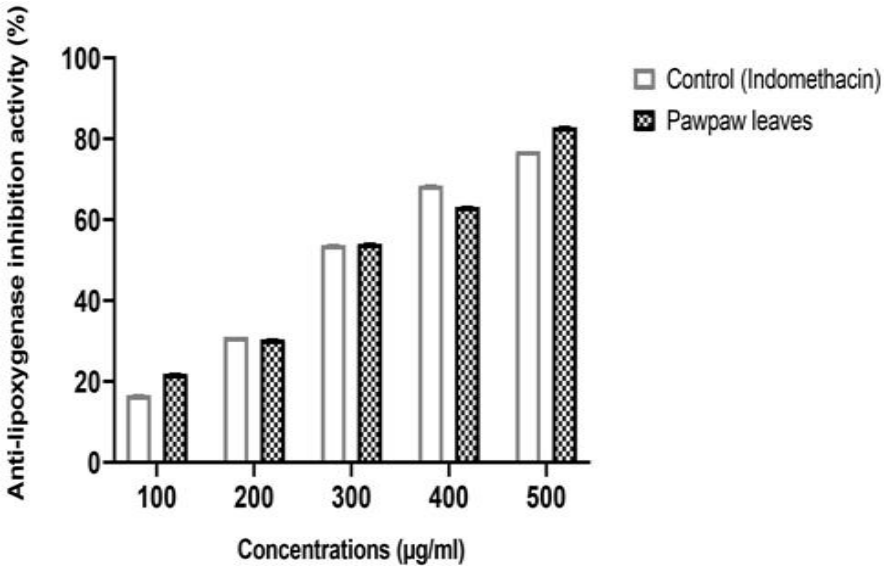

Figure 3.1: Anti-lipoxygenase inhibitory activity of *C. papaya* L.

Table 3.1: The MIC (µg/ml) of the *C. papaya* L. extract and standard indomethacin against ALOX enzyme activities.

	Control (Indomethacin)	***C. papaya* L.**
Anti-lipoxygenase activity	0.35±0.01	0.5±0.00*

Values are presented as mean±SD (n=3). Where SD: Standard deviation; MIC: Minimum inhibitory concentrations; *p< 0.05 when compared with control.

3.2 Protein Denaturation Inhibition Activity Analysis

Figure 3.2 and Table 3.2 depict the result for *C. papaya* L. extract as well as that of reference standard (aspirin) of protein denaturation (PD) inhibitory activity and its minimum inhibitory concentration (MIC). The *C. papaya* L. extract showed high PD inhibitory activity at 300, 400, and 500 µg/ml concentrations when compared with reference standard used, although the MIC of the *C. papaya* L. extract revealed a significant ($p<0.05$) decrease when compared with reference standard used. Therefore, at higher concentrations, the *C. papaya* L. extract exhibited potent PD inhibitory activity.

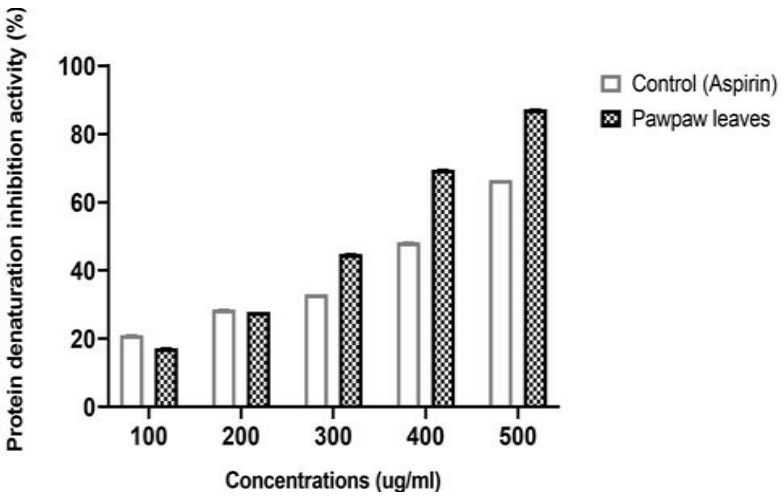

Figure 3.2: Protein denaturation inhibitory activity of *C. papaya* L.

Table 3.2: The MIC (µg/ml) of the *C. papaya* L. extract and standard aspirin against protein denaturation activity.

	Control (aspirin)	*C. papaya* L.
Protein denaturation activity	0.43±0.03	0.35±0.02*

Values are presented as mean±SD (n=3). Where SD: Standard deviation; MIC: Minimum inhibitory concentrations; * p< 0.05 when compared with control.

3.3 Membrane Stabilizing Activity Analysis

The membrane stabilizing activity and it minimum inhibitory concentration (MIC) of *C. papaya* L. extract as well as that of reference standard (aspirin) were as shown in Figure 3.3 and Table 3.3. It was observed that the *C. papaya* L. extract showed high membrane inhibitory activity at 300, 400, and 500 µg/ml concentrations when compared with reference standard used, although the MIC of the *C. papaya* L. extract revealed a significant (p<0.05) decrease when compared with reference standard used. Therefore, at higher concentrations, the *C. papaya* L. extract exhibited potent membrane stabilizing inhibitory activity.

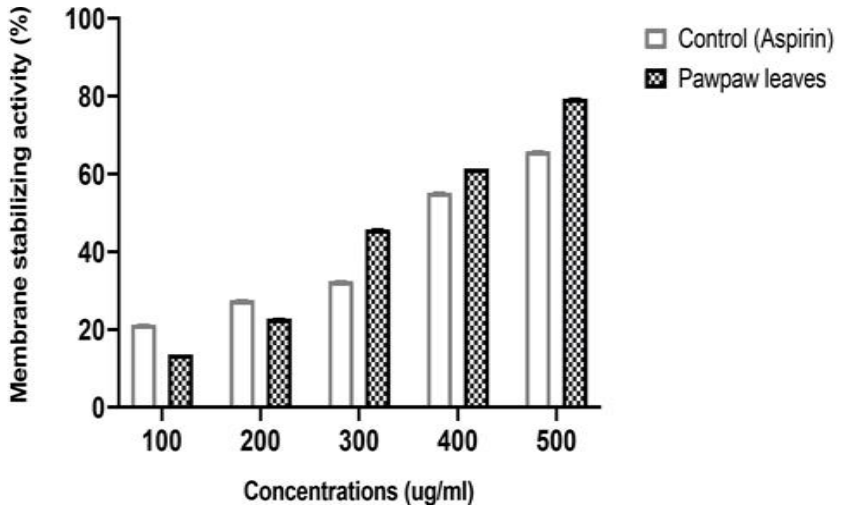

Figure 3.3: Membrane stabilizing inhibitory activity of *C. papaya* L.

Table 3.3: The MIC (µg/ml) of the *C. papaya* L. extract and standard aspirin against membrane stabilizing activity.

	Control (aspirin)	*C. papaya* L.
Membrane stabilizing activity	0.45±0.00	0.34±0.01*

Values are presented as mean±SD (n=3). where SD: Standard deviation; MIC: Minimum inhibitory concentrations; * p< 0.05 when compared with control.

3.4 Urease Inhibition Activity Analysis

The Urease inhibition activity and it minimum inhibitory concentration (MIC) of *C. papaya* L. extract as well as that of reference standard (thiourea) were as shown in Figure 3.4 and Table 3.4. It was observed that the *C. papaya* L. extract showed a concentrations dependent increase in urease inhibitory activity but less when compared with the standard reference used, while the MIC of the *C. papaya* L. extract revealed a significant (p<0.05) increase when compared with reference standard. Therefore, the *C. papaya* L. extract may exhibit potent urease inhibitory activity.

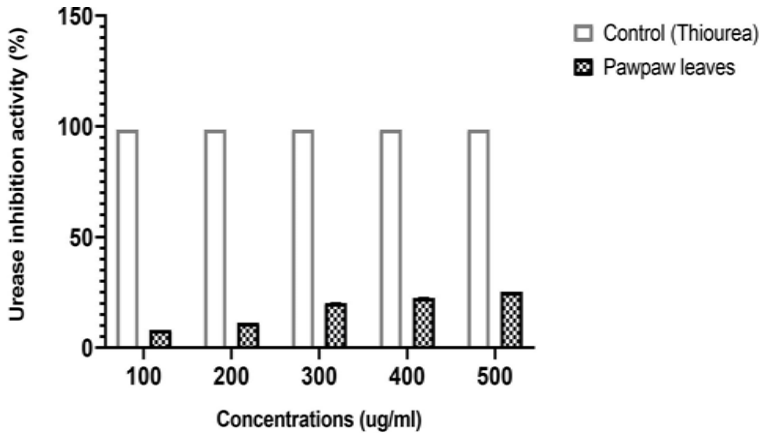

Figure 3.4: Urease inhibitory activity of *C. papaya* L.

Table 3.4: The MIC (µg/ml) of the *C. papaya* L. extract and standard thiourea against urease inhibition activity.

	Control (thiourea)	*C. papaya* L.
Urease inhibition activity	88.31±0.00	182.70±0.01*

Values are presented as mean±SD (n=3). Where SD: Standard deviation; MIC: Minimum inhibitory concentrations; * p< 0.05 when compared with control.

3.5 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis revealed the chemical composition of *C. papaya* L. which is presented in Table 3.5 with 90% or greater spectral matching accuracy compared to those of NIST Library mass Spectra data base and mass spectra. The result shows 18 bioactive compounds with a molecular weight (MW) ranging from 122g to 414g, peak Area percentage ranging from 0.80 to 14.19, and the compound weight percentage ranging from 0.00 to 16.13.

Table 3.5: Gas Chromatography-Mass Spectrometry (GC-MS) Analysis result of Bioactive compounds present in *C. papaya* L.

Peak #	RT	Compound Detected	Molecular Formula	Molecular Weight	Peak Area %	Composition by weight %	Mass fragmentation
1	2.54	5-Acetylpyrimidine	$C_6H_6N_2O$	122	3.31	2.28	43, 107, 122
2	8.00	Undecylenic acid	$C_{11}H_{20}O_2$	184	8.67	15.00	41, 55, 184
3	12.48	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	2.29	3.71	43, 57, 187
4	14.23	10-Undecenal	$C_{11}H_{20}O$	168	1.97	4.47	41, 68, 136
5	16.60	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	1.58	3.55	65, 149, 222
6	17.58	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280	1.50	2.97	51, 77, 289
7	25.00	Oleic acid	$C_{18}H_{34}O_2$	282	14.18	16.13	41, 55, 282
8	30.00	10-Undecenoyl chloride	$C_{11}H_{19}ClO$	202	1.22	2.52	41`, 67, 322
9	31.97	Phytol	$C_{20}H_{40}O$	296	7.09	4.90	43, 71, 296
10	35.51	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.18	2.69	43, 73, 284
11	36.25	n-Hexadecanoic acid	$C_{10}H_{16}$	256	9.48	6.31	43, 73, 256
12	37.62	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292	2.13	3.98	43, 68, 292
13	39.94	11-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	0.80	2.25	42, 58, 294
14	41.00	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	5.51	5.96	55, 67, 342
15	41.50	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	10.25	8.74	43, 98, 358
16	44.23	γ-Sitosterol	$C_{29}H_{50}O$	414	11.82	7.19	43, 55, 414
17	44.99	Squalene	$C_{30}H_{50}$	410	2.36	3.78	41, 69, 410

3.6 Clustering Analysis

The results of the clustering analysis are presented in Figure 3.6, which shows the various compounds and their weights. Canonical smiles and compound identifier (CID) numbers were generated from the NCBI PubChem database for each compound, as shown in Table 3.6. These identifiers were then used in clustering analysis to group the compounds according to their molecular weights. The color-coded legend (Figure 3.6) depicts the range of molecular weights for each compound, with each color representing a different weight range.

Table 3.6: Compound CID and canonical smiles of *C. papaya* L. bioactive compounds.

S/N	COMPOUND NAMES	CID NO.	CANNONICAL SMILES
1.	5-Acetylpyrimidine	572308	CC(=O)C1=CN=CN=C1
2.	Undecylenic acid	5634	C=CCCCCCCCCC(=O)O
3.	1-Octanol, 2-butyl-	19800	CCCCCCC(CCCC)CO
4.	10-Undecenal	8187	C=CCCCCCCCCC=O
5.	Diethyl Phthalate	6781	CCOC(=O)C1=CC=CC=C1C(=O)OCC
6.	13-Hexyloxacyclotridec-10-en-2-one	6536948	CCCCCCC1CC=CCCCCCCCC(=O)O1
7.	Oleic acid	445639	CCCCCCCCC=CCCCCCCCC(=O)O
8.	10-Undecenoyl chloride	38042	C=CCCCCCCCCC(=O)Cl
9.	Phytol	5280435	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
10.	Octadecanoic acid	5281	CCCCCCCCCCCCCCCCCC(=O)O
11.	n-Hexadecanoic acid	985	CCCCCCCCCCCCCCCC(=O)O
12.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	62603	CC(C)(C)C1=CC(=CC(=C1O)C(C)(C)C)CCC(=O)OC
13.	11-Octadecenoic acid, (Z)-	5282761	CCCCCCC=CCCCCCCCCCC(=O)O
14.	cis-13-Octadecenoic acid	5312441	CCCCC=CCCCCCCCCCCC(=O)O
15	Octadecanoic acid, 2,3-dihydroxypropyl ester	24699	CCCCCCCCCCCCCCCCCC(=O)OCC(CO)O
1.	γ-Sitosterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
18.	Squalene	638072	CC(=CCCC(=CCCC(=CCCC=C(C)CCC=C(C)CCC=C(C)C)C)C)C

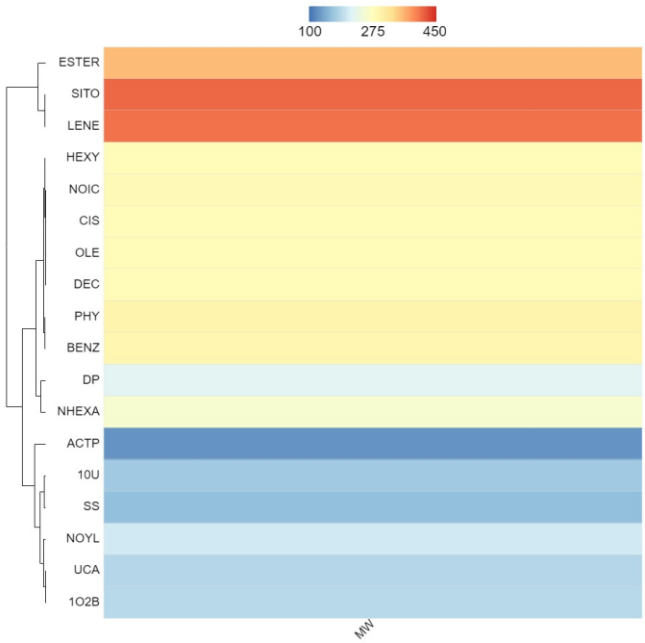

Figure 3.6: Clustering analysis of the major phytochemical constituents of *C. papaya* L.

3.7 Molecular Docking

Schrodinger Maestro 12.8 was utilized to dock the ligands identified with GC-MS against the target proteins. The docking result shows that a minimum of seven (7) of the bioactive compounds were able to bind well to the active site of the target proteins (Table 3.7). Octadecanoic acid, 2,3-dihydroxypropyl ester has the highest binding affinity of -8.453kcal/mol against the FFAR1 receptor, Phytol has the highest binding affinity of -6.990kcal/mol against the PPARD receptor, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester has the highest binding affinity against the FABP4 and the PPARA receptors with binding affinities of -5.287kcal/mol and -6.473kcal/mol respectively. All except γ-Sitosterol and Squalene exhibited good binding affinities against one or more target (Table 3.7). The 2- dimensional structure of all the lead compounds with the receptors are illustrated in Figure 3.7(a, b, c, and d).

Table 3.7: Docking scores of the lead compounds of *C. papaya* L. docked against the target proteins: FFAR1 (PDB ID: 4PHU), FABP4 (PDB ID: 3P6H), PPARD (PDB ID: 1Y0S), and PPARA (PDB ID: 3KDU) receptors.

COMPOUND NAME	DOCKING SCORE (Kcal/mol)			
	FFAR1	FABP4	PPARA	PPARD
5-Acetylpyrimidine	-	-3.762	-4.677	-3.896
Undecylenic acid	-5.446	-1.978	-4.380	-3-325
1-Octanol, 2-butyl-	-5.446	-2.707	-4.129	-4.215
10-Undecenal	-3.780	-1.761	-3.374	-2.604
Diethyl Phthalate	-	-4.209	-5.125	-5.077
13-Hexyloxacyclotridec-10-en-2-one	-6.387	-	-	-5.809
Oleic acid	-6.278	-	-	-6.550
10-Undecenoyl chloride	-5.060	-2.430	-4.840	-3.477
Phytol	-7.280	-	-6.418	-6.990
Octadecanoic acid	-7.173	-	-	-5.745
n-Hexadecanoic acid	-6.886	-	-5.388	-5.382
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	-3.542	-5.287	-6.473	-6.875
11-Octadecenoic acid, (Z)-	-6.882	-	-	-6.535
cis-13-Octadecenoic acid	-7.954	-	-6.293	-
Octadecanoic acid, 2,3-dihydroxypropyl ester	-8.453	-	-	-
γ-Sitosterol	-	-	-	-
Squalene	-	-	-	-

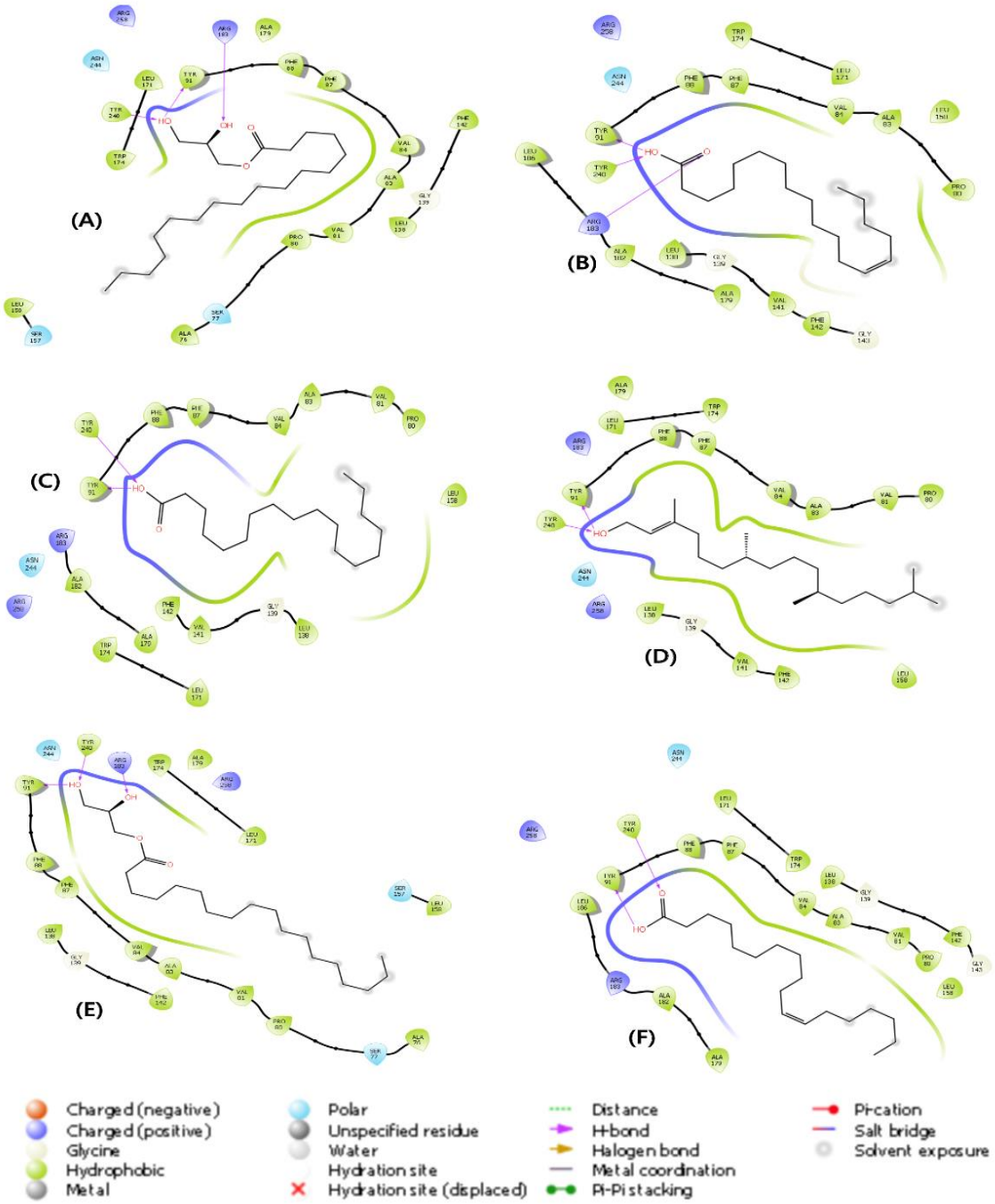

Figure 3.7a: 2D-molecular interactions of *C. papaya* L. lead compounds docked against FFAR1 active site: (A) Octadecanoic acid, 2,3-dihydroxypropyl ester (B) cis-13-Octadecenoic acid (C) Octadecanoic acid (D) Phytol (E) n-Hexadecanoic acid (F) 11-Octadecenoic acid,(Z)-

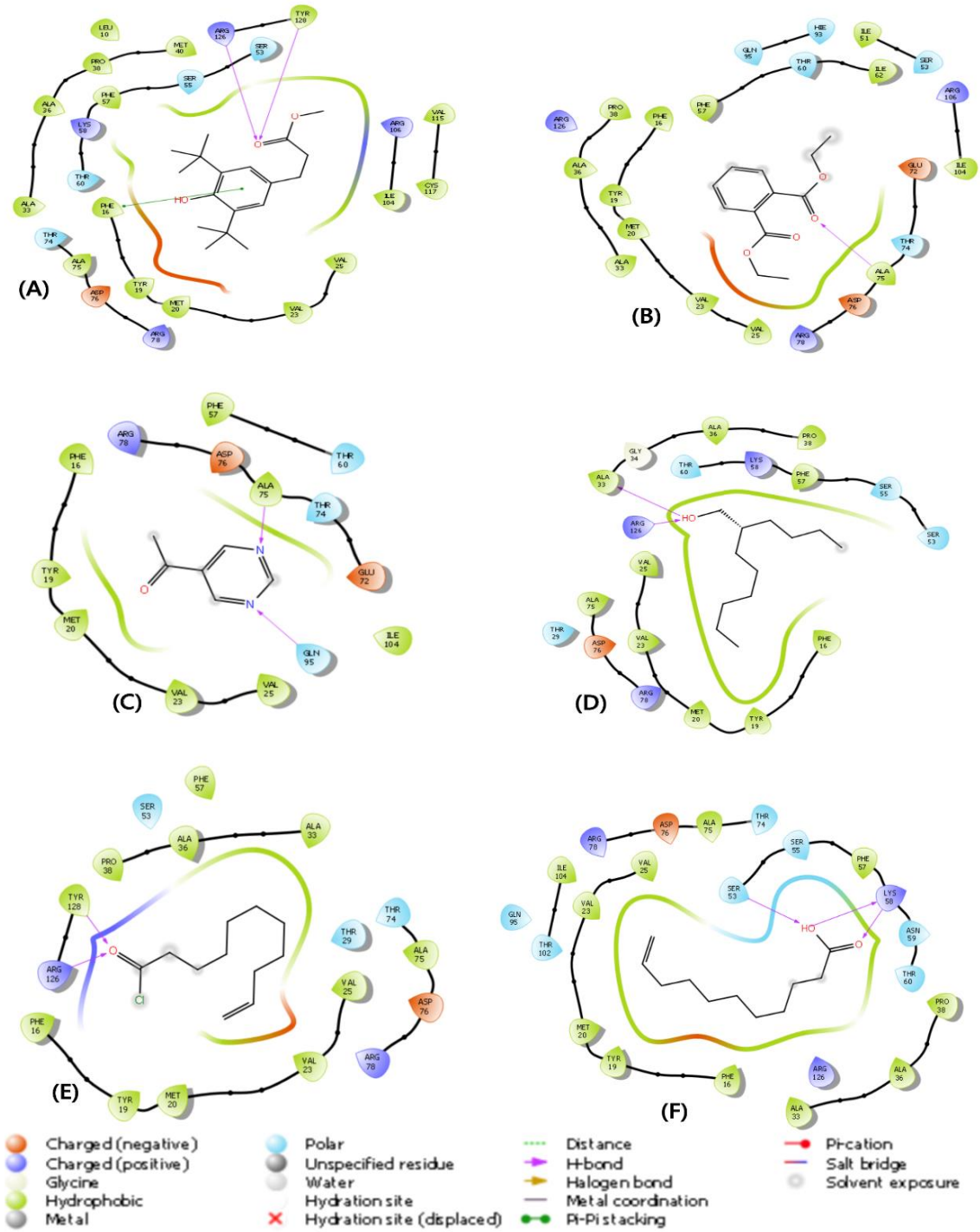

Figure 3.7b: 2D-molecular interactions of *C. papaya* L. lead compounds docked against FABP4 active site: (A) Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxyBenzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy (B) Diethyl Phthalate (C) 5-Acetylpyrimidine (D) 1-Octanol, 2-butyl- (E) 10-Undecenoyl chloride (F) Undecylenic acid.

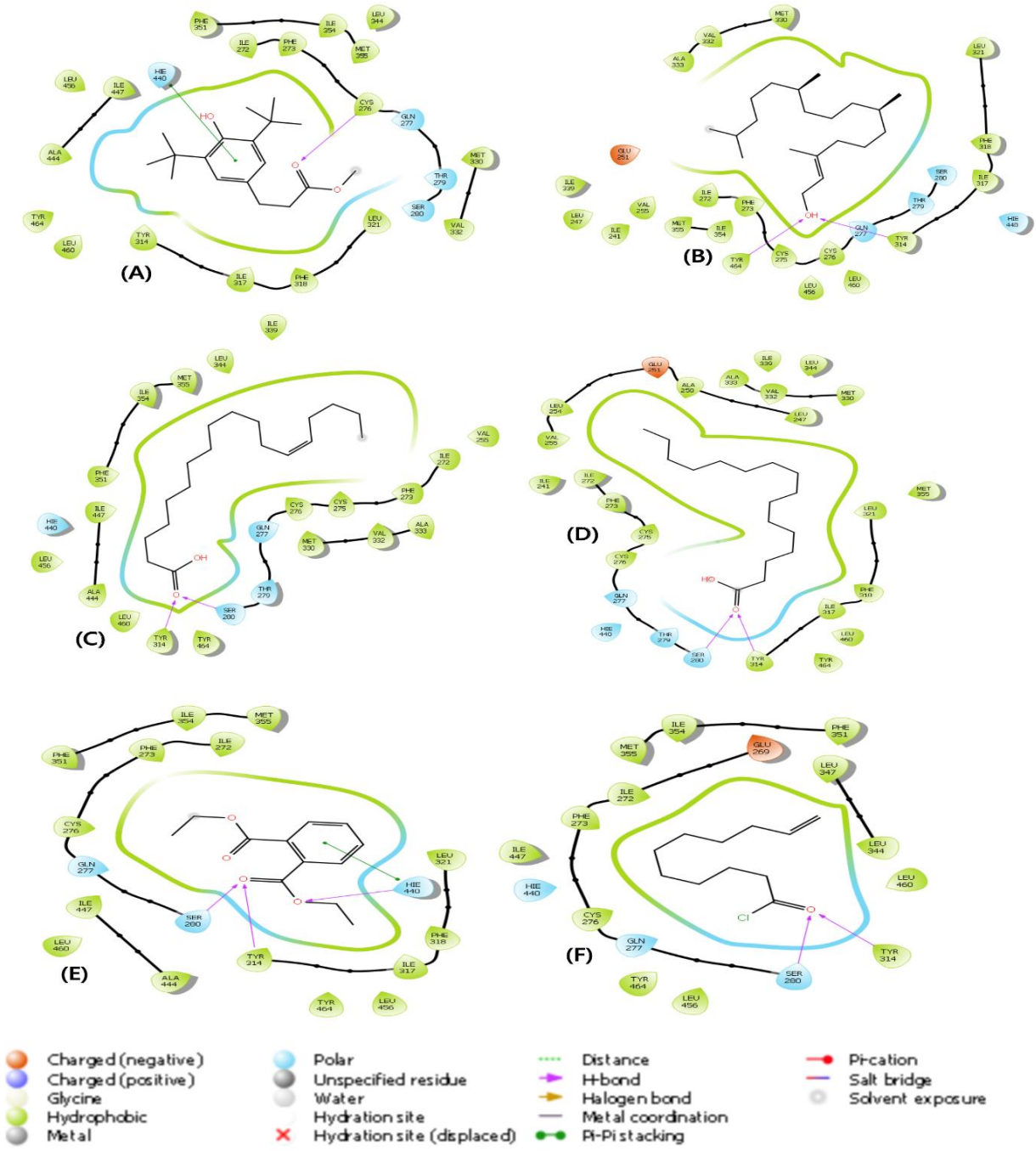

Figure 3.7c: 2D-molecular interactions of *C. papaya* L. lead compounds docked against PPARA active site: (A) Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxyBenzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy (B) Phytol (C) cis-13-Octadecenoic acid (D) n-Hexadecanoic acid (E) Diethyl Phthalate (F) 10-Undecenoyl chloride.

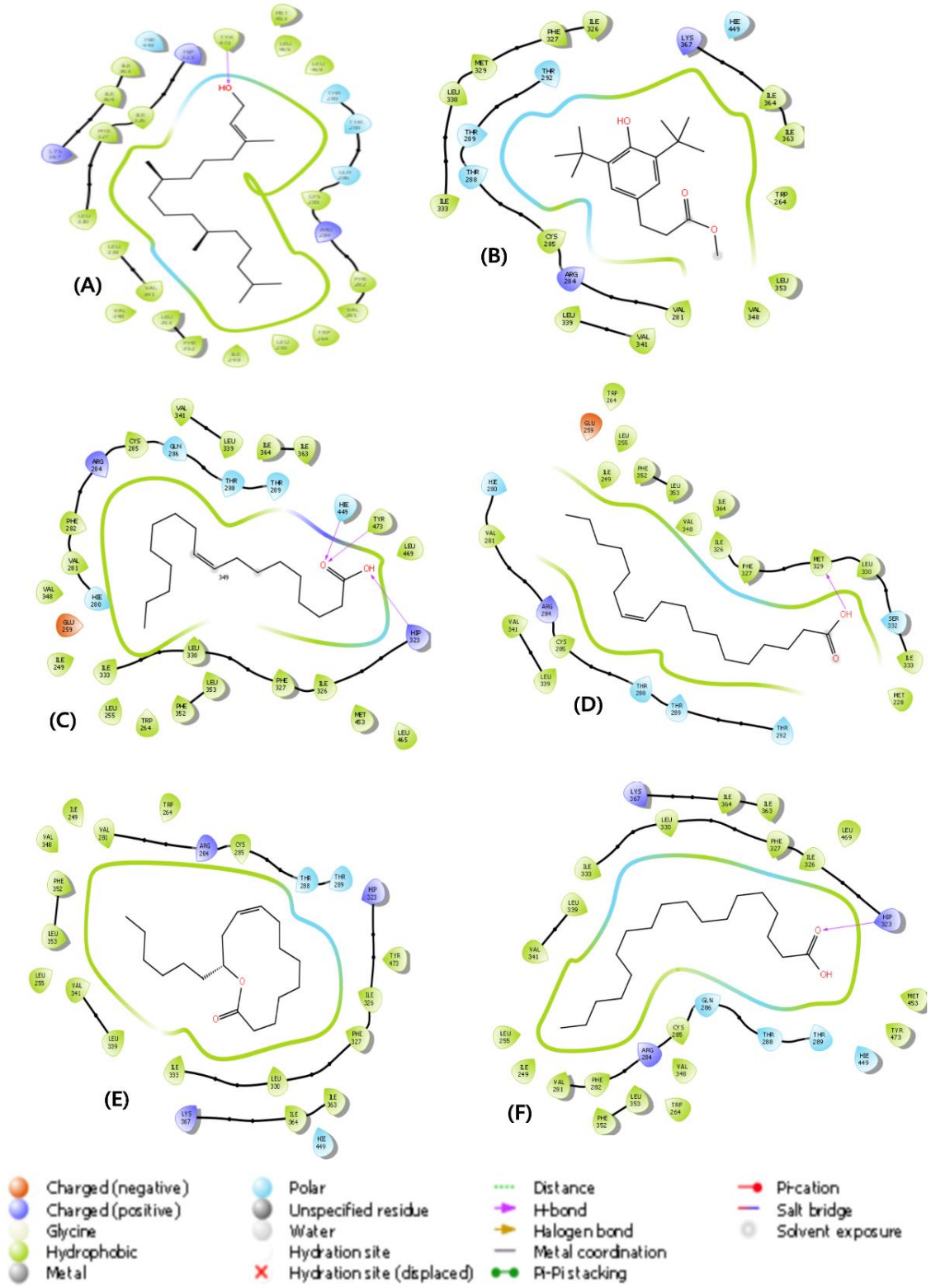

Figure 3.7d: 2D-molecular interactions of *C. papaya* L. lead compounds docked against PPARD active site: (A) Phytol (B) Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxyBenzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy (C) Oleic acid (D) 11-Octadecenoic acid, (Z)- (E) 13-Hexyloxacyclotridec-10-en-2-one (F) Octadecenoic acid.

Table 3.8: In-silico drug likeness prediction of the lead compounds from *C. papaya* L. docked against the protein receptors.

COMPOUND	MW	HBA	HBD	MLOGP	ROV
5-Acetylpyrimidine	122.12	3	0	-0.70	0
Undecylenic acid	184.28	2	1	2.76	0
1-Octanol, 2-butyl-	186.33	1	1	3.41	0
Diethyl Phthalate	222.24	4	0	2.39	0
13-Hexyloxacyclotridec-10-en-2-one	280.45	2	0	4.17	1
Oleic acid	282.46	2	1	4.57	1
10-Undecenoyl chloride	202.72	1	0	3.16	0
Phytol	296.53	1	1	5.25	1
Octadecanoic acid	284.48	2	1	4.67	1
n-Hexadecanoic acid	256.42	2	1	4.44	1
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292.4	3	1	4.12	0
11-Octadecenoic acid, (Z)-	282.46	2	1	4.57	1
cis-13-Octadecenoic acid	282.46	2	1	4.57	1
Octadecanoic acid, 2,3-dihydroxypropyl ester	358.60	4	2	3.63	0

4.0 DISCUSSION

Gastrointestinal ulcers are a significant health concern, affecting millions worldwide. These ulcers result from an imbalance between protective and damaging factors in the gut.[3] Conventional treatments can have adverse effects, leading to the exploration of alternative therapies, such as plant-derived compounds.[13] *Carica papaya* L., or papaya leaf, has shown promising potential due to its rich phytochemical composition and diverse biological activities, including anti-inflammatory, antimicrobial, and gastroprotective effects.[14] The study investigated bioactive compounds from *C. papaya* L. leaf extract, identified through GC-MS, for their potential to target key proteins in gastrointestinal ulcer pathogenesis. It evaluated their anti-lipoxygenase, protein denaturation, membrane stabilizing, and urease inhibition activities, and used in-silico docking to assess interactions with FFAR1, FABP4, PPARA, and PPARD, which are associated with the pathogenesis of gastrointestinal ulcers. The *C. papaya* L. leaf extract exhibited significantly higher inhibitory activity compared to the reference standard, indomethacin, in the anti-lipoxygenase assay. This suggests that the extract has the potential to suppress the inflammatory processes involved in the pathogenesis of gastrointestinal ulcers. Furthermore, the extract demonstrated high protein denaturation inhibitory activity, outperforming the reference standard, aspirin. This is particularly notable, as protein denaturation can disrupt the integrity of the gastric mucosal barrier, contributing to ulcer formation.[15] The extract also exhibited high membrane inhibitory activity, again outperforming aspirin, indicating its potential to maintain the integrity and stability of gastric mucosal cells. While the urease inhibition activity of the extract was less potent than the reference standard, thiourea, it still exhibited a concentration-dependent increase in this activity, which is relevant given that Helicobacter pylori-produced urease is a key factor in the development of gastrointestinal ulcers.[16]

GC-MS, a safe analytical method, was utilized to determine the concentration of individual chemicals in the sample. The GC-MS analysis of the *C. papaya* L. leaf extract revealed a diverse array of bioactive compounds, including fatty acids, alcohols, esters, and terpenes, which possess various pharmacological properties. Octadecanoic acid, a prominent fatty acid in the extract, has been shown to exhibit anti-inflammatory effects by inhibiting the production of pro-inflammatory mediators.[17] Another compound, Octadecanoic acid, 2,3-dihydroxypropyl ester, showed antioxidant and cytoprotective activities, contributing to the maintenance of gastric mucosal integrity. The extract also contained cis-13-Octadecenoic acid, a monounsaturated fatty acid with anti-inflammatory and gastroprotective properties, as well as

the terpene compound Phytol, which exhibited anti-inflammatory, antioxidant, and gastric cytoprotective effects. Additionally, the extract included other fatty acids, alcohols, and esters, such as n-Hexadecanoic acid, Pentadecanoic acid, and Octadecanoic acid, ethyl ester, which have also been associated with anti-inflammatory, antioxidant, and gastroprotective activities.[18] Collectively, these bioactive compounds in the *C. papaya* L. leaf extract could contribute to its potential therapeutic effects against gastrointestinal ulcers.

The computational docking studies conducted in this research were crucial in elucidating the potential binding interactions between the identified bioactive compounds from the *C. papaya* L. extract and the key target proteins FFAR1, FABP4, PPARA, and PPARD. These proteins have been recognized as important therapeutic targets in the management of gastrointestinal ulcers, as they play pivotal roles in the regulation of various physiological processes involved in the pathogenesis of this condition.[19-22] The docking results revealed that several of the bioactive compounds exhibited strong binding affinities to the active site of the FFAR1 protein. Octadecanoic acid, 2,3-dihydroxypropyl ester demonstrated the highest binding affinity, with a docking score of -8.453kcal/mol, suggesting its potential to modulate FFAR1-mediated signaling pathways. Cis-13-Octadecenoic acid, Phytol, Octadecanoic acid, n-Hexadecanoic acid, 11-Octadecenoic acid, (Z)-, 13-Hexyloxacyclotridec-10-en-2-one, and Oleic acid also showed promising binding interactions with FFAR1. The docking studies also revealed promising binding interactions between the *C. papaya* L. extract bioactive compounds and the FABP4 protein. Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester demonstrated the highest binding affinity to FABP4, with a docking score of -5.287kcal/mol. Other bioactive compounds, such as Diethyl Phthalate, 5-Acetylpyrimidine, 1-Octanol, 2-butyl-, 10-Undecenoyl chloride, Undecylenic acid, and 10-Undecenal, also exhibited strong binding interactions with the FABP4 protein. Several bioactive compounds from the *C. papaya* L. extract also exhibited strong binding affinities to the active sites of the PPARA and PPARD proteins. Compounds such as 5-Acetylpyrimidine, Undecylenic acid, 1-Octanol, 2-butyl-, 10-Undecenal, Diethyl Phthalate, 13-Hexyloxacyclotridec-10-en-2-one, Oleic acid, 10-Undecenoyl chloride, Phytol, Octadecanoic acid, n-Hexadecanoic acid, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester, and 11-Octadecenoic acid, (Z)-, demonstrated strong binding interactions with the active sites of PPARA and PPARD. The binding of these compounds to FFAR1, FABP4, PPARA, and PPARD may lead to the regulation of various physiological processes involved in the pathogenesis of gastrointestinal ulcers, such as gastrointestinal motility, secretion, inflammatory responses, energy metabolism, cell proliferation, and mucosal integrity.[19-22]

The assessment of drug-likeness is a crucial step in the evaluation of bioactive compounds for their potential as therapeutic agents.[23] The drug-likeness of a compound is a measure of its suitability for drug development, taking into account various physicochemical and structural properties that are associated with oral bioavailability and drug-like behavior.[24] The drug-likeness of the bioactive compounds identified in the *C. papaya* L. extract was evaluated based on Lipinski's Rule of Five, a set of guidelines widely used to predict the oral bioavailability of drug candidates. Lipinski's Rule of Five states that a compound is more likely to be orally active if it has a molecular weight (MW) less than 500 g/mol, a logarithm of the octanol-water partition coefficient (LogP) less than 5, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors.[25] The analysis of the bioactive compounds revealed that the majority of the identified compounds adhered to Lipinski's Rule of Five, suggesting their potential for oral bioavailability and drug-like properties. The adherence of the majority of the bioactive compounds to Lipinski's Rule of Five suggests their potential as suitable candidates for further drug development and optimization. These compounds exhibit physicochemical properties that are typically associated with good oral bioavailability and drug-like behavior,

making them promising starting points for the development of therapeutic agents for the treatment of gastrointestinal ulcers.

CONCLUSION

The findings of this study demonstrate the promising therapeutic potential of *C. papaya* L. extract and its bioactive compounds for the management of gastrointestinal ulcers. The extract exhibited potent anti-inflammatory, protein stabilizing, membrane stabilizing, and urease inhibitory activities, which are crucial mechanisms in the treatment of gastrointestinal ulcers. The in silico docking studies further revealed that several bioactive compounds within the extract displayed favorable binding interactions with key target proteins involved in the pathogenesis of gastrointestinal ulcers, including FFAR1, FABP4, PPARA, and PPARD. These results provide a strong rationale for the further investigation of *C. papaya* L. extract and its bioactive compounds as novel therapeutic agents for gastrointestinal ulcers. Additional in vivo and clinical studies are warranted to fully elucidate the mechanisms of action, pharmacokinetic properties, and therapeutic efficacy of these natural compounds in the treatment of this prevalent gastrointestinal disorder. The successful development of these natural-based therapies could offer a promising alternative to current treatment options, with the potential to improve patient outcomes and quality of life.

LIST OF ABBREVIATIONS

GI ulcers: Gastrointestinal Ulcer

FFAR1: Free Fatty Acid Receptor 1

FABP4: Fatty Acid-Binding Protein 4

PPARD: Peroxisome Proliferator-Activated Receptor Delta

PPARA: Peroxisome Proliferator-Activated Receptor Alpha

GPR40: G protein-coupled receptor 40

GC-MS: Gas Chromatography-Mass Spectrometry

PDB: Protein Data Bank

MIC: Minimum Inhibitory Concentration

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