



Phytochemical profiling, antioxidant potential, and in silico evaluation of *Carica papaya* seed oil

Sabir Khan^a, Zeemal Seemab Amin^b, Bushra Shakoor^c, Ali irfan^{d,e,*},
Muhammed Tilahun Muhammed^f, Katarzyna Kotwica-Mojzych^g, Yousef A. Bin Jardan^{h,**},
Mariola Glowackaⁱ, Shazia Perveen^j, Mariusz Mojzych^{i,**}

^a Department of Basic and Applied chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore 53000, Pakistan

^b School of Biochemistry, Minhaj University Lahore, Quaid-e-Azam industrial State, Lahore 54000, Pakistan

^c Synthetic and Natural Products Discovery (SNPD) Laboratory, Department of Chemistry, Government College Women University, Faisalabad 38000, Pakistan

^d Department of Chemistry, Government College University Faisalabad, Faisalabad-38000, Pakistan

^e The Green Institute of Chemical, Biomedical and Environmental Sciences, Lahore 54000, Pakistan

^f Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Suleyman Demirel University, Isparta, Turkey

^g Department of Histology, Embryology and Cytophysiology, Medical University of Lublin, Radziwillowska 11, 20-080 Lublin, Poland

^h Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

ⁱ Faculty of Health Sciences, Collegium Medicum, The Mazovian Academy in Plock, Pl. Dabrowskiego 2, 09-402 Plock, Poland

^j Department of Zoology, The Women University Multan, Matital Campus, Multan-60000, Pakistan

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ABSTRACT

Carica papaya seeds, traditionally used in folk medicine, have garnered scientific interest due to their potential therapeutic properties. The study investigates the antioxidant potential and compound composition of *Carica papaya* seed oil. Utilizing GC–MS analysis, we identified 42 significant compounds in the n-hexane fraction of the oil, with notable constituents including benzyl nitrile (3.43 %), pentadecanoic acid 14-methyl-methyl ester (5.23 %), and n-hexadecanoic acid (7.11 %). The antioxidant activity of the oil was assessed using DPPH radical scavenging assays, revealing a maximum inhibition activity of 61.13 % at a concentration of 100 mg/mL, compared to 76.23 % for the standard vitamin C. For further precise binding pose predictions, Schrödinger's Glide module was used for molecular docking. Docking scores and molecular interaction profiles were used to evaluate binding interactions after the produced ligands were docked into the receptor grid. The greatest docking score (–6.657) was shown by 6-chloro for 5B6N, 1H-Indole-2-carboxylic acid, which formed hydrogen bonds with PHE 207 and VAL 187, two essential residues for ligand stabilization. Further, the molecular dynamics simulation study disclosed that the complexes of the relatively active phytoconstituents with the target structures were stable. These findings highlight the therapeutic potential of *Carica papaya* seed oil as a natural antioxidant, suggesting its viability for further research and application in health-related fields.

1. Introduction

The *Carica papaya*, commonly called papaya, is a tropical fruit famous for its intense flavor and various health benefits (Fig. 1). Papaya originated in Central America and is known not only for being eaten but also for its active medicinal compounds [1]. Although the fruit itself contains tremendous nutrients such as vitamins A, C, and E, it is the seeds and the oil extracted from these seeds that have favorably demonstrated a great potential in natural medicine [2].

Papaya seeds have been utilized in folk medicine for centuries, particularly in areas where the papaya tree grows abundantly. Indigenous people from different regions around the world have been using papaya seeds for their anthelmintic effects (expelling intestinal parasites) and digestive health (possible health benefits) for generations. This traditional wisdom encouraged scientific interest in studying papaya seeds, resulting in a substantial body of scientific research and literature dedicated to evaluating the therapeutic potential of papaya seeds [3,4].

* Corresponding author at: Department of Chemistry, Government College University, Faisalabad 38000, Pakistan.

** Corresponding authors.

E-mail addresses: raialiirfan@gmail.com (A. irfan), ybinjardand@ksu.edu.sa (Y.A. Bin Jardan), m.mojzych@mazowiecka.edu.pl (M. Mojzych).

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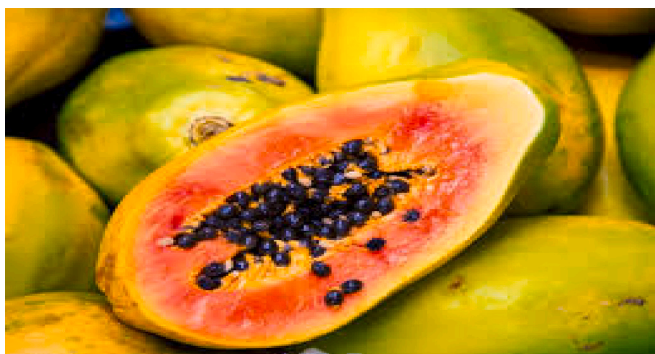


Fig. 1. Papaya fruit seeds.

One of the greatest notable products derived from papaya seeds is papaya seed oil, gained through the cold-pressing or solvent extraction of the seeds [5]. Papaya seed oil has a rich, golden color and a distinctive odor due to its varied phytochemicals. Papaya seed oil contains a variety

of bioactive compounds like flavonoids, phenolic acids, and essential fatty acids that have varying medicinal effects [6].

In recent years, the medicinal properties of papaya seed oil (PSO) have aroused interest from the academic community. Research has sought to describe the pharmacological activities of PSO and therapeutic possibilities in an array of health and wellness areas. From microbial and antioxidant properties to anti-inflammatory and wound-healing effects, there may be a wide range of biological activities for PSO that present great potential for human health [7]. One of the most recognizable characteristics of papaya seed oil is its antimicrobial activity. Many studies have shown its effectiveness against a wide array of microorganisms, including bacteria, fungi, and parasites. The antimicrobial activity of papaya seed oil also applies to drug-resistant organisms, suggesting that papaya seed oil may provide a natural alternative to antimicrobial drugs. Additionally, the antioxidant properties of papaya seed oil allow it to scavenge free radicals, reduce oxidative stress, and protect against cellular damage associated with numerous chronic diseases [8–10].

Additionally, papaya seed oil displays substantial anti-inflammatory effects by modulating inflammatory pathways and reducing the

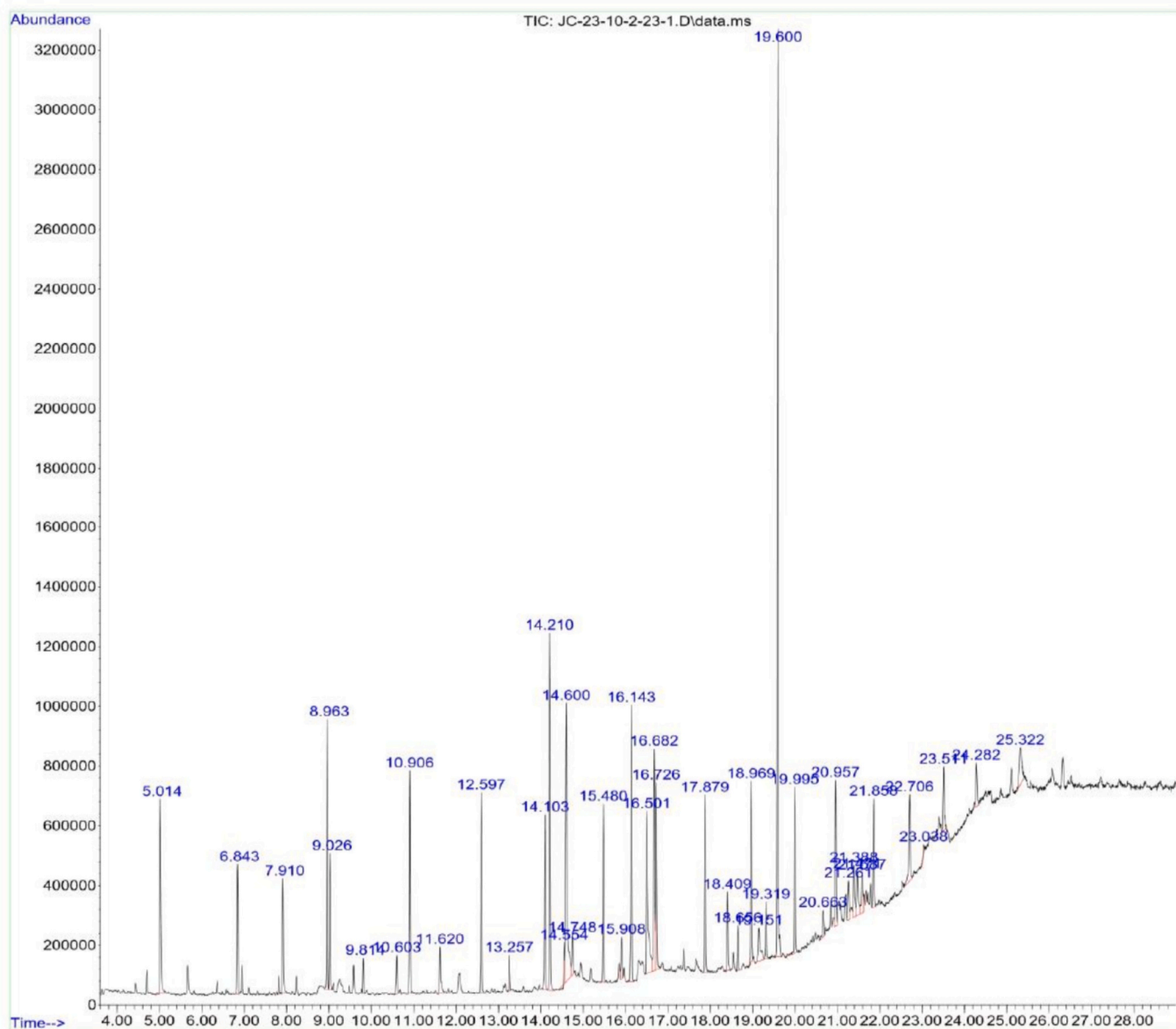
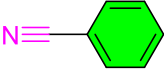
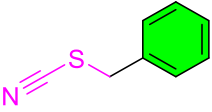
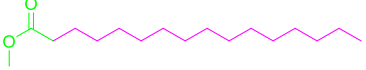
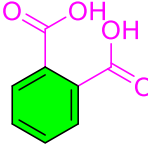
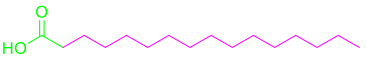
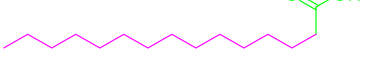
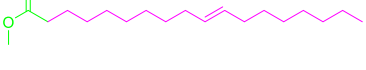
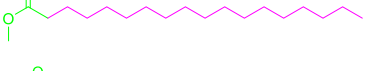
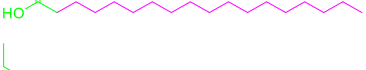
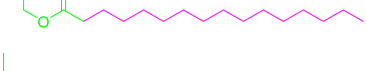
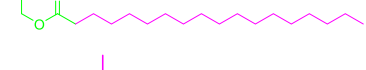
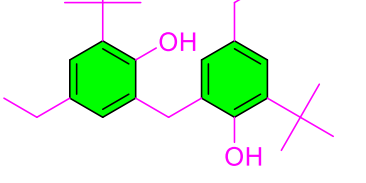
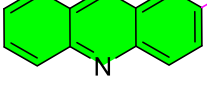
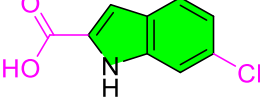


Fig. 2. Chromatogram illustrating the n-hexane extract of *carica papaya* oil.

Table 1
GC–MS data of n-hexane extract of *Carica papaya* oil.

Sr. no	Compound name	Molecular Formula	Molecular Mass	Retention Time (Min)	Peak Area (%)	2D Structures
1	Benzyl nitrile	C ₈ H ₇ N	117.14	5.014	3.43	
2	Thiocyanic acid, phenylmethyl ester	C ₈ H ₇ NS	149.21	7.910	1.89	
3	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₉ H ₃₆ O ₂	296.48	14.210	5.23	
4	1,2-Benzenedicarboxylic acid (Phthalic acid)	C ₈ H ₆ O ₄	166.13	14.554	0.36	
5	n-Hexadecanoic acid (Palmitic acid)	C ₁₆ H ₃₂ O ₂	256.42	14.600	7.11	
6	Pentadecanoic acid	CH ₃ (CH ₂) ₁₃ CO ₂ H	242.40	14.748	0.78	
7	10-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.48	15.908	0.73	
8	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5	16.143	4.31	
9	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5	16.501	4.67	
10	Hexadecanoic acid, butyl ester	C ₂₀ H ₄₀ O ₂	312.5	16.682	4.00	
11	Octadecanoic acid, butyl ester	C ₂₂ H ₄₄ O ₂	340.6	18.409	1.24	
12	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	C ₂₅ H ₃₆ O ₂	368.55	18.656	0.71	
13	2-Ethylacridine	C ₁₅ H ₁₃ N	207.27	19.151	1.14	
14	Carbonic acid, 2-ethylhexyl tridecyl ester	C ₂₂ H ₄₄ O ₃	356.6	19.319	0.94	
15	1H-Indole-2-carboxylic acid, 6-chloro-	C ₉ H ₆ ClNO ₂	195.60	21.858	1.97	

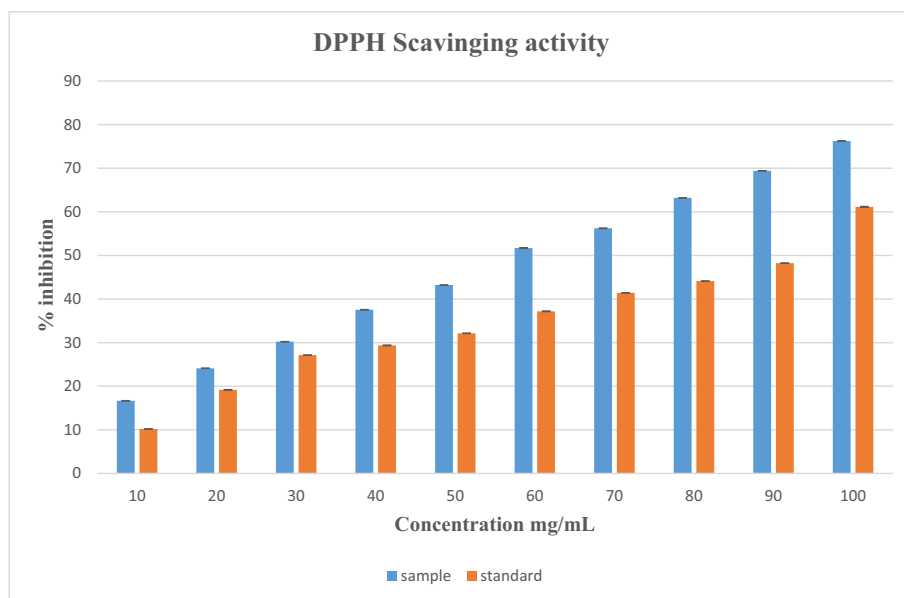


Fig. 3. Free radical scavenging activity of Papaya seed oil. Values are expressed in Mean $\pm$ SD of three replicates.

Table 2

Percentage Inhibition activity of *Carica papaya* oil. Values are expressed in Mean $\pm$ SD of three replicates, and the results are compared with two-way ANOVA. All the values are statistically significant, $p < 0.05$.

Concentration (mg/mL)	Percentage Inhibition activity (Mean $\pm$ S-D)	
	Standard	Sample
10	16.67 $\pm$ 0.02	10.19 $\pm$ 0.01
20	24.11 $\pm$ 0.009	19.17 $\pm$ 0.05
30	30.22 $\pm$ 0.02	27.13 $\pm$ 0.003
40	37.55 $\pm$ 0.03	29.36 $\pm$ 0.07
50	43.21 $\pm$ 0.01	32.13 $\pm$ 0.026
60	51.7 $\pm$ 0.08	37.18 $\pm$ 0.06
70	56.22 $\pm$ 0.03	41.43 $\pm$ 0.002
80	63.19 $\pm$ 0.005	44.11 $\pm$ 0.03
90	69.41 $\pm$ 0.002	48.22 $\pm$ 0.03
100	76.23 $\pm$ 0.04	61.13 $\pm$ 0.09

inflammatory responses associated with many diseases. This anti-inflammatory activity—along with its antimicrobial and antioxidant activity—makes papaya seed oil a viable option for treating inflammatory disorders such as arthritis, dermatitis, and gastrointestinal disorders [11].

Beyond its potential therapeutic value, papaya seed oil's cosmetic uses have gained widespread recognition, primarily in the realm of skincare. Its emollient qualities, as well as its capacity for nourishing and hydrating the skin, make it a desirable addition to any natural skincare product [12]. Additionally, its antimicrobial and anti-inflammatory properties pay to its worth in treating numerous skin conditions, from acne and eczema to wounds and burns [13,14].

This study aims to investigate the antioxidant ability of papaya seed oil using GC–MS analysis. In this study, we will examine the bioactive constituents of papaya seed oil and assess their potential therapeutic ability to induce oxidative stress. This study will use molecular docking to assess the bioactive potential of papaya phytochemical compounds against multiple target proteins (1HD2, 4MMM, and 5B6N) through the innovative use of the Glide module of the Schrödinger software, which has not previously been conducted with these target proteins. This study will contribute to the current understanding of the potential medicinal effects of papaya seed oil and its potential uses in the pharmaceutical and nutraceutical industries.

Table 3

Top 5 docked compounds showing the strongest binding affinities with each protein target.

Protein	Compound Name	Docking Score	Key Amino Acid Interactions	Interaction Types
1HD2	Benzyl nitrile	−5.215	ARG127, CYS47, THR44, PHE120	H-bonds, π - π stacking
	1H-Indole-2-carboxylic acid, 6-chloro-	−4.794	THR44, GLY46, CYS47	H-bonds
	1,2-Benzenedicarboxylic acid	−4.745	ARG127, GLY46, CYS47	H-bonds
	Ascorbic acid (control)	−4.372	THR44, GLY47	H-bonds
	Thiocyanic acid	−4.178	—	—
4MMM	1H-Indole-2-carboxylic acid, 6-chloro-	−5.897	ARG127, THR44, GLY46, PHE79	H-bonds, π - π stacking
	1,2-Benzenedicarboxylic acid	−5.133	THR44, GLY46, PHE79	H-bonds, π - π stacking
	2-Ethylacridine	−5.051	PHE79, THR44, GLY46, ARG127, PHE120	π - π stacking
	Benzyl nitrile	−4.910	CYS47, GLY46, THR44, ARG127	H-bonds, π - π stacking
	Ascorbic acid (control)	−4.811	THR44, ARG127, PHE207, PHE186, VAL187, SER186, GLY205, LYS204	H-bonds
5B6N	1H-Indole-2-carboxylic acid, 6-chloro-	−6.657	—	H-bonds, π - π stacking
	2-Ethylacridine	−5.506	—	—
	Phenol, 2,2'-methylenebis[...]	−5.432	PHE207, SER186, VAL187, SER186, GLY205, LYS204	H-bonds
	Ascorbic acid (control)	−4.838	—	H-bonds
	Thiocyanic acid	−4.789	—	—

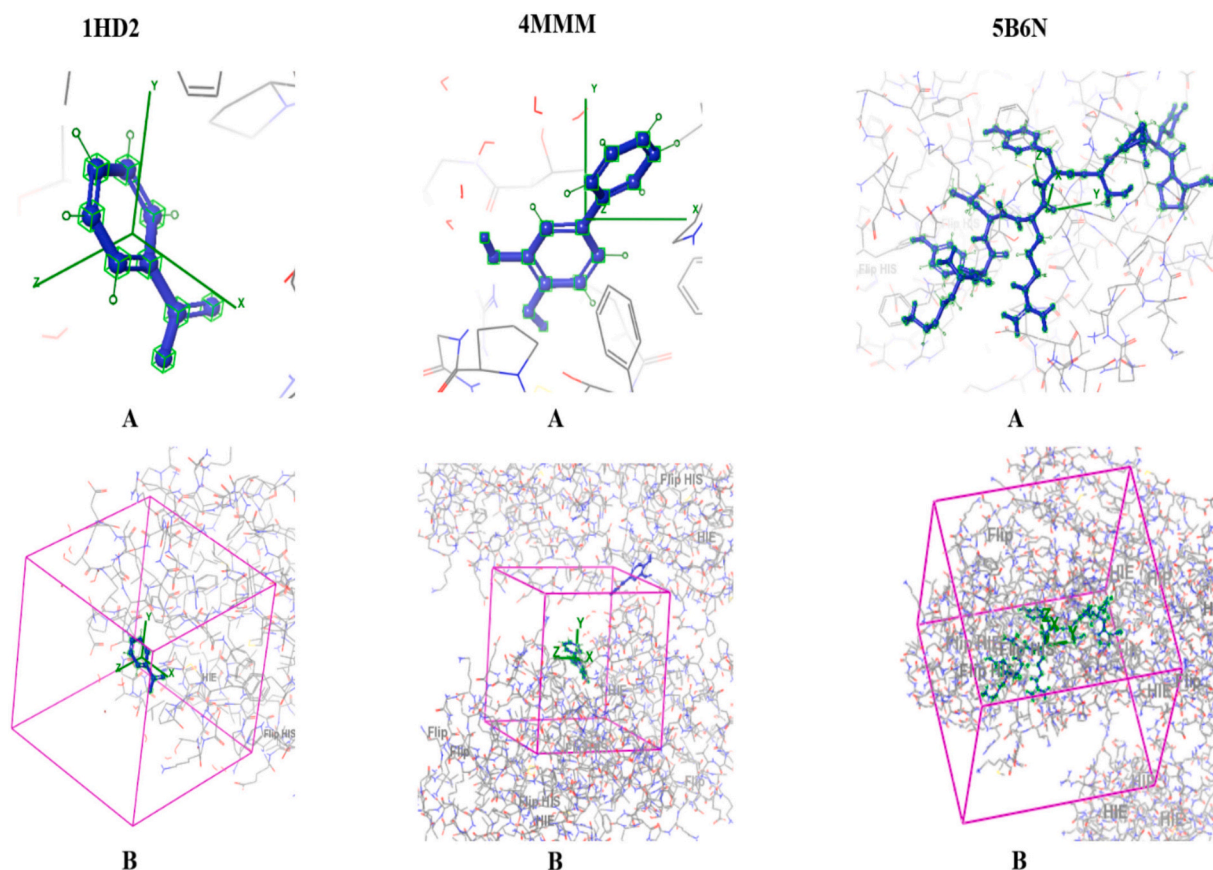


Fig. 4. (A) receptor grid on the ligands (B) receptor grid generation on proteins.

2. Materials and methods

2.1. Sample collection

Seeds of *Carica papaya* were obtained from Lahore, Punjab, Pakistan, in May 2023. All chemicals used were analytical-grade chemicals from Sigma Aldrich. The experimental work was conducted at the University of Central Punjab and TTI Testing Laboratories, Lahore.

2.2. Extraction of papaya seed oil

Fresh *Carica papaya* seeds weighing approximately 15 g were thoroughly washed in distilled water to remove any surface dust particles and to prevent bacteria. The seeds were then air-dried to remove moisture content; this is important because moisture can lead to beer and bacteria, and mold. After they dried, we removed the outer protective layer of the seeds by hand and de-hulled and ground the seeds to powder in a home-use grinder. The initial seed powder then underwent defatting completely by washing the seed powder multiple times in n-hexane in the ratio of 1:10 (w/v). As we repeated the washing process, we used smaller amounts of n-hexane to thoroughly remove all oily contents from the seed powder. Later, the oil extracted in this way was transferred into an Eppendorf tube and stored at 4 °C for analysis [15,16].

2.3. GC–MS analysis of papaya oil

The analytical instrument utilized in this study was an Agilent Technologies GC system featuring a GC-7890 A/MS-5975C model coupled with a DB-5MS column. The column dimensions were 30 m in

length, 0.25 μm in diameter, and 250 μm in thickness of film. The analytical procedure commenced with the oven temperature set at 50 °C for 1 min, followed by a gradual increase to 120 °C at a rate of 25 °C/min, maintained for 5 min. For analysis, a 1 μL sample was introduced into the system. Helium gas with a purity of 99.9 % served as the carrier gas, flowing at a rate of 1 mL/min, while the sample injection temperature was held at 230 °C. The split ratio was set at 20 throughout the experiment. Ionization mass was achieved using 70 eV, and mass spectra were recorded over a range of 55–416 m/z for a total duration of 26 min. Compounds eluted from the column were identified by comparing their mass spectra, with each compound exhibiting unique electronic signals. The calibration of the mass-to-charge ratio was conducted by correlating the obtained mass spectrum (fingerprint) for each molecule. Subsequently, the mass spectra for individual compounds were cross-referenced with databases such as PubChem and the NIST library for identification purposes [17].

2.4. Antioxidant activity

The assessment of free radical scavenging activity in *Glycine max* oil was conducted through the reduction in absorbance of a methanol solution containing DPPH (2,2-diphenyl-1-picrylhydrazyl). To evaluate its antioxidant potential, a stock solution of 1 mg DPPH in 50 mL methanol was prepared. Ascorbic acid served as the standard, and stock solutions (1 mg/mL) of ascorbic acid and papaya seed oil were utilized. Approximately 20 μL of each concentration (ranging from 10 to 100 $\mu\text{L/mL}$) dissolved in 10 % DMSO (dimethyl sulfoxide) was incubated with 20 μL of DPPH in darkness for 30–40 min. Subsequently, absorbance readings were taken using an ELISA microplate reader at 490 nm. [44] The percentage inhibition of free radicals was then calculated using the

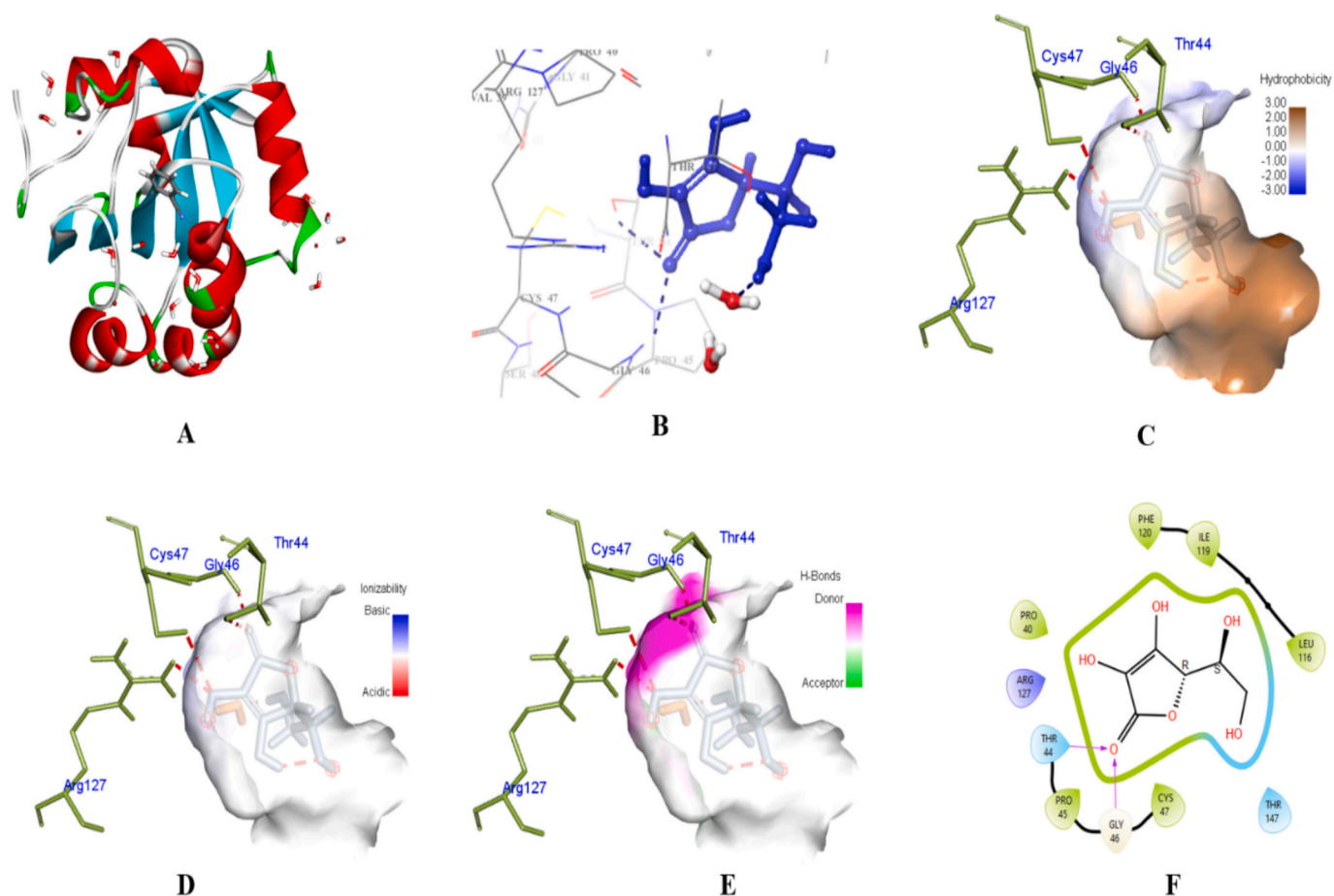


Fig. 5. Interaction of 1HD2 protein with ascorbic acid (A) structure of protein (B) protein-ligand interactions (C) hydrophobic interaction (D) Ionizability (E) H-Bond donors and acceptors (F) 2D diagram of ligand.

following formula:

$$\text{DPPH Percent Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \times 100$$

In this formula, DMSO was utilized as the blank reference [18].

2.5. Molecular docking

2.5.1. Protein selection

The choice of the proteins, 1HD2, 4MMM, and 5B6N, as the target of molecular docking was made by taking into consideration the established signatures of protein pathways related to disease-related oxidative stress pathway and inflammatory pathway. 1HD2 protein A crystal structure of human peroxiredoxin 5 (PRDX5), an important antioxidant enzyme that converts hydrogen peroxide and peroxynitrite into water in a process called reduction, avoiding oxidative damage in the cell [19]. PRDX5 dysregulation has been linked with neurodegenerative disorders, injury, and aging diseases. The oxidized variant of human peroxiredoxin 1 (PRDX1), the other representative of the peroxiredoxin family of redox homeostatic enzymes, is the 4MMM protein. PRDX1 is abundantly upregulated in inflammatory diseases and cancer, and oxidative deactivation is highly associated with redox dysregulation [20]. Finally, the

crystal structure of human lipoxygenase (15-LOX-2), the iron-containing enzyme transforming catalysis lipid peroxidation, which is closely

linked to the inflammatory process, atherosclerosis, and tumor formation, is labeled 5B6N [21]. Its contribution towards the generation of pro-inflammatory lipid mediators is associated with diseases with the basis on oxidative stress. Thus, the protein targets will be pertinent models to create antioxidant and therapeutic potentials of phytochemicals discovered in *Carica papaya* seed oil, specifically in conditions attributed to redox disturbance and inflammation.

2.5.2. Protein preparation

The Glide module of Schrödinger enabled researchers to create the receptor grid that established the docking operations active site. The designated active site determination relied on PDB co-crystallized ligands and binding residues reported in the literature for analysis. The suitable dimensions for the docking space required the selection of an active site location to position the grid center properly. Ligand-binding amino acids necessary for component function were included, while the remaining areas were removed from the defined grid box. Ligand flexibility for navigating the binding site was enhanced when a value of 0.8 was applied to van der Waals scaling for nonpolar atoms during docking.

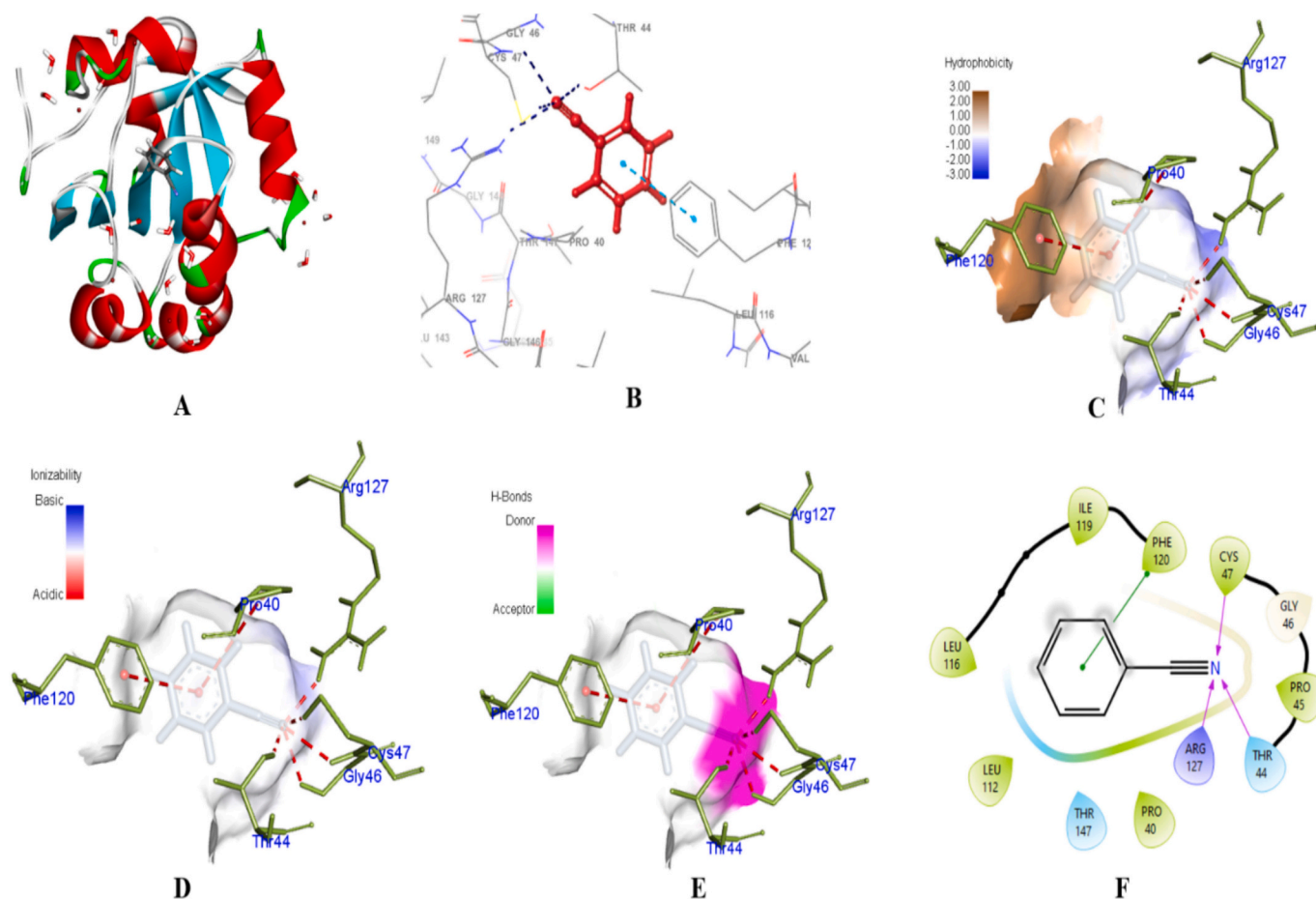


Fig. 6. Interaction of 1HD2 protein with benzyl nitrile (A) structure of protein (B) protein-ligand interactions (C) hydrophobic interaction (D) Ionizability (E) H-Bond donors and acceptors (F) 2D diagram of ligand.

The simulation required receptor grids, which included ligand parameters, to proceed [22].

Energy minimization of the protein structure was achieved through constrained minimization using the OPLS4 force field to advance docking stability by enabling hydrogen atom mobility only. The process prevents modifications to the active site structure by removing all steric clashes as well as unwanted conformations detected within the original crystal structure. The refined protein structure obtained from the previous step was saved for the upcoming grid generation process [23].

2.5.3. Glide grid generation

The active site designation for docking operations was established by creating the receptor grid through the Glide module of Schrödinger. The identified active site was determined by using PDB structure co-crystallized ligands or binding residues obtained from literature reports. Docking space needed a suitable ligand space, so the grid center was placed at the active site location. Only the essential amino acids participating in ligand binding received inclusion in the defined grid box, as additional areas were removed. A value of 0.8 for van der Waals scaling applied to nonpolar atoms during docking enabled ligands to navigate the binding site with increased flexibility.

Receptor grids containing the ligand parameters were used for simulations that followed [23].

2.5.4. Ligand preparation

The therapeutic compounds of papaya were selected according to their therapeutic potential and obtained from PubChem through the SMILES format. The 2D chemical structures underwent conversion into 3D optimized geometries through the LigPrep module of Maestro, which

ensured high accuracy for future docking procedures. The chemical compounds kept their original structure after preserving neutral charge states rather than accepting tautomer formation. The OPLS4 force field optimized the bond lengths and angles, and torsions to eliminate any steric strain for energy minimization [24].

2.5.5. Molecular docking

To guarantee precise binding pose predictions, Schrödinger's Glide module was used for molecular docking. Docking scores and molecular interaction profiles were used to evaluate binding interactions after the produced ligands were docked into the receptor grid. The docking scores represent the ligand-protein binding affinity; more binding is indicated by more negative scores. Van der Waals forces, hydrophobic interactions, π - π stacking, and hydrogen bonds—all of which support the stability of the ligand-receptor complex were investigated in the interactions [25]. The top-scoring ligand-protein complexes became visible through Discovery Studio and Maestro from Schrödinger for a detailed assessment of all molecular interactions [26]. Further evaluation proceeded with optimal compounds following the sorting of binding scores from docking data. The degree of hydrophobicity is indicated by the color of the surfaces. Low, medium, and high hydrophobicity are represented by the colors blue, white, and brown, respectively [27]. The red areas of the ionizability map characterize electron-deficient acidic regions, while blue areas define basic, electron-rich regions. Binding stability depends on these charge distributions since they control electrostatic interactions that occur between ligand and protein [28]. Whereas the green areas show hydrogen bond acceptors, the pink areas show hydrogen bond donor sites. Hydrogen bonds between the inhibitor and protein become stronger when the colored zones appear. This

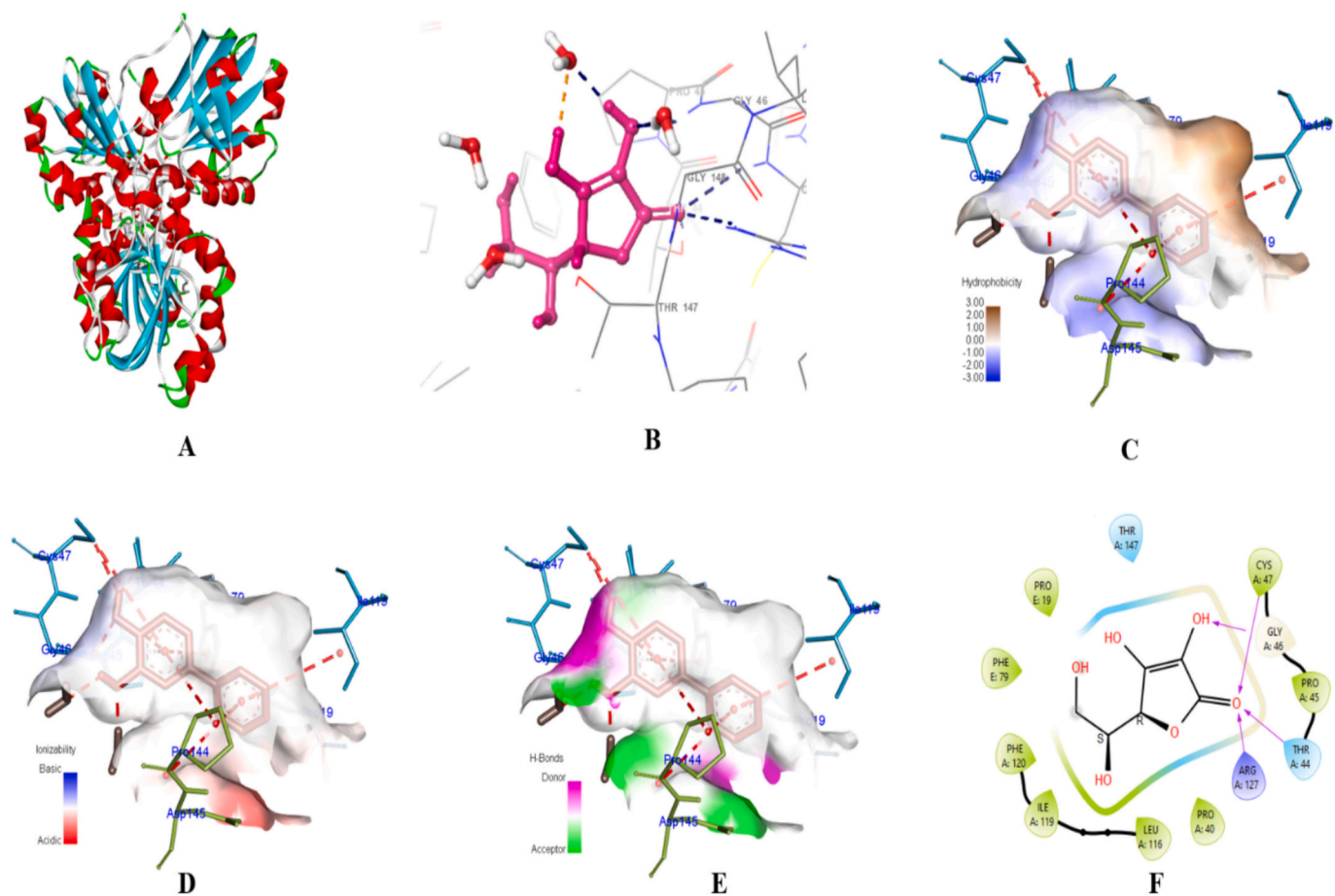


Fig. 7. Interaction of 4MMM protein with ascorbic acid (A) structure of protein (B) protein-ligand interactions (C) hydrophobic interaction (D) Ionizability (E) H-Bond donors and acceptors (F) 2D diagram of ligand.

signifies enhanced ligand binding with specific protein targets [29].

2.6. Molecular dynamic simulations

The stability of the complexes with the lowest binding energy and a higher number of hydrogen bonds in the docking study was evaluated by using MD simulations analysis. The compound topologies were built through the CGenFF server [30]. The protein structure topologies were built through GROMACS via the appropriate commands. Thereafter, the protein and the complex structures were made ready for the simulation via the related commands. MD simulation was performed by using CHARMM as a force field. The complexes between the target structures and compounds were put into a tricubic box and solvated by TIP3P water. Thereafter, ions from the NaCl salt were utilized to neutralize the system charge. Then, system energy minimization was conducted with the steepest descent method for 50,000 steps. After that, system equilibration was undertaken by applying NVT/NPT at 100 kPa pressure and 300 K temperature. After the simulation system elements were ready, a 200 ns simulation was undertaken through GROMACS 2024 [31]. The related plots were sketched through QtGrace, and analysis was performed thereafter [43].

3. Results and discussion

3.1. GC–MS analysis

In the given study, about 42 important compounds of medicinal and industrial use are found in the given n-hexane fraction of *Carica Papaya* oil (Fig. 2). Only important compounds are discussed here based on our

objectives. Some of these compounds are benzyl nitrile (3.43 %), Pentadecanoic acid, 14-methyl-, methyl ester (5.23 %), n-Hexadecanoic acid (7.11 %), Pentadecanoic acid (0.78 %), and 2-Ethylacridine (1.14 %), etc. (Table 1).

The overall percentage of the detected 42 compounds was found to be about 99 %. The peak area for each compound is proportional to its amount. Although the peak area of a few medicinal compounds is small, the minimum amount of these compounds is of exceptional importance, as many of these compounds are used as standard drugs at medical and industrial levels.

The compounds identified through GC–MS analysis exhibit potential antioxidant properties, which are crucial in mitigating oxidative stress-induced damage. Among these compounds, fatty acid derivatives such as n-Hexadecanoic acid (Palmitic acid), n-Octadecanoic acid (Stearic acid), and their ester derivatives (e.g., Butyl palmitate, Butyl stearate) have shown promise as antioxidants, and these compounds were reported in *Parinari polyandra* [32]. These compounds possess structural features that enable them to scavenge reactive oxygen species (ROS) and prevent oxidative damage to cellular components. Additionally, phenolic compounds such as Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-, commonly found in plant-based sources, exhibit potent antioxidant activity by donating hydrogen atoms or electrons to neutralize free radicals [33]. Furthermore, an unsaturated fatty acid derivative, n-Octadecenoic acid methyl ester, has a double bond in the hydrocarbon chain, and this allows for greater antioxidant effectiveness through the ability to donate electrons to stabilize free radicals. This study demonstrates the potential of the molecules studied as natural antioxidants and their significant role in the treatment of oxidative stress-related diseases like cancer, inflammation, diabetes, and heart disease, and promotes

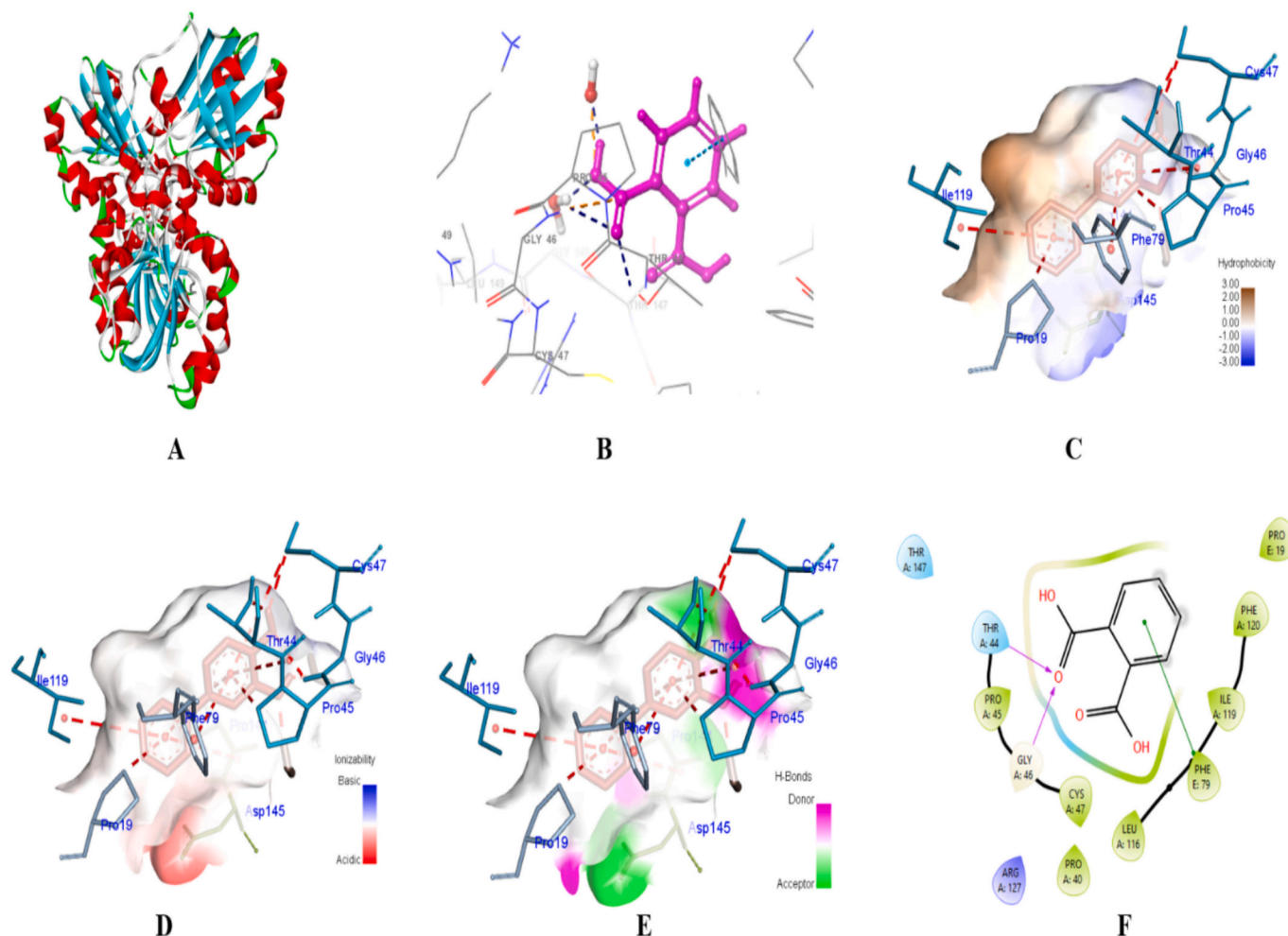


Fig. 8. Interaction of 4MMM protein with 1,2-Benzenedicarboxylic acid (A) structure of protein (B) protein-ligand interactions (C) hydrophobic interaction (D) Ionizability (E) H-Bond donors and acceptors (F) 2D diagram of ligand.

overall health [34,35].

Multiple compounds found in our GC–MS analysis could potentially act as anticancer agents, providing good prospects for cancer prevention and treatment. Phenolic compounds in particular, such as Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl- have been linked to anticancer properties by their role in modifying signaling pathways related to cell proliferation, apoptosis, and metastasis [36]. Additionally, 1*H*-Indole-2-carboxylic acid, 6-chloro-, a derivative of the naturally occurring indole compound, has shown anticancer activity by obstructing cell growth and inducing apoptosis in cancer cells [37,38]. Moreover, certain fatty acid derivatives like n-Hexadecanoic acid (Palmitic acid) and n-Octadecanoic acid (Stearic acid) have been studied for their anticancer effects, warranting further research to understand their action mechanisms and usefulness for improving treatment against specific types of cancers [39]. These conclusions highlight the various repertoire of compounds recognized in the GC–MS investigation and their probable as anticancer agents, warranting additional inquiry in preclinical and clinical studies.

The compounds identified using GC–MS analysis may possibly have potential antibacterial activity, which could provide alternative therapies for the treatment of bacterial infections. In the case of antibacterial activity, for example, 2-Ethylacridine demonstrated antibacterial activity against a wide array of microorganisms, including Gram-positive and Gram-negative bacteria [40]. Furthermore, some fatty acid derivatives such as n-Hexadecanoic acid (Palmitic acid) have confirmed antimicrobial effects by disordering bacterial cell membranes and

obstructing bacterial growth [41]. Furthermore, Carbonic acid, 2-ethyl-hexyl tridecyl ester, a derivative of carbonic acid, displays antibacterial activity against clinically related pathogens, making it a promising candidate for antibacterial drug progress [36]. The results of this study highlight the promising potential of the compounds identified in this work as natural antibacterial agents, presenting new avenues for the treatment of bacterial infections and the management of antibiotic resistance. Future work could focus on elucidating the mechanism of action and therapeutic potential of these compounds against bacterial pathogens, ultimately leading to the possible development of novel antibacterial therapies.

3.2. Antioxidant activity

The percentage DPPH activity observed for papaya seed oil at different concentrations (1–10 $\mu\text{L/mL}$). There is a gradual increase in percentage inhibition with an increase in concentration (Fig. 4.5). At each concentration, the radical scavenging activity of the sample is nearest to the standard, although it is less than the standard.

The highest percentage radical scavenging activity observed for papaya seed oil was (61.13 ± 0.09) as compared to that of Vitamin C (76.23 ± 0.04) and the smallest inhibition activity was found to be (10.19 ± 0.01) and (16.67 ± 0.02) for sample and standard respectively, while the IC_{50} value for the sample was 42.87 mg/mL as compared to the standard which is 39.28 mg/mL as indicated in Fig. 3 and Table 2.

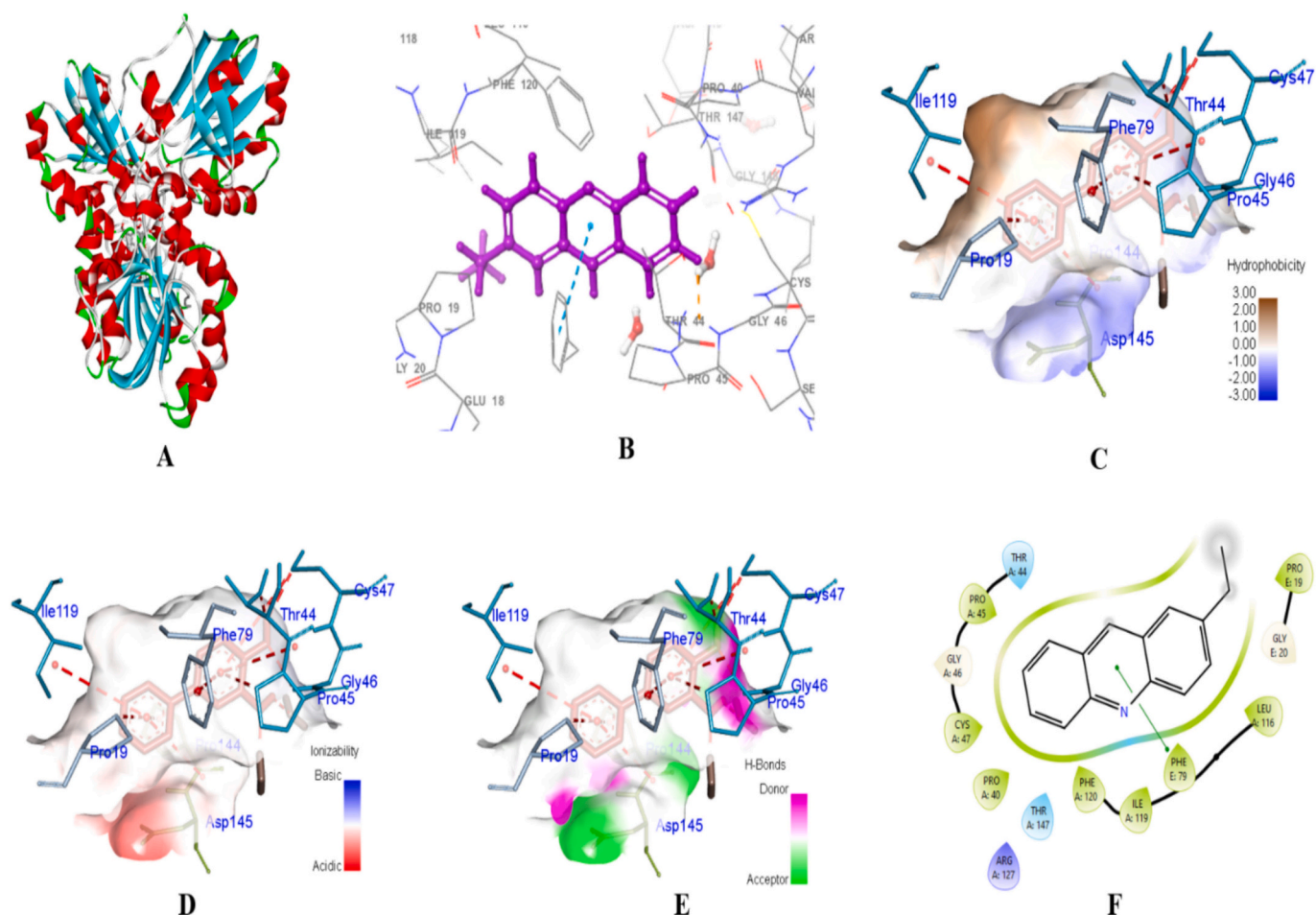


Fig. 9. Interaction of 4MMM protein with 2-Ethylacridine (A) structure of protein (B) protein-ligand interactions (C) hydrophobic interaction (D) Ionizability (E) H-Bond donors and acceptors (F) 2D diagram of ligand.

3.3. Molecular docking

The analysis of bioactive compounds from papaya through molecular docking was performed using Schrödinger's Glide module against three target proteins (1HD2, 4MMM, and 5B6N). Unlike prior studies, our research integrates structure-based virtual screening with phytochemical profiling of papaya seed oil, providing novel insights into its therapeutic potential through high-precision docking simulations using Schrödinger's Glide. The docking scores along with interaction profiles helped determine binding affinity values between molecules, but these findings were compared with ascorbic acid as the control substance (Table S1). A deeper investigation of ligand binding stability and selectivity occurred through the evaluation of molecular interactions like hydrogen bonding, together with hydrophobic interactions. The docking results were shown as 2D interaction diagrams and protein-ligand interactions within Maestro software operated by Schrödinger [42]. To make the table more readable, instead of all the compounds with the best docking scores against each protein target (1HD2, 4MMM, and 5B6N), the top five records are given in the updated [Table 3](#). The molecular interactions, including hydrophobic contacts and ionization behavior, hydrogen bond networks, and surface properties of molecules, could be explored using Discovery Studio [43]. A grid was created around the binding region of protein 1HD2, 4MMM, and 5B6N, as shown in [Fig. 4](#), for specific docking.

The docking results for 1HD2 show that benzoyl nitrile achieves a strong binding affinity at -5.215 while connecting with ARG 127, CYS 47, and THR 44 through hydrogen bonds, per Fig. 6. The docking score of benzoyl nitrile remains strong because this compound adapts well

within the protein binding space. Benzoyl nitrile achieves better binding stability while becoming more effective in protein-ligand interactions because of its hydrophobic contact with PHE 120 and other residues. The docking score for ascorbic acid turned out to be moderate at -4.372 . The strong bonding interactions between the ligand and CYS 47 and GLY 46 through hydrogen bonds contributed to weaker binding compared to other molecules, as depicted in [Fig. 5](#) [44].

Discovery Studio revealed that electrostatic interactions strengthen pocket ligand stability through its ionizability analysis. The oxygen atoms of PHE 79 and LEU 51, along with other hydrophobic residues, functioned to avoid solvent interaction through non-polar stabilization according to hydrophobic interaction mapping results. The ligand structure contains oxygen atoms that act as very effective acceptor sites while forming bonds with hydrogen donor residues present in the active site, per research on hydrogen bond acceptors and donors [45]. The development of ligand-protein complexes received vital stabilization through hydrophobic interactions. 2-Ethylacridine created robust π - π stacking connections with PHE 79, as the computer simulation delivered an evaluation of -5.051 . Active site stability of bound ligands depends on these non-covalent bonds, which remain essential for effective drug-binding performance. The molecular surface of the 4MMM-2-Ethylacridine complex, as presented in Fig. 10, revealed PHE E:79's π - π stacking function that proved ligand stability in the binding pocket, as shown in Fig. 9. Because 1,2-Benzenedicarboxylic acid formed several hydrogen bonds with THR A:44 and GLY A:46, stabilizing the ligand inside the active site, it demonstrated the highest binding affinity (-5.133) for 4MMM as shown in Fig. 8. The significance of these hydrogen bonds, which support the ligand's robust engagement with the receptor, was

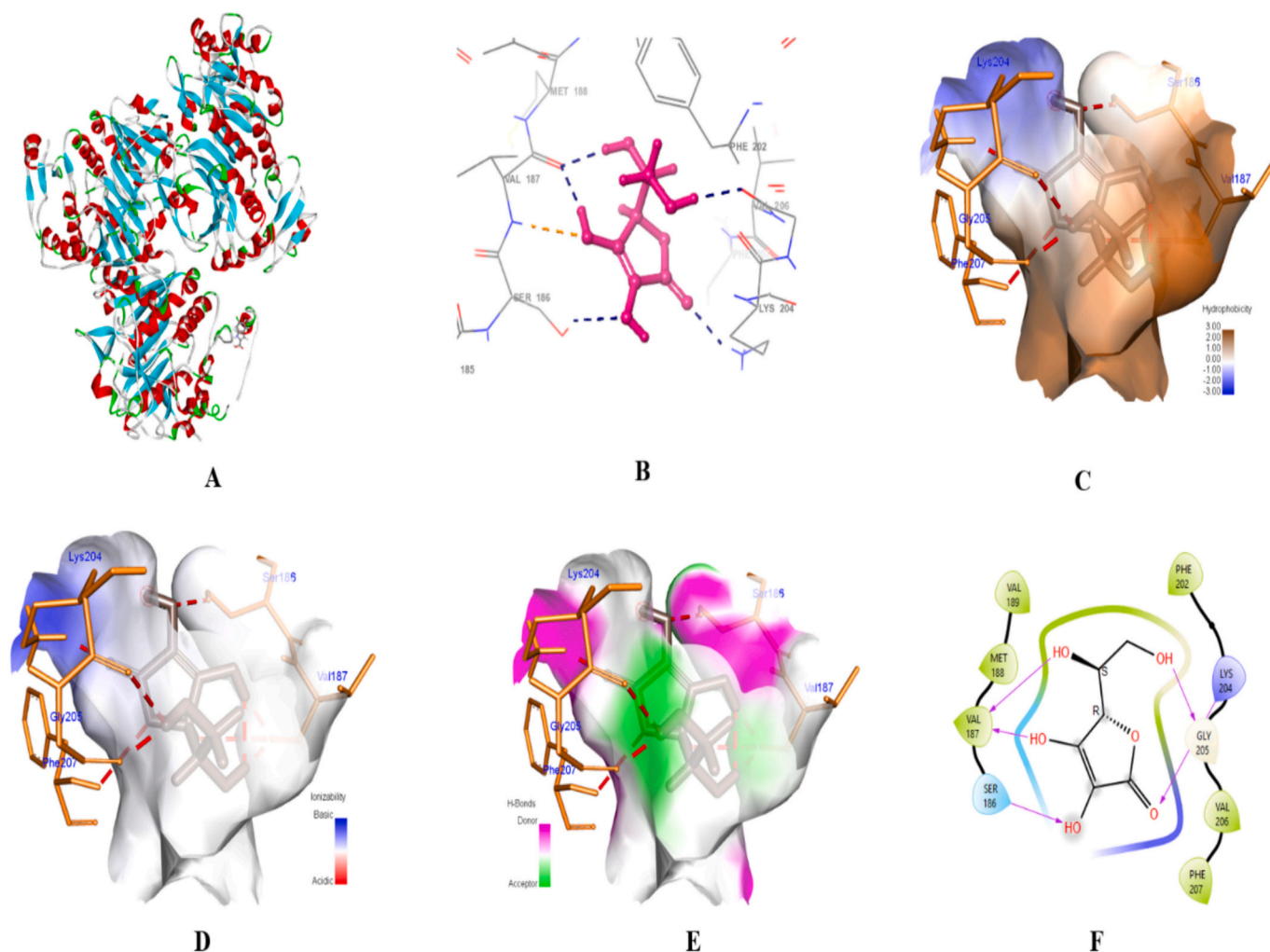


Fig. 10. Interaction of 5B6N protein with ascorbic acid (A), structure of protein (B), protein-ligand interactions (C), hydrophobic interaction (D), Ionizability (E), H-Bond donors and acceptors (F), 2D diagram of ligand.

brought to light by the Schrödinger docking visualization. Furthermore, electrostatic interactions that reinforce ligand stability within the pocket were discovered by ionizability analysis in Discovery Studio. Hydrophobic residues like PHE 79 helped to prevent undesired solvent interactions by contributing to non-polar stabilization, as demonstrated by hydrophobic interaction mapping. Oxygen atoms on the ligand structure served as strong acceptor sites, effectively connecting with hydrogen donor residues in the active site, according to the hydrogen bond donor and acceptor study. As compared to 1,2-Benzenedicarboxylic acid, ascorbic acid showed lesser binding (-4.811), forming hydrogen bonds with CYS A:47 and ARG A:127 while demonstrating less interaction specificity as shown in Fig. 7. As a stronger candidate than ascorbic acid, the molecular surface representation verified that van der Waals forces and π - π stacking contribute to the binding stability of 1,2-Benzenedicarboxylic acid.

The greatest docking score (-6.657) was shown by 6-chloro for 5B6N, 1H-Indole-2-carboxylic acid, which formed hydrogen bonds with PHE 207 and VAL 187, two essential residues for ligand stabilization, as shown in Fig. 11. Strong ligand-receptor interactions are made possible by these interactions' proper placement within the binding pocket, according to the hydrogen bonding network study from Schrodinger. Furthermore, the complex's overall stability was improved by π - π stacking interactions with PHE 207, which helped explain its high docking score. The study of hydrophobic interactions revealed that residues like ALA 248 and LEU 245 were essential for preserving ligand

stability through nonpolar interactions. Additionally, charged residues in the binding pocket affected ligand orientation, encouraging greater interactions with the ligand's electron-rich functional groups, according to ionizability mapping [46]. Numerous oxygen and nitrogen atoms in the ligand structure functioned as potent hydrogen bond acceptors, creating stable connections with polar residues in the protein, according to the hydrogen bond donor and acceptor study. Ascorbic acid, on the other hand, had a docking score of -4.838 and formed hydrogen bonds with VAL 187, SER 186, and GLY 205, but it did not demonstrate any notable π - π stacking or hydrophobic interactions, as shown in Fig. 10. The top-scoring ligand has excellent electrostatic and hydrophobic complementarity with the active site, which supports its potential as a powerful inhibitor, according to the molecular surface representation in Discovery Studio [47]. In the Fig. 12 the 2D diagram of the protein-ligand complex through Schrodinger showed five different types of binding forces: H-bonds appear in purple, while π - π stacking is green and hydrophobic forces are light green along with salt bridges that are blue and metal coordination that is displayed in gray. The color scheme indicates how charged and polar, and hydrophobic residues participate in binding ligands. The tool provides critical information about ligand stability together with molecular complementarity and binding affinity, which is vital for drug discovery processes and optimization procedures.

Strong binding affinity through molecular interactions of these high-ranking bioactive chemicals suggests they may become useful for therapeutic applications. Molecular dynamics (MD) simulations should be

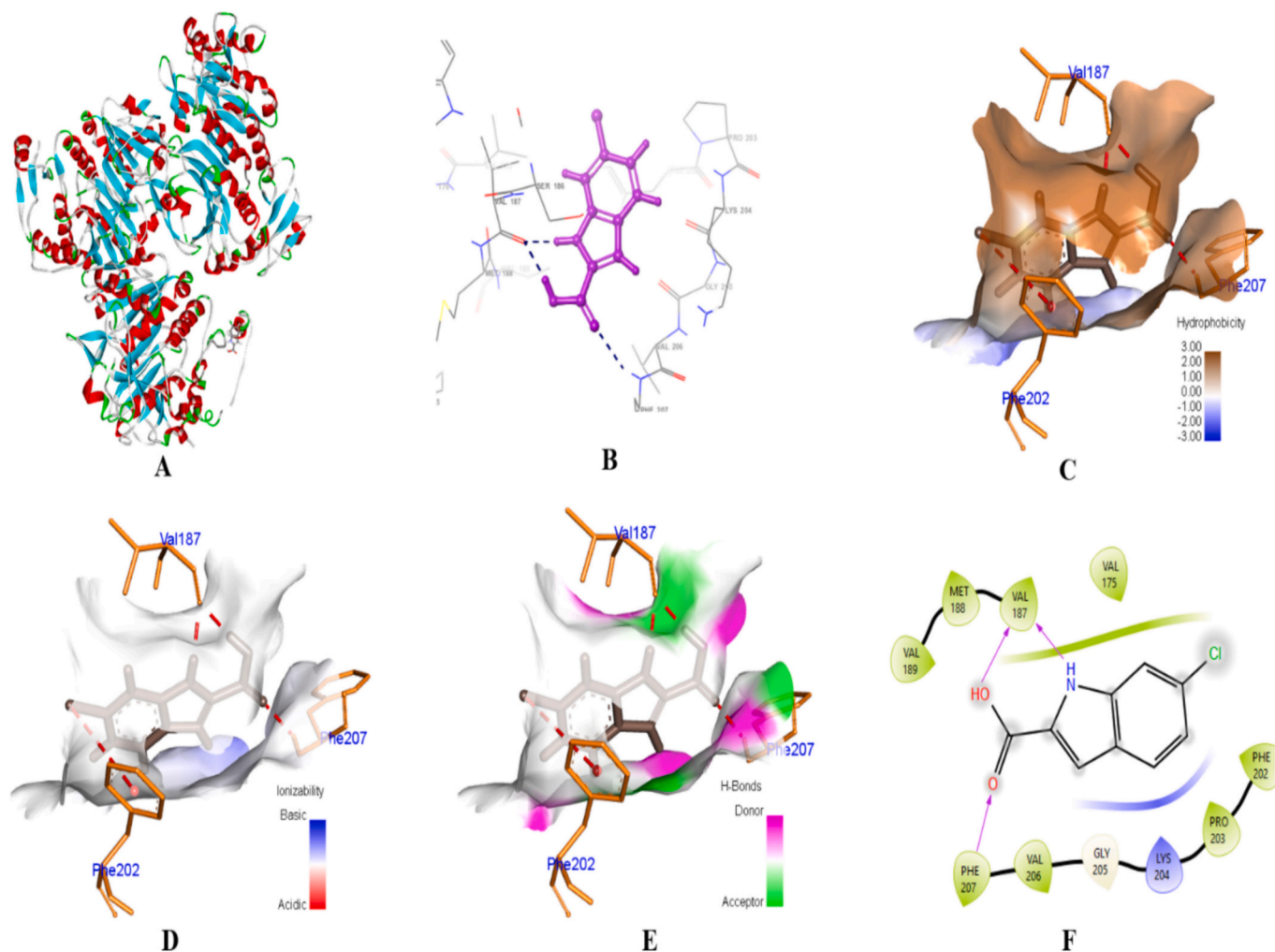


Fig. 11. Interaction of 5B6N protein with 1H-Indole-2-carboxylic acid, 6-chloro (A) structure of protein (B) protein-ligand interactions (C) hydrophobic interaction (D) Ionizability (E) H-Bond donors and acceptors (F) 2D diagram of ligand.

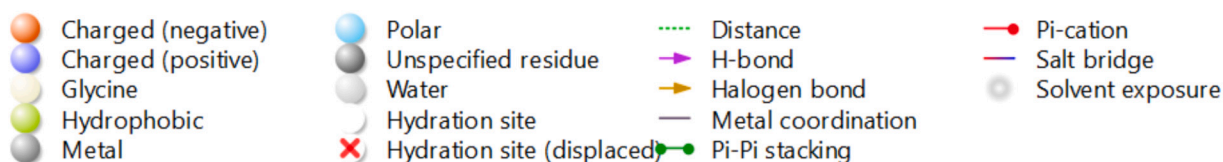


Fig. 12. Explanation of different colors and interactions in a 2D diagram of a docking study.

utilized to check binding stability while free energy calculations should enhance predictions about ligand binding. Both procedures need to be included in next investigative stages following in vitro and in vivo testing of high-scoring compounds. Future drug development initiatives will receive backing from additional analyses which will confirm the therapeutic capability of papaya-derived chemical compounds [48].

Literature docking scores of standard inhibitors were taken into account to put the docking performance of identified bioactive compounds into perspective. In past research, it was demonstrated that antioxidant standards such as ascorbic acid and other phenolic-based inhibitors have the potential to show docking energies (1HD2 and 5B6N) of -3.8 – -5.2 kcal/mol [49,50]. The docking score of some flavonoids and indole-based derivatives recorded in the structure-based drug discovery has reached values of about -6.0 kcal/mol [51]. In our study, 1H-Indole-2-carboxylic acid, 6-chloro- showed a docking score to be higher than most of these that have been documented -6.657 with 5B6N and 5.897 with

4MMM. Similarly, benzyl nitrile displayed a docking score of -5.215 with 1HD2 as compared with ascorbic acid with a docking score of -4.372 in the same system. This indicates that the components used in the *Carica papaya* seed oil have good binding affinities, and can be a useful lead in the creation of another inhibitor.

3.4. MD simulations

The docking study disclosed that the phytoconstituents had the potential to bind to the three target structures, but at different levels. Benzyl nitrile exhibited the highest binding affinity with the 1HD2 structure. Similarly, 1H-Indole-2-carboxylic acid, 6-chloro-1, exhibited the highest binding affinity with 4MMM and 5B6N structures. Hence, the stability of the three complexes was evaluated through MD simulation analysis. For this purpose, root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and hydrogen

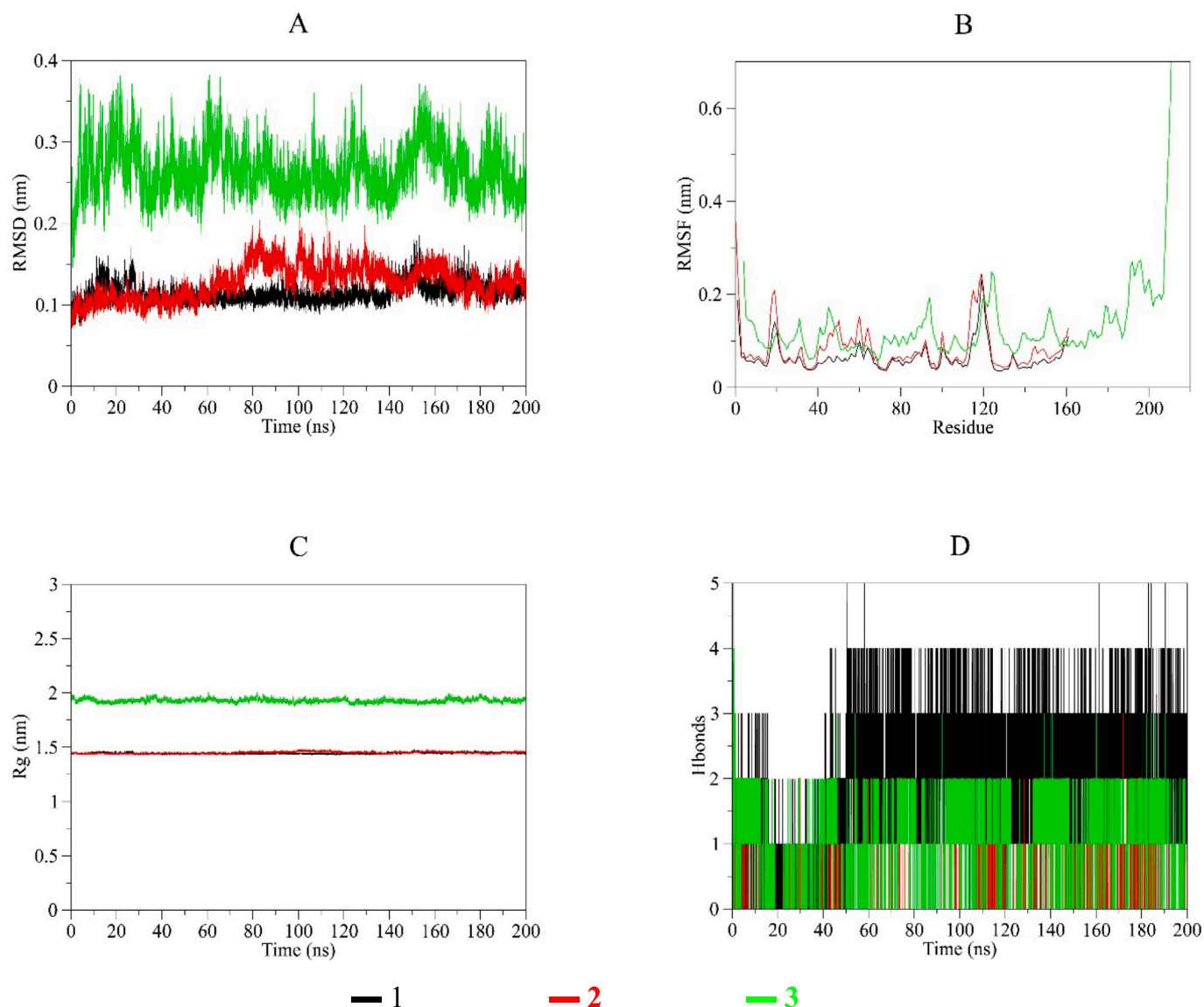


Fig. 13. RMSD (A), RMSF (B), Rg (C), and hydrogen bond number plots (D) of benzyl nitrile complexed with 1HD2 (1), 1H-Indole-2-carboxylic acid, 6-chloro-1 complexed with 4MMM (2), and 1H-Indole-2-carboxylic acid, 6-chloro-1 complexed with 5B6N (3).

bond number plots of the three complexes were drawn to understand the relative stability of the complexes. RMSD plots of protein structures or their complexes are used to assess the stability of complexes after compounds are bound to the apo structures [52]. Structure 1 (benzyl nitrile complexed with 1HD2) and 2 (1H-Indole-2-carboxylic acid, 6-chloro-1 complexed with 4MMM) gave similar RMSD values during the simulation. On the other hand, structure 3 (1H-Indole-2-carboxylic acid, 6-chloro-1 complexed with 5B6N) gave higher RMSD values throughout the simulation relative to the rest two complexes. Structures 1 and 2 gave RMSD values below 0.2 nm during the simulation time, implying formation of stable complexes for the two structures. Structure 1 gave lower RMSD values and fewer changes during the simulation. Hence, structure 1 is anticipated to have slightly higher stability relative to structure 2. Structure 3 had higher RMSD changes and values, including above 0.3 nm in some time intervals (Fig. 13A). Therefore, structure 3 is anticipated to have lower stability relative to the rest of the complexes.^[45]

RMSF plots of protein structures or their complexes are used to measure the per-residue perturbation of them during a simulation time. A lower RMSF value is a reflection of higher conservation for the related region of the structure [53]. Structures 1 and 2 had similar RMSF plots in

general. On the other hand, structure 3 gave higher RMSF values during the simulation time (Fig. 13B). A significant RMSF rise was observed for structure 2 at the N-terminal end. Similarly, a significant RMSF rise was observed at the C-terminal end of structure 3. Rg plots are utilized to have an idea about the relative stiffness of protein structures or their complexes [54]. Structures 1 and 2 gave similar Rg plots with an average of nearly 1.45 nm during the simulation time. Hence, the compactness of structures 1 and 2 is anticipated to be like each other. On the other hand, structure 3 gave higher Rg values with nearly 1.94 nm average (Fig. 13C). Therefore, structure 3 is expected to bear lower stiffness relative to the rest of the complexes.

Hydrogen bonding between compounds and their target structure is important in intermolecular binding. The number of H-bonds plays a crucial role in keeping the compounds to remain in the binding site of the target structures [55]. The H-bond number plots of the three complexes depicted that the highest number of H-bonds was observed in structure 1. The dominant number of H-bonds for benzyl nitrile in its interaction with 1HD2 was found to be three, especially after the 50 ns period (Fig. 13D). Benzyl nitrile formed a higher number of H-bonds to 1HD2 relative to the 1H-Indole-2-carboxylic acid, 6-chloro-1, with the other target structures in the docking study too. The number of H-bonds

with 5B6N was higher than that of 4MMM. The predominant H-bond with 5B6N was detected to be two. On the other hand, the predominant H-bond with 4MMM was detected to be a single one (Fig. 13D). In general, the number of H-bonds in the simulation was lower than the number obtained from the docking study. Together with this, the relative number of H-bond differences was similar in both methods. To wrap up, the MD simulation study disclosed that the complexes could be stable during the 200 ns simulation time. In addition, structures 1 and 2 might have slightly higher stability relative to structure 3.

4. Conclusion

Our study confirms that *Carica papaya* seed oil possesses substantial antioxidant properties, attributed to its rich composition of bioactive compounds such as n-hexadecanoic acid and pentadecanoic acid methyl ester. Molecular docking analyses further support these findings, revealing promising binding affinities of key constituents with molecular targets involved in inflammation, microbial resistance, and metabolic disorders, the greatest docking score (−6.657) was shown by 6-chloro for 5B6N, 1H-Indole-2-carboxylic acid, which formed hydrogen bonds with PHE 207 and VAL 187, two essential residues for ligand stabilization. The oil's antioxidant capacity, with a maximum DPPH radical scavenging activity of 61.13 %, underscores its potential utility in mitigating oxidative stress and associated disorders. These insights pave the way for the development of advanced delivery systems—such as nano-emulsions, liposomes, or transdermal gels—to enhance bioavailability and therapeutic efficacy. Future research should explore its application in treating diseases such as skin infections, diabetes, and gastrointestinal conditions, where natural bioactive compounds offer a safer and more sustainable therapeutic approach.

5. Prospects

Future research should focus on exploring the full pharmacological potential of the identified compounds through in vivo studies and clinical trials. Additionally, investigating the synergistic effects of these compounds in combination could enhance the therapeutic efficacy of papaya seed oil. The development of standardized extraction and formulation techniques will be crucial for the commercial application of papaya seed oil in various health-related products.

Author contribution

S.K. Conceptualization, Methodology, Software, Formal analysis, Writing - original draft. Writing—review and editing, Supervision, Investigation; **Z.S.A.** Validation, Formal analysis, Investigation, Data curation, Supervision, Resources; **B.S.** Methodology, Validation, Software, Investigation, **A.I.** Conceptualization, Formal analysis, Funding acquisition, Data curation, Project administration, Writing - review & editing; **M.T.M.** Data curation, Software, Writing - review & editing; **K-K-M**; Investigation, Resources, Writing - original draft. **Y.A.B.J.** Methodology, Validation, Formal analysis; **M.G.** Software, Validation, Investigation; **S-P**: Validation, Data curation, Visualization, Writing - review & editing; **M.M.** Funding acquisition, Formal analysis, Visualization, Project administration, Writing - review & editing.

All authors have read and agreed to the published version of the manuscript.

Clinical trial declaration

This is to certify that this study involved in vitro assays, with the protocols and references provided in the manuscript. Therefore, this study does not involve any clinical trials.

CRediT authorship contribution statement

Sabir Khan: Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Zeemal Seemab Amin:** Validation, Supervision, Resources, Investigation, Formal analysis, Data curation. **Bushra Sha-koor:** Validation, Software, Methodology, Investigation. **Ali irfan:** Writing – review & editing, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Muhammed Tila-hun Muhammed:** Writing – review & editing, Software, Data curation. **Katarzyna Kotwica-Mojzych:** Writing – original draft, Resources, Investigation. **Yousef A. Bin Jordan:** Validation, Methodology, Formal analysis. **Mariola Glowacka:** Validation, Software, Investigation. **Sha-zia Perveen:** Writing – review & editing, Visualization, Validation, Data curation. **Mariusz Mojzych:** Writing – review & editing, Visualization, Project administration, Funding acquisition, Formal analysis.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All the data related to this study are contained in the manuscript, and more data related to this study can be accessed upon a reasonable request to the corresponding authors or zeemalseemab279@gmail.com

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