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NUTRITIONAL AND CLINICAL EVALUATION OF CARICA PAPAYA

Original Research

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ABSTRACT

Background: Medicinal plants have been used for centuries in traditional systems of medicine for the management of metabolic and inflammatory disorders. *Carica papaya*, a member of the Caricaceae family, is widely recognized for its nutritional and therapeutic potential. The fruit contains diverse phytochemicals and proteolytic enzymes that have been associated with antioxidant, anti-inflammatory, anti-diabetic, and wound healing properties. Despite growing interest, comprehensive evaluation integrating phytochemical profiling, biological assays, and molecular docking remains limited.

Objective: To investigate the phytochemical composition, in-vitro biological activities, and in-silico molecular interactions of different solvent extracts of *Carica papaya*.

Methods: Fresh *C. papaya* fruits were procured, air-dried, powdered, and extracted separately using ethanol, distilled water, n-hexane, and chloroform. Extracts were concentrated using a rotary evaporator. Phytochemical screening was performed using standard qualitative procedures. Antioxidant activity was assessed by DPPH radical scavenging assay, anti-inflammatory activity by bovine serum albumin denaturation method, and anti-diabetic activity by α-amylase inhibition assay. Absorbance readings were recorded using a UV–visible spectrophotometer and ELISA reader. GC–MS analysis was conducted for ethanol and n-hexane extracts. Protein profiling was performed using SDS-PAGE. Molecular docking of identified ligands with TAR DNA-binding protein was carried out using AutoDock Vina.

Results: Ethanol extract exhibited 93.62% antioxidant inhibition at 0.5 mg/mL compared with 97.43% for ascorbic acid. Chloroform extract showed 94.07% inhibition at the same concentration. In anti-inflammatory assay, ethanol extract demonstrated 42.91% inhibition at 0.5 mg/mL, whereas diclofenac showed 80.47%. Anti-diabetic activity revealed 89.47% inhibition for ethanol extract and 87.50% for n-hexane extract at 0.5 mg/mL, compared to 93.87% for metformin. GC–MS identified 26 compounds in ethanol extract, with 10-octadecenoic acid methyl ester (28.47%) and 5-hydroxymethylfurfural (25.35%) as predominant constituents. Docking analysis showed binding affinities up to −6.0 kcal/mol for selected ligands.

Conclusion: *Carica papaya* extracts demonstrated significant antioxidant, anti-inflammatory, and anti-diabetic activities supported by phytochemical diversity and molecular docking interactions. These findings highlight its potential as a promising source of bioactive compounds for further pharmacological development.

Keywords: Alpha-Amylase Inhibitors, Antioxidants, Carica papaya, Gas Chromatography-Mass Spectrometry, Molecular Docking Simulation, Phytochemicals, Proteases.

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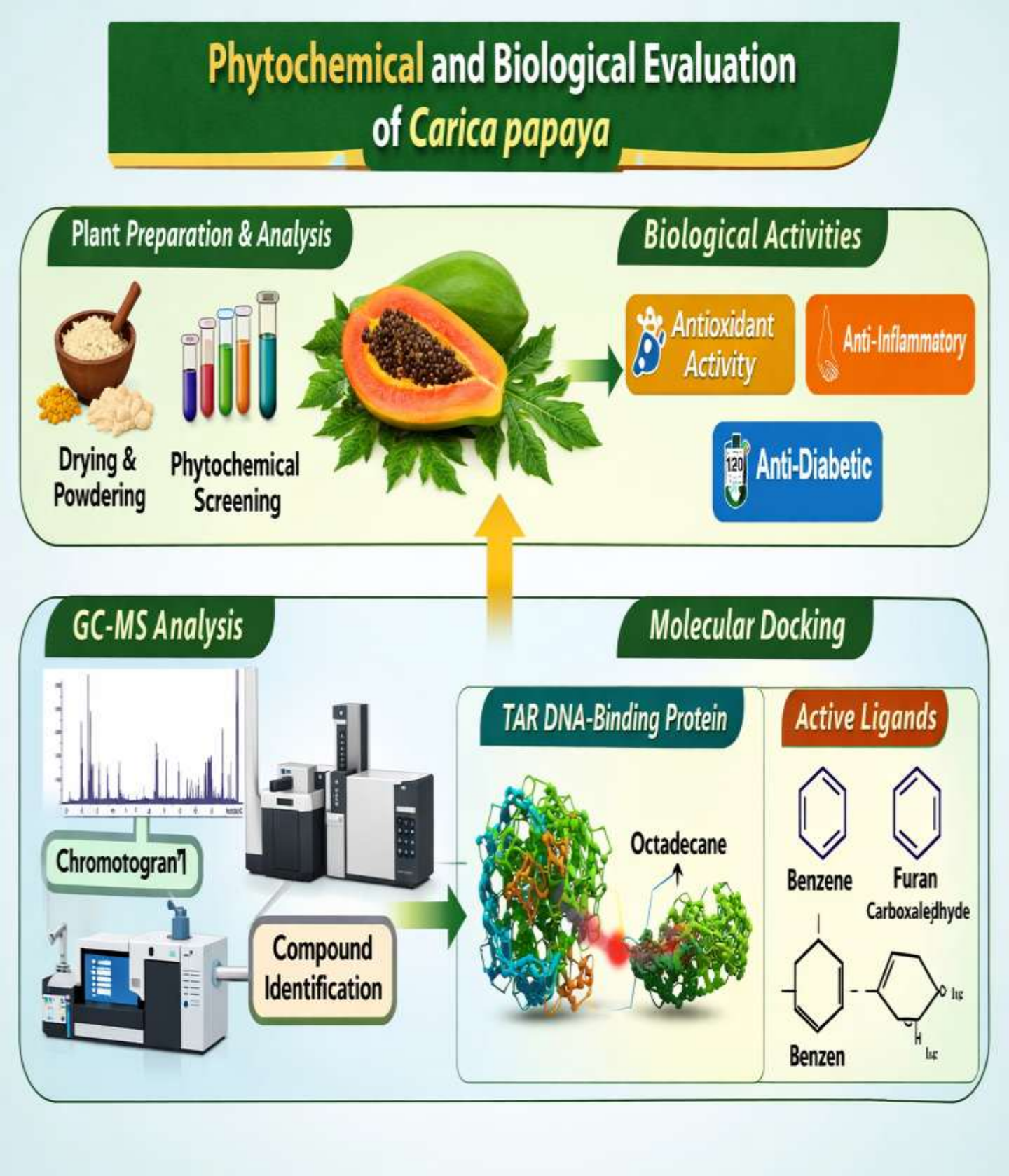

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INTRODUCTION

Since antiquity, humanity has relied on plants as primary therapeutic agents to combat infectious and chronic diseases. Long before the emergence of synthetic pharmaceuticals, medicinal flora formed the backbone of healthcare systems in ancient civilizations such as China, Greece, Egypt, and Persia, where plant extracts were systematically used to treat infections and inflammatory disorders (1,2). The transition to synthetic drug development following the Industrial Revolution revolutionized medicine, enabling the large-scale production of antibiotics, antivirals, chemoprotective agents, and other pharmacological therapies (3). However, despite their transformative impact, the widespread and often indiscriminate use of synthetic medications has contributed to escalating antimicrobial resistance and concerns regarding adverse effects. The progressive evolution of resistant bacterial and viral strains has renewed global scientific interest in plant-derived bioactive compounds as potential therapeutic alternatives or adjuncts (4). Medicinal plants are characterized by their diverse repertoire of secondary metabolites, including phenolics, flavonoids, alkaloids, steroids, and glycosides, which serve ecological defense roles in plants and exhibit pharmacological activities in humans (5,6). These phytochemicals contribute to antioxidant, anti-inflammatory, antimicrobial, and anticancer effects, and may act either independently or synergistically with conventional drugs (7). Such synergism has been proposed as a strategy to enhance therapeutic efficacy while potentially mitigating drug resistance and toxicity. In this context, plant-based therapies are increasingly viewed not as replacements but as complementary interventions integrated within evidence-based medicine. Among the medicinally significant plants, *Carica papaya* L., a member of the Caricaceae family, has attracted considerable attention. Commonly cultivated in tropical and subtropical regions, papaya is valued both as a nutritional fruit and as a therapeutic resource (8). The plant is characterized by a soft, hollow stem, large lobed leaves, and fleshy fruits containing numerous bioactive seeds. Beyond its dietary value, papaya contains proteolytic enzymes such as papain and chymopapain, as well as bioactive constituents including carpaines, benzyl isothiocyanate (BITC), benzyl glucosinolates, lycopene, lutein, and zeaxanthin (9). These compounds are distributed across various plant parts—fruit, peel, seeds, leaves, and latex—each contributing distinct pharmacological properties. Emerging evidence suggests that *C. papaya* exhibits a wide spectrum of biological activities. Its antioxidant potential is attributed to phenolic and flavonoid constituents capable of scavenging free radicals and reducing oxidative stress, a key contributor to chronic diseases such as cardiovascular disorders, neurodegeneration, cancer, and diabetes (10,11).

In vitro and in vivo studies demonstrate significant radical scavenging activity, particularly in seed extracts, underscoring the therapeutic potential of plant by-products that are often discarded (12). Similarly, papaya-derived compounds have demonstrated anti-inflammatory effects in experimental models, including carrageenan-induced paw edema and formaldehyde-induced arthritis, supporting its traditional use in inflammatory conditions (13,14). Given the central role of chronic inflammation in metabolic syndrome, atherosclerosis, and malignancy, these findings warrant systematic exploration. Furthermore, papaya has been investigated for antidiabetic properties. Experimental studies in streptozotocin-induced diabetic models reveal significant reductions in serum glucose and lipid parameters following administration of papaya leaf extracts, suggesting modulation of metabolic pathways linked to insulin resistance (15,16). The fruit's fiber content and antioxidant profile may further contribute to glycemic control and lipid regulation, factors integral to the prevention of metabolic syndrome and cardiovascular complications (17). In addition, anticancer potential has been proposed, with lycopene implicated in modulation of cell-cycle progression and oxidative mechanisms, while papain has been explored for its proteolytic and possible tumor-suppressive properties (18,19). Despite extensive traditional use and a growing body of experimental research, the therapeutic claims surrounding *C. papaya* remain heterogeneous, with variability in extraction methods, plant parts studied, and experimental models employed. Moreover, while antioxidant and anti-inflammatory properties are frequently cited, the mechanistic pathways linking these activities to clinical outcomes are not fully delineated. There remains a need for an integrated, evidence-based synthesis that evaluates the pharmacological potential of *C. papaya* within the broader context of oxidative stress, inflammation, metabolic disorders, and malignancy. Given the rising burden of chronic diseases driven by oxidative stress, inflammation, and metabolic dysregulation, and the increasing resistance to conventional pharmacotherapies, it is imperative to systematically evaluate plant-based alternatives with established ethnomedicinal relevance. Therefore, the objective of the present study is to critically examine the phytochemical composition and biological activities of *Carica papaya*, with particular emphasis on its antioxidant, anti-inflammatory, antidiabetic, and anticancer properties, in order to assess its therapeutic potential and identify gaps for future translational research.

METHODS

An experimental in-vitro laboratory-based study was conducted to evaluate the phytochemical composition and selected biological activities of *Carica papaya* fruit extracts, alongside protein profiling and molecular docking analyses. The study was carried out in a

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controlled laboratory setting following standard biosafety and chemical handling protocols. As the investigation involved plant materials and in-vitro assays without human or animal participants, formal ethical approval and informed consent were not applicable; however, the study adhered to institutional laboratory safety and research integrity guidelines. Analytical-grade chemicals were used throughout the experiments. The reagents included 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), ascorbic acid, distilled water, bovine serum albumin (BSA), diclofenac sodium, metformin, α-amylase enzyme, sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), phosphate buffer solution, ethanol, n-hexane, and chloroform. All reagents were procured from certified suppliers and used without further purification. The instrumentation employed included a UV–visible spectrophotometer for absorbance measurements, an ELISA reader for microplate-based assays, a rotary evaporator for solvent removal under reduced pressure, a hot air oven for controlled drying, a gas chromatography–mass spectrometry (GC–MS) system for compound profiling, and a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) apparatus for protein separation. Fresh *Carica papaya* fruits were procured from the local market, visually inspected to exclude damaged or spoiled specimens, and transported to the laboratory under hygienic conditions. Botanical identification was confirmed based on morphological characteristics; however, no herbarium voucher number was documented. The fruits were washed thoroughly with distilled water to remove surface contaminants. Edible portions were separated, sliced into small pieces, and air-dried at room temperature under shade to prevent degradation of heat-sensitive phytoconstituents. The dried material was pulverized into a fine powder using liquid nitrogen to minimize enzymatic degradation and oxidative loss of bioactive compounds. The powdered samples were stored in airtight containers at low temperature until extraction.

For extract preparation, 25 g of the dried powder was macerated separately in 250 mL of ethanol, chloroform, n-hexane, and distilled water, representing solvents of varying polarity to ensure comprehensive extraction of phytochemicals. The mixtures were kept at room temperature for ten days with intermittent shaking to enhance solvent penetration and extraction efficiency. After maceration, the extracts were filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure at controlled temperature. The concentrated extracts were further dried to constant weight and stored in amber-colored containers at 4°C until further analysis. Preliminary phytochemical screening was conducted using standard qualitative procedures to detect the presence of proteins, alkaloids, flavonoids, and saponins. Established colorimetric and precipitation-based methods were applied for each class of compounds, and observations were recorded based on characteristic color changes or precipitate formation. Although qualitative analysis provided insight into phytochemical classes, quantitative estimation was not performed in the present design. The antioxidant activity of the extracts was evaluated using the DPPH free radical scavenging assay. Briefly, different concentrations of each extract were mixed with DPPH solution and incubated in the dark to prevent photodegradation. Absorbance was measured at 517 nm using a UV–visible spectrophotometer. Ascorbic acid served as the reference standard. Percentage inhibition of DPPH radicals was calculated using standard formulae, and IC_{50} values were determined where applicable. Anti-inflammatory activity was assessed using the bovine serum albumin (BSA) denaturation assay. Reaction mixtures containing BSA and varying concentrations of extracts were incubated under controlled conditions, followed by heat-induced denaturation. Absorbance was measured spectrophotometrically, and percentage inhibition of protein denaturation was calculated relative to diclofenac sodium as the standard anti-inflammatory drug.

Anti-diabetic activity was evaluated through the α-amylase inhibition assay. Extracts at different concentrations were incubated with α-amylase enzyme and substrate under buffered conditions. The reaction was terminated using NaOH where required, and absorbance was recorded. Metformin was used as a reference control. Percentage inhibition of α-amylase activity was calculated to estimate the extracts' potential to modulate carbohydrate metabolism. Protein profiling of the papaya extracts was performed using standard SDS-PAGE techniques. Samples were prepared with loading buffer, denatured, and subjected to electrophoretic separation on polyacrylamide gels under reducing conditions. Protein bands were visualized and documented to assess molecular weight distribution patterns. For in-silico analysis, molecular docking studies were conducted to explore potential interactions between selected phytochemicals and target proteins relevant to oxidative stress, inflammation, or diabetes. Three-dimensional structures of target proteins were retrieved from the Protein Data Bank (PDB), while ligand structures were obtained from the PubChem database. Docking simulations were performed using AutoDock Vina, and binding affinities were expressed in kcal/mol. Visualization and interaction analysis were carried out using appropriate molecular graphics software. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using suitable software (e.g., SPSS or GraphPad Prism, if applicable). One-way analysis of variance (ANOVA) followed by post-hoc testing was applied to determine statistical significance among groups, with a p-value <0.05 considered statistically significant.

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RESULTS

Qualitative phytochemical screening of *Carica papaya* extracts demonstrated variable distribution of secondary metabolites across solvents of different polarity. Alkaloids were detected in all extracts, including ethanol, n-hexane, chloroform, and distilled water. Proteins were identified in ethanol, n-hexane, and chloroform extracts but were absent in the aqueous extract. Flavonoids were present exclusively in the n-hexane and chloroform extracts, while ethanol and aqueous extracts tested negative. Saponins were observed in ethanol, n-hexane, and chloroform extracts but were absent in the distilled water extract. These findings indicated that semi-polar and non-polar solvents extracted a broader spectrum of phytoconstituents compared to the aqueous fraction. Gas chromatography–mass spectrometry (GC–MS) analysis of the ethanol extract identified 26 bioactive compounds with varying retention times and relative peak areas. The most abundant compound was 10-octadecenoic acid, methyl ester (28.47%, retention time 14.825 min), followed by 5-hydroxymethylfurfural (25.35%, 7.946 min). Other prominent constituents included n-hexadecanoic acid (4.95%, 13.589 min), 4H-pyran-4-one derivative (4.57%, 7.032 min), D-erythro-pentose (4.40%, 10.781 min), 9-octadecenoic acid (4.27%, 17.825 min), and octadecanoic acid (4.26%, 14.910 min). Minor components such as erythritol (0.25%), 2-furancarboxaldehyde (0.26%), and ethyl 2,3-epoxybutyrate (0.02%) were also detected. The chromatographic profile confirmed the presence of fatty acids, esters, aldehydes, alcohols, and phenolic derivatives. The antioxidant activity assessed using the DPPH radical scavenging assay demonstrated concentration-dependent inhibition across all extracts. At 0.5 mg/mL, chloroform extract showed 94.07% inhibition, followed by ethanol extract at 93.62%, aqueous extract at 88.30%, and n-hexane extract at 83.51%, whereas ascorbic acid exhibited 97.43% inhibition. At the lowest tested concentration (0.03125 mg/mL), ethanol extract retained 85.42% inhibition, chloroform 83.94%, aqueous 73.21%, and n-hexane 72.19%, while ascorbic acid showed 87.63%. The percentage inhibition decreased progressively with decreasing concentration in all groups.

Anti-inflammatory activity evaluated through the protein denaturation assay also demonstrated concentration-dependent inhibition. At 0.5 mg/mL, ethanol extract showed 42.91% inhibition, chloroform extract 37.63%, n-hexane extract 35.86%, and aqueous extract 27.82%, whereas diclofenac (standard) demonstrated 80.47% inhibition. At 0.03125 mg/mL, inhibition decreased to 29.75% (ethanol), 22.59% (chloroform), 21.59% (n-hexane), and 16.25% (aqueous), compared to 68.29% for diclofenac. The ethanol fraction consistently exhibited the highest anti-inflammatory activity among plant extracts across all concentrations tested. The anti-diabetic activity assessed by α-amylase inhibition revealed substantial inhibitory potential in all extracts. At 0.5 mg/mL, ethanol extract demonstrated 89.47% inhibition, n-hexane extract 87.50%, chloroform extract 84.82%, and aqueous extract 78.64%, while metformin (standard) showed 93.87% inhibition. At the lowest concentration (0.03125 mg/mL), ethanol extract maintained 79.59% inhibition, n-hexane 79.21%, chloroform 74.42%, and aqueous 63.47%, compared to 82.61% for metformin. A dose-dependent reduction in inhibitory activity was observed across all fractions. Protein profiling using SDS-PAGE revealed multiple protein bands in each extract, indicating the presence of diverse protein constituents with varying molecular weights. Distinct band patterns were observed across fractions, suggesting differences in protein composition based on solvent polarity. In silico docking analysis demonstrated binding interactions between identified ligands and the target TAR DNA-binding protein. Octadecane exhibited a best binding affinity of −5.0 kcal/mol in the top-ranked pose, with RMSD lower and upper bounds of 0.000 Å. Other docking modes ranged from −4.9 to −4.7 kcal/mol. The ligand 5-(4-nitrophenyl)-2-furaldehyde showed stronger interaction, with a highest binding affinity of −6.0 kcal/mol in the primary docking mode and subsequent modes ranging from −5.9 to −5.1 kcal/mol. RMSD values varied across conformations, indicating different spatial orientations within the binding site.

Table 1: Phytochemical screening of C. papaya

Tests	**Plant extracts**			
	Ethanol	n-Hexane	Chloroform	Distilled water
Proteins	+	+	+	-
Alkaloids	+	+	+	+
Flavonoids	-	+	+	-
Saponins	**+**	**+**	**+**	**-**

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Table 2: Ethanol extract identified compounds by GCMS

Sr. No.	Compound Name	Molecular Weight (g/mol)	% Area	Molecular Formula	Retention Time (min)
1	2-Furancarboxaldehyde	96.08	0.26	$C_5H_4O_2$	4.914
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)	144.12	0.52	$C_6H_8O_4$	5.096
3	1,3-Cyclohexanedione	112.13	1.54	$C_6H_8O_2$	5.363
4	Propanamide, N-acetyl-	115.13	1.51	$C_5H_9NO_2$	6.251
5	L-Alanine, N-acetyl	207.23	0.68	$C_{11}H_{13}NO_3$	6.460
6	Erythritol	122.12	0.25	$C_4H_{10}O_4$	6.524
7	1,2,3,4-Butanetetrol	122.12	1.77	$C_4H_{10}O_4$	6.775
8	4H-Pyran-4-one, 2,3-dihydro-3,5-…	144.12	4.57	$C_6H_8O_4$	7.032
9	5-Hydroxymethyl furfural	126.11	25.35	$C_6H_6O_3$	7.946
10	Thiocyanic acid, phenylmethyl ester	149.21	0.94	C_8H_7NS	8.995
11	Butylated Hydroxytoluene (BHT)	220.35	2.59	$C_{15}H_{24}O$	10.172
12	2-Isopropoxyethyl propionate	160.21	3.48	$C_8H_{16}O_3$	10.573
13	D-erythro-pentose, 2-deoxy-	134.13	4.40	$C_5H_{10}O_4$	10.781
14	Butanoic acid, 2-oxo-	102.09	1.98	$C_4H_6O_3$	10.856
15	d-Mannitol, 1,4-anhydro-	164.16	0.39	$C_6H_{12}O_5$	12.129
16	Ethyl 2,3-epoxybutyrate	130.14	0.02	$C_6H_{10}O_3$	12.391
17	Pentadecanoic acid, 14-methyl-	270.41	0.51	$C_{16}H_{30}O_3$	13.317
18	n-Hexadecanoic acid	256.42	4.95	$C_{16}H_{32}O_2$	13.589
19	10-Octadecenoic acid, methyl ester	296.50	28.47	$C_{19}H_{36}O_2$	14.825
20	Octadecanoic acid	284.50	4.26	$C_{18}H_{36}O_2$	14.910
21	Eicosanoic acid	312.50	0.55	$C_{20}H_{40}O_2$	16.034
22	Hexadecanoic acid, 2-hydroxy-1-(…)	516.80	2.17	$C_{31}H_{52}N_2O_4$	16.873
23	Bis(2-ethylhexyl) phthalate	390.60	0.70	$C_{24}H_{38}O_4$	16.975
24	9-Octadecenoic acid	282.50	4.27	$C_{18}H_{34}O_2$	17.825
25	Octadecanoic acid, 2-hydroxy-1-(…)	371.60	1.32	$C_{22}H_{45}NO_3$	17.932
26	9-Octadecenamide	281.50	0.42	$C_{18}H_{35}NO$	18.232

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Table 3: Anti-oxidant activity of C. papaya

Samples	**% Inhibition Anti-oxidant activity**				
	Concentration mg/mL				
	0.5 mg/ml	0.25 mg/ml	0.125 mg/ml	0.0625 mg/ml	0.03125 mg/ml
Ethanol	93.62	91.97	88.73	87.63	85.42
Aqueous	88.3	84.62	81.95	78.34	73.21
Chloroform	94.07	91.52	89.63	86.23	83.94
n-hexane	83.51	80.41	78.67	74.57	72.19
Ascorbic acid	**97.43**	**95.41**	**92.29**	**89.67**	**87.63**

Table 4: Anti-inflammatory activity of C. papaya

Sample/Extract	**0.5 mg/mL**	**0.25 mg/mL**	**0.125 mg/mL**	**0.0625 mg/mL**	**0.03125 mg/mL**
Ethanol Extract	42.91	39.87	34.57	31.64	29.75
Aqueous Extract	27.82	25.84	22.67	19.68	16.25
Chloroform Extract	37.63	32.17	30.89	27.41	22.59
n-Hexane Extract	35.86	31.85	28.19	24.69	21.59
Dicloran (Standard)	80.47	76.63	72.54	70.48	68.29

Table 5: Anti-diabetic inhibition (%) of C. papaya extracts compared with Metformin

Sample	**% Inhibition Anti-diabetic activity**				
	Concentration mg/Ml				
	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml	0.03125mg/ml
Ethanol	89.47	86.28	84.86	81.49	79.59
Aquous	78.64	74.57	71.94	69.86	63.47
Chloroform	84.82	83.14	81.57	79.13	74.42
n-hexane	87.5	85.91	83.62	81.85	79.21
Metformin	**93.87**	**90.19**	**89.48**	**85.63**	**82.61**

Table 6: Molecular Docking Interaction of TAR DNA-Binding Protein with Ligand

Mode	**Binding Affinity (kcal/mol)**	**RMSD Lower Bound (Å)**	**RMSD Upper Bound (Å)**
1	-5.0	0.000	0.000
2	-4.9	2.257	9.037
3	-4.9	0.848	7.018

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Mode	Binding Affinity (kcal/mol)	RMSD Lower Bound (Å)	RMSD Upper Bound (Å)
4	-4.9	1.360	7.654
5	-4.8	0.941	2.182
6	-4.8	1.711	3.171
7	-4.7	1.990	5.049
8	-4.7	1.948	5.814
9	-4.7	1.619	8.163

Table 7: Binding Energies of TAR DNA-Binding Protein with Ligand

Mode	Binding Affinity (kcal/mol)	RMSD Lower Bound (Å)	RMSD Upper Bound (Å)
1	-6.0	0.000	0.000
2	-5.9	2.667	3.223
3	-5.8	3.519	7.082
4	-5.8	2.638	6.494
5	-5.5	1.900	2.632
6	-5.5	3.156	6.918
7	-5.4	20.864	22.311
8	-5.1	21.414	23.585
9	-5.1	2.039	2.950

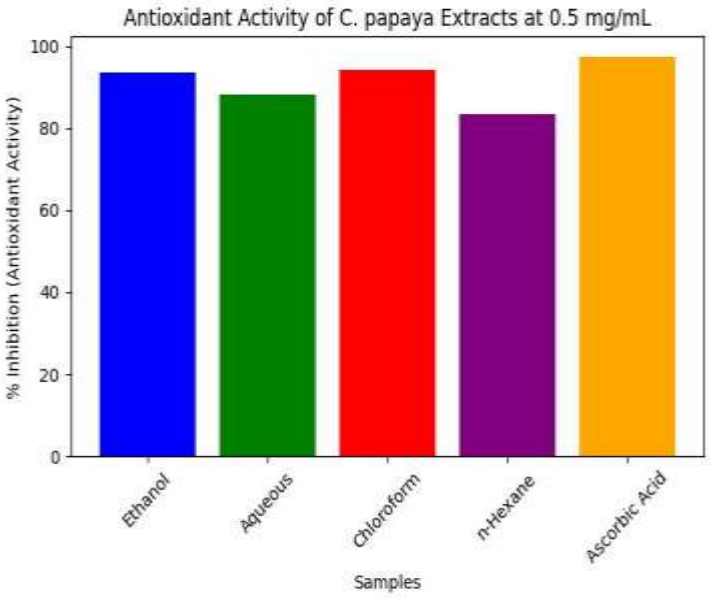


Figure 1 Antioxidant Activity of C. Papaya Extracts at 0.5mg/mL

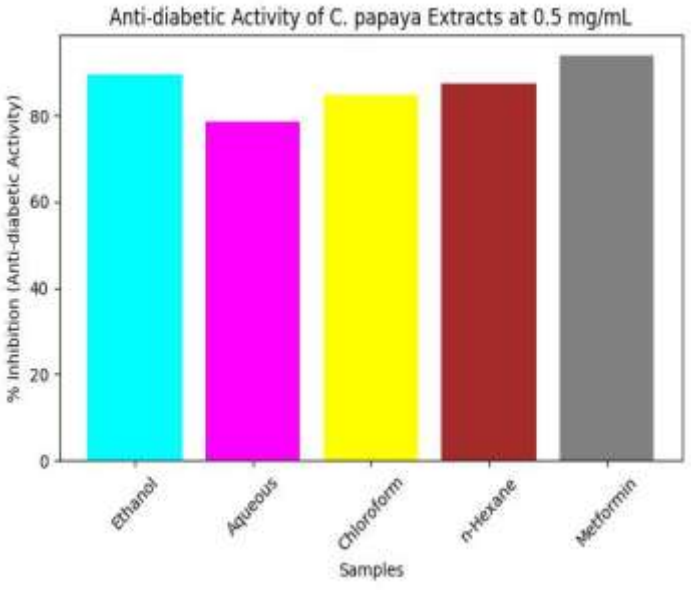
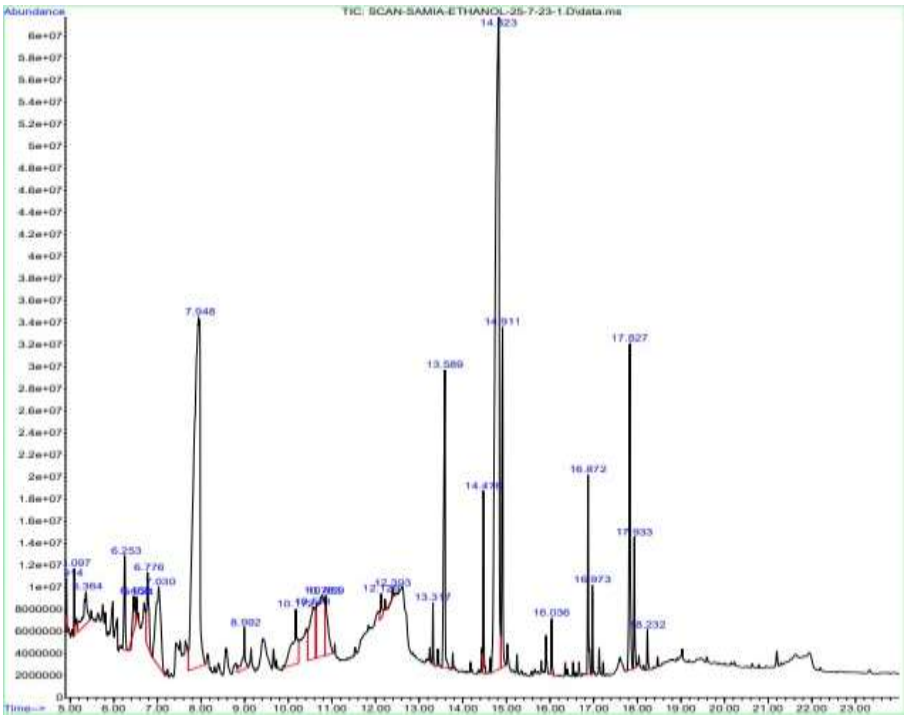
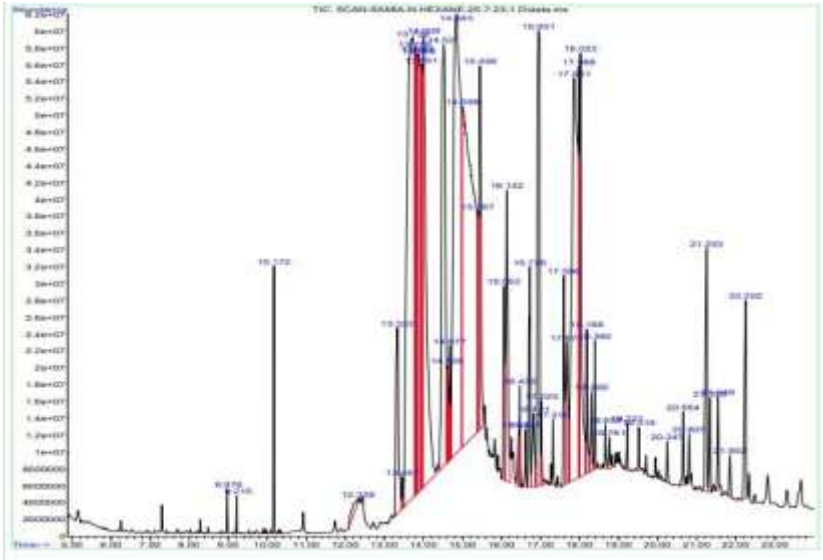


Figure 1 Anti-diabetic Activity of C. Papaya Extracts at 0.5 mg/mL



Chromatogram of ethanol extract of C. papaya

Figure 3 Chromatogram of Ethanol Extract of C. Papaya



Chromatogram of n-hexane extract of C. papaya

Figure 4 Chromatogram of n-hexane Extract of C. Papaya

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DISCUSSION

The present investigation demonstrated that *Carica papaya* extracts possessed notable antioxidant, anti-inflammatory, and anti-diabetic activities, with variations observed according to solvent polarity and concentration. These findings aligned with the established understanding that papaya is a nutritionally dense tropical fruit enriched with vitamins A, C, and E, B-complex nutrients, essential minerals, dietary fiber, and a wide range of phytochemicals (15). The detection of flavonoids, alkaloids, saponins, and proteins across different solvent fractions supported the biochemical complexity of the fruit and suggested that solvent selection played a decisive role in phytochemical recovery. The strong DPPH radical scavenging activity observed particularly in chloroform and ethanol extracts indicated substantial antioxidant potential. This outcome corresponded with previous reports that papaya fruit and seeds contain phenolic compounds, carotenoids, tocopherols, and benzyl isothiocyanate, all of which contribute to free radical neutralization and oxidative stress mitigation (16). Antioxidant activity is clinically relevant, as oxidative stress is implicated in the pathogenesis of cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, and malignancies. The high inhibition percentages recorded even at lower concentrations suggested that papaya extracts may exert protective cellular effects under oxidative conditions. However, the absence of calculated IC_{50} values limited precise quantitative comparison with standard antioxidants and other published data. The anti-inflammatory activity demonstrated moderate inhibition of protein denaturation, with ethanol extract showing comparatively higher activity among the tested fractions. Chronic inflammation is a recognized contributor to metabolic syndrome, cardiovascular disorders, and carcinogenesis. The anti-inflammatory effect observed in this study may be attributable to the presence of flavonoids, saponins, and proteolytic enzymes such as papain and chymopapain. Previous investigations have highlighted the therapeutic relevance of papaya-derived cysteine proteases in modulating inflammatory pathways and promoting tissue repair (17-19). Nevertheless, the magnitude of inhibition remained lower than that of the standard drug, indicating that while papaya extracts exhibited biological activity, their potency may be moderate when compared with conventional anti-inflammatory agents.

Similarly, the α-amylase inhibitory activity supported the potential anti-diabetic role of *C. papaya*. The dose-dependent inhibition pattern observed across all extracts was consistent with earlier experimental findings suggesting that plant-derived phytochemicals can modulate carbohydrate metabolism and improve glycemic control. The presence of bioactive compounds such as phenolics and glucosinolates may contribute to enzyme inhibition and improved metabolic regulation. Given the increasing global burden of diabetes mellitus and the adverse effects associated with long-term pharmacotherapy, plant-based alternatives continue to attract attention as adjunctive strategies. However, in-vitro enzyme inhibition does not necessarily translate into clinical glycemic control, and further in-vivo and clinical validation remains essential. The GC–MS analysis revealed the presence of fatty acids, esters, aldehydes, and phenolic derivatives, including 10-octadecenoic acid methyl ester and 5-hydroxymethylfurfural as predominant constituents. Fatty acids and their derivatives have been associated with anti-inflammatory and antioxidant properties, potentially contributing to the observed bioactivities. The molecular docking results demonstrated moderate binding affinities between selected compounds and the target protein, indicating possible biochemical interactions at the molecular level. Although docking studies provided supportive mechanistic insights, binding affinity values in the reported range suggested modest interaction strength, warranting cautious interpretation. Computational predictions, while informative, require biological validation through enzyme kinetics or cellular assays to confirm functional relevance. The nutritional profile of papaya further strengthened its position as a functional food. The presence of proteases such as papain and chymopapain, which constitute a significant proportion of the fruit's protein content, underscored its digestive and therapeutic potential (20,21). Papain's proteolytic, amylolytic, and mild lipolytic activities explained its longstanding use in digestive disorders and food processing industries. In addition, the reported hepatoprotective effects—evidenced in previous literature by reductions in ALT, AST, and ALP levels and enhancement of endogenous antioxidant enzymes—supported its broader systemic benefits.

Several strengths characterized the present study. The use of multiple solvents of varying polarity allowed comprehensive phytochemical extraction. The assessment of three distinct biological activities provided a multidimensional evaluation of therapeutic potential. Integration of in-vitro assays with GC–MS profiling and molecular docking added mechanistic depth and strengthened the analytical framework. However, important limitations were evident. The study was restricted to in-vitro assays, limiting translational inference. Quantitative phytochemical estimation was not performed, and IC_{50} values were not calculated, reducing comparative precision. Statistical parameters, including p-values and confidence intervals, were not detailed in the reported findings, limiting assessment of reproducibility and significance. Additionally, the specific target protein selected for docking was not clearly justified in relation to the biological assays performed, which may affect mechanistic coherence. The absence of herbarium authentication details and standardized extraction yields may also influence reproducibility. Future research should focus on quantitative phytochemical analysis, determination of IC_{50} values, and inclusion of robust statistical modeling. In-vivo experimental studies and controlled clinical trials are required to

confirm therapeutic efficacy and safety. Mechanistic studies exploring molecular signaling pathways, enzyme kinetics, and gene expression modulation would further elucidate the pharmacological basis of observed effects. Standardization of extract preparation and dose optimization would enhance translational applicability (22). Overall, the findings reinforced the concept that *Carica papaya* is not merely a nutritional fruit but a bioactive reservoir with potential antioxidant, anti-inflammatory, and anti-diabetic properties. While the results were promising, they remained preliminary and should be interpreted within the context of methodological constraints. Continued systematic investigation is warranted to clarify its role within evidence-based complementary medicine.

CONCLUSION

The present study demonstrated that *Carica papaya* extracts obtained using different solvents possess significant biological potential, particularly in terms of antioxidant, anti-inflammatory, and anti-diabetic activities. Phytochemical screening and chromatographic profiling confirmed the presence of bioactive constituents, including phenolic compounds, alkaloids, and flavonoids, which likely contributed to the observed pharmacological effects. Among the tested fractions, the ethanol extract consistently exhibited superior biological activity, highlighting the importance of solvent selection in maximizing therapeutic yield. These findings support the growing recognition of *C. papaya* as a promising natural source of bioactive compounds with potential applications in the development of novel adjunct therapies for metabolic and inflammatory disorders. While the results provide encouraging preliminary evidence, further in-depth experimental and clinical investigations are essential to validate efficacy, explore antibacterial potential, and establish its role in managing broader conditions such as respiratory inflammation.

AUTHOR CONTRIBUTIONS

Author	Contribution
Samia Rubab Ashraf*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Fatima Mushtaq	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Abbas Shahid	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Rabia Khaliq	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Aanifa Firdous	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Haseeb Anwar Tarar	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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