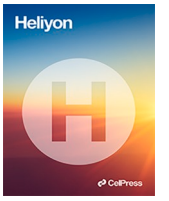
Heliyon 10 (2024) e39635

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Prospects of *Carica papaya* in the treatment of human viral infections: A comprehensive and a systematic review

Masooma [a], Ariba Qaiser [a], Dr Sajid Ali [b], Dr Sobia Manzoor [a,*]

[a] *Atta-ur-Rehman School of Applied Biosciences, National University of Sciences and Technology H-12 Islamabad, Islamabad Capital Territory, Pakistan*

[b] *Department of Biotechnology Abdul Wali Khan University Mardan, Pakistan*

A R T I C L E I N F O

Keywords:
Carica papaya extract
Leaves
Fruits
Viruses
Antiviral agent
Virus titer
Anti dengue

A B S T R A C T

Background: Viruses cause various human diseases, some of which become pandemic outbreaks lack effective treatments and vaccines. This study gathered data on Carica papaya's antiviral effects against various human viruses, highlighting its potential as a natural treatment. Through *in-vitro*, *in-vivo*, and clinical study analysis, we illustrated the potential of Carica papaya in combating viral infections and its potential role in cure of common viral diseases.

Method: ology: Research papers on antiviral activity of Carica papaya were identified by using specific keywords in PUBMED, Google Scholar, and ScienceDirect databases. Articles published between January 2015 and March 2024 were screened for inclusion. Eligible studies utilized Carica papaya leaves (powder or juice) or fruits to investigate their effects against human viral infections. Each selected study applied either qualitative or quantitative antiviral assays to assess the efficacy of Carica papaya in combating human viruses.

Results: Fifteen studies went through assessment for antiviral properties of Carica papaya, 7 (46.67 %) of them displayed significant activity against the dengue virus, while 1 study (6.67 %) demonstrated moderate/less effectiveness against dengue serotype-2. Two studies (13.33 %) found no anti-dengue effects. Additionally, Carica papaya exhibited strong antiviral activity against SARS-CoV-2 in 2 studies (13.33 %). One study (6.67 %) showed inhibition of both dengue serotype-2 and chikungunya, and 1 study (6.67 %) each demonstrated inhibitory effects against human immunodeficiency virus and Zika virus infections.

Conclusion: The present study listed human diseases for which Carica papaya revealed significant antiviral effects against Dengue virus, Severe acute respiratory syndrome, Human immunodeficiency virus-I, Chikungunya virus and Zika virus suggesting its potential as a treatment candidate for all these viruses. However, the majority of the research involved *in-vitro* screening, with minimal *in-vivo* and clinical testing. Further *in-vivo* studies and clinical trials should be conducted to explore the potential of Carica papaya in the treatment of human viral infections.

* Corresponding author.
E-mail addresses: raziimasoooma@gmail.com, masoomarazi6@gmail.com (Masooma), qaiser.ariba.aq@gmail.com (A. Qaiser), dr.sobiamanzoor@asab.nust.edu.pk (D.S. Manzoor).

https://doi.org/10.1016/j.heliyon.2024.e39635
Received 6 July 2024; Received in revised form 16 October 2024; Accepted 18 October 2024
Available online 29 October 2024

1. Introduction

Carica papaya Linn, commonly known as Pawpaw or Papaya, belongs to the Caricaceae family and is renowned for its nutritious qualities [1](2). Papaya is rich in essential nutrients, including vitamins C, A, and E, also vital minerals like; magnesium (Mg) and potassium (K). Additionally, it contains enzymes like papain and chymopapain, which aid in digestion, accelerate wound healing, and diminish inflammation. Papaya also supports cardiovascular health, boosts immune system, and possesses medicinal properties for treating hypertension, digestive disorders, and skin ailments. Various components of Carica papaya (CP), including its leaves, fruits, and bark, are often utilized for the treatment of viral diseases [2]. Ripe papaya fruits serves as a nutrient reservoir, rich in essential vitamins, they further include essential minerals like iron (Fe), Mg and K, manganese (Mn) and zinc (Zn) [3]. Moreover, papain is also recognized for its efficacy in addressing digestive and gastrointestinal diseases [1], (4).

CP contains specialized cells called laticifers, which are present throughout the plant and contribute to its pharmacological significance along with its active components [5]. Each part of the plant contributes to its therapeutic value, showing promising effects in areas such as anti-inflammatory [6], antibacterial and antifungal [7](2). It is also beneficial in fertility regulation [6], liver protection, wound healing, blood pressure regulation, tumor growth inhibition [5], dysentery and chronic diarrhea [7](2). Papaintha, an enzyme found in papaya, is renowned for its therapeutic effects on allergies and sports injuries [5], assisting in digestion [5](2), improve heart health, regulate cholesterol levels, and alleviate abdominal disorders [5]. Numerous pharmacological activities have been associated with CP, encompassing anti-microbial and anti-fungal properties to anti-thrombocytopenic, anti-viral, anti-gout, antihypertensive, analgesic, and hepato-protective activities [8], (9).

The treatment of viral illnesses experience significant challenges because viruses mutate quickly, become resistant to drugs, and effective antiviral medicines are not always available [10]. Overcoming these obstacles is crucial for developing new and effective ways to combat viral infections. Creating and distributing vaccines is typically a lengthy process, requiring extensive research, testing, and regulatory approval. Furthermore, viruses undergo mutations that can change their structure and characteristics, including the proteins targeted by vaccines. When significant genetic changes occur, the virus may become less recognizable to the immune system, reducing the effectiveness of vaccines in preventing infection [10]. Therefore, until both secure and efficient vaccines are developed for new viral illnesses like SARS (severe acute respiratory syndrome Coronavirus 2), MERS (Middle East respiratory syndrome), ZIKV (Zika virus), and Ebola virus, it is imperative to prioritize the development of effective treatments [10], (11). In cases where vaccines are unavailable, focusing on vaccine development remains crucial, though complex and time-consuming. Meanwhile, antiviral drugs serve as valuable treatment options, providing immediate relief and treatment to the infected individuals [10].

Plant extracts by working together often produce medicinal effects. Scientists are investigating they have antiviral properties, and some have shown potential in fighting viruses, offering hope for new treatments against viral infections [12]. Carica papaya extracts (CPE) have demonstrated efficacy against viruses like SARS-CoV-2 and Dengue (DENV) [13] and Human Immunodeficiency virus (HIV) [14], offering a cost-effective and less harmful alternative to synthetic drugs.

In the las few decades numerous studies including; lab studies, pre-clinical trials and clinical trials were reported on the effect of CPE. Therefore, we made the decision to create a systematic review for the purpose to investigate the antiviral properties of CP against different human viruses by analyzing previous researches for reliable evidences.

2. Methods

2.1. Search strategy

The literature search for this article was conducted using Google Scholar, ScienceDirect, and PubMed. In Google Scholar, the advanced search keywords were "Antiviral Carica papaya against viruses" with at least one word "leaves or fruits." ScienceDirect searches used "Antiviral Carica papaya" AND "leaves" AND "fruits" AND "Viruses" (with open access and archived articles). PubMed searches used "Antiviral activity of Papaya AND viruses." Searches in all databases were conducted from January to March 2024. Those articles aligning with the aim of this review were subjected for detailed screening.

2.2. Inclusion criteria

This review included research articles with titles containing "Carica papaya" and a human virus, focusing on in-vitro experiments, in-vivo studies, and clinical trials investigating CP's antiviral properties. Articles published between January 1, 2015 and March 30, 2024 were considered. Only original research papers with freely accessible full texts and abstracts were included. Selected studies had to use CP leaves (powder or juice) or fruits against human viruses and employ either qualitative or quantitative antiviral assays to assess efficacy of CP leaves or fruit against viruses.

2.3. Exclusion criteria

Our study excluded research articles that lacked both Carica papaya and specific human viruses in their titles. We omitted non-full-text papers, articles without free abstracts and free full texts, and those published before January 2015 after March 2024. Duplicate records and studies not using antiviral assays were also excluded. Additionally, case reports, books, observational, cross-sectional, longitudinal studies, simple reviews, systematic reviews, meta-analyses, and other irrelevant documents were also omitted.

2.4. Data extraction

Data from individual studies were extracted using a structured form to systematically collect relevant information from individual studies, designed by the authors (Masooma and Ariba). The extracted information included the experimental locations, specific CP components used, the active constituents within each CP extract, concentration levels, treatment stages (pre-, co-, or post-infection treatment), and the respective infection models. Additionally, extracted data included: IC_{50} or EC_{50} values of CP extracts against virus targets, the assay methods for evaluating viral titers after treatment, and the outcomes of each selected study.

3. Results

3.1. Study selection

The literature search determined the following number of articles from each database: PubMed (n = 24), Google Scholar (n = 3790), and Science Direct (n = 0). After excluding articles lacking CP and the name of human virus(es) in their title, duplicates, and those without abstracts, a total of 45 abstracts were screened. Abstracts of in silico studies and review articles were subsequently excluded, leaving 22 full-text articles for screening after applying exclusion criteria a total of 15 articles were included in this study. The diagram shown in Fig. 1, illustrating the schematic diagram of the search strategy for relevant articles. Among the 15 studies, 3 involved animal experiments (mice and rats), 1 was from human trials (adult patients aged 18 years or older), 1 study involved both cell lines and animal experiments, and 10 studies were based on cell lines only.

3.2. Antivirasl benefits of Carica papaya leaves and fruit

Out of the 15 studies reviewed; CP leaf powder extracts demonstrated antiviral activity in 11 studies. Notably, only 3 studies documented the efficacy of CP leaf juice (CPLJ) extracts. In addition, in only one study the pulp extract of CP fruit was proved to have antiviral activity against Zika strains, hindering the virus's ability to enter host cells.

The varied extracts derived from different constituents of CP, as summarized in Fig. 2, showcase their usage against various human

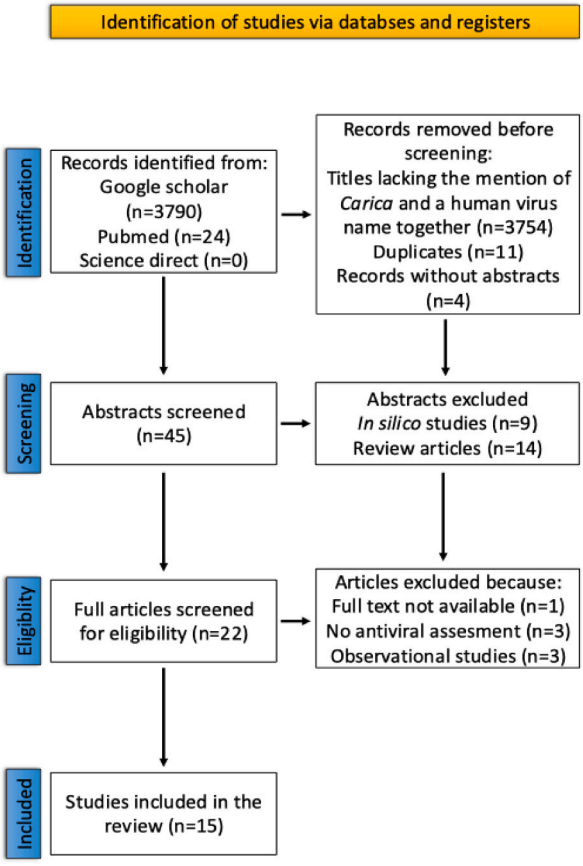


Fig. 1. Flow diagram following the Preferred Reporting Items for Systematic Reviews (PRISMA), illustrating the selection process for included studies.

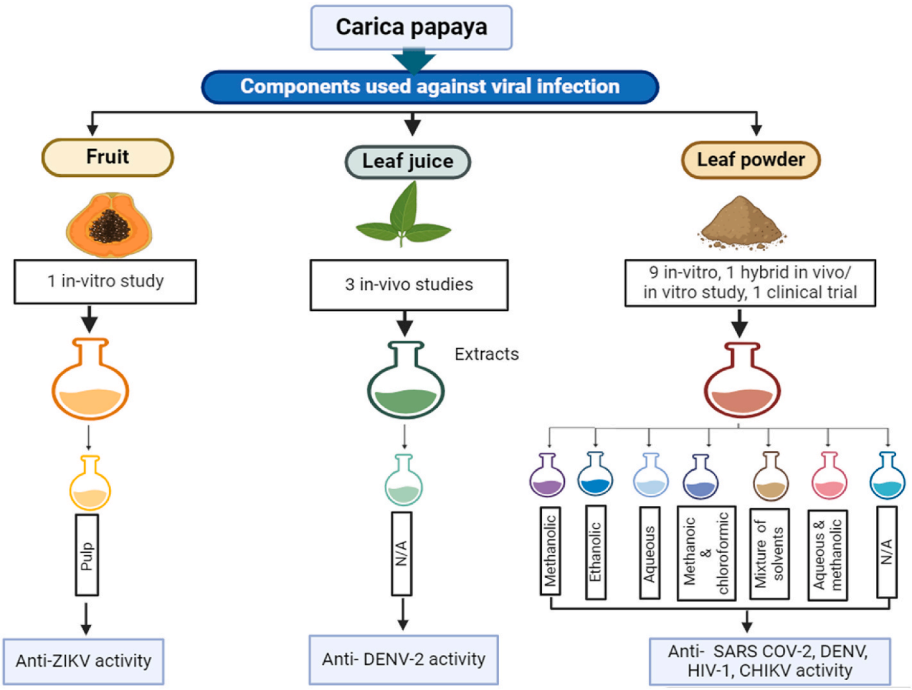


Fig. 2. Schematic flow diagram outlining diverse extracts derived from various components of Carica papaya utilized in combating human viruses.

viruses. The active antiviral chemical compounds effective against DENV identified are illustrated in Fig. 3. In 1 in-vitro study, the pulp extract of CP fruit effectively inhibited ZIKV infection by targeting the early stage, particularly attachment of the viral life cycle [15]. The CPLJ extracts were utilized in 3 in-vivo studies [16](17) [17]. However, the specific extracts used in these studies were not available (NA). Prominently, both the studies by Norahmad et al. and Mohd et al. did not exhibit a significant effect on DENV-2 [17] (16). In contrast, in another in-vivo study by Mohd et al. CPLJ extract exhibited promising inhibition of DENV-2 replication [18]. A

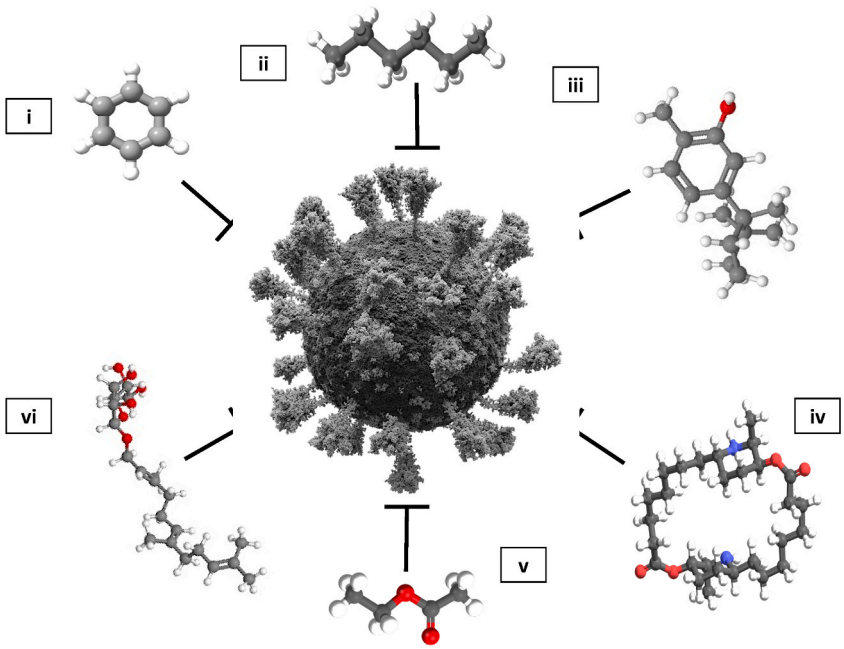


Fig. 3. Antiviral chemical compounds of Carica papaya. Papaya derived antiviral chemical compounds inhibit viral structural and non-structural proteins (not shown) i. n-Benzene, ii. N-Hexane, iii. Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-, iv. Carpaine, v. Ethyl acetate, vi. β-D-Mannofuranoside, farnesyl.

total of 9 in-vitro studies, 1 hybrid in-vivo/in-vitro study and 1 clinical trial utilized extracts from CP leaf powder. Among these, methanol was used as the solvent in 3 in-vitro studies [19](8) [14]. Ethanol was chosen in 2 other studies [20](21) demonstrating anti-SARS-CoV-2 and DENV-2 activity. Besides, an aqueous-based extraction approach was used in both a hybrid in-vivo/in-vitro study, which inhibited DENV-2, and an in-vitro study, which inhibited both DENV-2 and CHIKV [21](23). In 1 in-vitro study by Joseph et al. a combination of methanol and chloroform was utilized as extraction solvents, with only the chloroform extract showing moderate/less effectiveness against DENV-2 [22]. Thao et al. employed a mixture of organic solvents (n-hexane, ethyl acetate, and n-butanol) for extraction, where only n-hexane illustrated potent antiviral effects across all DENV serotypes (DENV 1–4) [23]. In 1 in-vitro study, a combination of methanol and aqueous-based extraction approach was employed, with methanol extract was the most efficient in preventing DENV-2 replication [24]. Moreover, in the clinical trial conducted by Sathyapalan et al. the exact extract was not available (NA). However, caripill tablets demonstrated inhibition of DENV replication by clearance of DENV non-structure protein-1 (NS1) [25].

Most of the studies, about 10/15 (66.66 %) focused on the effect of CPLE on DENV, particularly DENV-2. Two studies (13.33 %) investigated if these extracts could combat SARS-CoV-2. Furthermore, there was 1 (6.66 %) study each on DENV along with CHIKV, ZIKV, and HIV-1.

Tables 1–4 summarize the antiviral efficacy of CP against multiple human viruses. The highest efficacy was observed against DENV infections reported in 7/15 studies (46.67 %) [18](19) [26](22) [23](26) [25]. CPLE showed moderate/less effectiveness against DENV-2 in 1/15 study [22]. 2/15 studies (13.33 %) found no anti-DENV activity but noted improvements in overall health of infected models [16](18). 2/15 studies conducted on SARS CoV-2 showed potent activity of CP on SARS CoV-2 infection (13.333 %) [8](20). 1/15 in-vitro study showed inhibition of HIV replication (6.67 %) [14]. Additionally, 1/15 study (6.67 %) reported inhibition of both DENV-2 and CHIKV [27] and 1/15 study (6.67 %) showed that CP fruit pulp inhibited ZIKV infection [15].

4. Discussion

Over the time in viral resistance against antiviral drugs are increased and new strategies are required to be developed to combat this problem (3). Plant extracts can be particularly valuable because they contain bioactive compounds that can act on multiple stages of the viral replication cycle. This multi-target approach can be more effective in inhibiting viral infections and diminishing the risk of resistance development. Though synthetic drugs are effective yet, highly costly, cause significant side effects, and development of antiviral drug resistance highlight the need for alternative treatments (3). Herbal extracts offer a promising solution, with many plants demonstrating antiviral activities against several viruses: HIV, HSV (Herpes Simplex Virus), rabies virus, SARS, MERS, poxvirus, DENV, and influenza viruses [28]. There is a global need for the discovery of potential antiviral compounds sourced from medicinal plants and herbal extracts [29].

A large number of the studies in this review assess the ability of CP to combat the dengue virus. The mechanism of action of papaya on dengue was proposed by Sathyapalan et al., 2020.where a significant reduction in blood NS1 levels was observed when the patients were given CPLE by day five. NS1of dengue is essential for infection and replication in host cells. Notably, NS1 clearance was faster in the treatment group, suggesting an antiviral effect [25].(31). In DENV, NS1 is essential for the synthesis of viral RNA, particularly formation of the negative strand, and it plays a structural function in the assembly of the viral replication complex in the Endoplasmic Reticulum. Its interactions with other non-structural proteins, especially NS4B are vital for efficient viral RNA replication [30]. Besides, the NS2B-NS3 protease, which consists of two components, NS3 (a serine protease that performs the enzymatic functions necessary for viral replication) and NS2B (a cofactor that activates NS3) essential for dengue virus replication. This protease is a major target in the development of antiviral drugs because it is necessary for breaking down the viral polyprotein into individual proteins needed for the production of new virion. One flavonoid found in Carica papaya is quercetin, which showed promising inhibitory activity against the NS2B-NS3 protease, making it an efficient choice for the development of strong antiviral treatments [31], (32).

CPLE treatment notably increased platelet counts showed a significant antiviral effect particularly in primary dengue patients. The clinical trials of this antiviral candidate are ongoing, one of antiviral therapies we reviewed, the Caripill tablets made from Carica leaf powder exhibited efficacy for DENV treatment across the clinical outcomes accelerating NS1 clearance [25]. Similarly, in another study 250 patients received CPLE tablets showed a quicker increase in platelet counts compared to the control group (received only symptomatic and supportive treatment without CPLE. This suggests that CPLE may have a positive effect on platelet recovery in dengue patients [33].

Out of the 3 *in-vivo* studies incorporated in our review, 2 studies [16](18) reported that CPLJ had no significant impact on DENV plasma NS1 levels or viral RNA but reduced morbidity by providing relief in dengue-infected mice. Mohd et al. noted CPLJ's capacity to reduce DENV replication in the liver and its potential immunomodulatory effects [18]. In reverse, in another study CPLJ lowered the level of liver enzyme (Serum Glutamate Pyruvate Transaminase (SGPT)) and abruptly boosted platelet and white blood cell counts, 24 h post administration of CPLJ in the bodies of dengue patients, as these signs generally refer to a severe dengue infection. The patients suffering from dengue benefited by a speedy recovery and avoid hospital stays. Following studies should concentrate on understanding the complex processes behind these effects and confirming the safety and efficacy of CPLJ to be used as therapy for dengue fever. However, this study did not investigate the anti-dengue effect of CPLJ [34], (35).

Among the 10 *in-vitro* studies reviewed, 5 focused on DENV [19](21) [22](25) [24]. Amin et al. found that co-treatment with Carica papaya leaf (CPL) powder exhibited strong antiviral activity against DENV [19] while Agfata et al. explored the antiviral potential of various fractions from papaya leaf extract against DENV infection. Particularly, the n-hexane fraction showed significant antiviral activity against DENV by inhibiting non-structural-1 and 3 (NS1 and NS3) helicases through its high saponin content, which hindered viral RNA replication by binding to viral proteins. In contrast, despite containing flavonoids with known antiviral properties, the

Table 1
Summary of antiviral benefits associated with *Carica papaya* extract in clinical trial.

Study	Virus	Part of Plant	Voucher specimen number	Drug Dose (μg/mL)	Duration	Pre/Post/Co-Infection treatment	Antiviral Compound	EC_{50}	IC_{50}	Target	Method of evaluation of viral titter	Outcome
Sathyapalan et al., 2020 [25]	DENV	Leaves	NA	Tablets 1100 mg	5 days (three times a day)	Post-infection treatment	Caripill tablets	N/A	N/A	NS1	qRT PCR	Clearance of viral NS1, increased platelet counts and immunomodulatory effects were observed

DENV, Dengue virus; N/A, not available; IC50, 50 % inhibition concentration; EC50, Half maximal effective concentration; qRT-PCR, quantitative real time polymerase chain reaction; NS1, Non-structural protein 1.

Table 2
Summary of Antiviral Benefits Associated with *Carica papaya* Extracts in *in-vivo* studies.

Study	Virus	Part of Plant	Voucher specimen number	Drug Dose (μg/mL)	Duration	Pre/Post/Co-Infection treatment	Antiviral Compound	EC_{50}	IC_{50}	Target	Method of evaluation of viral titter	Outcome
Mohd et al., 2018 [16]	DENV-2 NGC	Leaf juice	007/10	500 & 1000 mg/kg/day	3 days	Post-infection treatment	N/A	N/A	N/A	NS1	qRT-PCR	No significant effect of CPLJ was observed.
Norahmad et al., 2019 [17]	DENV -2	Leaf juice	007/10	500 or 1000 mg/kg	3 days	Post-infection treatment	N/A	N/A	N/A	N/A	qRT-PCR	No effect
Mohd et al., 2021 [18]	DENV -2	Leaf juice	007/10	500 and 1000 mg/kg/day	3 days	Post-infection treatment	N/A	N/A	N/A	N/A	qRT-PCR	FCPLJ presented a specific reduction in viral replication within the liver only.

DENV, Dengue virus; NGC, New guinea C; N/A, not available; IC50, 50 % inhibition concentration; EC50, Half maximal effective concentration; qRT-PCR, quantitative real time polymerase chain reaction; CPLJ, Carica Papaya leaf juice; FCPLJ, frozen CP leaf juice.

Table 3
Summary of Antiviral Benefits Associated with *Carica papaya* Extracts in *in-vitro* studies.

Study	Virus	Part of Plant	Voucher specimen number	Drug Dose (μg/mL)	Duration	Pre/Post/ Co-Infection treatment	Antiviral Compound	EC_{50}	IC_{50} μg/mL	Target	Method of evaluation of viral titter	Outcome
Adel et al., 2022 [8]	SARS-CoV-2	Leaves	PHG-PCP-382	N/A	N/A	Co-Infection treatment	1. n-hexane 2. Ethyl acetate 3. n-butanol	N/A	1. 1.98 2. 138.1 3. >406.4	Mpro, PLpro, RdRp, & COMT	Crystal violet assay	n-hexane inhibited the virus infected cells with the highest selectivity and without harming normal cells.
Jadaun et al., 2023 [14]	HIV-1 (HIV-1UG070 & HIV-1VB028)	Leaves	DP01	1000–1500	1 h.	Co & post-infection treatments	N/A	1VB028) = 1.03 (1UG070) = 1.25 (mg/ml)	N/A	HIV protease	Luciferase Gene Assay	Viral replication was inhibited & also the level of intracellular ROS in HIV-1 infected cells was decreased.
Joseph et al., 2015 [22]	DENV-2	Leaves	N/A	N/A	5 days	Pre & post-infection treatment	N/A	(Methanolic extract) = 0 (Chloroform extract) > 1	N/A	N/A	PFU assay	Only the chloroform extract was moderate/less effective against DENV with no cytotoxicity.
Patil et al., 2022 [27]	DENV-2 & CHIKV	Leaves	D. DUN 25636 & MDU 3201	12.5–100	5 days	Pre, Co, & Post-infection treatment	N/A	N/A	N/A	N/A	FFU assay, RT-PCR, Immunofluorescence assay	Silver nanoparticles of CPLE resulted in complete reduction of DENV-2. Papaya leaves powder inhibited CHIKV, significantly.
Bere et al., 2021 [24]	DENV -2	Leaves	AWB-JKUATBH/ 001C-2020	10–40	60 min	Post-infection treatment	N/A	N/A	9.20	NS5 protein	FFU assay	Silver- nanoparticles of methanol extract, were the most effective in inhibiting DENV-2 replication by greater than 90 %.
Thao et al., 2022 [23]	DENV1- 4	Leaves	HD01	250, 125, 62.5, 31.25, 15.625, 7.81, & 3.90	N/A	Co-infection treatment	1. n-hexane 2. Ethyl acetate 3. n-butanol	N/A	15.625–31.25	N/A	Plaque assay	Only n-hexane demonstrated potent antiviral effect across all DENV serotypes without any cytotoxicity.
Haddad et al., 2020 [15]	ZIKV	Fruit	N/A	2000	2 h.	Co-infection treatment	Pulp extract	N/A	300	Early stage (particularly attachment) of viral life cycle	PFU Assay	CP fruit pulp inhibited ZIKV infection without harming cell viability and significantly.
Amin et al., 2023 [19]	DENV -2	Leaves	GC.Herb. Bot.2236	20, 80, 200, 150 & 300	4–16 h.	Co-infection treatment	N/A	N/A	N/A	N/A	PFU Assay	Methanolic extracts exhibited potent antiviral action against DENV.
Hariono et al., 2022 [20]	SARS-CoV-2	Leaves	N/A	750, 125 & 1000	N/A	Pre-infection Treatment	Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-(S)- and β-manno-furanoside, farnesyl-	N/A	1. 0.02 2. 0.06 3. 1888	1. 3CLpro 2. PLpro 3. TMPRSS2	N/A	The compounds displayed higher affinity for the main proteases (3CLpro and PLpro) than for TMPRSS2 also non-toxic towards Vero cells.

(*continued on next page*)

Table 3 (*continued*)

Study	Virus	Part of Plant	Voucher specimen number	Drug Dose (μg/mL)	Duration	Pre/Post/ Co-Infection treatment	Antiviral Compound	EC_{50}	IC_{50} μg/mL	Target	Method of evaluation of viral titter	Outcome
Agfata et al., 2021 [26]	DENV-2 NGC	Leaves	N/A	20	N/A	Pre-infection Treatment	1. n-hexane 2. Benzene 3. n-butanol	N/A	N/A	NS1, NS3 helicase and NS5	FFU assay	The n-hexane fraction extracted from papaya leaves showed significant antiviral activity against dengue without causing cell toxicity. Saponins present in this extract effectively hindered DENV replication by binding to NS1 and inhibiting NS3 helicase.

DENV, Dengue virus; NGC, New guinea C; N/A, not available; IC_{50}, 50 % inhibition concentration; EC_{50}, Half maximal effective concentration; NS1, Non-structural protein 1; NS3, Non-structural protein 3; NS5, Non-structural protein 5; Mpro, main protease; PLpro, Papain-like protease; RdRp, RNA dependent RNA polymerase; COMT, Catechol-O-methyltransferase; SARS-CoV-2, Severe acute respiratory syndrome Coronavirus 2; HIV, Human immune deficiency virus; ZIKV, Zika virus; CHIKV, Chikungunya virus; ROS, reactive oxidative stress; PFU, plaque forming unit; FFU, focus forming unit; 3CLpro, 3-chymotrypsin-like protease; TMPRSS2, transmembrane protein serine 2.

Table 4
Summary of Antiviral Benefits Associated with *Carica papaya* Extracts in hybrid *in-vivo/in-vitro* study.

Study	Virus	Part of Plant	Voucher specimen number	Drug Dose (μg/mL)	Duration	Pre/Post/Co-Infection treatment	Antiviral Compound	EC50	IC_{50}	Target	Method of evaluation of viral titter	Outcome
Sharma *et al.*, 2019 [21]	DENV-2 NGC	Leaves	N/A	100 or 200 (cell lines). 200 mg/kg or 40 mg/ml (rats)	48 h (cells) 6 days (rats)	Post-infection treatment	N/A	N/A	N/A	Enve-lope and NS1 proteins	FACS	CPLE reduces envelope and NS1 protein expression in DENV-infected cells, leading to decreased viral entry and replication, reduced erythrocyte damage, and increased platelet count in rats.

DENV, Dengue virus; NGC, New guinea C; N/A, not available; IC_{50}, 50 % inhibition concentration; EC_{50}, Half maximal effective concentration; NS1, Non-structural protein 1; CPLE, CP leaf extract; FACS, fluorescence activated cells sorting.

benzene fraction showed ineffectiveness due to lower concentrations of active compounds. Similarly, the n-butanol fraction, containing flavonoids and saponins, lacked antiviral efficacy, possibly due to the presence of carbohydrates facilitating DENV entry into cells [26].

Research findings by Joseph et al. reported that methanol extracts of CPL did not inhibit DENV-2, and chloroform extracts were moderate/less effective with no cytotoxicity. These findings suggest that under the experimental conditions employed, these extracts may not specifically target DENV-2 or significantly restrict its replication cycle [22]. Thao et al. demonstrated that the n-hexane fraction had potent antiviral effects across all DENV serotypes with high safety levels [23]. Bere et al. observed that the methanolic crude and silver nanoparticles (AgNPs) synthesized from methanolic extracts of CP L significantly inhibited DENV-2 replication [24], as these nanomaterials are proved to be more efficient for drug delivery [36](26). In another similar study, Metal nanoparticles (NPs) synthesized through eco-friendly process by using extracts of medicinal plants have indicated potential anti-dengue applications. Such NPs are simple, cost-effective, and free of hazardous wastes. The recent progress in the phyto-synthesized metal NPs as anti-dengue applications has encouraged us to investigate natural resources for the dengue control. However, to fully realize their potential, further research is needed to understand their mechanisms of action, assess their environmental impact, and ensure their safe application [36].

Two *in-vitro* studies exhibited antiviral activity of CPLE against SARS-CoV-2. Adel et al. found that the n-hexane fraction of CPL, rich in antiviral compounds like sterols and flavonoids, was effective and non-cytotoxic, while the ethyl acetate fraction was cytotoxic and the n-butanol fraction lacked strong antiviral effects. The computational analyses also supported these findings by showing good binding affinity of the compounds to viral proteins [8]. Specific CPLE compounds, such as phenol and β-mannofuranoside, farnesyl-, which targeted key viral proteases "3CLpro and PLpro", demonstrated safe against SARS-CoV-2, identified by Hariono et al. [20]. Likewise, the docking analysis of 40 CP compounds revealed that 20 interact favorably with multiple SARS-CoV-2 protein targets, including 3-chymotrypsin-like protease (3CLpro), Papain-like protease (PLpro), RNA-dependent RNA polymerase (RdRp) Endonuclease (EndoU), S1 and S2 regions of the spike protein. According to the average binding energy, the 20 compounds interact with the protein in a sequence of higher to lower binding affinity as follows: S1 > 3CLpro > EndoU > RdRp > PLpro > S2 (with the highest observed for the S1 region), suggesting their potential as antiviral agents. Diverse compounds of CP hold promise in combating SARS-CoV-2, as proteins 3CLpro, EndoU, RdRp, and PLpro are involved in viral replication and immune evasion, whereas the S1 and S2 regions of the spike protein facilitate viral attachment and entry into host cells. However, it's essential to note that these findings are based on computational analyses and have not yet been validated through *in-vivo* and *in-vitro* research [37].

Despite being a major global health burden, there is currently no vaccine or cure for HIV. In 1 laboratory setting, Methanolic extracts of CPL inhibited viral replication. Methanolic extracts of CPL reduced viral replication in 1 *in-vitro* study. Moreover, these extracts reduced the level of intracellular reactive oxygen species (ROS) in HIV-1 infected cells, indicating potential ROS scavenging capabilities [14]. In another study, the anti-HIV activity of CP extract (CPE) was explored. The toxicity and anti-HIV-1 effects of methanolic and aqueous extracts of CP aerial parts were investigated. Both methanol and aqueous extracts of CP exhibited anti-HIV-1 activity. The aqueous extract demonstrated higher anti-HIV-1 activity as compared to the methanol extract because of its ability to extract more active anti-HIV components [38], (39).

Patil et al. assessed the effectiveness of CPLE against both CHIKV and DENV in laboratory settings. It was reported that silver nanoparticles generated from CPL completely reduced DENV-2, while methanol extracted nanoparticles of CPL indicated significant anti-DENV-2 activity. CHIKV suppression was shown by CPL powder [27]. However, Keramagi and Skariyachan investigated the binding potential of compounds extracted from CP against potential drug targets associated with CHIKV. They discovered that Kaempferol displayed promising interactions with CHIKV non-structural protein-3 (NSP3) and CHIKV envelope protein (E2). Additionally, Chymopain, exhibited significant binding affinity towards CHIKV NSP-3 and E2 proteins, forming notable hydrogen bonds. These findings collectively suggest the potential anti-CHIKV activity of CPLE, requiring subsequent examination and potential development as antiviral agents [40].

There isn't a vaccine or a reliable treatment for ZIKV. Creating antiviral strategies that involve the use of nutritional supplements to prevent ZIKV infection is still critical. CP fruit pulp extract effectively reduced Zika virus infection by primarily targeting the early stage of the viral life cycle, specifically the attachment phase, also preserved cell viability [15]. On the other hand, a different study focused on quercetin as a potent inhibitor of ZIKV NS2B-NS3pro, an enzyme critical in cleaving the Zika polyprotein into functional subunits during replication. This suggests that CP fruit, encompassing quercetin among other bioactive compounds, may exert its antiviral effects against ZIKV by hindering viral replication. Quercetin, a flavonoid found in various parts of the CP plant, including its fruits, emerges as a promising inhibitor of a pivotal viral enzyme (NS2B-NS3pro) involved in ZIKV replication [41].

Sharma et al., 2019 analyzed the effectiveness of CPLE against DENV-infected THP-1 cells and rats with DENV-induced thrombocytopenia, the only hybrid study in this review. CPLE exhibited antiviral effects by reducing the expression of the envelope and NS1 proteins, thereby inhibiting DENV replication on infected cells. In thrombocytopenic rats, CPLE increased platelet counts and boosted thrombopoietin (TPO) and interleukin-6 (IL-6) levels, both of which are vital for platelet production and immune response. These findings suggest CPLE could serve as both an antiviral treatment and a platelet-boosting therapy for dengue patients [21]. In accordance, Banjan et al., 2024 identified three key phytocompounds "rutin, myricetin 3-rhamnoside, and kaempferol 3-(2″-rhamnosylrutinoside)" from CPLE that were effective against dengue-associated thrombocytopenia. These compounds were shown to bind to the active site of the DENV NS1 protein, which is known to stimulate toll-like receptor 4 (TLR4) and cause platelet aggregation, leading to thrombocytopenia. By potentially disrupting the NS1-TLR4 interaction, these phytocompounds could reduce platelet aggregation and reduce thrombocytopenia. Further *in-vitro* studies are needed to confirm their therapeutic efficacy and pharmacological potential [42].

This study focused on assessing antiviral activity of CP against a limited number of viruses, including SARS-CoV-2, ZIKV, CHIKV,

HIV, and mostly DENV-2. While this provides novel insights, the effects of CP on other significant viruses, such as influenza, Ebola, Hepatitis B and C viruses (HCV, HBV) remain unexplored. Clinically, if CP proves effective against more viruses, it could contribute to developing new antiviral treatments and offer broader protection, especially in regions with high viral prevalence. However, the analysis was limited by insufficient databases, potentially missing relevant data. Future research is required to investigate CP's antiviral effects on more viruses, conduct *in-vivo* studies and clinical trials, and perform broader database searches to assess its safety, efficacy, and optimal dosage as a potential antiviral drug.

Funding

Not applicable.

CRediT authorship contribution statement

Masooma: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Ariba Qaiser:** Writing – review & editing, Writing – original draft, Validation, Formal analysis. **Dr Sajid Ali:** Visualization, Validation, Investigation. **Dr Sobia Manzoor:** Visualization, Supervision, Project administration, Investigation, Formal analysis.

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.

Acknowledgement

We thank Momna Arooj Malik (National University of Sciences and Technology) and Arzoo Rehman (National University of Sciences and Technology) for excellent technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39635.

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