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Review Article *Open Access*

ENHANCED PRODUCTION OF ANTI-DENGUE SECONDARY METABOLITES IN CARICA PAPAYA CELL SUSPENSION CULTURES USING ELICITORS: A REVIEW

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Abstract *Dengue fever is among the most important mosquito-borne viral infections in the world, impacting millions of people each year in tropical and sub-tropical areas. However, with the spread of the disease in the world and the lack of specific antiviral therapy, we need to find new therapeutic methods. Carica papaya L. is a plant that is widely used in traditional medicine for the treatment of dengue and is also known for its high content of phytochemical compounds, which is why it has become a potential medicinal plant. This review aims to determine the potential of C. papaya as a source of anti-dengue secondary metabolites and investigate the biotechnological strategies to increase the production of secondary metabolites by using plant cell culture technology. This review covers the taxonomy, botanical characteristics, distribution, and phytochemical makeup of Carica papaya, emphasizing important bioactive compounds like flavonoids, alkaloids, phenolic acids, and proteolytic enzymes. It brings into focus several key metabolites, such as quercetin and kaempferol (due to inhibition of viral NS2B-NS3 protease and NS5 polymerase enzymes and their anti-dengue properties) and chlorogenic acid and carpaine (because of anti-dengue action through modulation of the immune system and platelet protection). Additionally, the biology and replication of the dengue virus are also discussed in order to rationalize molecular targets of these compounds. It also reviews the drawbacks of whole plant extraction, and it considers cell suspension cultures of C. papaya as a sustainable approach for metabolite production while paying attention to the use of elicitors like methyl jasmonate, salicylic acid, chitosan, and yeast extract for the improvement of the biosynthesis of flavonoids and phenolics. Overall, elicitor-assisted cell culture is a promising methodology for the production of anti-dengue metabolites and the establishment of plant-based antiviral drug development*.

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Introduction

Dengue fever is one of the fastest-growing mosquito-transmitted viral diseases of the 21st century, and a significant and increasing global public health hazard (Ross, 2010). Now endemic to over 100 countries, the disease is caused by the dengue virus (DENV), which is transmitted to humans principally by *Aedes aegypti* mosquitoes. In recent years, there has been a spread and rapid expansion of the global burden of dengue. In 2024, the world saw a dengue epidemic for the first time as there were more than 14,406,542 cases and more than 11,000 deaths ("WHO," 2025). The rise of dengue has been attributed to rapid urbanization, population growth, lack of vector management, climate change, and environmental conditions conducive to the presence of Aedes mosquitoes. In the last two decades, Pakistan has witnessed several big Dengue epidemics. They have seen notable outbreaks, such as in Lahore with over 21,000 cases with more than 350 deaths in 2011, a record outbreak with more than 53,000 cases and about 95 deaths all over the country in 2019, and in 2021, 48,906 cases and 183 deaths were reported nationally. All per-province PRICE collection reports suggest that the overall burden of the disease is highest in Punjab province, followed by Khyber Pakhtunkhwa, Sindh, and Islamabad (Tabassum et al., 2023). Further, the clinical burden gets magnified by the lack of a specific anti-dengue therapy. It is managed mainly supportively by providing fluid replacement and symptomatic treatment, and in severe cases, a hospital stay is required (Tayal, Kabra, & Lodha, 2023).

Natural products derived from plants are increasingly recognized as therapeutic sources to overcome drawbacks in the development of synthetic antiviral drugs. Plants have been a major source of new drugs, with nearly one quarter of all modern medicines obtaining their lead in some way from plants (Newman & Cragg, 2020). Plant secondary metabolites are compounds that include flavonoids, alkaloids, terpenoids, tannins, and phenolic acids, and are synthesized by plants as a response to environmental stress and pathogens. They have a wide range of biological activities in humans, such as antiviral, antibacterial, anti-inflammatory, antioxidant, and anticancer properties. Furthermore, plant-based antivirals are likely to have multi-molecular targets, limiting resistance development compared to single-target synthetic drugs (Adel, 2021). *Carica papaya* L. is one of the medicinal plants that has attracted significant scientific attention, as it is rich in phytochemical constituents and has been traditionally used for medicinal purposes. Papaya is native to Central America but cultivated all over the tropics and has been traditionally used for treating fever, malaria, digestive problems, and viral infections such as Dengue Fever (Koul et al., 2022).

It is rich in bioactive components in its leaves, including flavonoids (quercetin, kaempferol), alkaloids (carpaine), phenolic acids, saponins, carotenoids, and proteolytic enzymes (e.g., papain). These metabolites have antioxidant, immune modulatory, anti-inflammatory, and antiviral activity (Sandhya Rani et al., 2023). Of note, flavonoids of C. papaya have been found to inhibit important dengue viral enzymes such as NS2B–NS3 protease and NS5 polymerase that aid in viral replication. Besides, extracts from papaya leaves can be used to help recover platelets, which is also a crucial aspect in dengue, caused by the viral infection (Zandi et al., 2011). Although promising for therapy, the therapeutic application of C. papaya is constrained by low and variable yield of metabolites, seasonal limitations, and the influence of the environment on the phytochemical composition. With these challenges, it is hard to produce them on a large scale, on a standardized basis. Plant cell suspension culture technology has proved to be an alternative that enables metabolite production in a controlled and sustainable manner, irrespective of the environmental conditions. Culture cells of C. papaya preserve biosynthetic pathways, especially those of phenylpropanoid metabolism, which has been confirmed using transcriptomic studies, suggesting that culture cells would be a good source for in vitro production systems (Jamaluddin, Mohd Noor, & Goh, 2017). In addition, secondary metabolite production may be significantly increased by activation of the plant defence signalling pathways, such as ROS signalling, MAPK cascades, and transcriptional regulation of biosynthetic genes, through the use of elicitation strategies in the form of methyl jasmonate, salicylic acid, chitosan, and yeast extract (Jamaluddin et al., 2017). This review not only incorporates data related to the global burden of dengue but also includes information on phytochemical studies of C. papaya and plant cell biotechnology with emphasis on elicitor-induced production of anti-dengue secondary metabolites. It also points out important research needs and suggests a mechanistic approach to maximize the yields of metabolites from plant cell suspension cultures and elicitation techniques.

Taxonomy, Botanical Description, and Geographical Distribution of Carica papaya

Taxonomic Classification

Carica papaya L. is papaya, the only member of the genus Carica and a member of the family Caricaceae. One of the most economically important fruit crops in the tropics and subtropics has nutritional, medicinal, and industrial value. It is one of the most economically important fruit crops in tropical and subtropical regions, recognized for its nutritional, medicinal, and industrial significance. Although papaya was historically classified under various plant families, including Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae, modern taxonomic studies have firmly placed it within the Caricaceae family, which comprises approximately 35 latex-producing species divided into four genera: *Carica*, *Jarilla*, *Jacaratia*, and *Cyclicomorpha*, distributed mainly across tropical regions of the world (Koul et al., 2022; L. Kumar & Srinivasan, 1944).

Morphological Features

Carica papaya is a fast-growing, soft-stemmed tree that can reach a height of 2 to 10 meters. It has a single hollow trunk marked with leaf scars and produces large, deeply lobed green leaves at the top, giving it an umbrella-like appearance. All parts of the plant, leaves, stem, fruit, and roots, contain a white milky juice called latex, which is rich in the enzymes papain and chymopapain (Joachim M. Dotto). The leaves are the most important part of the plant from a medicinal perspective, as they are the richest source of anti-dengue compounds such as quercetin, kaempferol, and carpaine. The plant produces three types of flowers: male, female, and hermaphrodite, and the fruit is a large, oval-shaped berry with juicy yellow-orange flesh and black seeds inside (Wadekar et al., 2021).

Global Distribution and Cultivation

Papaya originally came from Central America and was spread to Asia, Africa, and the Pacific Islands by European explorers in the 16th century. Today, it is grown in more than 60 tropical and subtropical countries around the world. It grows best in warm temperatures between 21°C and 33°C and requires well-drained soil and plenty of sunlight (Morton, 1987). According to a recent papaya production report (2020), India is the world's largest producer of

papaya, generating 13.9 million tonnes (mt) per year, or 43% of global papaya production. In contrast, the United States is the world's largest consumer of papaya (Koul et al., 2022). Importantly, the countries that produce the most papaya, such as India, Indonesia, Bangladesh, Thailand, and Pakistan, are also the countries most heavily burdened by dengue fever, which makes *C. papaya* a highly relevant and locally accessible source of anti-dengue medicine (Mohit Kumar, 2022).

Phytochemistry of Carica papaya

Carica papaya L. has long been recognized not only as a nutritional fruit but as a rich reservoir of biologically active compounds distributed across all its parts — fruit, leaf, seed, bark, and latex (Adel, 2021). This plant's medicinal status is firmly established in a time of extensive scientific research over the past few decades in relation to its many phytochemicals that are under investigation.

The compounds that give these properties are called secondary metabolites, which are organic compounds that the plant produces in response to environmental selective pressures (biotic and abiotic), including herbivory, microbial attack, UV radiation, and competition with neighbouring plants. They act as a chemical defense system in the plant, assisting in tissue repair. These compounds also have a wide range of biological activities in humans, such as anti-inflammatory, anti-microbial, antioxidant, anticancer, and antiviral.

(Ashutosh Sharma & 2020).

Major Classes of Secondary Metabolites in *Carica papaya*

Phytochemical studies have found various secondary metabolites in various parts of *C. papaya*, such as alkaloids, flavonoids, phenolic acids, tannins, saponins, terpenoids, steroids, glycosides, carotenoids, glucosinolates, and isothiocyanate (Joachim M. Dotto). Many of the therapeutic uses of papaya can be attributed to these metabolites.

Alkaloids are given the name of nitrogen-containing compounds that possess many pharmacological activities. The most remarkable alkaloids found in C. papaya leaves are carpain, pseudocarpaine, and dehydrocarpaine. These compounds exhibit antitumor activity, showing signs of antibacterial activity and antiviral activity, along with activity as antioxidants and cardioprotectives. Carpain, specifically, has been highlighted for its purported effects on cancerous cells, alongside some parasites, and its ability to interfere with the male survival and reproduction of certain pathogens is likely to help Papaya Leaf Extracts serve as anti-viral agents (Mohit Kumar, 2022).

Flavonoids are polyphenolic compounds with well-established antioxidant and anti-inflammatory properties. The leaves of papaya are known to contain significant amounts of flavonoids, including quercetin, kaempferol 3-rutinoside, quercetin 3-rutinoside, myricetin 3-rhamnoside, and kaempferol 3-(2G-rhamnosylrutinoside). These compounds scavenge free radicals and help reduce cellular oxidative damage. Several flavonoids have also demonstrated antiviral activity by inhibiting viral entry, replication, and enzyme function, while their anti-inflammatory properties help reduce tissue damage during infections (S. S. Kumar, Krishnakumar, & John, 2022).

Phenolic acids such as ferulic acid and caffeic acid contribute to the plant's antioxidant capacity and play a role in UV protection and pathogen resistance. Phenolics, flavonoids, and alkaloids represent the key phytochemicals behind papaya's well-documented antioxidant, antibacterial, anticancer, anti-inflammatory, antiulcer, antidiabetic, and hepatoprotective activities.

Isothiocyanates, particularly benzyl isothiocyanate (BITC), are sulfur-containing compounds found mainly in the seeds and latex. These compounds are produced when glucosinolate precursors are broken down enzymatically. BITC exhibits strong antimicrobial, antifungal, antiparasitic, anticancer, and antioxidant activities. Several studies have also reported antiviral effects of isothiocyanates through interference with viral replication and enhancement of host defense mechanisms.

Saponins are glycosidic compounds with soap-like properties. They contribute to the plant's immune modulation, anti-inflammatory action, and are involved in membrane permeabilization, which explains their antimicrobial effects.

Sterols and Triterpenes, including β-sitosterol, are found mainly in the bark and seeds. β-Sitosterol is known for its cholesterol-lowering effects and has been investigated for anti-inflammatory and antiproliferative activities.

Proteolytic Enzymes, while technically proteins rather than classic secondary metabolites, are among the most commercially and medicinally significant compounds in papaya. The latex from unripe papaya fruit contains the enzymes papain, caricain, and chymopapain, along with a mixture of cysteine endopeptidases, chitinases, and an inhibitor of serine protease.

Carotenoids, particularly **β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin**, are abundant in ripe papaya fruits. These pigments act as strong antioxidants, preventing oxidative stress in tissues and enhancing immune system function; β-carotene is also a precursor of vitamin A that is vital for visual function, immunity, and epithelial function.

However, three other major classes of secondary metabolites, namely, **tannins, glycosides, and fatty acids**, have been detected in different parts of the C. papaya. Tannins, primarily present in leaves, bark, and roots, have well-established antimicrobial, antidiarrhea, and wound healing activities, which they exhibit due to their capacity of reacting with proteins and biological macromolecules. The leaves,

seeds, and bark contain glycosides, of which the most important are carposide and quercetin 3-rutinoside, and their pharmacological activity (such as the antitumor effect and gastrointestinal activity) is exhibited after enzymatic hydrolysis of active ingredients. The fatty acids, mainly in seeds, are primarily oleic (~72 %) acid, along with palmitic acid and linoleic acid, which possess cardiovascular protective, anti-inflammatory, and antifungal activity.

Distribution of Metabolites Across Plant Parts

Another characteristic of the phytochemistry of Carica papaya is that the secondary metabolites are accumulated in different parts of the plant, which is the reason for its various uses in medicine. The leaves include a high level of alkaloids (carpain), xanthophyll, flavonoids (kaempferol), phenolic acids (ferulic acid), carposide, and high levels of vitamins C and E, and the bark glucose, fructose, sucrose, xylitol, and β-sitosterol. The latex is packed with papain, chymopapain, caricain, lysozyme enzymes and peptidases A & B, whereas the roots and stems have low concentration of alkaloids, phenolics and terpenoid compounds (Bere, Mulati, Kimotho, & Ng'ong'a, 2021).

The unripe fruit is exceptionally rich in alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, and health-promoting carotenoids like lycopene and β-carotene, which bring about significant health benefits along with some digestive enzymes as well. Seeds possess a wide range of bioactive phytochemicals, such as flavonoids, tannins, phenols, alkaloids, glycosides, saponins, isothiocyanate, and benzyl glucosinate, which are especially effective in antimicrobial and antifertility activities (Table 1). It has also been found that phenolic compounds such as flavonoids and phenolic acids are more concentrated in young leaves when compared with mature leaves (Joachim M. Dotto).

Table 1. The diverse phytochemical composition of *C. papaya* explains its extensive use in traditional medicine and highlights its potential as a valuable source of bioactive compounds for pharmaceutical and nutraceutical application

Class	Key compounds	Plant part(s)	Primary activity	Reference
Alkaloids	Carpain, dehydrocarpaine pseudocarpaine,	seeds, Leaves	Antitumor, antibacterial, Antiviral, cardiotonic,	(Adel, 2021)
Flavonoids	Quercetin, kaempferol, myricetin, rutin, quercetin 3-rutinoside, Quercetin	Fruit, Leaves,	Antioxidant, anti-inflammatory, anticancer; antiviral — quercetin and kaempferol inhibit dengue virus NS2B-NS3 protease and NS5 polymerase; active against HIV and HSV	(N. Sharma et al., 2019) (Anshu Sharma et al., 2022) (Srivastava, Jaiswal, Kharkwal, Dubey, & Srivastava, 2025)
Phenolic acids	Ferulic acid, chlorogenic acid, caffeic acid	Leaves, fruit peel	Free radical scavenging, antimicrobial; chlorogenic acid inhibits viral replication in dengue patients	(Kong et al., 2021)
Isothiocyanates	Benzyl isothiocyanate (BITC)	Seeds, Latex	Antimicrobial, antifungal, anticancer,	(Adel, 2021)
Carotenoids	β-Carotene, β-cryptoxanthin, lycopene,	Ripe fruit, leaves	Antioxidant, eye health, provitamin A,	(Belinskaia et al., 2020)
Saponins	Oleanolic acid, glycosides	Bark, Leaves	Anti-inflammatory, antimicrobial, and immune modulation,	(Liyanagamage, Jayasinghe, Attanayake, & Karunaratne, 2021)
Proteolytic enzymes	Papain, chymopapain, caricain, glycyl endopeptidase,	unripe fruit, Latex	Protein digestion, wound healing, anticancer, antiviral (chymopapain active against CHIKV, caricain against dengue)	(Masooma, Qaiser, Ali, & Manzoor, 2024)
Sterols	campesterol, β-Sitosterol	Bark, seeds	Anti-inflammatory, hepatoprotective,	(Demel & De Kruyff, 1976)

			Cholesterol-lowering,	

Table 1: Summary of key bioactive compound classes from *Carica papaya*, their anatomical distribution, primary therapeutic activities, and associated viral targets.

Dengue Virus: Taxonomy, Structure and Genome Organization

Dengue virus (DENV) is a positive-sense single-stranded RNA virus of the family Flaviviridae, which is transmitted by the mosquito (*Aedes aegypti*). Geographical gaps in distribution have been observed, and there has been a rise in the occurrence of dengue cases in the last 20-30 years. Nowadays, the disease occurs in over 125 countries, and is primarily tropical/sub-tropical throughout Asia, Latin America, Africa, and part of Europe (Guzman et al., 2010).

The virus has 4 closely related serotypes (DENV-1 to DENV-4), all of which can cause mild Dengue fever, severe dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS)(Kularatne, 2015). They usually have a very high fever, very severe headache (behind the eyes), muscle & joint pain/aches, nausea & vomiting, skin rash, and fatigue. In severe disease, there may be increased vascular permeability, thrombocytopenia, bleeding, and shock, which may be due to an overactive immune response and antibody-dependent enhancement (ADE) in secondary infections (Halstead, 1988). The growth of the Dengue virus inside the host cell involves several steps that are coordinated. Once the virus is inside, the viral RNA will be released into the cytoplasm of the cell, then translated into a single large polyprotein. The viral NS2B-NS3 protease complex proteolyses this polyprotein to form the structural and non-structural proteins (Liu et al., 2018). The non-structural proteins then assemble on the modified endoplasmic reticulum (ER) membranes to become the replication complex. The NS5 RNA-dependent RNA polymerase copies the viral RNAs to create new genomes, and the NS3 helicase unwinds the viral RNA and the NS5 methyltransferase caps the viral RNA for stability and translation in this complex. Viral RNA is made in the host cell and packaged into immature virions, which are assembled in the ER, matured in the secretory pathway, and secreted from the host cell in order to infect new cells (Malavige, Fernando, Fernando, & Seneviratne, 2004).

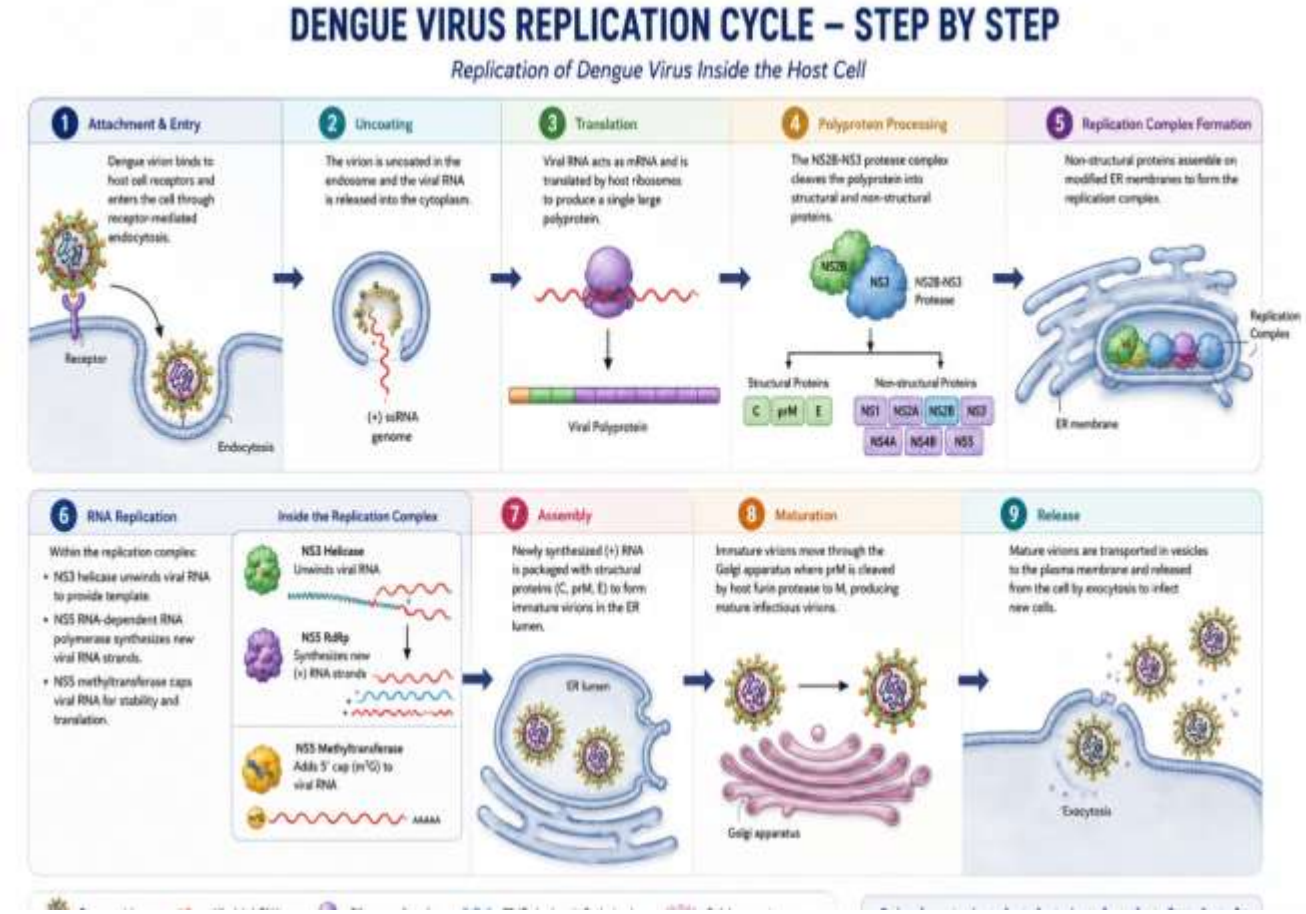

Figure 1: Schematic representation of the step-by-step Dengue virus replication cycle within a host cell.

Anti-Dengue Activity of Carica papaya ***Secondary Metabolites***

Due to the lack of specific anti-dengue treatment drugs, a considerable scientific effort has been made on secondary metabolites derived from plants as potential drugs to treat dengue. *Carica papaya* is one of the most researched plants of these, and several of its bioactive compounds have also been shown to have anti-dengue action via distinct and well-defined mechanisms.

Flavonoids: Inhibition of Viral Replication Enzymes

The effect of *C. papaya* as an anti-dengue is most widely studied with the flavonoids quercetin and kaempferol. Numerous papaya leaf extracts are rich in these compounds, which have demonstrated strong inhibitory activity against the NS2B-NS3 protease and NS5 polymerase, both enzymes necessary for dengue virus replication (N. Sharma et al., 2019). Maturation and assembly of the virus depend on the ability of the NS2B-NS3 protease to cleave the viral polyprotein to functional non-structural proteins, which is an essential requirement for virion maturation and assembly. The binding energy of Quercetin is the highest, able to form 6 hydrogen bonds with the binding site amino acids in NS2B-NS3, and prevent the protease activity and stop the viral reproduction. Kaemperol also acts on NS5 polymerase, which is responsible for RNA synthesis in the viral replication process, interfering with the generation of new viral RNA strands (Ashutosh Sharma & 2020).

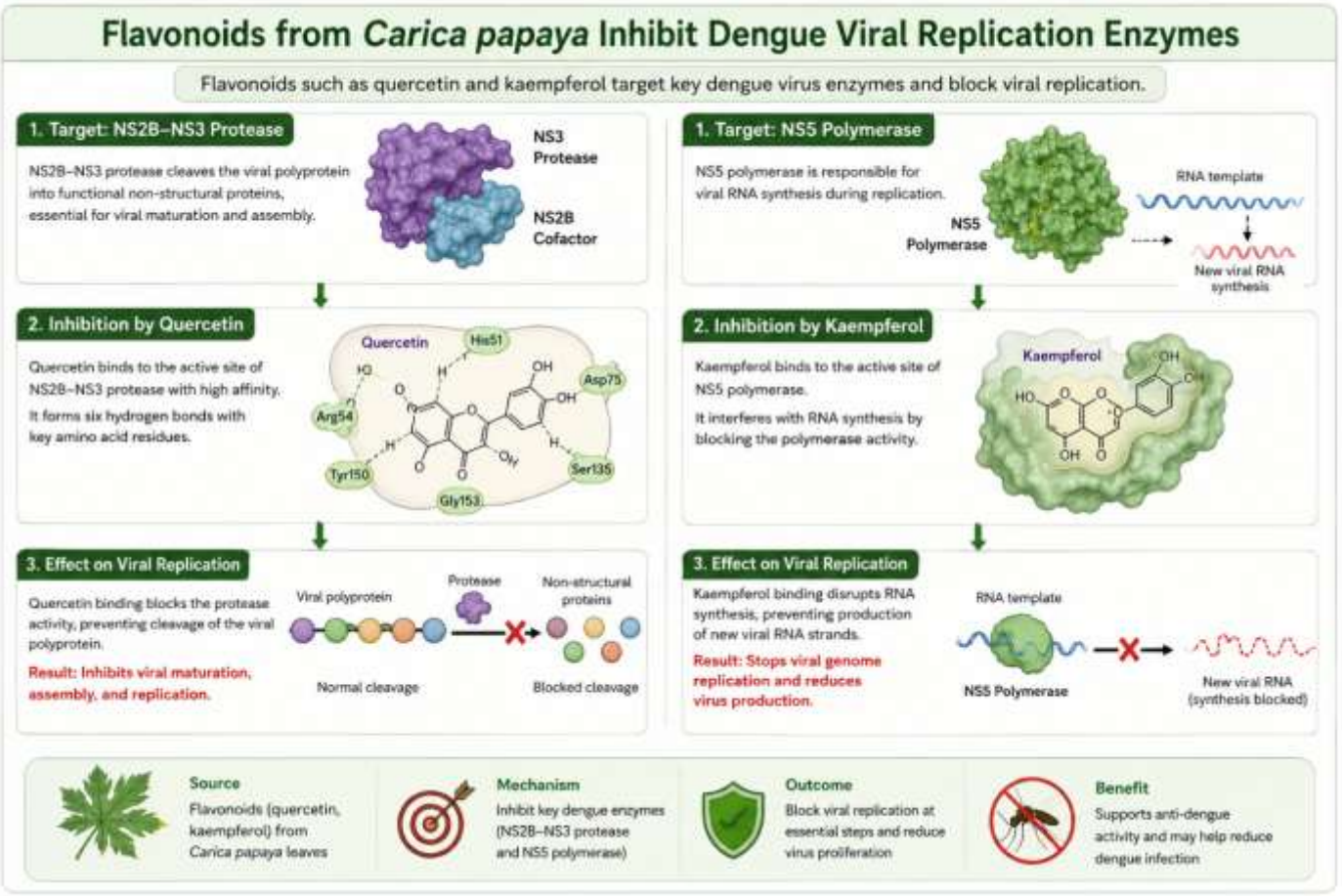

Figure 2: Molecular mechanisms of dengue virus enzyme inhibition by *Carica papaya*-derived flavonoids.

Alkaloids: Direct Antiviral and Platelet-Protective Action

Carpaine, which is the primary alkaloid in the leaves of *C. papaya*, functions in a complementary (and separate) manner. In-vitro studies show that carpaine has the ability to prevent the infection of DENV-2 and the virus replication, and silico studies show the interference of carpaine in the early stages of virus entry into host cells. In addition to direct antiviral effects, several bioactive components such as carpaine, dehydrocarpaine I, dehydrocarpaine II, chymopapain, and papain have a strong influence on platelet counts, one of the most dangerous signs of dengue infection being thrombocytopenia (Patil et al., 2022). Carpaine has also been demonstrated to increase the differentiation of megakaryocytes and to increase the receptor CD110, thus stimulating platelet production and preventing or diminishing the drastic platelet loss of dengue hemorrhagic fever (Nandini et al., 2021).

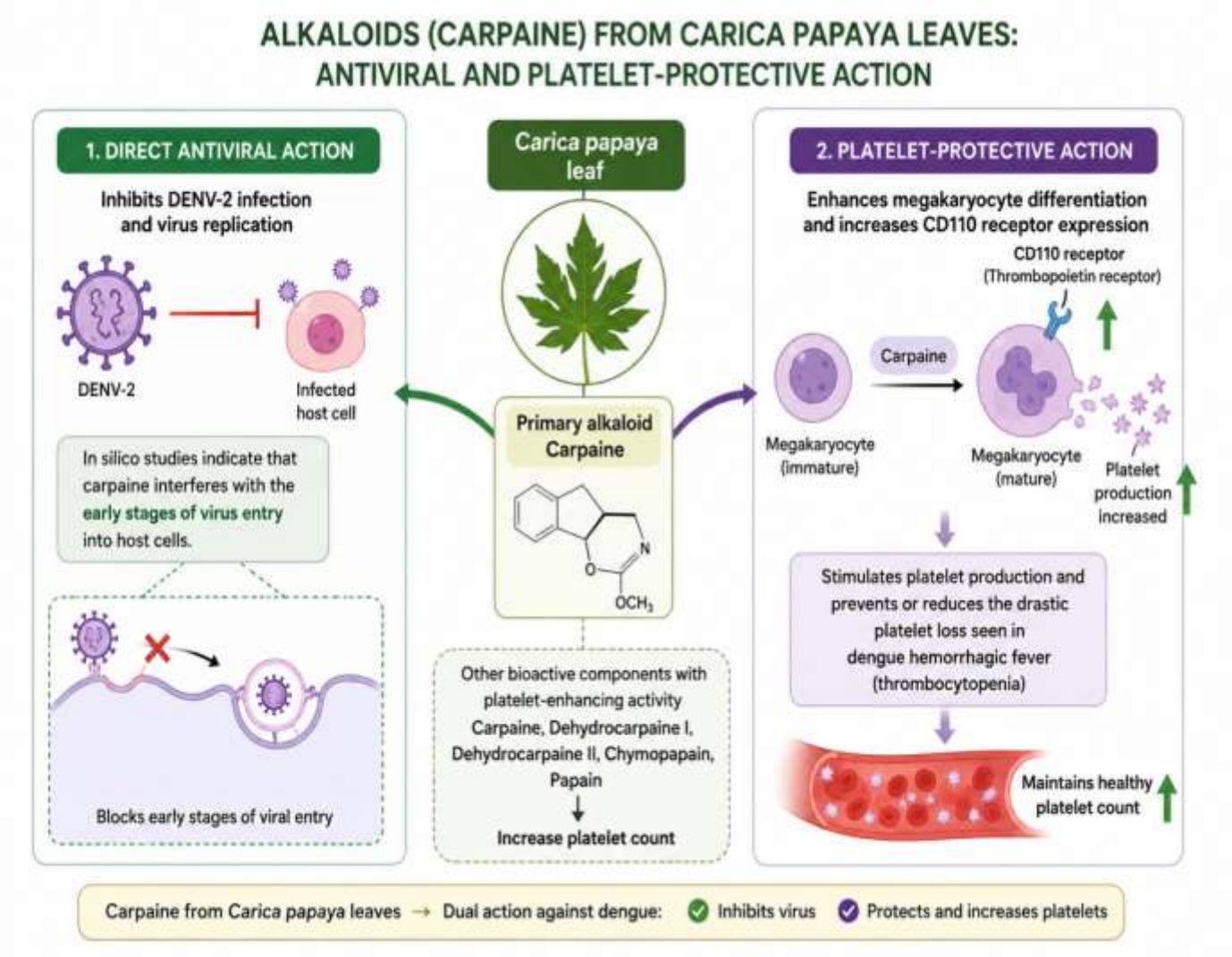

Figure 3: Dual antiviral and platelet-protective mechanisms of carpaine from *Carica papaya* leaves during dengue infection.

Phenolic Acids: Suppression of Viral Protein Expression

The anti-dengue activity of papaya leaves is because they contain chlorogenic acid, protocatechuic acid, and other phenolics that inhibit the functional proteins of the virus structural proteins (Dai & Mumper, 2010). Extracts of papaya leaves that contain kaempferol, quercetin, and chlorogenic acid have shown potential inhibitory activity for both the NS3 and NS5 proteins of DENV-2, and these phenolics also decrease the oxidative stress in infected cells, which reduces damage to host cells and promotes immune recovery (Subenthiran et al., 2013).

Immunomodulatory and Antioxidant Mechanisms of Carica Papya against Dengue

In addition to direct anti-viral activities, metabolites of C. papaya work in a combined manner to modulate the immune response of the host. Carica papaya contains bioactive compounds, including Proteolytic enzymes like papain, chymopapain, and caricain, which might exhibit anti-dengue effects via effects on immune modulation, inflammation, antioxidant activity, and antiviral mechanisms (N. Sharma et al., 2019). An increase of flavonoids in cellular membranes helps to protect the cytopathic effects of the virus towards the host cells, and increase carotenoids and vitamin C dose neutralise free radicals produced during the inflammatory reaction of the Dengue infection. Overall, the anti-dengue properties exhibited by C. papaya are not derived from any single compound acting by a single mode but rather the combined effect of its flavonoids, alkaloids, and phenolics acting on several key phases of the dengue viral life cycle, such as entry, replication, modulation of the host immune system, and platelet recovery (Ashutosh Sharma & 2020).

Low and Inconsistent Yield from Whole Plants: The Challenge:

Low and Inconsistent Yield from Whole Plants. Despite this enormous medicinal use, the bioactive

compound extraction from the whole papaya plant has several serious restrictions:

- **Seasonal dependency:** Papaya is only available in tropical and sub-tropical regions. Due to its dependency, its supply to the market is inconsistent.
- **Geographical constraints:** Papaya is not a crop that can be grown in all geographies, which influences its raw material availability.
- **Low natural yield:** The level of target secondary metabolites is also very low in the tissues of plants, and depends on the plant age, soil, climate, and time of harvesting.
- **Sustainability issues in commercialization:** The strong demand for medicinal plant material might be resulting in unsustainable harvesting.

Standardization for pharmaceutical use is difficult as the chemical composition of plant extracts varies greatly depending on growth conditions and can be inconsistent. Such restrictions would render using whole-plant extraction exclusive to antiviral compound production in papaya for large-scale pharmaceutical production impractical.

Plant Cell Suspension Cultures: Biotechnological Solution

An elegant solution to this problem is plant tissue culture technology. In particular, Secondary Metabolites can be manufactured at any time of the year, anywhere in any part of the world by plant cells or small clusters of plant cells in cell suspension cultures, which are a special form of controlled whole-plant cultivation. For secondary metabolite production, friable callus is used to initiate and maintain suspension cultures, which have significant advantages in terms of scaling up and ease of manipulation; the important parameters that need to be taken into account are nutrient requirements, agitation, and culture conditions (Murthy, Lee, & Paek, 2014). Genome-wide transcriptome profiling of C. papaya embryogenic callus has identified several genes, particularly involved in the synthesis of Secondary Metabolites, such as genes involved in the phenylpropanoid biosynthesis pathway are highly expressed, thus having important implications for Cell factory Applications. This implies that the genetic apparatus for valuable production of secondary metabolites is already in place in C. papaya cells in culture, but with a proper strategy, this apparatus can be stirred up and amplified (Y. Chen et al., 2020).

***Establishment of Cell Suspension Cultures in* Carica papaya**

Selection of a Suitable Explant

For Carica papaya, a suitable explant that is capable of actively dividing and friable callus would be used to establish cell suspension culture, and callus culture would be initiated from such an explant. Callus induction and plant regeneration from various explants like leaf, petiole, stem segment, shoot tip, lateral bud, zygotic embryo, and anther have been proven to be effective. Of the above, young leaf and petiole tissue are frequently selected because of their good regenerative capabilities and potential for the growth of proliferating callus cultures. One study reported that leaf explants yielded soft, spongy, white callus while petiole explants produced hard, friable, dark callus (Manasa, Mahendra, Sampathkumar, & Sudarshana, 2019).

Surface Sterilization of Explant

Explant surface sterilization is done before culture initiation to ensure a reduction of microbial contaminants. The first step is to wash the excised tissue thoroughly in running tap water, sometimes with a few drops of liquid washing detergent, to remove dust and other material that is not firmly attached. Then it is washed briefly in 70% ethanol (30s), which is enough to kill surface fungi & spores but not to cause damaging phytotoxic effects on delicate tissues. This is followed by either treatment with 0.1% mercuric chloride ($HgCl_2$) or 20-30% sodium hypochlorite (NaOCl) solution for 10-20 minutes, depending on the tender quality of the explant (George, Hall, & De Klerk, 2008). For papaya explant, the surface sterilization with NaOCl at 1% and $HgCl_2$ at 0.1% for 4 minutes was reported to produce the maximum survival percentage of 48.96% and 45.31%, respectively (HASAN, 2018).

Inoculation onto Callus Induction Medium

Sterilized explants are used to inoculate the solid Murashige and Skoog (MS) medium containing a suitable plant growth regulator (PGR) for callus induction. It was concluded that auxin–cytokinin interaction is important to trigger dedifferentiation and callus formation in Carica papaya. Several treatments that include different combinations of NAA, BAP (BA), and kinetin have been reported to successfully induce callus from different papaya explants. Culturing of petiole explants on MS medium containing NAA (0.5 – 10.5) μM and BA (0.5 – 5.0 μM) resulted in good formation of callus, and shoot regeneration was best achieved on MS medium with 2 μM BA and 0.1 μM NAA. In a similar way, stem segment explants were successfully induced to form callus on MS medium with 1.0 mg L^{-1} NAA and 0.1 mg L^{-1} kinetin (Mohammed & Yusuf, 2026). Another study showed that optimized combinations of growth regulators were important with MS medium containing 3.0 mg L-1 NAA, 0.5 mg L-1 kinetin, and 1.5 μM thidiazuron (TDZ), showing the highest callus induction frequency for leaf explants and the genotype (M. H. Chen, Wang, & Maeda, 1987).

Preparation of Cell Suspension Culture

After repeated subculturing, friable and fast-growing callus is used for the establishment of the cell suspension cultures. About 1-3 g of the friable callus is placed in the liquid culture medium, usually liquid MS, Gamborg's B5, or Nitsch and Nitsch medium, containing or not containing the same or similar concentrations as in the callus induction medium of

the growth regulators. Cultures are grown on Rotary Shakers at 100-120 rpm at a controlled temperature (25 ± 2°C). When aggregated cells are agitated, the cells break up, the cells aggregate, and individual cells are released to the culture media, creating a homogenous culture. During culture, cell aggregates grow, and they are transferred after a certain time to fresh culture liquid, inducing active growth of cultures again (Sambrook, 2012). Suspension cultures have been approved with success in somatic embryogenesis in *C. papaya* in which developing somatically derived embryos from embryogenic cells in callus cultures have better embryo maturation and regeneration than solid cultures. These cell suspension systems are useful to produce biomass and secondary metabolites on a large-scale, in genetic transformation studies, and to improve papaya biotechnologically (M. H. Chen et al., 1987).

Elicitors — Classification, Mechanisms, and Signaling Pathways

Elicitation is the use of biotic or abiotic agents to induce or stimulate the plant defence reactions and thus, increase the secondary metabolite biosynthesis and accumulation. Elicitors activate complex signaling networks, which mimic stress and induce the production of defense-related compounds (Huang, Yang, Hu, & Zhang, 2016).

Classification of Elicitors

Biotic Elicitors

Biotic elicitors are derived from living organisms, such as fungal extracts, yeast extract, bacterial polysaccharides, chitosan, and microbial cell wall fragments. These compounds simulate pathogen attack mechanisms and aid in triggering plant defense (Ramirez-Estrada et al., 2016).

Abiotic Elicitors

Abiotic factors are involved in the induction of stress-related metabolic response, which includes nanoparticles, ultraviolet radiation, heavy metals, salinity and temperature stress, jasmonic acid, and salicylic acid (Isah et al., 2024).

Endogenous Elicitors

Endogenous elicitors are plant signaling molecules that are produced in response to tissue damage or stress. These include peptide signals, or damage-associated molecular patterns (DAMPs), and oligogalacturonides (Boller & Felix, 2009).

Type	Examples
Biotic	chitosan, yeast extract, Fungal extracts (*Aspergillus*, *Fusarium*)
Abiotic	salicylic acid (SA), UV-B, heavy metals ($CuSO_4$, $AgNO_3$), jasmonic acid (JA)
Endogenous	Systemin, oligogalacturonides

Table 2: Classification of elicitors

Key Elicitors Used in Plant Cell Cultures

Methyl jasmonate is among the best elicited agents to increase alkaloid, phenolics, flavonoids, and terpenoids production by plant cell cultures. SA works as a signaling molecule in SAR and triggers the accumulation of phenolic compounds. Chitosan is known to trigger defense responses by receptor-mediated signaling and is one of the most employed compounds for increasing production of secondary metabolites (Bavi, Khavari-Nejad, Najafi, & Ghanati, 2022). Metabolite accumulations are stimulated in cultured cells, and defense-linked pathways of response are stimulated by yeast extract and fungal polysaccharides. The level of some heavy metal like Ag^{+}, $Cu^{2+,}$ and $Cd^{2+,}$ may serve as abiotic elicitors because they produce the oxidative stress signal (Apel & Hirt, 2004). Nanoparticles have proven to be strong elicitors with potential to boost secondary metabolite biosynthesis via ROS generation and signal transduction pathways (Bere et al., 2021).

Molecular Mechanism of Elicitor Action

Elicitor Recognition and Receptor Binding

When plant cells recognize an elicitor molecule as a stress or pathogen signal, the enhancement of Secondary metabolite production by the elicitor begins. Elicitors can be endogenous chemicals produced upon wounding of plants or chemical compounds or factors derived from microorganisms and the environment. There are certain receptors on the plasma membrane of the plant cell that recognize these molecules. During activation of the plant defense system, the interaction between elicitors and their receptor triggers a succession of intracellular signal transduction that leads to activation of the plant defense system (Boller & Felix, 2009).

ROS Burst and Signal Transduction

A primary response after elicitor detection is an increase in calcium ions (Ca^{2+}) in the cell's cytoplasm, along with the generation of reactive oxygen species (ROS) like hydrogen peroxide. The oxidative burst is an important signalling event to let the cell know that there are stress factors present. While excess ROS is harmful, the controlled production of ROS serves as a second messenger that carries defence signals throughout the cell (Apel & Hirt, 2004).

MAPK Cascade Activation

The ROS burst then triggers the activation of several protein kinases, such as mitogen-activated protein kinase (MAPK) cascades. These signaling proteins work by allowing the transfer of phosphate groups between proteins – this amplifies the elicitor signal. MAPK activation is mediated by a series of phosphorylation reactions, and the resulting MAPK activation influences gene expression in the nucleus (Eulgem & Somssich, 2007).

Transcription Factor Activation—MYC2 and WRKY

His activation of signaling pathways in the nucleus leads to the activation of transcription factors involved in the expression of MYC2, WRKY, MYB, and bHLH proteins. These transcription factors specifically bind to the promoter region of the

defense-related genes, and in turn regulate their expression (Eulgem & Somssich, 2007).

Activation of Biosynthetic Genes

This allows the expression of genes that encode the generation of some of the enzymes required to make secondary metabolites. Overexpression of biosynthetic genes leads to the overproduction of enzymes, which participate in the various biosynthetic pathways like phenylpropanoid biosynthesis, terpenoid biosynthesis, alkaloid biosynthesis, and flavonoid biosynthesis (Ashutosh Sharma & 2020). Therefore, plant cells synthesize and have a higher level of production of secondary metabolites such as flavonoids, terpenoids, alkaloids, phenolic compounds, and other defense-related molecules. Therefore, plant cells synthesize and have a higher level of production of secondary metabolites such as flavonoids, terpenoids, alkaloids, phenolic compounds, and other defense-related molecules (Ramirez-Estrada et al., 2016).

Hence, one can consider elicitation as a kind of stimulatory step on the plant defense chain, which involves elicitor perception, a subsequent activation of signal transduction pathways, transcription factors, and a final increase in the expression of biosynthetic genes for secondary metabolite production.

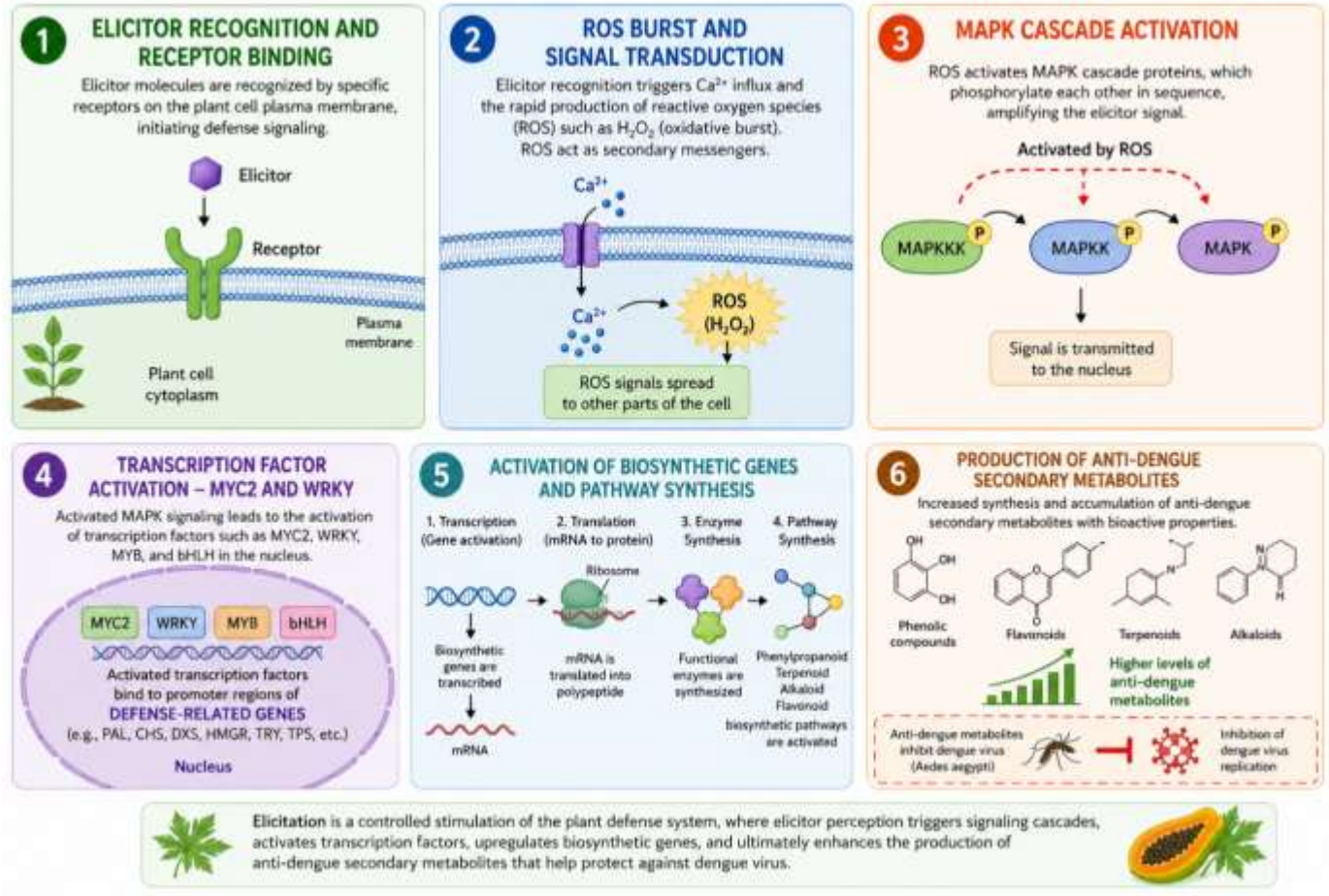

Figure 4: Signaling cascade and molecular mechanism of elicitor-induced defense responses in *Carica papaya* for anti-dengue metabolite production.

Hence, you can consider elicitation as a kind of stimulatory step on the plant defense chain, which involves elicitor perception, a subsequent activation of signal transduction pathways, transcription factors, and a final increase in the expression of biosynthetic genes for secondary metabolite production.

Factors Affecting Elicitor Efficiency

Elicitor Concentration

Elicitor concentrations have a great effect on metabolite accumulation, with high concentrations leading to growth inhibition or cell death (Ramirez-Estrada et al., 2016).

Duration and Timing of Elicitor Application

Physiological state of the culture, as well as exposure time, can dramatically impact elicitor responsiveness and metabolite production (Boller & Felix, 2009).

Synergistic Elicitation

When two or more elicitors are applied together, often synergistic increases of secondary metabolite yield are observed when compared to a single application.

Historical and Comparative Evidence of Elicitor Responses in Plant Cell Culture Systems

Methyl jasmonate (MeJA) among chemical elicitors has been consistently the most effective. The optimum concentration has also been found in callus cultures of Allium cepa in which a 13.9-fold increase in quercetin content was observed with 100 µM MeJA, while higher concentrations led to a decrease in the content (Mendoza, Cuaspud, Arias, Ruiz, & Arias, 2018). MeJA at 1.0mM increased the production of kaempferol by 6.8-fold after 120 hours in the context of Jatropha curcas, and MeJA 50µM in Linum album cell cultures resulted in higher amounts of quercetin (2-fold) and catechin (2.42-fold) after 48 hours (Zaragoza-Martinez et al., 2017). Salicylic acid (SA) has also been reported as a very potent inducing agent of the metabolites of the phenylpropanoid biosynthetic pathway. Within the Elicitors analyzed, the inductions with 50 mg/L SA for 96 hours cell suspension cultures of Phoenix dactylifera, and with 300 µM SA in the cell cultures of *Thevetia peruviana* have the highest content of total phenolics (317.9 ± 28.7 mg GAE/100 g DW), of caffeic acid (31.4 ± 3.8 µg/g DW) or kaempferol, respectively (13.6 ± 1.6 µg/g DW)(Mendoza et al., 2018).

As for biotic elicitors, the total flavonoid amounts of Isatis tinctoria hairy root cultures were increased by 7.08 fold in 150 mg/l of chitosan treatment after 36 hours, and in Zataria multiflora cell suspension culture, chitosan could increase the content of quercetin by 1.4-fold, gallic acid by 6.3-fold, and rutin by 1.4-fold, and enhance the enzyme activities of PAL and TAL (Zaragoza-Martinez et al., 2017) (Bavi et al., 2022). The eco-friendly B. (Arthrobacter) sp. CNP27, at 100-500 mg/L, was able to boost the content of phenylpropanoids by 2.5-fold and of flavonoids by 2.0-fold in E. purpurea cell suspension cultures after 48 h, and even at 50 mg/L, boosted the accumulation of flavonoids in *Limonium algarvense* callus culture by 3-fold (Goncharuk, Saibel, Zaitsev, & Zagoskina, 2022) (Lescano, Cziáky, Custódio, & Rodrigues, 2025). Further, treatments with MeJA (50 – 100µM), SA (50 – 300µM), chitosan (50 – 150mg/L), and yeast extract (50 – 500mg/L) to the cell culture of C. papaya in its exponential growth stage can be emphasized as potential applications for enhancing the accumulation of anti-dengue secondary metabolites.

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Statements and Declarations

Data Availability statement

All relevant data are within the manuscript file.

Author's Contribution Statement

MZS, MJA, MN, and GZJ collected data and wrote manuscript equally. KH, SARS, SM, NH, SY make final editing. All authors have read the final manuscript and approve its submission.

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Conflict of interest

The investigation was undertaken without any financial conflicts of interest or any other commercial relationships that could be seen as such by any of the authors.