



Potentially Enhanced Carpaine Extraction from *In Vitro* Samples Comparing to *In Vivo* Samples of *Carica papaya* L.: A Review

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ABSTRACT

In addition to being a fruit with diverse applications, *Carica papaya* L. is highly valued in the pharmaceutical industry for its potential as a source of medicinal compounds. It is also a commonly cultivated fruit in Malaysia. To meet the increasing demand for planting materials and to provide adequate resources for further research and development such as cell culture studies and the production of bioactive compounds, innovation in propagation and culture techniques is essential. Numerous studies on papaya tissue culture have demonstrated that somatic embryos can regenerate whole plants, indicating that papaya is a suitable candidate for somatic embryogenesis. An efficient papaya regeneration system has also been established to facilitate the extraction of bioactive compounds from both *in vivo* and *in vitro* sources. Therefore, it is crucial to develop an effective plant regeneration system for *Carica papaya* cultures prior to chemical extraction, as this ensures an adequate and sustainable supply of raw material. Moreover, the utilization of embryogenic cell suspension cultures holds great potential for the production of high-value compounds for pharmaceutical applications. Hence, the successful establishment of cell suspension cultures for the production of valuable secondary metabolites is both timely and essential, particularly for determining the optimal culture conditions that maximize the yield of bioactive compounds from plant cell cultures.

Keywords: Biotechnology, Compound, Secondary metabolite, Somatic embryogenesis, Tissue culture.

INTRODUCTION

In Malaysia, *Carica papaya* L. (*C. papaya*), commonly known as papaya or betik, is widely utilized for its edible fruits, nutritional benefits, and applications in traditional herbal medicine. *C. papaya* is an herbaceous plant with a soft stem that can reach up to 8 m in height under both natural and cultivated conditions. It is a large, short-

lived, woody herb with a basal diameter exceeding 30 cm and a height ranging from 5 to 30 cm (Boshra & Tajul, 2013). The species typically produces male, female, or hermaphroditic flowers throughout a flowering period of 9 to 12 months (Boshra & Tajul, 2013). Papayas are versatile fruits used both as desserts and as medicinal agents in the treatment of ailments such as cancer, cardiovascular diseases, and aging-related disorders (Lam et al., 2024; Sanghani et al., 2023; Soib et al., 2020). The papaya is extensively cultivated in Malaysia for both local consumption and export, serving as a vegetable, dessert fruit, and a source of medicinal compounds. Consequently, it represents a commercially and economically important fruit crop.

The Caricaceae family comprises four genera: *Jacaratia*, *Jarilla*, *Vasconcellea*, and *Carica papaya* L (Yogiraj et al., 2014). This species is widely distributed across tropical and subtropical regions, where it is a common and economically valuable fruit crop. Cultivation of the dioecious papaya typically begins with seedlings derived from open-pollinated flowers, resulting in a range of genotypes exhibiting variation in traits such as flavour, size, shape, and disease susceptibility (Rajan et al., 2025). *Carica papaya* is a major fruit crop in several Southeast Asian countries, including Thailand, Malaysia, Indonesia, and the Philippines (Nguyen, 2016). In Malaysia, papaya is extensively cultivated by small-scale farmers, particularly in the states of Johor, Perak, and Selangor.

Plant biotechnology enables the production of complex natural compounds through the use of plant cell cultures. These metabolites are valuable in daily life as fragrances, flavourings, and pharmaceuticals. Researchers are actively developing plant cell cultures capable of synthesizing such high-value compounds. The fermentation-based production of plant-derived chemicals is a well-established industrial process, and plant cells can similarly be cultivated in aerated suspension cultures (Anandan et al., 2012). The ability to regenerate and propagate plants from single cells, tissues, or organs under sterile and controlled environmental conditions known as *in vitro* plant cell and tissue culture provides an essential tool for research and production. *In vitro* cultures serve as model systems for investigating physiological, biochemical, genetic, and structural aspects of plant development (Saiman et al., 2012). Various types of explants can be utilized to induce *in vitro* regeneration of desired plant materials (Table 1).

Metabolic engineering of papaya for enhanced compound production should be conducted in parallel with conventional breeding efforts to incorporate chemical elicitors. This integrated approach offers a more direct and efficient route for achieving targeted biochemical modifications. A reliable, efficient, and reproducible plant regeneration system is a prerequisite for any successful enhancement platform (Solórzano-cascante et al., 2018).

Metabolic engineering can be used to modify biosynthetic pathways and improve the yield of specific compounds in papaya. For alkaloids, genes involved in carpaine biosynthesis can be upregulated to increase alkaloid concentrations in leaves or latex (Hoa Vien & Van Loc, 2017). The production of phenolic compounds can be enhanced by modulating key enzymes in the phenylpropanoid pathway, such as phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS). This approach promotes flavonoid biosynthesis and increases phenolic accumulation (Tokusoglu et al., 2003).

Similarly, terpenoid and carotenoid production can be improved by manipulating genes in the isoprenoid pathway. Genes such as phytoene synthase (PSY) and lycopene β -cyclase (LCYB) play important roles in increasing carotenoid accumulation in fruits (Julsing et al., 2006). The content of benzyl isothiocyanate (BITC) can also be enhanced through the regulation of glucosinolate degradation pathways, thereby increasing BITC levels in papaya seeds (Jiao et al., 2010). In addition, papain production may be increased by improving protein expression systems in plant tissues or cell cultures (Vij & Prashar, 2015).

These metabolic modifications can be achieved through various strategies, including gene editing, transcriptional regulation, and pathway reconstruction. Such approaches can be applied in whole plants as well as in vitro systems, including cell and tissue cultures (de Rosas et al., 2017).

Several studies have reported successful papaya regeneration through organogenesis and embryogenesis. For instance, Gurupkar et al., 2024; Homhuan et al., 2008; and Kabir et al., 2016 demonstrated that papaya exhibits high responsiveness to somatic embryogenesis from immature embryos, petioles, and hypocotyls.

Conventional papaya breeding, however, presents several challenges, including the difficulty of introducing and inducing specific elicitors within plants and the lengthy process required for compound selection and analysis. Non-traditional breeding and biotechnological approaches offer alternative methods for introducing desired metabolites into crop species. The development of an efficient enhancement protocol requires not only the proliferation of induced plant materials but also the generation of suitable target materials, such as somatic embryos (Mohamad Fhaizal et al., 2013). Effective plant regeneration from tissue cultures is therefore essential for the success of chemical enhancement technologies.

Establishing a robust plant regeneration system before implementing chemical augmentation strategies in papaya is essential for several reasons. First, it provides a controlled and reproducible biological platform, such as callus, shoots, roots, or somatic embryos, for studying secondary metabolism and elicitor responses under defined conditions (Gurupkar et al., 2024; Jimenez-Garcia et al., 2013). A well-established regeneration system also ensures tissue viability and responsiveness. Only healthy and physiologically competent tissues can effectively perceive and respond to elicitor treatments, thereby maximizing the potential for secondary metabolite production (Murthy et al., 2014; Thomas, 2013).

In addition, regeneration systems facilitate the selection of the most suitable developmental stage for elicitation. Different tissue types and growth stages often exhibit varying capacities for synthesizing and accumulating secondary metabolites. Consequently, identifying the optimal stage can improve the effectiveness of elicitation strategies (Murthy et al., 2014). Another important advantage is the promotion of genetic and metabolic stability. Uniform regenerated tissues reduce biological variability and enhance the reproducibility of experiments designed to evaluate elicitor-induced changes in metabolite production (Uddin, 2019).

A reliable regeneration system also supports large-scale metabolite production. Regenerated tissues can be propagated in suspension cultures or bioreactors, enabling the application of elicitor treatments on a commercial or industrial scale (Mengarda et al., 2018; Ramakrishna & Ravishankar, 2011). Furthermore, regenerated tissues provide a baseline metabolic profile prior to elicitation. This baseline serves as an important reference for quantifying and validating metabolic changes induced by chemical augmentation treatments (Armengaud et al., 2009).

Without a stable regeneration system, chemical elicitation would be biologically inconsistent, technically unreliable, and scientifically invalid (Solórzano-cascante et al., 2018). Previous studies on papaya regeneration through somatic embryogenesis and organogenesis support the feasibility of developing such systems. High frequencies of somatic embryogenesis have also been observed in other *Carica* species (Aluthge et al., 2021; Cabrera-ponce et al., 1996; Eeswara, 2014; Kabir et al., 2016; Rajesh et al., 2020).

Plant cell cultures are increasingly employed for the production of bioactive compounds that have diverse applications in daily life, including fragrances, flavourings, and pharmaceuticals. Researchers are actively developing plant cell culture systems capable of synthesizing high-value secondary metabolites. The industrial production of plant-derived compounds through fermentation is well established, and similarly, plant cells can be cultivated in suspension cultures maintained by continuous aeration.

The *in vitro* plant cell and tissue culture technique enables the regeneration and propagation of whole plants from single cells, tissues, or organs under sterile and controlled environmental conditions. *In vitro* cultures serve as valuable model systems for studying various physiological, biochemical, genetic, and structural aspects of plant biology. Furthermore, different types of starting plant materials (explants) can be utilized and manipulated through multiple approaches to induce *in vitro* regeneration of selected plant species (Table 1).

Table 1. Types of cultures used to promote *in vitro* regeneration of selected plant materials based on the source explant

No.	Types of Culture	Type of Starting Material	References
1.	Plant	Seedlings or mature plants: Whole plants or seedlings used as the initial source material for explant collection.	(Karyati et al., 2013)
2.	Embryo	Mature or immature embryos: Embryonic tissues serving as explants for the initiation of embryogenic cultures.	(Fransiska et al., 2025)
3.	Organ	Plant organs: Organs such as leaves, stems, roots, or buds used directly for organogenesis or callus induction.	(Gurupkar et al., 2024)
4.	Tissue or Callus	Tissues derived from plant organs: Callus or differentiated tissues obtained from organ explants and used for further culture or regeneration.	(Fhaizal et al., 2013)
5.	Suspension or Cell	Cultures of isolated cells or small cell aggregates: Dispersed single cells or small clusters maintained in liquid (suspension) media.	(Anandan et al., 2012)
6.	Protoplast	Protoplast cultures: Cultures of isolated cells with cell walls removed, used for somatic hybridization or genetic transformation.	(Shimizu et al., 2010)
7.	Another culture or Haploid	Anthers and/or immature pollen grains: Male gametophytic tissues cultured to obtain haploid cells or callus lines for haploid and doubled-haploid plant production.	(Balavijayalakshmi & Ramalakshmi, 2014)

PLANT TISSUE CULTURE

The initial cultured explant can undergo organogenesis, differentiation, or dedifferentiation by manipulating environmental conditions and plant growth regulators (Gurupkar et al., 2024). Plant tissue culture techniques hold great potential for the commercial vegetative propagation of economically important crops as well as species with future commercial value. Micropropagation supports the development of microcuttings from axillary buds of apically dominant plants, the induction of shoots from nodal segments, the separation of axillary shoots on rooting media, and the formation of small rooted plantlets (Chan et al., 2009).

Micropropagation is defined as the true-to-type propagation of a selected genotype through *in vitro* culture techniques and the regeneration of plants from organs, tissues, cells, or protoplasts. According to (Bindu & Podikunju, 2017; Mccubbin & Staden, 2003; Patel et al., 2013; Patil et al., 2007; Rajan et al., 2025; Setargie et al., 2015), *in vitro* plant propagation (Table 2) can be divided into distinct stages that describe not only the micropropagation process but also the morphogenic events occurring in the chosen explant or initial plant material under aseptic conditions.

Table 2. Stages of *in vitro* plant propagation

Stage	Description	Objective	Activity
0	Stock plant selection and adaptation	Reduction of endogenous tissue contaminants: Minimization or elimination of internal microbial contaminants to ensure aseptic culture conditions.	Definition of the stock or mother plant and preparation of explant sources: Identification and preparation of healthy, disease-free donor plants for the collection of explants.
I	Establishment	Establishment of aseptic <i>in vitro</i> cultures: Promotion of sterile growth environments to support explant survival and proliferation.	Explant selection, decontamination, and <i>in vitro</i> establishment: Selection of suitable explants, removal of exogenous contaminants, and adaptation of plant tissues to <i>in vitro</i> culture conditions.
II	Multiplication	Induction of somatic embryogenesis: Stimulation of embryogenic development from somatic cells for whole-plant regeneration. Enhancement of axillary bud development: Promotion of axillary meristem growth to facilitate shoot multiplication. Induction of adventitious shoot formation: Initiation of shoot organogenesis from non-meristematic tissues.	Induction of organogenesis or tissue differentiation: Promotion of <i>in vitro</i> cell and tissue organogenesis leading to the formation of new meristematic structures. Multiplication of shoots: Rapid proliferation and differentiation of multiple shoots from the established explants.
III	Rooting	Regeneration of <i>in vitro</i> plantlets: Development of complete plants from cultured tissues or organs under controlled conditions.	Induction of rhizogenesis: Stimulation of root formation in newly regenerated shoots under <i>in vitro</i> or <i>ex vitro</i> conditions.
IV	Acclimatisation	Stimulation of autotrophic metabolism: Enhancement of photosynthetic and metabolic competence in regenerating tissues.	Acclimatization (hardening): Gradual adaptation of vegetative structures to external environmental conditions.
V	Field	Improvement of normal morphogenesis in <i>in vitro</i> regenerants: Optimization of growth conditions to promote normal structure, physiology, and development of regenerated plants.	Transfer to open growth (regeneration): Growth and development of hardened plantlets under greenhouse or field conditions.

In addition, micropropagation is employed to support long-term germplasm conservation through cryopreservation and the maintenance of disease-free stock under controlled environmental conditions (Al-shara et al., 2018). In cases where conventional breeding has been unsuccessful, this form of *in vitro* vegetative propagation can be used to establish stable plant lines. Furthermore, it holds great potential for the large-scale propagation of economically important crops. The micropropagation of superior or selected plant genotypes has produced positive results, offering significant benefits to both horticulture and agriculture.

Currently, increasing attention is being directed toward the advancement of *in vitro* techniques that enable the multiplication and breeding of economically valuable food, industrial, and medicinal plants. However, the genetic integrity of plants regenerated *in vitro* can be compromised during clonal micropropagation, which is often used for germplasm preservation and expansion. As summarized in Table 3, the cellular and tissue

organization of the initial explant plays a critical role in determining the success of *in vitro* propagation and in minimizing the occurrence of somaclonal variation or genetic off-types.

Table 3. Cell and tissue organization influencing *in vitro* plant propagation

No.	Type of Tissue	Description	References
I	Organised	▪ Cultures consisting of nearly whole plants, embryos, seeds, pre-formed buds, and plant organs are referred to as organized cultures. ▪ The structural organization of individual cells, tissues, and organs is maintained throughout the culture process. ▪ These cultures closely resemble <i>in vivo</i> vegetative propagation methods such as cuttings, divisions, runners, or axillary bud and shoot formation. ▪ When the organizational structure remains intact, the progeny produced are genetically and morphologically identical to the original plant material.	(Miller & Drew, 1990)
II	Unorganised	▪ When cells and/or tissues are isolated from organized plant structures and induced to undergo dedifferentiation and active cell division, growth occurs in the form of callus tissue. ▪ When the callus is dispersed into small aggregates or single cells in a liquid medium, it forms a suspension culture, representing a non-organized tissue structure. ▪ Growth in such cultures is typically stimulated by high concentrations of auxins and/or cytokinins in the nutrient medium. ▪ Genetic stability in unorganized cultures is often low, increasing the likelihood of somaclonal variation.	(Abreu et al., 2014)
III	Combination of organized and unorganised (Intermediately Organized Cultures)	▪ These cultures are intermediate between Type I (organized) and Type II (unorganized) systems. Cells within an isolated organ or tissue first undergo dedifferentiation, forming layers of callus tissue through cell division, from which organs, pro-embryos, or embryos may rapidly develop. ▪ Organized structures can arise from previously non-organized cultures, either spontaneously or through the application of specific culture techniques. ▪ The progeny derived from intermediately organized cultures is often not completely identical to the original plant material, indicating partial genetic or epigenetic variation.	(Fransiska et al., 2025)

SECONDARY METABOLITES IN PLANTS

Plants naturally synthesize a wide range of primary and secondary metabolites that play essential roles in their growth and development, including self-defence, fertilization, and differentiation (Tugizimana et al., 2013). Primary metabolism encompasses all biochemical activities involved in the anabolic and catabolic pathways responsible for absorption, respiration, transport, and differentiation. These primary metabolic processes are universal among plant cells and are fundamental to life (Drew & Demain, 1977).

In contrast, secondary metabolites are structurally diverse compounds that, although not directly essential for growth and reproduction, play important ecological and physiological roles (Guaadaoui et al., 2014). They contribute to the colour, flavour, and fragrance of plants and serve as sources of valuable natural products, including pharmaceuticals, herbal medicines, pesticides, dyes, flavours, and perfumes. Unlike primary metabolism, which involves conserved pathways across all cells, secondary metabolism consists of distinct and specialized biosynthetic processes that vary among cell types and species (Anulika et al., 2016).

One major class of secondary metabolites is alkaloids. Historically, the biological functions of many secondary metabolites were poorly understood, and they were initially regarded as mere waste products. However, these compounds have been used in medicine for millennia, and today they continue to serve as vital therapeutic agents, either in their natural form or after chemical modification (Mazid et al., 2011; Patra et al., 2013; Turnbull et al., 2018). Plant secondary metabolites remain a valuable source of bioactive molecules for both academic and pharmaceutical research, particularly as lead compounds for novel drug discovery.

Among the most pharmacologically significant groups of secondary metabolites are the polyketides, which exhibit remarkable structural and functional diversity. Polyketide natural products display a wide spectrum of biological activities, including antibacterial, antifungal, antiparasitic, anticancer, and agrochemical properties (Hanson, 2003; Mohiuddin, 2019). Due to this broad range of bioactivities, polyketides are among the most economically, therapeutically, and industrially important natural products.

Polyketides are typically classified according to their chemical structure and differ substantially from primary metabolites such as sugars, amino acids, nucleotides, or chlorophyll (Mohiuddin, 2019). They are not directly required for fundamental plant growth processes such as photosynthesis, nutrient assimilation, respiration, or the biosynthesis of macromolecules (proteins, RNA, DNA). Over 50,000 distinct secondary metabolites have been identified in plants, each exhibiting unique structural characteristics and often restricted to specific taxa, plant organs, or developmental stages (Panche et al., 2016).

To distinguish naturally occurring compounds from those synthesized in laboratories, biologists often refer to these plant-derived substances as natural products. Although many secondary metabolites can be chemically synthesized, laboratory yields are often low due to the complexity of their biosynthetic pathways. These metabolites are produced by enzyme-mediated reactions within plant cells and can accumulate to high concentrations in specific tissues or cellular compartments (Cotton & Gibson, 1965; Pant et al., 2015; Ramanathan et al., 2014).

Alkaloids, for instance, constitute a diverse group of nitrogen-containing secondary metabolites, many of which are derived from amino acids, purine, or pyrimidine bases (pseudo-alkaloids) (Mohamad Bukhori et al., 2014). A notable example is piperidine, derived from the amino acid lysine, which serves as the structural core for various alkaloids characterized by a distinctive bitter taste. Alkaloids were among the first bioactive compounds to be isolated in pure form, primarily due to their salt-like nature in plants, as the presence of nitrogen renders them basic. Consequently, alkaloids are commonly extracted using water or weak acid solutions, followed by basification to recover the crystalline form (Tambe et al., 2021).

Although secondary metabolites are widespread, not all plants produce every type of metabolite. Certain compounds are restricted to specific species or plant families, and even within a plant, their occurrence is often limited to particular organs, tissues, or developmental stages (Yang et al., 2018). Many secondary metabolites also function as signalling molecules, such as plant hormones, which regulate metabolic processes, coordinate cellular activities, and orchestrate the overall growth and development of the plant (Pant et al., 2015).

PAPAYA AND ITS IMPORTANCE IN TISSUE CULTURE

Papaya is one of the most extensively cultivated tropical crops, particularly among smallholder farmers in Malaysia and other tropical and subtropical regions. Its high demand arises not only from its value as a fruit for

consumption but also from its use as a source of bioactive compounds with therapeutic potential, such as papain, chymopapain, and carpaine (Vij & Prashar, 2015). Traditionally, papaya planting materials are propagated through seeds, and cultivar improvement is achieved via conventional breeding. However, these methods often result in low productivity due to the use of genetically variable planting materials, suboptimal cultivation techniques, and inadequate management practices (Rajan et al., 2025). To overcome these limitations, various tissue culture techniques have been developed to enable micropropagation of superior genotypes.

Researchers have successfully documented plant regeneration in papaya from diverse explant sources through both organogenesis and somatic embryogenesis. Somatic embryogenesis in papaya has been reported from multiple tissues, including polyembryonic cultures, anthers, hypocotyls, roots, seeds, immature embryos, petioles, *in vitro* lamina and stems, integuments of immature seeds, and axillary buds (Table 1). Several studies have also demonstrated the successful use of papaya suspension cultures to induce somatic embryogenesis as part of efficient micropropagation systems (Table 2).

The success of papaya tissue culture largely depends on the composition of the culture medium and the exogenous application of plant growth regulators (PGRs). Commonly used PGRs include 2,4-Dichlorophenoxyacetic acid (2,4-D), 6-Benzylaminopurine (BAP), Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), Gibberellic Acid (GA₃), and Naphthaleneacetic Acid (NAA). The optimal type and concentration of these regulators, as well as the nutrient composition of the medium, vary depending on the cultivar and specific culture conditions. Proper optimization of these parameters is essential for successful *in vitro* regeneration (Bindu & Podikunju, 2017; Fitch & Manhardt, 1990; Chen et al., 1987; Yu et al., 2000).

An established papaya tissue culture system can serve as a reliable and sustainable source of biomass for the production and extraction of valuable secondary metabolites, particularly carpaine, which possesses notable pharmacological potential.

CARPAINE AS A BIOACTIVE SECONDARY METABOLITE IN *Carica papaya* L.

In metabolomics research, the identification and characterization of secondary metabolites across plant species are of great significance due to their diverse biological activities and pharmacological potential (Tugizimana et al., 2013). In this context, the secondary metabolites of *C. papaya* L. have attracted particular interest. Papaya is a native perennial species widely cultivated in tropical and subtropical regions worldwide. Among the various classes of phytochemicals, alkaloids are of special importance, with more than 5,000 structurally diverse members currently under investigation for their biological properties. These compounds classified as true alkaloids, protoalkaloids, and pseudoalkaloids have been studied since 1819, when W. Meissner first coined the term alkaloid (Burdick, 1971; Tokusoglu et al., 2003; Setyawati et al., 2016; Soib et al., 2020; Vien & Loc, 2017).

One such secondary metabolite is carpaine, a macrocyclic lactone alkaloid found predominantly in papaya leaves, but also present in the roots, seeds, bark, and fruit peel, in decreasing order of concentration (1000 to 1500 ppm) (Avinash Shinde & Hase, 2020; Rajarajeswari & Prathibha, 2018; Wang et al., 2015). Structurally, carpaine is a dimeric alkaloid comprising two identical piperidine rings bridged by two ester linkages and featuring a lactone moiety attached to the α -position. As described by (Govindachari et al., 1965), the molecule exhibits considerable conformational flexibility, with both piperidine rings capable of adopting stable chair conformations (Figure 1).

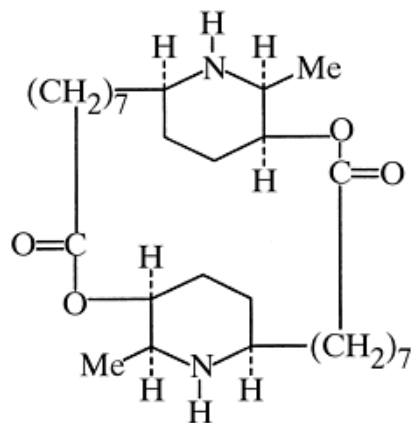


Figure 1. Chemical structure of carpaine (Vien & Loc, 2017).

Despite its pharmacological significance, the extraction of pure carpaine from papaya leaves is often challenging due to the co-extraction of several structurally related artifacts and impurities, including ψ -carpaine, ψ -carpamic acid, pseudocarpaine, novel carpaine-derived macrocyclic ethers, monoamides, and dehydrocarpaine I and II. Consequently, the yield of pure carpaine remains low (Lam et al., 2024; Patil & Kavimandan, 2024; Rajarajeswari & Prathibha, 2018; Vien & Loc, 2017). Various extraction methods have been investigated, including supercritical fluid extraction (SFE) (Mohamad Bukhori et al., 2014) and conventional liquid–liquid extraction techniques (Julianti et al., 2014; Rajarajeswari & Prathibha, 2018; Tak et al., 2024; Vien & Loc, 2017).

The extraction and analysis of metabolites from *C. papaya* have been conducted on several plant parts, including seeds, roots, and leaves, primarily from *in vivo* material. Among these, the leaves have consistently been reported as the richest source of carpaine, serving as the principal contributor to the plant's alkaloid content (Lam et al., 2024; Mohamad Bukhori et al., 2014; Patil & Kavimandan, 2024; Vien & Loc, 2017; Wang et al., 2015). However, the limited yield from *in vivo* sources suggests the potential of developing *in vitro* papaya explants as alternative and sustainable sources for carpaine production.

Papaya plants are known to produce several economically important biochemical compounds, including papain, chymopapain, and carpaine (Teixeira et al., 2007). Some of these compounds, such as carpaine, can also be synthesized in *in vitro* plant cell cultures, which serve as promising biotechnological platforms for metabolite production (Table 4). Carpaine, in particular, has been identified in papaya latex and leaves and exhibits a wide range of pharmacological activities. At a dosage of 2.0 mg/kg, carpaine has been reported to decrease cardiac output, stroke volume, and blood pressure, primarily by reducing heart rate. However, at higher concentrations, it may cause vasoconstriction. Furthermore, carpaine has been described as possessing diuretic, antihelminthic, and anti-amoebic properties, and may also inhibit the growth of *Mycobacterium tuberculosis* (Balavijayalakshmi & Ramalakshmi, 2014; Dubey et al., 2025; Radhakrishnan et al., 2017; Rajarajeswari & Prathibha, 2018).

The broad spectrum of carpaine's bioactivities highlights its significant industrial and pharmacological importance, with applications across multiple domains. In pharmaceutical and medical contexts, carpaine exhibits hypotensive and bradycardic effects, indicating its potential use in the management of hypertension and cardiac arrhythmias; additionally, its anti-inflammatory, antimicrobial, and antihelminthic properties support its application in natural therapeutic products and in the treatment of intestinal parasitic infections in both humans and animals (Balavijayalakshmi & Ramalakshmi, 2014). In the nutraceutical and herbal supplement industry, carpaine is incorporated into herbal teas and dietary formulations aimed at promoting general wellness, immune support, detoxification, and the improvement of cardiovascular, liver, and digestive health (Avinash Shinde & Hase, 2020). Furthermore, in veterinary applications, carpaine has been explored for use in livestock health management, where it may help combat infections and enhance gastrointestinal function, particularly within organic and natural animal husbandry practices (Zunjar et al., 2016).

Given these applications, the extraction of carpaine is of considerable value owing to its high market demand, therapeutic potential, and natural origin. As a bioactive alkaloid, carpaine represents a natural alternative to synthetic drugs for managing hypertension, inflammation, and parasitic infections (Balavijayalakshmi & Ramalakshmi, 2014; Rajarajeswari & Prathibha, 2018; Teng et al., 2019). The increasing global interest in plant-based medicines and functional foods further enhances the commercial viability of carpaine-rich products.

From an economic perspective, carpaine extraction from papaya leaves could significantly enhance the profitability of papaya cultivation by valorizing agricultural by-products. Moreover, from a sustainability standpoint, utilizing the whole plant for multiple purposes such as fruit production and metabolite extraction supports resource-efficient and eco-friendly agricultural practices.

Table 4. Pharmacological properties and significance of *Carica papaya* L. compounds

No.	Compounds	Chemical Class	Main Source (Plant Part)	Pharmacological Properties	Industrial/Therapeutic Significance	References
1.	Carpaine	Alkaloid (macrocyclic lactone)	Leaves, seeds, roots, bark, latex	Hypotensive, bradycardic, diuretic, antihelminthic, anti-amoebic, antimicrobial, anti-inflammatory, antiparasitic	Used in cardiovascular drugs; potential lead compound for antihypertensive and anti-parasitic formulations; utilized in nutraceutical and veterinary products	(Burdick, 1971)
2.	Papain	Proteolytic enzyme (cysteine protease)	Latex of unripe fruit	Proteolytic, anti-inflammatory, antioxidant, wound-healing, digestive aid	Widely used in pharmaceuticals, food industry, leather softening, and wound debridement preparations	(Avinash Shinde & Hase, 2020)
3.	Chymopapain	Proteolytic enzyme	Latex of unripe fruit	Anti-inflammatory, proteolytic, enzymatic tissue dissolving	Used in enzymatic treatment of herniated intervertebral discs; potential anti-inflammatory therapeutic	(Saran & Choudhary, 2013)
4.	Benzyl isothiocyanate	Isothiocyanate	Seeds, leaves	Anticancer, antibacterial, antifungal, antioxidant	Potential chemopreventive compound; applied in natural pesticide formulations and anticancer research	(Jiao et al., 2010b)
5.	Flavonoids	Polyphenolic compounds	Leaves, fruit	Antioxidant, anti-inflammatory, hepatoprotective, anticancer	Used in nutraceutical and functional food formulations; potential antioxidant supplements	(Nugroho et al., 2017)
6.	Phenolic acids	Phenolic compounds	Leaves, fruit peel	Antioxidant, antimicrobial, anti-aging	Contributes to skin care formulations, natural	(Sri Harini et al., 2016)

7.	Lycopene and β -carotene	Carotenoids	Fruit pulp	Antioxidant, anticancer, cardioprotective, provitamin A activity	preservatives, and nutraceutical products Used in dietary supplements, cosmetic formulations, and as natural food colorants	(Soib et al., 2020)
8.	α -amylase			Deleterious activity towards <i>Callosobruchus maculatus</i> .		(Agada et al., 2021)
9.	Lipase			Enantioselective biocatalyst, Biocatalyst		(Anjum et al., 2024)
10.	Pectin			Pectin methylesterase softening process.		(Onyeyirichi et al., 2015)
11.	Monoamides and diamide			Anticancer agents		(Dong et al., 2012)
12.	Choline			Stimulant action of acetylcholine on smooth muscle.		(Sudi et al., 2022)

EXTRACTION OF CARPAINE FROM *IN VITRO* AND *IN VIVO* SAMPLES OF *Carica papaya* L.

According to (Miller & Drew, 1990) and (Renukdas et al., 2003), the formulation of culture media is the most crucial factor for the successful initiation and maintenance of *in vitro* cultures derived from tissue explants of most plant species. It is well recognized that the composition of the culture medium significantly influences the outcome of *C. papaya* L. tissue culture and mass propagation (Chaudhary & Prakash, 2019). The course of *in vitro* morphogenesis is often determined by the use of a particular culture medium containing varying concentrations and types of plant growth regulators.

Several studies have reported the isolation of the alkaloid carpaine from the leaves of *C. papaya* L. varieties such as Uzbekistan, Nigerian, and Solo (Azad et al., 2012; Carlos et al., 2023; Nwachujor et al., 2019). However, the quantities of carpaine extracted from these varieties were relatively low, ranging from 0.025% to 0.4%, and the isolates were relatively impure, containing other compounds such as dehydrocarpaine I and dehydrocarpaine II (Mohamad Bukhori et al., 2014; Soib et al., 2020; Vien & Loc, 2017).

The major alkaloid isolated from *C. papaya* leaves is carpaine, along with a minor nitrogenous base compound, pseudocarpaine, which is a stereoisomer of carpaine. This stereoisomer, however, is present only in trace amounts (approximately 10%) (Govindachari & Narasimhan, 1965). Previous studies on carpaine isolation have yielded both low quantities and low purity. Therefore, it is pertinent to investigate whether the reduced yield of isolated carpaine results from limitations in the extraction and purification processes or from factors regulating secondary metabolism at multiple levels, including genetic, enzymatic, transport, and compartmentalization mechanisms.

Samples may be obtained from both *in vivo* and *in vitro* sources of *C. papaya* to determine the influence of material origin on carpaine yield. The methods described by (Burdick, 1971; Govindachari et al., 1965; Julianti et al., 2014; Mohamad Bukhori et al., 2014; Ramanathan et al., 2014) can be employed for the isolation of carpaine from *C. papaya* leaves, although the yields are generally low. Hence, modifications to the conventional acid-base extraction procedure may be undertaken to improve both purity and yield.

Alternatively, the supercritical fluid extraction (SFE) method may be utilized to compare its efficiency with that of traditional liquid-liquid extraction, with the aim of determining which method produces carpaine of higher purity and greater yield. Table 5 presents a comparison of carpaine quantities extracted from *C. papaya* leaves using these two different extraction techniques.

Table 5. Amount and percentage yield of carpaine extracted from *in vivo* and *in vitro* leaves of *Carica papaya* L. using two different extraction methods

Samples	Types of Extraction		References
	Acid/Base	Supercritical Fluid	
<i>In vivo</i> leaves	0.256 g (0.051%)	1.923 g (0.961%)	(Mohamad Bukhori et al., 2014)
	0.175 g (0.012%)		(Nwachujor et al., 2019)
<i>In vitro</i> leaves	0.003 g (0.012%)	0.016 g (0.32%)	(Mohamad Bukhori et al., 2014)

The yield of carpaine isolated using the acid/base extraction method from *in vivo* and *in vitro* leaves of *Carica papaya* L. was 0.256 g (0.051%) and 0.003 g (0.012%), respectively. In comparison, the yield obtained using the supercritical fluid extraction (SFE) method was 1.923 g (0.961%) from *in vivo* leaves and 0.016 g (0.32%) from *in vitro* leaves. These results indicate a significant improvement in extraction efficiency compared to previously reported yields for Nigerian and Solo varieties (0.175 g or 0.012%) (Azad et al., 2012; Carlos et al., 2023; Nwachujor et al., 2019). The yields achieved were approximately 1:5 and 1:50 times higher for the acid/base and SFE methods, respectively.

To justify those *in vitro* leaves yielding 0.003 g (0.012%) from 50 g of biomass are superior to *in vivo* leaves yielding 0.256 g (0.051%) from 500 g, several factors are considered, namely efficiency, productivity per biomass,

sustainability, and scalability. Although the absolute yield from *in vivo* leaves is greater, the *in vitro* system demonstrates higher relative yield efficiency when normalized per gram of biomass. Moreover, *in vitro* production offers sustainable and controllable conditions, enabling consistent compound synthesis independent of environmental fluctuations and providing a scalable platform for industrial metabolite production.

$$\begin{aligned} \text{In vitro: } 0.003 \text{ g from } 50 \text{ g} &= 0.00006 \text{ g per g of tissue} \\ \text{In vivo: } 0.256 \text{ g from } 500 \text{ g} &= 0.000512 \text{ g per g of tissue} \end{aligned}$$

Although *in vivo* samples exhibit higher absolute and relative yields of carpaine, this does not necessarily translate to superior overall productivity when other factors such as controlled and sustainable production systems, scalability, consistency, suitability for metabolic engineering and elicitation, and conservation of plant resources are considered. Furthermore, the use of younger leaves, as opposed to older leaves reported in previous studies, appears to enhance carpaine accumulation (Avinash Shinde & Hase, 2020; Rajarajeswari & Prathibha, 2018; Susilawati et al., 2021). This observation is consistent with the description of secondary metabolite formation processes provided by (Le Bot et al., 2009).

Naturally, *in vivo* plants produce a wide range of secondary metabolites, particularly in their roots, which often serve as the first line of defence against soil-borne pathogens and herbivores. The production and accumulation of defensive compounds in the roots, followed by their transport to aerial parts, represent an adaptive strategy for survival under biotic stress (Anulika et al., 2016). In contrast, *in vitro* cultures typically synthesize lower levels of secondary metabolites under aseptic conditions. However, upon exposure to contaminants or elicitors mimicking pathogen attack, these cultures may enhance metabolite biosynthesis as part of their induced defence response (Pant et al., 2015). This phenomenon supports the hypothesis that *in vitro* systems can be strategically manipulated to improve secondary metabolite yield through elicitation or metabolic engineering approaches.

At the intercellular level, plant organelles also contribute to secondary metabolite biosynthesis, including carpaine production (Mohamad Bukhori, 2003). During extraction, centrifugation is employed to remove solid particles, leading to plasmolysis of organelles and subsequent release of intracellular compounds into the medium, thereby increasing the amount of recoverable carpaine. In the isolation process, solvents such as ethanol, water, and acetic acid (v/v/v) are typically used for acid-base extraction, whereas SFE utilizes carbon dioxide (CO₂) as the mobile phase. Supercritical fluid extraction offers several advantages over conventional methods: extraction occurs at low temperatures, minimizing compound degradation; the absence of oxygen reduces oxidative losses; and since CO₂ is gaseous, no residual solvent remains in either the extract or raffinate (Mohamad Bukhori et al., 2014; Shams et al., 2015). Consequently, SFE minimizes compound loss during post-processing and purification, resulting in higher yield and purity of the extracted carpaine (Table 5).

CONCLUSION

The establishment of an effective regeneration method for *Carica papaya* is crucial for the extraction of desired bioactive compounds through tissue culture. Reliable and efficient plant regeneration protocols are a prerequisite for the successful development of compound enhancement systems from initiated cells. Prior to implementing such enhancement strategies, robust tissue culture procedures for regeneration must first be optimized for the target crop. Although *in vivo* systems typically yield a higher absolute quantity of compounds, *in vitro* systems offer a more sustainable, controllable, and scalable platform. They enable consistent production independent of environmental fluctuations and are highly amenable to metabolic engineering and elicitation approaches. Despite the comparatively lower yield percentages, *in vitro* systems require less biomass input and can be adapted for continuous production, thereby representing a more efficient and promising long-term strategy for the sustainable biosynthesis of high-value secondary metabolites.

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REFERENCES

- Abreu, I. S., Carvalho, C. R., & Clarindo, W. R. (2014). Massal induction of *Carica papaya* L. golden somatic embryos and somaclone screening by flow cytometry and cytogenetic analysis. *Cytologia*, 79(4), 475–484.
- Agada, R., Thagriki, D., Esther Lydia, D., Khusro, A., Alkahtani, J., Al Shaqha, M. M., Alwahibi, M. S., & Soliman Elshikh, M. (2021). Antioxidant and anti-diabetic activities of bioactive fractions of *Carica papaya* seeds extract. *Journal of King Saud University – Science*, 33(2), 101342.
- Al-Shara, B., Mat Taha, R., & Rashid, K. (2018). Biotechnological methods and limitations of micropropagation in *Carica papaya* L. production: A review. *The Journal of Animal & Plant Sciences*, 28(5), 1208–1226.
- Aluthge, A. D. R. P., Nagahawaththa, S. M. S., Basnayake, B. M. V. S., Hettiarachchi, C., & Senanayake, D. M. J. B. (2021). Regeneration of *Carica papaya* “Rathna” variety through somatic embryogenesis from immature zygotic embryos. *Asian Journal of Research and Review in Agriculture*, 3(1), 185–192.
- Anandan, R., Sudhakar, D., Balasubramanian, P., & Gutierrez-Mora, A. (2012). *In vitro* somatic embryogenesis from suspension cultures of *Carica papaya* L. *Scientia Horticulturae*, 136, 43–49.
- Anjum, S., Ali, H., Naseer, F., Abduh, M. S., Qadir, H., Kakar, S., Waheed, Y., & Ahmad, T. (2024). Antioxidant activity of *Carica papaya* and *Persea americana* fruits against cadmium-induced neurotoxicity, nephrotoxicity, and hepatotoxicity in rats with a computational approach. *Journal of Trace Elements in Medicine and Biology*, 81, 127324.
- Anulika, N. P., Ignatius, E. O., Raymond, E. S., Osasere, O.-I., & Abiola, A. H. (2016). The chemistry of natural product: Plant secondary metabolites. *International Journal of Technology Enhancements and Emerging Engineering Research*, 4(8), 1.
- Armengaud, P., Sulpice, R., Miller, A. J., Stitt, M., Amtmann, A., & Gibon, Y. (2009). Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in *Arabidopsis* roots. *Plant Physiology*, 150(2), 772–785.
- Avinash Shinde, A., & Hase, D. (2020). Isolation of carpaine from *Carica papaya* leaves by using LCMS. *Journal of Medicinal Plants Studies*, 8(4), 1–5.
- Azad, A. K., Rabbani, G., & Amin, L. (2012). Plant regeneration and somatic embryogenesis from immature embryos derived through interspecific hybridization among different *Carica* species. *International Journal of Molecular Sciences*, 13, 17065–17076.
- Balavijayalakshmi, J., & Ramalakshmi, V. (2014). Contractile responses of the uterus in isometric tension induced by oxytocin, CaCl₂ and depolarizing solution. *RSC Advances*, 4(2), 3248300.
- Bindu, B., & Podikunju, B. (2017). Tissue culture protocol for *in vitro* propagation of papaya (*Carica papaya* L.). *Journal of Krishi Vigyan*, 6(1), 205–212.
- Boshra, V., & Tajul, A. Y. (2013). Papaya: An innovative raw material for food and pharmaceutical processing industry. *Health and the Environment Journal*, 4(1), 68–75.

- Burdick, E. M. (1971). Carpaine: An alkaloid of *Carica papaya*: Its chemistry and pharmacology. *Economic Botany*, 25(4), 363–365.
- Cabrera-Ponce, J. L., Vegas-Garcia, A., & Herrera-Estrella, L. (1996). Regeneration of transgenic papaya plants via somatic embryogenesis. *In Vitro Cellular and Developmental Biology – Plant*, 32, 86–90.
- Carlos, L., Granero, M. L., & Regalado, J. (2023). *In vitro* long-term cultures of papaya (*Carica papaya* L. cv. Solo). *Horticulturae*, 9, 1–17.
- Chan, L. K., Keng, C. L., Yee, L. S., & Pin, P. L. (2009). Micropropagation of *Gynura procumbens* (Lour.) Merr., an important medicinal plant. *Journal of Medicinal Plants Research*, 3(3), 105–111.
- Chaudhary, K., & Prakash, J. (2019). Effect of 2,4-D and picloram on somatic embryogenesis in *Carica papaya* var. P-7-9. *Plant Tissue Culture & Biotechnology*, 29(1), 25–32.
- Chen, M. H., Wang, P. J., & Maeda, E. (1987). Somatic embryogenesis and plant regeneration in *Carica papaya* L. tissue culture derived from root explants. *Plant Cell Reports*, 6, 348–351.
- Cotton, R. G. H., & Gibson, F. (1965). The biosynthesis of phenylalanine and tyrosine: Enzymes converting chorismic acid into prephenic acid and their relationships to prephenate dehydratase and prephenate dehydrogenase. *Biochimica et Biophysica Acta*, 100(1), 76–88.
- de Rosas, I., Ponce, M. T., Malovini, E., Deis, L., Cavagnaro, B., & Cavagnaro, P. (2017). Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of Malbec and Bonarda grown under high temperature conditions. *Plant Science*, 258, 137–145.
- Dong, Y., Nakagawa-Goto, K., Lai, C. Y., Morris-Natschke, S. L., Bastow, K. F., Kim, Y., Lee, E. Y. H. P., & Lee, K. H. (2012). Antitumor agents 289. Design, synthesis, and anti-breast cancer activity in vivo of 4-amino-2H-benzo[h]chromen-2-one (ABO) and 4-amino-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (ATBO) analogues with improved water solubility. *Journal of Natural Products*, 75(3), 370–377.
- Drew, S. W., & Demain, A. L. (1977). Effect of primary metabolites on secondary metabolism. *Annual Review of Microbiology*, 31, 343–356.
- Dubey, A., Samanthula, K. S., Dash, S. S., & Anjali, A. (2025). Role of *Carica papaya* in thrombocytopenia. *Journal of Natural Remedies*, 25(4), 758–770.
- Eeswara, J. (2014). Somatic embryogenesis in papaya (*Carica papaya* L.) cv. Rathna. *Journal of the National Science Foundation of Sri Lanka*, 36(1), 41–50.
- Fitch, M. M. M., & Manshardt, R. M. (1990). Somatic embryogenesis and plant regeneration from immature zygotic embryos of papaya (*Carica papaya* L.). *Plant Cell Reports*, 9, 320–324.
- Fransiska, Efendi, D., & Purwoko, B. S. (2025). Induction of embryogenic callus and somatic embryo from anther culture in papaya (*Carica papaya* L.). *Journal of Breeding and Genetics*, 57(2), 529–540.
- Govindachari, T. R., Nagarajan, K., & Viswanathan, N. (1965). Carpaine and pseudocarpaine. *Tetrahedron Letters*, 24, 1907–1916.
- Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., & Hamal, A. (2014). What is a bioactive compound? A combined definition for a preliminary consensus. *International Journal of Nutrition and Food Sciences*, 3(3), 174–179.

- Gurupkar, V., Sidhu, S., Nanaso, P., Sukhjinder, G., Mann, S., & Singh, M. (2024). Optimized protocol for high-frequency papaya propagation: Morpho-stereomicroscopic analysis and genetic fidelity assessment. *Plant Cell, Tissue and Organ Culture*, 156(81), 1–27.
- Hanson, J. R. (2003). Secondary metabolites: An introduction to natural products chemistry. In E. W. Abel (Ed.), *Natural products: The secondary metabolites*. Royal Society of Chemistry.
- Hoa Vien, D. T., & Van Loc, T. (2017). Extraction and quantification of carpaine from *Carica papaya* leaves of Vietnam. *International Journal of Environment, Agriculture and Biotechnology*, 2(5), 2394–2397.
- Homhuan, S., Kijwijan, B., Wangsomnuk, P., & Bodhipadma, K. (2008). Variation of plants derived from indirect somatic embryogenesis in cotyledon explants of papaya. *ScienceAsia*, 34, 347–352.
- Jiao, Z., Deng, J., Li, G., Zhang, Z., & Cai, Z. (2010). Study on the compositional differences between transgenic and non-transgenic papaya (*Carica papaya* L.). *Journal of Food Composition and Analysis*, 23(6), 640–647.
- Jimenez-Garcia, S. N., Vazquez-Cruz, M. A., Guevara-Gonzalez, R. G., Torres-Pacheco, I., Cruz-Hernandez, A., & Feregrino-Perez, A. A. (2013). Current approaches for enhanced expression of secondary metabolites as bioactive compounds in plants for agronomic and human health purposes: A review. *Polish Journal of Food and Nutrition Sciences*, 63(2), 67–78.
- Julianti, T., Oufir, M., & Hamburger, M. (2014). Quantification of the antiplasmodial alkaloid carpaine in papaya (*Carica papaya*) leaves. *Planta Medica*, 80(13), 1138–1142.
- Julsing, M. K., Koulman, A., Woerdenbag, H. J., Quax, W. J., & Kayser, O. (2006). Combinatorial biosynthesis of medicinal plant secondary metabolites. *Biomolecular Engineering*, 23, 265–279.
- Kabir, M. H., Rahman, M. Z., & N, K. M. A. (2016). Somatic embryogenesis and plant regeneration from zygotic embryo in *Carica papaya* L. cv. Red-Lady. *Plant*, 4(6), 45–50.
- Karyati, Ipor, I. B., & Ismail, J. (2013). Composition and diversity of plant seedlings and saplings at early secondary succession of fallow lands in Sabal, Sarawak. *Acta Biologica Malaysiana*, 2(2), 85–94.
- Lam, T. Q., Tran, A. T. Q., Le Anh Phan, T., Zitzmann, F., Van Ho Phan, N., & Nguyen, K. T. (2024). Enhancing extraction efficiency of carpaine in *Carica papaya* L. leaves: Coupling acid-base extraction with surfactant-assisted micro-flotation. *RSC Advances*, 14(39), 28768–28778.
- Le Bot, J., Bénard, C., Robin, C., Bourgaud, F., & Adamowicz, S. (2009). The “trade-off” between synthesis of primary and secondary compounds in young tomato leaves is altered by nitrate nutrition: Experimental evidence and model consistency. *Journal of Experimental Botany*, 60(15), 4301–4314.
- Mazid, M., Khan, T. A., & Mohammad, F. (2011). Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, 3(2), 232–249.
- Mccubbin, M. J., & van Staden, J. (2003). A modified technique for *in vitro* propagation of papaya (*Carica papaya* L.). *South African Journal of Botany*, 69(3), 287–291.
- Mengarda, L. H. G., Lopes, J. C., Alexandre, R. S., & Otoni, W. C. (2018). Induction of somatic embryogenesis in hermaphrodite papaya from cotyledon leaves of *in vitro* seedlings or adult plant-derived explants. *Fruits*, 73(4), 209–217.

- Miller, R. M., & Drew, R. A. (1990). Effect of explant type on proliferation of *Carica papaya* L. *in vitro*. *Plant Cell, Tissue and Organ Culture*, 21, 39–44.
- Mohamad Bukhori, M. F. (2003). *A study on cell suspension cultures for micropropagation and the production of carpaine from papaya (Carica papaya L. var. Eksotika)* [Master's thesis]. University of Malaya.
- Mohamad Bukhori, M. F., Abdul Rahman, N., Khalid, N., Rashid, A. H., & Mohd Diah, M. (2014). The supercritical fluid extraction of alkaloids from papaya (*Carica papaya* L. var. Eksotika) leaves. *Borneo Journal of Resource Science and Technology*, 4(2), 35–49.
- Mohamad Fhaizal, M. B., Jin, S., Pillai, V., & Rahman, N. A. (2013). Improved protocol for high frequency plant regeneration through somatic embryogenesis in *Carica papaya*. *Research in Biotechnology*, 4(5), 9–19.
- Mohiuddin, A. K. (2019). Chemistry of secondary metabolites. *Annals of Clinical Toxicology*, 2(1), 1014.
- Murthy, H. N., Lee, E. J., & Paek, K. Y. (2014). Production of secondary metabolites from cell and organ cultures: Strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell, Tissue and Organ Culture*, 118, 1–16.
- Nguyen, T. T. T. (2016). *Investigation of bioactive compounds with anti-cancer potential in Carica papaya leaves* [Doctoral dissertation]. The University of Queensland.
- Nugroho, A., Heryani, H., Choi, J. S., & Park, H. J. (2017). Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity. *Asian Pacific Journal of Tropical Biomedicine*, 7(3), 208–213.
- Nwaehujor, C. O., Ode, J. O., Ekwere, M. R., & Udegbonam, R. I. (2019). Anti-fertility effects of fractions from *Carica papaya* (pawpaw) Linn. methanol root extract in male Wistar rats. *Arabian Journal of Chemistry*, 12(7), 1563–1568.
- Onyeyirichi, I., Mohammed, G., Bala, M. M., Bala, S., & Aliyu, N. (2015). Chemical composition of chloroform extract of *Carica papaya* seed oil. *International Journal of Modern Biochemistry*, 4(1), 15–22.
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5, 1–15.
- Pant, B. D., Pant, P., Erban, A., Huhman, D., Kopka, J., & Scheible, W. R. (2015). Identification of primary and secondary metabolites with phosphorus status-dependent abundance in *Arabidopsis*, and of the transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation. *Plant, Cell & Environment*, 38, 172–187.
- Patel, J. R., Patel, R. M., Patel, S. R., & Singh, V. (2013). *In vitro* micropropagation of papaya var. Red Lady. *International Journal of Plant Protection*, 6(2), 449–456.
- Patil, P., Vastrad, N., Dinesh, M. R., & Bantwal, A. R. (2007). A revised protocol for *in vitro* propagation of *Carica papaya* using lateral buds from field-grown trees. *Journal of Horticultural Science*, 2(2), 99–103.
- Patil, S., & Kavimandan, B. (2024). A review on isolation and purification of carpaine from *Carica papaya* L. leaves. *African Journal of Biomedical Research*, 27(3), 4759–4763.
- Patra, B., Schluttenhofer, C., Wu, Y., Pattanaik, S., & Yuan, L. (2013). Transcriptional regulation of secondary metabolite biosynthesis in plants. *Biochimica et Biophysica Acta*, 1829(11), 1236–1247.

- Radhakrishnan, N., Lam, K. W., & Norhaizan, M. E. (2017). Molecular docking analysis of *Carica papaya* Linn constituents as antiviral agent. *International Food Research Journal*, 24(4), 1819–1825.
- Rajan, P., Kang, J. H., Natraj, P., Lim, C. K., Kim, H. B., & Kim, S. C. (2025). Advances, challenges, and future prospects of *in vitro* propagation in papaya (*Carica papaya* L.). *Plant Cell, Tissue and Organ Culture*, 161(36), 1–14.
- Rajarajeswari, K., & Prathibha, D. (2018). *Carica papaya* leaf extract: Potential therapeutic effects—A review. *International Journal of Allied Medicinal Sciences and Clinical Research*, 6(2).
- Rajesh, C. K., Sudhakar, D., Kumar, K. K., Kavitha, C., Karthikeyan, G., & Soorianathasundaram, K. (2020). Somatic embryogenesis in papaya (*Carica papaya* L. cv. TNAU Papaya CO.8). *Current Journal of Applied Science and Technology*, 39(3), 18–26.
- Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, 6(11), 1720–1731.
- Ramanathan, S., Parthasarathy, S., Murugaiyah, V., Magosso, E., Tan, S. C., & Mansor, S. (2014). Understanding the physicochemical properties of mitragynine, a principal alkaloid of *Mitragyna speciosa*, for preclinical evaluation. *Molecules*, 19(4), 4915–4927.
- Renukdas, N., Mohan, M. L., Khuspe, S. S., & Rawal, S. K. (2003). Influence of boron on somatic embryogenesis in papaya. *Biologia Plantarum*, 47(1), 129–132.
- Saiman, M. Z., Mustafa, N. R., Schulte, A. E., Verpoorte, R., & Choi, Y. H. (2012). Induction, characterization, and NMR-based metabolic profiling of adventitious root cultures from leaf explants of *Gynura procumbens*. *Plant Cell, Tissue and Organ Culture*, 109, 465–475.
- Sanghani, H. V., Shirke, S. D., & Kurne, P. U. (2023). *In silico* studies on phytochemical carpaine in papaya leaves. *IJARIIIE*, 9(4), 2879–2885.
- Saran, P. L., & Choudhary, R. (2013). Drug bioavailability and traditional medicaments of commercially available papaya: A review. *African Journal of Agricultural Research*, 8(25), 3216–3223.
- Setargie, A., Mekbib, F., & Abraha, E. (2015). *In vitro* propagation of papaya (*Carica papaya* L.). *World Journal of Agricultural Sciences*, 11(2), 84–88.
- Setyawati, D., Andayani, S., & Yanuhar, U. (2016). Characterization of fraction of *Carica papaya* L. leaves ethyl acetate extract to African catfish *Clarias gariepinus* leucocytes using UV-Vis, FTIR and GC-MS methods. *International Journal of ChemTech Research*, 9(9), 247–253.
- Shams, K. A., Abdel-Azim, N. S., Tawfik, W. A., Hassanein, H. D., Saleh, M. A., & Hammouda, F. M. (2015). Green extraction techniques: Effect of extraction method on lipid contents of three medicinal plants of Apiaceae. *Journal of Chemical and Pharmaceutical Research*, 7(4), 1080–1088.
- Shimizu, Y., Maeda, K., Kato, M., & Shimomura, K. (2010). Isolation of anthocyanin-related MYB gene, GbMYB2, from *Gynura bicolor* leaves. *Plant Biotechnology*, 27, 481–487.
- Soib, H. H., Ismail, H. F., Husin, F., Abu Bakar, M. H., Yaakob, H., & Sarmidi, M. R. (2020). Bioassay-guided different extraction techniques of *Carica papaya* (Linn.) leaves on *in vitro* wound-healing activities. *Molecules*, 25(3), 1–13.

- Solórzano-Cascante, P., Sánchez-Chiang, N., & Jiménez, V. M. (2018). Explant type, culture system, and encapsulation affect indirect somatic embryogenesis and regeneration in *Carica papaya* L. *Frontiers in Plant Science*, 9, 1–12.
- Sri Harini, R., Senthilkumar, N., Saivikashini, A., Keerthana, G., Kumaresan, K., & Murugesan, S. (2016). Profiling metabolites of *Carica papaya* Linn. variety CO7 through GC-MS analysis. *Journal of Pharmacognosy and Phytochemistry*, 5(2), 200–203.
- Sudi, S., Chin, Y. Z., Wasli, N. S., Fong, S. Y., Shimmi, S. C., How, S. E., & Sunggip, C. (2022). Carpaine promotes proliferation and repair of H9c2 cardiomyocytes after oxidative insults. *Pharmaceuticals*, 15(2), 1–14.
- Susilawati, Y., Saptarini, N. M., Ramadhania, Z. M., Halimah, E., & Ulfah, N. N. (2021). Isolation and determination of carpaine alkaloid in papaya (*Carica papaya* L.) leaf extract by thin-layer chromatography scanner. *International Journal of Applied Pharmaceutics*, 13(4), 234–238.
- Tak, S. T., Zamakshshari, N. H., Bukhori, M. F. M., Madihlagan, E., Wahi, R., & Anchah, L. (2024). Acidified water extraction increases carpaine yield from *Carica papaya* L. leaf. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 32(4), 231–244.
- Tambe, V. S., Waichal, D. D., & Chanshetty, R. R. (2021). Bioactivity-enhanced isolated carpaine from *Carica papaya* leaves for platelet stimulating activity. *Indian Journal of Pharmaceutical Sciences*, 83(4), 723–731.
- Teixeira da Silva, J. A., Rashid, Z., Tan Nhut, D., Sivakumar, D., Gera, A., Teixeira Souza Jr., M., & Tennant, P. F. (2007). Papaya (*Carica papaya* L.): Biology and biotechnology. *Tree and Forestry Science and Biotechnology*, 1(1), 47–73.
- Teng, W. C., Chan, W., Suwanarusk, R., Ong, A., Ho, H. K., Russell, B., Rénia, L., & Koh, H. L. (2019). *In vitro* antimalarial evaluations and cytotoxicity investigations of *Carica papaya* leaves and carpaine. *Natural Product Communications*, 14(1), 33–36.
- Thomas, H. (2013). Senescence, ageing and death of the whole plant. *New Phytologist*, 197(3), 696–711.
- Tokusoglu, O., Unal, M. K., & Yildirim, Z. (2003). HPLC–UV and GC–MS characterization of the flavonol aglycons quercetin, kaempferol, and myricetin in tomato pastes and other tomato-based products. *Acta Chromatographica*, 13, 1–12.
- Tugizimana, F., Piater, L., & Dubery, I. (2013). Plant metabolomics: A new frontier in phytochemical analysis. *South African Journal of Science*, 109, 1–11.
- Turnbull, J. M., Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 1–26.
- Uddin, M. (2019). Environmental factors on secondary metabolism of medicinal plants. *Research and Advances in Pharmacy and Life Sciences*, 1(2), 1–19.
- Vij, T., & Prashar, Y. (2015). A review on medicinal properties of *Carica papaya* Linn. *Asian Pacific Journal of Tropical Disease*, 5(1), 1–6.
- Wang, X., Hu, C., Ai, Q., Chen, Y., Wang, Z., & Ou, S. (2015). Isolation and identification of carpaine in *Carica papaya* L. leaf by HPLC-UV method. *International Journal of Food Properties*, 18(7), 1505–1512.

- Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 762.
- Yogiraj, V., Goyal, P. K., Chauhan, C. S., Goyal, A., Vyas, B., Goyal, K., & Chauhan, S. (2014). *Carica papaya* Linn: An overview. *International Journal of Herbal Medicine*, 2(5), 1–8.
- Yu, T., Yeh, S., Cheng, Y., & Yang, J. (2000). Efficient rooting for establishment of papaya plantlets by micropropagation. *Plant Cell, Tissue and Organ Culture*, 61, 29–35.
- Zunjar, V., Dash, R. P., Jivrajani, M., Trivedi, B., & Nivsarkar, M. (2016). Antithrombocytopenic activity of carpaine and alkaloidal extract of *Carica papaya* Linn. leaves in busulfan induced thrombocytopenic Wistar rats. *Journal of Ethnopharmacology*, 181(2016), 20-25.

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