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REVIEW

Annona deceptrix as a potential biofactory for secondary metabolites using plant cell and tissue cultures

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Abstract

Plant biotechnology is a powerful tool that has enabled the transformation of plant cells into small-scale biofactories to produce secondary metabolites. These compounds can be synthesized in laboratory settings on a large scale, independent of spatial, resource, or environmental constraints. Identifying plant species with promising phytochemical profiles is crucial to obtaining bioactive products with high market demand and commercial value. The purpose of the present review is to highlight the potential of *Annona deceptrix* as a biotechnological resource, based on findings from previous studies conducted on other *Annona* species. Despite belonging to a botanical family with a well-established track record in the production of characteristic and biologically active secondary metabolites—particularly those with agrochemical relevance—*A. deceptrix* remains underutilized and understudied. Exploring its biotechnological potential is essential, as establishing this species *in vitro* would allow the development of callus production protocols, characterization of its cell growth kinetics, and the subsequent extraction of high-quality bioactive compounds. These extracts could serve as innovative solutions to challenges across various industries, ultimately leading to the development of marketable final products.

Keywords: Annonaceae, bioactive compounds, callus cultures, cell suspension cultures, biofactory.

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1. Introduction

Secondary metabolites are plant-derived compounds synthesized in response to biotic and abiotic stress, functioning as key components of the plant's adaptive defense mechanisms (Upadhyay et al., 2025). These structurally complex natural products are widely utilized in cosmetics, pharmaceutical, fragrance, flavoring, and natural colorant industries (Joshi et al., 2024).

In vitro plant tissue culture techniques, particularly callus and cell suspension cultures, offer a sustainable and renewable alternative to produce these high-value metabolites (Gunaseelan et al., 2025). Compared to harvesting wild plant materials, callus cultures are more reliable and controllable for consistent metabolite isolation (Nordine, 2025). Commercial-scale production of phytochemicals through these systems represents an attractive solution to meet increasing demand from consumers seeking natural alternatives to synthetic compounds (Anuradha et al., 2025).

Within this context, species of the genus *Annona* have gained attention as rich sources of nutrients and secondary metabolites (Zubaidi et al., 2023). These include acetogenins (characteristic of the Annonaceae family), alkaloids, phenolic compounds, essential oils, cyclopeptides, carotenoids, amino acids, anthocyanins, vitamins, and minerals (Joseph et al., 2023). Such compounds have been extracted

from various plant parts, including roots, leaves, bark, seeds, peel, and fruit pulp (Corrêa de Souza et al., 2025) and have demonstrated a wide range of biological activities, such as antiparasitic, insecticidal, herbicidal, and therapeutic effects (Dey et al., 2024; Rangel et al., 2024).

Despite this potential, *Annona deceptrix* (Westra) H. Rainer remains an underutilized and endangered plant genetic resource. To date, only one study has reported on its phytochemical composition and antioxidant capacity (Pinoargote-Chang et al., 2025), and no data is available on the biological activity of its individual constituents. As a species adapted to challenging environmental conditions, *A. deceptrix* has evolved the ability to synthesize a variety of secondary metabolites, making it a promising candidate for *in vitro* biotechnological applications. Cultivating this species *in vitro* not only offers a method to exploit its chemical potential sustainably but also contributes to its conservation. Furthermore, as a member of the Annonaceae family, it holds the promise of yielding novel bioactive compounds of interest to science and industry.

The main objective of this review is twofold: first, to consolidate the existing studies on *Annona deceptrix*; and second, to compile and analyze reports on cell and tissue culture techniques applied to other *Annona* species, which may serve as references for developing *in vitro* systems aimed at secondary metabolite production in *A. deceptrix*.

2. Plant morphology, taxonomy, distribution, and genetic diversity of *Annona deceptrix* (Westra) H. Rainer

Annona deceptrix (Westra) H. Rainer (Magnoliales: Annonaceae), known as "anonilla" (De la Torre, 2008), is an endemic tree to the tropical moist lowland forest on the Ecuadorian coast (León Yánez et al., 2019). This species serves as a biological resource used for food (De la Torre, 2008), and wood harvesting (Erkens, 2021). It is listed as vulnerable in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species due to deforestation and agricultural expansion (Erkens, 2021).

Annona deceptrix is a small tree to 3 m tall with straight branches (Figure 1a). The leaves are elliptic to narrowly elliptic (Figure 1b), membranaceous to chartaceous, and covered with simple hairs, becoming rather densely glabrous with a brownish hue on larger veins. The inflorescences have an infra-axillary arrangement, consisting of a single rhipidium with 15 flowers (Figure 1c). The flowers are bisexual, yellowish or cream in color (Figure 1d). The fruits are cylindrical (Figure 1e), tapering toward the apex, yellowish-green, composed of 150-200 carpels. The seeds are brown (Figure 1f) (Westra, 1995).

The species was first described as *Raimondia*, but the studies carried out by Rainer (2001) led to its nomenclatural and taxonomic reclassification under the genus *Annona*, based on its morphological and anatomical characteristics.

Figure 1. *Annona deceptrix* (Westra) H. Rainer. A, tree; B, leaves; C, inflorescence; D, flower; E, fruits; F, seeds. (Pictures Miryan Pinoargote-Chang).

Currently, it is classified scientifically as *Annona deceptrix* (Westra) H. Rainer according to GBIF Backbone Taxonomy (GBIF Secretariat, 2022): Kingdom: Plantae; Phylum: Tracheophyta.; Class: Magnoliopsida; Order: Magnoliales; Family: Annonaceae; Genus: *Annona* L.; Species: *Annona deceptrix* (Westra) H. Rainer ≡ *Raimondia deceptrix* Westra.

There are 42 records (from 1990-2021) of specimens of the species on the Global Biodiversity Information Facility web site (GBIF, 2024) indicating its distribution across several Ecuadorian provinces: Manabí, Santo Domingo, Santa Elena, Esmeraldas, Guayas, Los Ríos, Pichincha (Figure 2), at altitudes ranging between 200 and 1200 m.a.s.l., (mostly around 500 m.a.s.l.). These records on GBIF have been compiled from eleven data sets, including Naturalis Biodiversity Center and Tropicos.

This species exhibits desirable agronomic characteristics and holds potential for genetic improvement, which has prompted numerous studies aimed at implementing effective conservation strategies.

A common approach to studying genetic diversity in plants involves the use of molecular markers. These markers have proven invaluable for assessing the genetic resources of plants, enhancing our understanding of the distribution and extent of genetic variation within and between species (Cavers & Dick, 2013; Ramesh et al., 2020). Recently developed marker technologies have enabled the unprecedented discovery of genetic variation, providing broader genome coverage. These markers have a wide range of applications in plant sciences, including genetic conservation, marker-assisted selection, and the study of population structure (Porth & El-Kassaby, 2014; Salgotra & Chauhan, 2023).

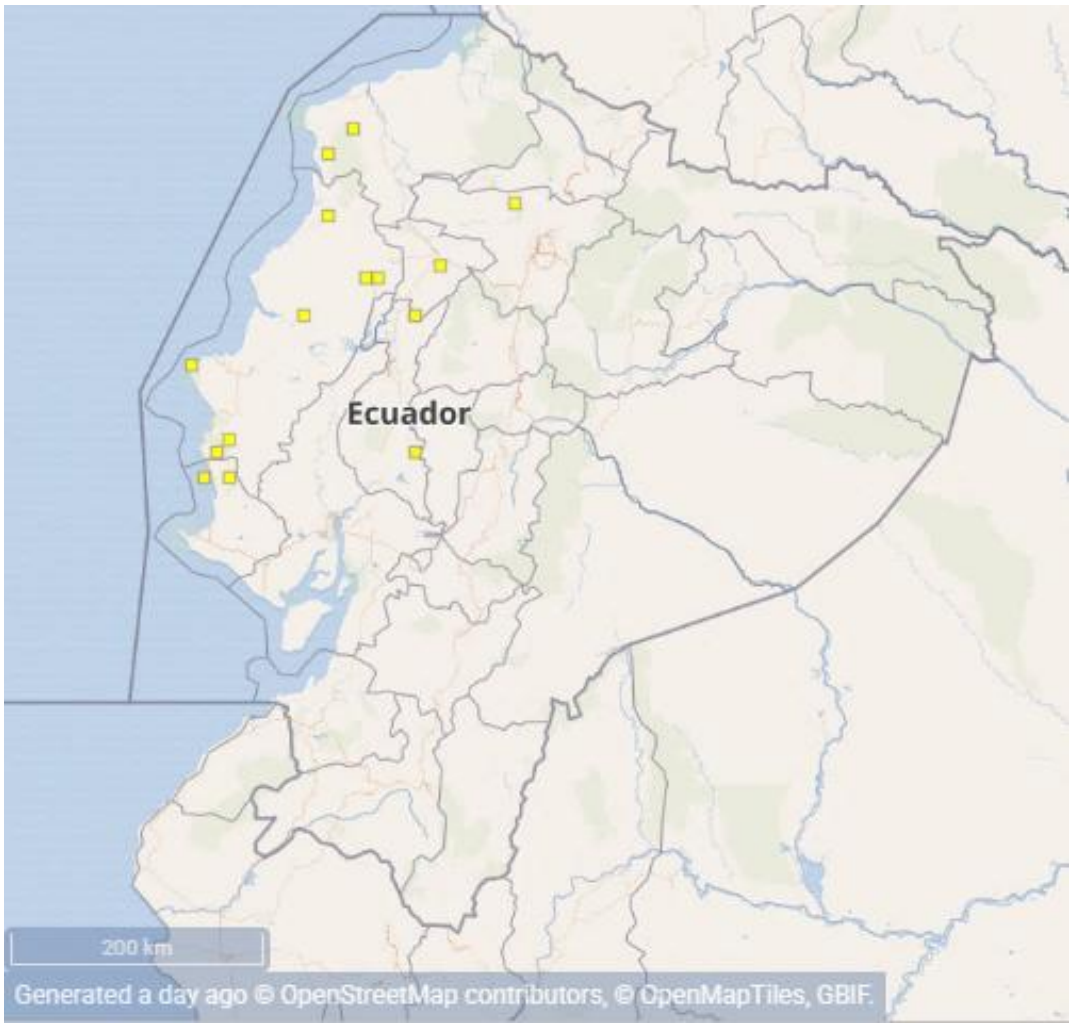

Figure 2. Geographic distribution of *Annona deceptrix* specimens (indicated by yellow square) in mainland Ecuador (1999–2021), based on GBIF data – Map created in May 2025.

To explore the genetic structure and population diversity of *A. deceptrix*, various populations along the Ecuadorian coast have been assessed. Pico-Mendoza et al. (2024) developed the first microsatellite markers for this species, isolating and characterizing 22 specific simple sequence repeat (SSR) loci. The loci exhibited observed heterozygosity ranging from 0.00 to 1.0 and expected heterozygosity from 0.00 to 0.93. The genetic differentiation observed suggests limited gene flow between populations, likely influenced by habitat fragmentation and natural geographic barriers that restrict genetic exchange. Such insights are crucial for analyzing genetic diversity in natural populations and other species of the *Annona* genus. In another recent study, 18 SSR markers were employed to assess 106 individuals from 11 natural populations of *A. deceptrix* in the Manabí province. This study reported a moderate level of genetic diversity (He = 0.445), identifying AD8, AD5, AD6, AD10, and AD1 as the most informative markers for the species. Moreover, the study found that 65% of the total genetic diversity resided within individuals, while only 11% was attributed to differentiation between populations (Pico-Mendoza et al., 2024).

The use of SSR markers has not only facilitated the identification of genetic variability at specific levels but also enabled the tracking of genetic changes over time. This capability is crucial for evaluating the effectiveness of implemented conservation strategies and adjusting conservation policies as needed (Ahmad et al., 2018). Preliminary research suggests a correlation between genetic variability in plant species and certain adaptive traits, which could significantly impact the selection of individuals for breeding and conservation programs (Leinonen et al., 2013). The genetic variability observed in *A. deceptrix* may be linked to its adaptive capacity to respond to environmental changes and the long-term survival of its natural populations. Genetic studies on this species not only help identify existing genetic diversity but also aid in selecting individuals with desirable adaptive characteristics for conservation, reproduction, and restoration of degraded populations, aligning with results reported in other plant species (Jara-Arancio et al., 2022; Shirk et al., 2014). Micropropagation has been explored as a method for conserving genetic material of *A. deceptrix*, complementing traditional conservation strategies and offering solutions for the long-term preservation of genetic diversity (Nevárez Loor et al., 2024).

The limited studies on the genetic diversity of *A. deceptrix* underscore the importance of international collaboration in the conservation of endangered species. Collaboration among research institutions, non-governmental organizations, and governments is essential for developing and implementing effective conservation strategies. Educating and raising awareness among local communities about the importance of conserving *A. deceptrix* is another crucial component of these conservation efforts.

3. Phytochemistry in *Annona* species and their biopesticide properties

Secondary metabolites are a diverse group of multifunctional organic compounds produced by plants, derived from primary metabolism, and essential for ecological interactions rather than basic survival (Erb & Kliebenstein, 2020). While these phytochemical compounds do not directly contribute to plant growth, metabolism, and development, they play crucial roles in defense, signaling, and interactions with the environment (Khare et al., 2020). Secondary metabolites are utilized as raw materials in various industries to produce pharmaceutical, cosmetics, food additives, and agrochemicals (Ozyigit et al., 2023). Many bioactive compounds, such as vincristine, ephedrine, morphine, aspirin, reserpine are in high global demand (Panchawat & Ameta, 2021), generating substantial interest in plant secondary metabolites within scientific and industrial communities (Guerriero et al., 2018).

Annona deceptrix is known to contain a variety of secondary metabolites, including catechins, triterpenes, tannins, alkaloids, flavonoids, amino acids, cardiotonic glycosides, anthocyanidins, reducing sugars, phenolic compounds, and saponins (Pinoargote-Chang et al., 2025). Moreover, this species exhibits antioxidant properties (Pinoargote-Chang et al., 2025). Other secondary metabolites such as ascorbic acid, carotenoids, flavonols, acetogenins, cyclopeptides, fatty acids, and essential oils, have also been identified in various other *Annona* species including *Annona diversifolia, A. foetida, A. glabra, A. macroprophyllata, A. montana, A. mucosa, A. muricata, A. purpurea, A. reticulata, A. scleroderma,* and *A. squamosa* (Anaya-Esparza et al., 2020; Ilango et al., 2022).

The secondary metabolites found in various *Annona* species, whether individually or in combination, are associated with a range of pharmacological activities, including antiangiogenic, cytotoxic, analgesic, anti-inflammatory, anticonvulsant, antidepressant, anxiolytic, neuroprotective, antihyperglycemic, vasorelaxant, antiulcer, and antipyretic (Anaya-Esparza et al., 2020; Ilango et al., 2022). Additionally, the fresh fruit of *Annona* species provides essential nutrients and minerals that are beneficial for human health, such as potassium, calcium, sodium, copper, iron, and magnesium (Ilango et al., 2022).

On the other hand, these compounds have ecological activities in nature, such as allelopathy, antifeedant activity, attraction, fungicidal, nematicidal, insect growth regulating, insecticidal, and repellent effects, making them a promising source for biopesticides (Souto et al., 2021). This contrasts with phosphate and chlorine based biopesticides, which pose significant health risks (primarily neurotoxic effects) and raise environmental concerns (Ayilara et al., 2023). Issues such as pest resistance to insecticides and the presence of agrochemicals residues in food could potentially be addressed using biopesticides (Ribeiro et al., 2013). Biopesticides are known for their biodegradability, which reduces their persistence in the environment compared to conventional chemical pesticides and treated surfaces (Gonçalves et al., 2022).

The secondary metabolites found in the extracts from seeds and leaves of *Annona* spp. demonstrate effective control of agricultural pests (Table 1). The biological activity of *Annona* spp. ethanolic seed extract is attributed to alkaloids, triglycerides, and primarily acetogenins, as well as their synergistic interactions and overall polarity, which contribute to the extract's activity (Ansante et al., 2015). Figure 3 illustrates the *in vitro* methodology employed to evaluate the biological activity of plant materials derived from various *Annona* species against agricultural pests. The process begins with the careful selection of the plant matrix—such as leaves, seeds, and stems—chosen based on its phytochemical profile and potential bioactivity. Once selected, the plant material undergoes extraction through a variety of possible methods, including maceration, percolation, or Soxhlet extraction. The choice of extraction technique depends on the physical and chemical characteristics of the matrix as well as the nature of the bioactive compounds of interest. The crude extracts obtained are then subjected to *in vitro* bioassays to assess their effects on target pest species. These assays are designed to evaluate the impact of the extracts on various developmental stages of the pests, ranging from egg to adult, providing insights into the potential of *Annona*-derived compounds as environmentally friendly alternatives for pest control in agriculture.

Annonaceous acetogenins represent a novel class of bioactive products, derived from long chain fatty acids in the polyketide pathway, characterized by their C35/C37 structure (Neske et al., 2020). These acetogenins exhibit lethal toxicity against all stages of arthropod development, including ovicidal, larvicidal, and nymphicidal effects (Souza et al., 2019), achieved through inhibition of ATP production via mitochondrial complex I (NADH: ubiquinone oxidoreductase), leading to cellular apoptosis (Tormo et al., 1999). Additionally, annonaceous acetogenins induce sublethal effects such as reduced fertility, inhibition of oviposition, and decreased offspring production in arthropods (Goncalvez et al., 2015). They also act as feeding deterrents and repellents (Fernandes et al., 2017) and diminish growth rates and food conversion efficiency (Souza et al., 2019) by damaging intestinal epithelial and digestive cells. This damage reduces the expression of genes associated with nutrient, metabolite, and non-electrolyte transport and absorption, while increasing the expression of genes linked to autophagy induction (Costa et al., 2016).

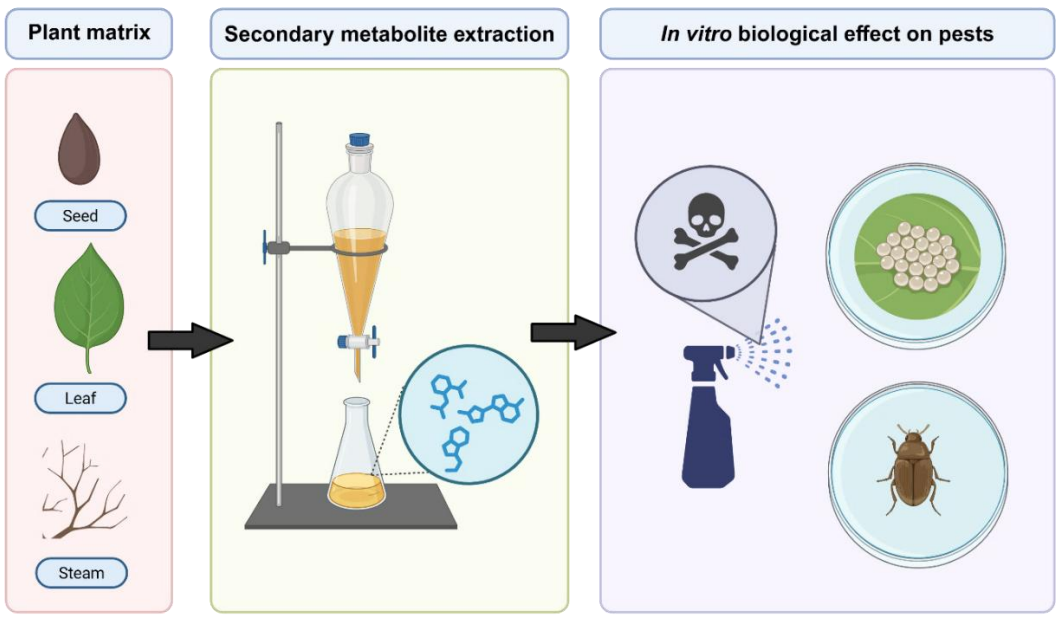

Figure 3. *In vitro* process to assess the biological activity of plant materials from various *Annona* species on agricultural pests. Created in BioRender. Pinoargote Chang, M. (2025) https://BioRender.com/nb0qvm0

Table 1
Biological activity of seed, steam and leaf extracts from various *Annona* species against agricultural pest

Plant species	Plant part	Extraction	Solvent	Secondary metabolite	Pest species	Pest life stage	Plant extract application	Biological activity	References
A. cherimola	Seeds	Percolation	Methanol	Acetogenins (squamocin, molvizarin and itrabin)	*Oncopeltus fasciatus* (Hemiptera: Lygaeidae)	Nymphs and adults	Topical	T	(Colom et al., 2008)
A. crassiflora	Seeds	Soxhlet	Chloroform-methane (2:1)	ND (Acetogenins presumably)	*Chrysodeixis includens* (Lepidoptera: Noctuidae)	Larvae	Contact and ingestion	T	(Massarolli et al., 2017)
A. diversifolia	Steam and leaves	Soxhlet	Ethanol	ND (Acetogenins and alkaloids presumably)	*Anastrepha ludens* (Diptera: Tephritidae)	Larvae	Contact	T	(González-Esquinca et al., 2012)
A. lutescens	Steam and leaves	Soxhlet	Ethanol	ND (Acetogenins and alkaloids presumably)	*Anastrepha ludens* (Diptera: Tephritidae)	Larvae	Contact	T	(González-Esquinca et al., 2012)
A. montana	Leaves and twigs	Percolation	Methanol	Acetogenins (squamocin, molvizarin and itrabin)	*Oncopeltus fasciatus* (Hemiptera: Lygaeidae)	Nymphs and adults	Topical	T	(Colom et al., 2008)
	Seeds	-	Methanol	Acetogenins (annonacin, cis - annonacin, cis -annonacin-10-one, asimicin, rolliniastatin-2, cherimolin-1, cherimolin-2, almuñequin, laherradurin, and itrabin)	*Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae)	Larvae	Ingestion	T	(Hidalgo et al., 2018)
A. mucosa	Seeds	-	Ethanol	ND (Acetogenins presumably)	*Duponchelia fovealis* Zeller (Lepidoptera: Crambidae)	Larvae	Contact	T, P, GI	(Gonçalves et al., 2022)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)	Larvae	Ingestion and contact	T, GI	(Ribeiro et al., 2014)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Myzus persicae* (Sulzer) (Hemiptera: Aphididae)	Adult	Contact	T	(Ribeiro et al., 2014)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Bemisia tabaci* MEAM 1 (Hemiptera: Aleyrodidae)	Eggs	Contact	O, N	(Soares et al., 2021)
		Maceration	Ethanol	Acetogenins	*Diaphorina citri* Kuwayama (Hemiptera: Liviidae)	Nymphs	Contact	T	(Ribeiro et al., 2015)
		Maceration	Ethanol	Acetogenin (bis-tetrahydrofuran rolliniastatin-1)	*Tetranychus urticae* Koch. (Acari: Tetranychidae)	Adult, eggs	Contact	T, OD, O	(Miotto et al., 2020)
		Maceration	Ethanol	Acetogenin (bis-tetrahydrofuran rolliniastatin-1)	*Helicoverpa armigera* (E12) (Lepidoptera: Noctuidae)	Larvae	Ingestion	T, GI	(Souza et al., 2017, 2019)
		Maceration	Ethanol	Acetogenins and alkaloids	*Corythucha gossypii* Fabricius (Hemiptera: Tingidae)	Nymphs	Contact	T	(Giraldo-Rivera & Guerrero-Álvarez, 2018)
		Maceration	Ethanol	ND (Acetogenins, alkaloids and triglycerides presumably)	*Zaprionus indianus* (Diptera: Drosophilidae)	Adult	Ingestion and topical	T	(Geisler et al., 2019)
		Maceration	Ethanol	Acetogenin (bis-tetrahydrofuran rolliniastatin-1)	*Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae)	Larvae	Ingestion	T, GI	(Ansante et al., 2015)

		-	Ethanol	Acetogenin (rolliniastatin-1)	*Palpita forficifera* Munroe (Lepidoptera: Crambidae)	Larvae	Ingestion	T, O	(Scheunemann et al., 2022)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Panonychus citri* (McGregor) (Acari: Tetranychidae)	Adult	Contact	T	(Ribeiro et al., 2014)
		Maceration	Methanol	Agetogenin (squamocin)	*Anticarsia gemmatalis* (Lepidoptera: Noctuidae)	Larvae	Ingestion	T, HCM	(Fiaz et al., 2018)
	Seeds and leaves	Maceration	Hexane, dichloromethane and ethanol	Alkaloids y acetogenins	*Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)	Adult	Contact	T	(Ribeiro et al., 2013)
A. muricata	Seeds	-	Methanol	Acetogenins (annonacin, cis - annonacin, cis -annonacin-10-one, asimicin, rolliniastatin-2, cherimolin-1, cherimolin-2, almuñequin, laherradurin, and itrabin)	*Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae)	Larvae	Ingestion	T	(Hidalgo et al., 2018)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Bemisia tabaci* MEAM 1 (Hemiptera: Aleyrodidae)	Adult	Contact	OD	(Soares et al., 2021)
	Steam and leaves	Soxhlet	Ethanol	ND (Acetogenins and alkaloids presumably)	*Anastrepha ludens* (Diptera: Tephritidae)	Larvae	Contact	T	(González-Esquinca et al., 2012)
A. squamosa	Leaves	Maceration	Ethanol	Alkaloids, flavonoids, glycosides, saponins, terpenoids	*Spodoptera exigua* Hübner (Lepidoptera: Noctuidae)	Larvae	Topical	T	(Casamina & F. Reyes, 2022)
	Seeds	Maceration	Hexane, acetone and methanol	ND (Acetogenins presumably)	*Spodoptera litura* F. (Noctuidae: Lepidoptera)	Larvae, eggs	Topical, ingestion	T, O	(Keerthi et al., 2023)
		Maceration	Methanol	ND (Acetogenins presumably)	*Spodoptera litura* F. (Noctuidae: Lepidoptera)	Larvae	Ingestion	T, HCM	(Muthu et al., 2023)
		-	Methanol	Acetogenins (annonacin, cis - annonacin, cis -annonacin-10-one, asimicin, rolliniastatin-2, cherimolin-1, cherimolin-2, almuñequin, laherradurin, and itrabin)	*Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae)	Larvae	Ingestion	T	(Hidalgo et al., 2018)
A. sylvatica	Seeds	Maceration	Ethanol	Triglycerides, alkaloids, and acetogenins	*Zabrotes subfasciatus* (Coleoptera: Chrysomelidae)	Adult	Contact, ingestion	T, OD	(Goncalvez et al., 2015)
		-	Ethanol	ND (Acetogenins presumably)	*Duponchelia fovealis* Zeller (Lepidoptera: Crambidae)	Larvae	Contact	T, P, GI	(Gonçalves et al., 2022)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)	Larvae	Ingestion and contact	T, GI	(Ribeiro et al., 2014)
		Maceration	Ethanol	ND (Acetogenins presumably)	*Myzus persicae* (Sulzer) (Hemiptera: Aphididae)	Adult	Contact	T	(Ribeiro et al., 2014)
A. vepretorum	Leaves	Maceration	Hexane and methanol	ND (acetogenins, steroids, terpenoids, and phenolic compounds presumably)	*Tetranychus urticae* Koch. (Acari: Tetranychidae)	Adult	Contact	T, FLR, R	(Fernandes et al., 2017)

Notes: *ND* no determinate, *T* toxicity, *GI* growth inhibition, *P* phagodeterrence, *O* ovicidal, *N* Nymphicidal, *HCM* histological changes in the midgut, *OD* oviposition deterrence, *FLR* fecundity and longevity reduction, *R* repellent. For more information prior to 2014 review Isman & Seffrin (2014).

It could be inferred that most of insecticidal action of annonaceous acetogenins is due to the relationship between the number and arrangement of adjacent bis-tetrahydrofuranic (THF) rings and hydroxyl groups (e.g., rolliniastatin-1), as well as the chemical nature of the terminal lactonic groups. Moreover, it has been suggested that the hydroxyl groups flanking the THF rings are of great influence on biological activity because the toxicity of these compounds decreases when these groups are blocked by acetylation or methoxy-methylation reactions (Di Toto Blessing et al., 2010). For this reason, the acetogenin bis-tetrahydrofuran rolliniastatin-1 is the major active component against arthropods and could be used as a chemical marker in the quality control of formulations derived from *Annona* spp. seeds (Ansante et al., 2015).

The efficacy of *Annona* spp. ethanolic seed extract is comparable to that of a commercial acetogenin-based bioinsecticide (containing 10,000 ppm of annonin as the main active ingredient) and flubendiamide-based chemical insecticide, suggesting that this plant extract could serve as an alternative control strategy for major agricultural pest (Souza et al., 2017). Additionally, the extract is easy and inexpensive to obtain through ethanol maceration and is non-phytotoxic (Souza et al., 2017). Crude extracts may also prove more effective than isolated compounds (Goncalvez et al., 2015).

The biological activity of acetogenins has been scarcely studied *in vivo*; therefore, further research is necessary to validate their potential and assess their effects on beneficial species (such as natural enemies and pollinators) within agroecosystems (Massarolli et al., 2017; Neske et al., 2020).

To identify acetogenins in an extract, Thin Layer Chromatography (TLC) with Kedde's reagent is used, wich detects the g-lactone-α, b-unsaturated subunit characteristic of acetogenins (Goncalvez et al., 2015). Fourier Transform Infrared Spectroscopy (FT-IR), Fourier Transform Raman (FT-Raman) Spectroscopy and UV-visible spectroscopy in methanol solutions are employed to characterize annonaceous acetogenin, such as squamocin, in the solid state (Hidalgo et al., 2020). Additional analytical techniques for the identification and quantification of acetogenins include ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), proton nuclear magnetic resonance (^{1}H NMR), and carbon-13 nuclear magnetic resonance (^{13}C NMR) (Bonneau et al., 2017; de Moraes et al., 2016).

The total phenolic content is determined using Folin-Ciocalteu method, while flavonoid content is measured via the aluminium trichloride colorimetric method (Ovando-Domínguez et al., 2019). Antioxidant capacity is assessed through radical scavenging assay, including 2,2-diphenyl-1-picrylhydracil radical (DPPH) and 2,2'-azino-bis- (3-ethylbenzothiazoline) -6-sulfonic acid (ABTS) (Pinoargote-Chang et al., 2025).

4. Micropropagation of *Annonas*

Nair and collaborators were pioneers in the *in vitro* propagation of *Annona squamosa* between 1983 and 1986. They produced the first *in vitro* haploid woody plant (n = 7) using *A. squamosa* anthers as explants. The organogenic response was indirect; haploid plantlets emerged from the callus formed on the explants. These plants were developed for use in genetic improvement programs (Nair et al., 1983).

During the 1980s, reports on *in vitro* propagation from leaf explants in woody species were scarce. However, Nair and colleagues published findings on the *in vitro* organogenesis of *A. squamosa* leaf explants. They achieved multiple shoot bud formation using Murashige & Skoog (1962) basal medium supplemented with cytokinins. Notably, they observed that BAP (6-benzylaminopurine) and KIN (kinetin) acted synergistically to promote vigorous shoots formation without the need for auxin supplementation. Additionally, they reported that leaf explants containing petioles and midribs favored shoot proliferation in these regions (Nair, Gupta, Shirgurkar, et al., 1984).

Attempts to produce *in vitro* triploid plants (3n = 21) of *A. squamosa* using the endosperm of germinated seeds as explants were unsuccessful. Although callus tissue was induced on basal medium supplemented with cytokinins, auxins, and gibberellins, and was periodically subcultured with some differentiation into shoots and roots, complete plantlets with both shoot and root systems were not obtained (Nair et al., 1986).

From the 1990s onwards, several researchers continued to explore the micropropagation of *Annona* species. Reports have documented *in vitro* propagation of the hybrid atemoya (*Annona cherimola* x *Annona squamosa*), *Annona cherimola*, *A. muricata*, *A. glabra*, *A. senegalensis*, *A. cauliflora*, *A. coriacea*, *A. bahiensis*, *A. silvatica A. purpurea*, *A. reticulata*, *A. deceptrix* (Table 2). Among the studies listed in Table 2, Murashige and Skoog (MS) is the most frequently used culture medium, followed by Woody Plant Medium (WPM), while Nitsch (N) is used less frequently. The most common explants used are nodal segments, followed by hypocotyls to a lesser extent. BAP is the most frequently used plant growth regulator (PGR), applied at concentrations ranging

from 0.5 mg L^{-1} to 5 mg L^{-1}, either alone or in combination with other PGRs. Successful *in vitro* regeneration of plantlets has been reported in the majority of these studies.

The incubation conditions commonly used for the micropropagation of *Annona* spp. typically involve temperature ranging from 25-28±1°C, a light intensity of 45 µmol m^{-2} s^{-1}, and photoperiod of 16 hours per day (Encina et al., 1994; Figueiredo et al., 2001; Lemos & Blake, 1996c; Nagori & Purohit, 2004; Padilla & Encina, 2004). The *in vitro* behavior of the *Annona* species has been investigated under various culture conditions, particularly assessing the effects of different additives to the basal media. For example, culture tubes sealed with cotton plugs and containing activated charcoal have resulted in the greatest bud length and dry weight (Santana et al., 2011b). Additionally, sugars such as glucose, sucrose, fructose, galactose, and maltose have been identified as viable carbon sources (Santana et al., 2011a). It has also been reported that the distal region of the hypocotyl produces the highest number of shoots when cultured on MS medium supplemented with cytokinins, biotin, and calcium pantothenate (Rasai et al., 1994).

Future advancements in the tissue culture of *Annona* species are focused on two main objectives: firstly, the large-scale micropropagation of selected genotypes; and second, the development of plant regeneration systems—such as organogenesis and somatic embryogenesis—to support agronomic improvement through breeding strategies involving ploidy manipulation (Encina et al., 2014). These biotechnological advances could significantly support the domestication, utilization, and consumption of wild species such as *A. deceptrix*. In this context, promising results have been reported for the micropropagation of *A. deceptrix* (Nevárez Loor et al., 2024), facilitating both its establishment under field conditions and its long-term preservation under *in vitro* conditions as a germplasm bank, as illustrated in Figure 4.

5. *In vitro* culture of calli and cell suspensions of *Annona* species for secondary metabolites production at flask scale

Callus culture

Calli are masses of unorganized plant cells formed in response to biotic and abiotic stimuli (Ikeuchi et al., 2019). Under controlled laboratory conditions, callogenesis in plant explants can be induced through the exogenous application of plant growth regulators (PGR) (Ikeuchi et al., 2013). Calli can proliferate *in vitro* indefinitely and possess totipotency, meaning they have the potential to regenerate an entire plant (Moscatiello et al., 2013). In addition, calli are pluripotent, as they can regenerate either roots or shoots depending on the levels of PGRs applied (Ikeuchi et al., 2019). Typically, basal medium supplemented with a high-auxin-to-low cytokinin ratio promote callus production from explants, while lower auxin concentrations favor root regeneration. Conversely, a high-cytokinin-to-low auxin ratio can stimulate shoot regenerate (Ikeuchi et al., 2019).

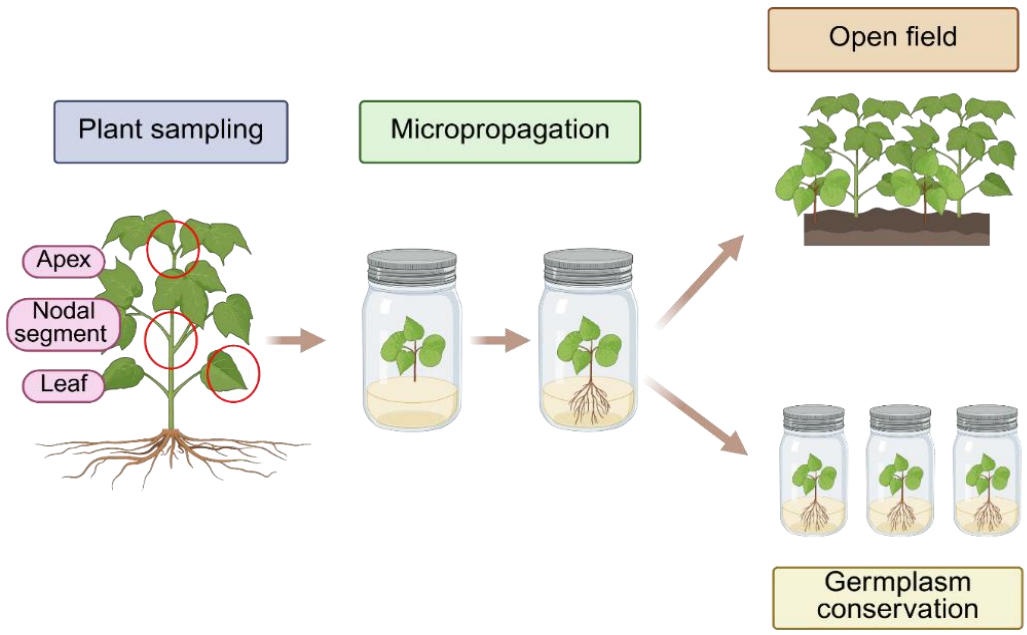

Figure 4. Overview of the micropropagation pathway of *Annona deceptrix*, from explant collection to *in vitro* rooting, field establishment, and conservation as a germplasm bank. Created in BioRender. Pinoargote Chang, M. (2025) https://BioRender.com/wmpnk5c

Table 2
Chronological reports of micropropagation in *Annona* species

Specie	Explant	Basal medium	PGR	Response	Reference
A. squamosa	Anther callus	N	BAP (2 mg L^{-1}) + IAA (0.1 mg L^{-1})	Haploid plantlets	(Nair et al., 1983)
Atemoya	Nodal segment	MS	KIN + BAP	Plantlets	(Nair, Gupta, & Mascarenhas, 1984)
A. squamosa	Leaf	MS	BAP (0.5 mg L^{-1}) + KIN (0.5 mg L^{-1})	Plantlets	(Nair, Gupta, Shirgurkar, et al., 1984)
A. squamosa	Endosperm callus	N	NAA (0.5 mg L^{-1}) + BAP (2 mg L^{-1})	Roots and shoots	(Nair et al., 1986)
A. cherimola	Hypocotyl	MS	BAP (2 mg L^{-1}) + NAA (0.5 mg L^{-1})	Shoots	(Jordan, 1988)
A. cherimola	Nodes and internodes	N	BAP (2 mg L^{-1}) + NAA (0.5 mg L^{-1}) + PVP + CH	Shoots	(Jordan et al., 1991)
Atemoya	Hypocotyl	MS	BAP (2 mg L^{-1})	Plantlets	(Rasai et al., 1994)
A. cherimola	Nodal segment	MS	BAP (0.66 µM) + ZEA (1.36 µM)	Plantlets	(Encina et al., 1994)
A. muricata	Hypocotyl Nodal segment	WPM	BAP (4 mg L^{-1}) + NAA (1 mg L^{-1})	Plantlets	(Lemos & Blake, 1996b)
A. squamosa	Hypocotyl Nodal segment	WPM	BAP (9 µM)	Plantlets	(Lemos & Blake, 1996a)
A. mucosa	Hypocotyl Epicotyl	MS	BAP (2.2 µM) + KIN (2.32 µM) BAP (8.8 µM) + NAA (0.54 µM)	Plantlets	(Figueiredo et al., 2001)
A. squamosa	Nodal segment	MS	BAP (0.6 mg L-1) + KIN (0.6 mg L^{-1})	Plantlets	(Farooq et al., 2001)
A. squamosa	Nodal segment	MS	BAP (1.5 mg L^{-1}) + CH (1.0 g L^{-1})	Plantlets	(Zobayed et al., 2002)
A. muricata	Nodal segment	MS	BAP (1 mg L^{-1}) + NAA (0.1mg L^{-1})	Plantlets	(Zobayed et al., 2002)
A. squamosa	Hypocotyl	MS	BAP (5 mg L^{-1})	Plantlets	(Nagori & Purohit, 2004)
A. cherimola	Nodal segment	MS	ZEA (2.28 µM)	Plantlets	(Padilla & Encina, 2004)
A. glabra	Nodal segment	MS	BAP (0.5 mg L^{-1})	Plantlets	(Deccetti et al., 2005)
A. senegalensis	Double nodal segment	MS	KIN (2 mg L^{-1}) + NAA (0.2 mg L^{-1})	Plantlets	(Rabou, 2006)
A. glabra	Nodal segment	WPM	IBA (4.9 µM) + AC (164.4 mM)	Plantlets	(Santana et al., 2008)
A. glabra	Nodal segment	WPM	BAP or KIN (1 mg L^{-1})	Plantlets	(Oliveira et al., 2008)
A. glabra, A. cauliflora, A. coriacea, A. bahiensis, A. silvatica	Nodal segment	WPM	BAP (8.87 µM)	Buds	(Santana et al., 2011a)
A. glabra	Nodal segment	WPM	no PGR, sucrose (58.42 mM)	Buds	(Santana et al., 2011b)
A. muricata *A. purpurea*	Hypocotyl	MS	BAP (2 mg L^{-1})	Shoots	(Ovando-Domínguez et al., 2019)
A. reticulata	Nodal segment	MS	BAP (2.5 mg L^{-1}) + IBA (0.3 mg L^{-1})	Plantlets	(Kudikala et al., 2020)
A. deceptrix	Nodal segment	WPM	BAP (1 mg L^{-1}) + GA_3 (0.25 mg L^{-1})	Plantlets	(Nevárez Loor et al., 2024)

Notes: *N* Nitsch & Nitsch (1969), *MS* Murashige & Skoog (1962), *WPM* Woody Plant Medium (Lloyd & McCown, 1981), *PGR* Plant Growth Regulators, *BAP* 6-Benzylaminopurine, *IAA* Indole-3-Acetic Acid, *KIN* 6-Furfurylaminopurine (kinetin), *NAA* 1-Naphthaleneacetic Acid, *PVP* Polyvinylpyrrolidone, *CH* casein hydrolysate, *ZEA* zeatin, *AC* activated charcoal, *IBA* Indole-3-Butyric Acid, GA_3 gibberellic acid.

Depending on the external stimuli that induce their formation, calli can be classified into two major types: wound-induced calli, which result from mechanical injury alone, and CIM-induced calli which develop after wounding followed by incubation on a callus-inducing medium (CIM) containing high levels of auxin (Ikeuchi et al., 2013). Morphologically, calli are categorized as either friable or compact. They can also be classified based on the type of organ they regenerate: root calli, shoot calli, or embryogenic calli, the latter capable of forming somatic embryos (Bidabadi & Mohan Jain, 2020). Furthermore, based on their culture history, calli are referred to as primary callus when they develop directly from the original explant, and secondary when obtained through subculturing of primary callus tissue (Bhatia, 2015).

Callus induction in *Annona* species has been documented since the early tissue culture studies in the genus. However, most of these studies primarily aimed at regenerating complete plantlets (Nair et al., 1983), rather than the production of callus per se. Nevertheless, some research has specifically focused on generating calli from *Annona* species for the production of secondary metabolites, which have been verified through phytochemical analysis. Successful callus induction has been reported in *Annona mucosa*, *A. muricata*, *A. purpurea*, and *A. squamosa*, using explants such as leaves, hypocotyls, and seeds, and culture media including MS, WPM, and B5. The growth regulators used include auxins such as picloram, 2,4-D, and NAA, as well as cytokinins such as thidiazuron (TDZ), BAP, kinetin (KIN), and zeatin (ZEA) (Barboza et al., 2014; dos Santos et al., 2015; Figueiredo et al., 2003; Wang et al., 2002).

These callus cultures have yielded secondary metabolites of interest, including the acetogenin squamocin, furofuranic lignans, alkaloids, flavonoids, phenols, and tannins. The antioxidant activity of polyphenolic compounds has been confirmed, and the antimicrobial activity of both polyphenols and alkaloids has also been evaluated (Table 3).

Cell suspension culture

Dedifferentiated cells that grow either freely or in small aggregates within a constantly agitated liquid medium, typically in flasks or bioreactors, are referred to as cell suspension cultures. Once callus cultures are established, the callus tissue is transferred to liquid medium to initiate the suspension culture phase (Moscatiello et al., 2013).

Plant cell suspension cultures offer several advantages, including improved safety, regulatory compliance, scalability, and reduced production costs (Wu et al., 2021). Moreover, they enable a more consistent, standardized, and often greater yield of secondary metabolites compared to conventional whole-plant or callus-based approaches (Marchev et al., 2020).

An overview of the process is presented in Figure 5, which provides a schematic representation of the cell suspension culture workflow. The diagram illustrates the transition from callus transfer to liquid medium, followed by metabolite production in shake flasks. It also outlines the subsequent steps of bioactivity testing and final product development, underscoring the biotechnological potential of this *in vitro* strategy.

The establishment and growth conditions for cell suspension cultures of *Annona mucosa* were first reported by Figueiredo & colleagues (2000). Supplementation of Murashige and Skoog (MS) basal medium with picloram significantly promoted the growth of cell suspensions, resulting in the highest biomass accumulation with minimal variation in cell morphology (Figueiredo et al., 2000). The growth kinetics of these cultures follow a typical three-phase pattern: a lag phase (0–8 days), an exponential growth phase (8–20 days), and a stationary phase (20–40 days) (Figueiredo et al., 2000).

Table 3
Successful cases of callus production to obtain secondary metabolites in *Annona* species

Specie	Basal medium	PGR	Concentration	Explant	Secondary metabolites identified	Bioactivity evaluated	References
Annona squamosa	B5	NAA + ZEA	5 mg L^{-1} + 4 mg L^{-1}	Seed	Acetogenin	NE	(Wang et al., 2002)
A. mucosa	MS	PIC	20.8 µM	Leaf	Furofuranic lignan	NE	(Figueiredo et al., 2003)
A. mucosa	WPM	PIC + KIN	2 µM + 0.02 µM	Hypocotyl	Alkaloids, flavonoids, tanins	Antimicrobial activity	(de Souza Barboza et al., 2015)
A. mucosa	MS	PIC + KIN	10 µM + 0.1 µM	Leaf	Alkaloids, flavonoids, phenols, tanins	Antimicrobial activity	(de Souza Barboza et al., 2015)
A. muricata	MS	2,4-D or NAA	3 mg L^{-1}	Hypocotyl	Phenols, flavonoids	Antioxidant activity	(Ovando-Domínguez et al., 2019)
A. purpurea	MS	2,4-D or NAA	3 mg L^{-1}	Hypocotyl	Phenols, flavonoids	Antioxidant activity	(Ovando-Domínguez et al., 2019)

Notes: *B5* (Gamborg et al., 1968), *MS* Murashige & Skoog (1962), *WPM* Woody Plant Medium (Lloyd & McCown, 1981), *PGR* Plant Growth Regulators, *NAA* 1-Naphthaleneacetic Acid, *ZEA* zeatin, *PIC* picloram, *KIN* 6-Furfurylaminopurine (kinetin), *2,4-D* 2,4-dichlorophenoxyacetic acid, *NE* not evaluated.

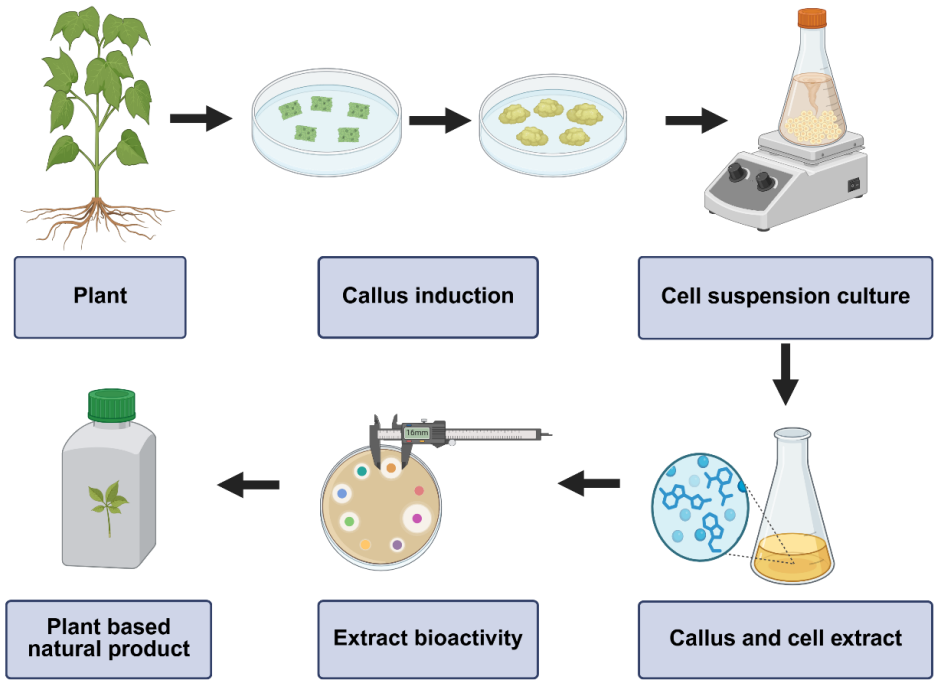

Figure 5. Schematic representation of plant cell suspension culture: from callus transfer to liquid medium to metabolite production in flasks, with bioactivity testing and final product development. Created in BioRender. Pinoargote Chang, M. (2025) https://BioRender.com/m1v5hos

The phytochemical profile of these suspensions has also been examined. Among the classes of secondary metabolites analyzed, only alkaloids were detected (De Souza Barboza et al., 2015). Moreover, the crude methanolic extract from *A. mucosa* cell suspension exhibited antimicrobial activity, demonstrated by its inhibitory effect against *Bacillus thuringiensis* (De Souza Barboza et al., 2015).

As previously noted, reports on *in vitro* cell cultures within the Annonaceae family are extremely limited and have primarily focused on *Annona mucosa* in Brazil. Although available data is scarce, these studies provide valuable foundation for future research on other *Annona* species with biotechnological potential, such as *Annona deceptrix*. Early findings on *A. mucosa* can inform the development of cell suspension protocols at the laboratory scale, which could later be optimized for use in bioreactor systems to enable the scalable production of valuable metabolites, including acetogenins, alkaloids, phenolic compounds, and other bioactive compounds.

6. Current and future challenges

The development of *in vitro* culture techniques for plant cells and tissues has significantly advanced plant biotechnology by enabling fundamental research, crop improvement, biodiversity conservation, and the production of industrially valuable metabolites (Häkkinen et al., 2024). In this context, plant cells are increasingly viewed as "green biofactories" for the environmentally sustainable production of biomass and specialized metabolites, particularly for the pharmaceutical, agrochemical, and cosmetic industries. This growing interest is largely driven by the increasing demand for natural and eco-friendly products.

In vitro technologies offer a resource-efficient alternative to conventional cultivation, eliminating the need to exploit wild plant populations or grow them in open fields. These methods reduce land use, water consumption, agronomic inputs, pesticide applications, and reliance on fossil fuels, making them especially appealing for sustainable production systems.

This growing interest in plant-based biotechnological applications has also shaped research trends in specific botanical families known for their rich metabolic profiles. Among these the Annonaceae family stands out due to its remarkable diversity of specialized metabolites. To explore how scientific attention toward this family has evolved over time, a bibliometric keyword co-occurrence analysis was conducted. Based on 3,263 articles indexed in Scopus (https://www.scopus.com/) between 2000 and 2025, the analysis revealed that the most frequently co-occurring keyword was *Annonaceae*, followed by terms such as plant extracts, cytotoxicity, drug isola-

tion, essential oils, molecular structure, and antioxidant and antibacterial activity (Figure 6). Notably, since 2015, there has been a marked increase in studies focusing on the secondary metabolism of Annonaceae, highlighting bioactive compounds such as flavonoids, essential oils, terpenes, acetogenins, coumarins, and others.

These findings underscore the biotechnological and industrial potential of the Annonaceae family, owing to its diverse array of bioactive metabolites with promising applications in pharmacology, agrochemistry, and related fields.

Given this background, it is essential to initiate targeted studies on *Annona deceptrix* focused on evaluating different culture media and combinations of plant growth regulators, particularly auxins and cytokinins, that promote efficient callus induction. Establishing a reliable protocol for the multiplication of friable callus induction is a key priority. Once friable calli are obtained, further experiments should be conducted to establish cell suspension cultures in flasks, including the assessment of cell growth kinetics, cell viability, and the determination of optimal subculturing intervals.

Subsequently, biomass production can be scaled up using bioreactor systems to obtain secondary metabolites of industrial relevance. Extracts derived from *in vitro* cultured cells can be subjected to biological assays. In the food industry, these include antimicrobial tests targeting pathogens such as *Escherichia coli, Staphylococcus aureus, and Clostridium* species. In the pharmaceutical sector, the extracts may be evaluated for cytotoxic against cancer cell lines, such as breast and colon cancer models, focusing particularly on their ability to induce programmed cell death (apoptosis), a critical mechanism in anticancer therapy. In the context of agricultural biotechnology, these extracts could be tested for activity against key pest species like *Spodoptera frugiperda*. Such assays should aim to determine whether the bioactive compounds act through toxic, antifeedant, repellent, or reproductive inhibitory mechanisms.

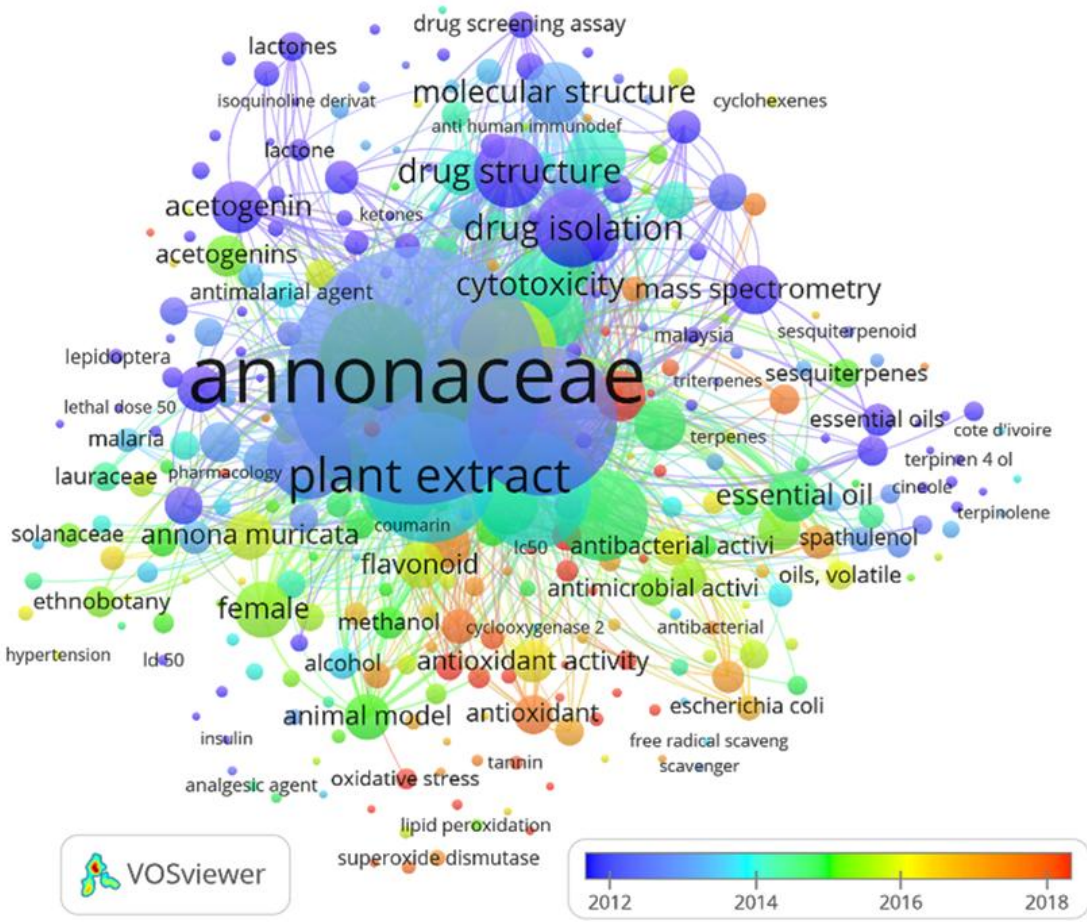

Figure 6. Keyword Co-occurrence Analysis. Term Overlay Visualization Over Time. The data were obtained from the Scopus database (search criteria: Keywords: 'Annonaceae'; Document Type: 'ALL'; Period: 2000 to 2025) and analyzed using VOSviewer (https://www.vosviewer.com/).

Once bioactivity is confirmed, additional studies should address the extract's shelf life and assess potential safety risks for human handlers. However, several challenges remain, including the limited baseline data on *A. deceptrix* in *in vitro* systems, the inherent complexity of metabolite profiling, and the scalability of production processes. Moreover, aligning the development of these extracts with current regulatory frameworks, particularly in the food and pharmaceutical sectors, represents a significant hurdle. Addressing these challenges will require a multidisciplinary approach, grounded in sustainable innovation and aligned with global environmental and public health goals.

7. Conclusions

Annona deceptrix represents a promising species for the *in vitro* production of bioactive secondary metabolites. However, to fully harness its biotechnological potential, further research is needed to establish efficient protocols for callus induction and cell suspension culture. Exploring and optimizing culture conditions, particularly through the strategic us of plant growth regulators and elicitors, may significantly enhance the biosynthesis of key metabolites such as acetogenins, triterpenes, alkaloids, and polyphenols. Additionally, the identification and characterization of these compounds will be essential for developing value-added applications. Conducting targeted biological assays will provide critical insight into the potential uses of *A. deceptrix* extracts in areas such as crop protection, pharmaceuticals, and food technology.

Overall, advancing this line of research will support the sustainable exploitation of *A. deceptrix* and contribute to its integration into future bio-based innovation strategies.

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