

CANCER FIGHT AND THE SECONDARY METABOLITES FROM ANNONACEAE DISTRIBUTED IN MEXICO

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Abstract

Worldwide, cancer remains one of the leading causes of death; however, chemotherapy can cause toxic effects in affected individuals. Additionally, cancer cells can develop multidrug resistance, enabling them to evade treatment and control. Therefore, the search for new and more targeted compounds remains one of the most active areas in the fight against this disease. Phytochemical and pharmacological studies have shown that members of the Annonaceae family contain some chemical compounds with cytotoxic and antitumor activity, including the proliferation inhibition at the multidrug resistance cancer cells. This review focuses on the knowledge of extracts and secondary metabolites from Annonaceae species distributed in Mexico and their potential as chemotherapeutic agents against cancer. It also delves into the molecular mechanisms and genes involved in the metabolic pathways that trigger apoptosis induced by these plant-derived compounds.

Keywords: Antitumor activity, cytotoxicity, phytochemistry.

Resumen

El cáncer es una de las principales causas de mortalidad a nivel mundial; sin embargo, la quimioterapia puede causar efectos tóxicos en las personas afectadas. Además, las células cancerosas pueden expresar mecanismos de resistencia a múltiples fármacos, lo que les permite evadir el tratamiento y el control. Por lo tanto, la búsqueda de compuestos nuevos y más específicos es una de las áreas más dinámicas en la lucha contra esta enfermedad. Estudios fitoquímicos y farmacológicos muestran que los miembros de la familia Annonaceae contienen algunos compuestos químicos con actividad citotóxica y antitumoral, incluyendo la inhibición de la proliferación en las células cancerosas resistentes a múltiples fármacos. Esta revisión se centra en el conocimiento de los extractos y metabolitos secundarios de especies de Annonaceae presentes en México y en su potencial como quimioterapéuticos contra el cáncer. También profundiza en los mecanismos moleculares y genes involucrados en las vías metabólicas que desencadenan la apoptosis inducida por estos compuestos derivados de plantas.

Palabras clave: Actividad antitumoral, citotoxicidad, fitoquímica.

According to the World Health Organization (WHO), cancer is the leading cause of mortality, and in 2020, nearly 10 million deaths were attributed to this disease. The most common causes were breast, lung, colon, rectal, and prostate cancer (WHO 2022). Unfortunately, this number is projected to increase 6.7 million by 2045 (WHO 2022, PAHO 2022).

In cancer cells, the acquisition of multidrug resistance (MDR) mechanisms is characterised by the expression of proteins that prevent cell death or apoptosis, which has long been considered the most significant barrier to cancer therapy (Bredel 2001). The medicinal properties of plants are attributed to the production of secondary metabolites (phenols, flavonoids, tannins, coumarins, steroids, alkaloids, and other compounds), whose ecophysiological function has been linked to the communication of plants with their environment. However, being chemically active, they can interact with receptors or target molecules of other biological systems, modifying their physiological processes and naturally exhibiting biological activities such as antifungal, antimicrobial, insecticidal, cytotoxic, etc. (Anaya & Espinosa-García 2006), making them compounds of great biotechnological interest to humans.

In the oncology field, more than 50 % of the drugs have a natural origin. These include plant-derived agents such as the alkaloids vinblastine and vincristine isolated from *Catharanthus roseus*, a plant native to Madagascar (Huang *et al.* 2004), paclitaxol (taxol), isolated from the bark of the Pacific Northwest tree *Taxus brevifolia* (Huang & Fan 2002), and camptothecin, isolated from the bark of *Camptotheca acuminata*, a precursor to the semi-synthetic drug topotecan (Brantley-Finley *et al.* 2003). Additionally, some of these natural products have been observed to disrupt or inactivate MDR mechanisms in cancer cells, thereby increasing intracellular concentrations of chemotherapeutics or enabling the activation of programmed cell death pathways.

Mexico is considered one of the countries with high biodiversity; however, the percentage of studied organisms that could serve as a basis for developing new drugs is relatively low. It is estimated that the number of molecules isolated so far does not even represent 10 % of the potential active ingredients that would be expected to be found in a country with Mexico's natural wealth (Alonso-Castro *et al.* 2011).

The Annonaceae family is widely distributed in Mexico, especially in the Central, Gulf, and Pacific states. Some of them are used for food, therapeutic purposes, insecticides, and in the conservation of agroecosystems, among others (De la Cruz-Chacón *et al.* 2016).

Materials and methods

We conducted a comprehensive review of the existing literature on the anticancer properties of the Annonaceae family that are reported in Mexico (De la Cruz-Chacon *et al.* 2016). For this review, an extensive search across the academic databases ScienceDirect, Medline/PubMed, and Google Scholar was performed. The search words (keywords) included were “Annonaceae”, “cancer”, “apoptosis”, “proliferation inhibition”, “cytotoxicity”, and “antitumor activity”. No restrictions were placed on the publication year. The chemical structures from the PubChem or ChemSpider databases were obtained and drawn using ChemDraw software.

Gene ontology enrichment analysis using the bioinformatics tool GOnet (<https://tools.dice-database.org/GOnet/>) was conducted (Pomaznoy *et al.* 2018) to identify cell gene networks influenced by secondary metabolites from Annonaceae species.

Cytotoxic and antitumoral activity of Annonaceae

In recent decades, research on the phytochemical and pharmacological properties of various Annonaceae species has increased, as numerous bioactive compounds have been isolated from extracts of leaves, roots, fruits, bark, and seeds. These compounds exhibit a wide range of biological activities, including the ability to induce apoptosis (programmed cell death) in cancer cells (Joseph *et al.* 2023, Nugraha *et al.* 2019).

This review focused on the Annonaceae species distributed in Mexico, with reports of active metabolites against cancer cells. Due to the explosion of literature in recent years, the most representative literature was selected. The

genus *Annona* is the most studied, possibly due to its economic importance and accessibility in commercial orchards, while other genera, such as *Stenanona*, *Sapranthus*, and *Desmopsis*, have scarce or no literature.

Anaxagorea A. St.-Hil. *Anaxagorea* is the only genus of the Annonaceae family with a disjunct distribution in South and Central America and Southeast Asia.

Anaxagorea guatemalensis Schltdl. The only species of the genus reported in our country is *A. guatemalensis*, and it is found in a humid tropical biome from southern Mexico to Central America. No specific literature was found on the cytotoxic or anticancer activity of *A. guatemalensis*; however, reports on other *Anaxagorea* species have been documented, suggesting that it may contain similar active compounds.

The phytochemical investigation of *Anaxagorea dolichocarpa* led to the isolation of eupolaramine and sampangine alkaloids, which exhibit concentration-dependent cytotoxic activity against K562 myelogenous leukemia cell lines, with an average inhibitory concentration (IC_{50}) of 18.97 and 10.95 $\mu\text{g/mL}$, respectively (Lúcio *et al.* 2011).

The bark hexane extract at a 50 $\mu\text{g/mL}$ achieved 89.02 % inhibition of cell proliferation in the colon cell line HCT-116 (Pinheiro *et al.* 2016). The essential oil from the leaves of *A. brevipes* exhibits complete antiproliferative activity (TGI) against breast cancer cell lines MCF-7 (TGI: 12.8 $\mu\text{g/mL}$), lung cancer cell lines NCI-H460 (TGI: 13.0 $\mu\text{g/mL}$), and importantly, against prostate cancer cells PC-3 (TGI: 9.6 $\mu\text{g/mL}$). On the other hand, in the non-cancerous keratinocyte cell line HaCat, the TGI reached 38.8 $\mu\text{g/mL}$, indicating good selectivity (De Alencar *et al.* 2016). Some of the components identified were β -eudesmol (13.16 %), α -eudesmol (13.05 %), γ -eudesmol (7.54 %), guaialol (5.12 %), caryophyllene oxide (4.18 %) and β -bisabolene (4.10 %).

Annona L. In Mexico, the *Annona* L. genus includes species with great agricultural potential, such as *A. muricata* (soursop), *A. cherimola* (custard apple), and *A. squamosa* (saramuyo), which are grown in commercial orchards or backyards. On the other hand, species such as *A. contreraasi*, *A. liebmanniana*, or *A. spraguei* are endemic or found in restricted areas (De la Cruz-Chacón *et al.* 2016); therefore, there are no studies on the activity of their secondary metabolites.

Annona cherimola Mill. In recent decades, a growing number of studies have focused on the phytochemical composition of *A. cherimola* extracts to explain their traditional uses and the responsible compounds for their biological activity.

Some compounds of interest have been isolated from root, seed, and pulp extracts (Durán *et al.* 2021). Thus, ethanolic extracts of *A. cherimola* seeds exhibited a cytotoxic effect (IC_{50}) on the acute myeloid leukemia KG-1, Monomac-1, and U937 cell lines. The extract exhibited dose- and time-dependent inhibitory activity, but not on normal mesenchymal stem cells (MSCs). Apoptosis was confirmed by the detection of phosphatidylserine on the outer cell membrane, DNA fragmentation, and the expression of proapoptotic proteins. Phytochemical analysis of the extract revealed the presence of bioactive compounds like phytosterols (Haykal *et al.* 2019).

Ethanolic seed extract at 20 $\mu\text{g/mL}$ significantly reduced the ability to form clones in the human SNU-1 gastric cancer cells, which are characterized by forming multicellular aggregates, and the doses of 50 $\mu\text{g/mL}$ induced apoptosis. The extract phytochemical analysis detected two acetogenins (annonacinone and annonacin) that could be largely responsible for their effect, as well as eight fatty acids, among them, oleic acid, which has been recognized as a good adjuvant in antitumor treatments (Macuer-Guzmán *et al.* 2023).

In the search for active molecules against colorectal cancer, the ethanolic seed extracts exhibited *in vitro* inhibitory activity against the T84, HCT-15 (drug-resistant), SW480, and HT-29 cell lines, as well as the cancer stem cells (CSCs). The lowest IC_{50} was found in T-84 line cells ($23.2 \pm 2.75 \mu\text{g/mL}$) and HCT-15 ($33.0 \pm 1.2 \mu\text{g/mL}$). The inhibitory effect on CSCs is noteworthy because, *in vivo*, these cells are often responsible for colorectal cancer recurrence and chemotherapy resistance. Furthermore, the extracts at 12 $\mu\text{g/mL}$ showed synergistic activity with 5 $\mu\text{g/mL}$ of 5-Fu (5-fluorouracil), the colon cancer drug of choice (Fuel *et al.* 2021).

The ethanolic leaf extract at a dose of 390.2 $\mu\text{g/mL}$ during 48 h was active against the triple-negative breast cancer cell line (MDA-MB-231) without toxic effects on normal murine mesenchymal stem cells. The proapoptotic effect was confirmed by increased cell and DNA fragmentation, as well as the translocation of phosphatidylserine to the outer layer of the cell membrane. The underlying molecular mechanism indicated involvement of the mitochondrial pathway, as shown by changes in mitochondrial permeability, upregulation of pro-apoptotic proteins, and downregulation of anti-apoptotic proteins. (Younes *et al.* 2020).

Cyclic peptides are an interesting class of molecules that exhibit a broad range of biological activities and can be used as scaffolds in the synthesis of new bioactive compounds. For example, the cherimolacyclopeptide A is a cyclic octapeptide isolated from the seeds of the *A. cherimola* and is cytotoxic against the human nasopharyngeal carcinoma KB cells, with an IC_{50} of 0.6 μM , whereas cherimolacyclopeptide B was less active with an IC_{50} of 45 μM (Figure 1) (Wélé *et al.* 2004, Dahiya & Dahiya 2021).

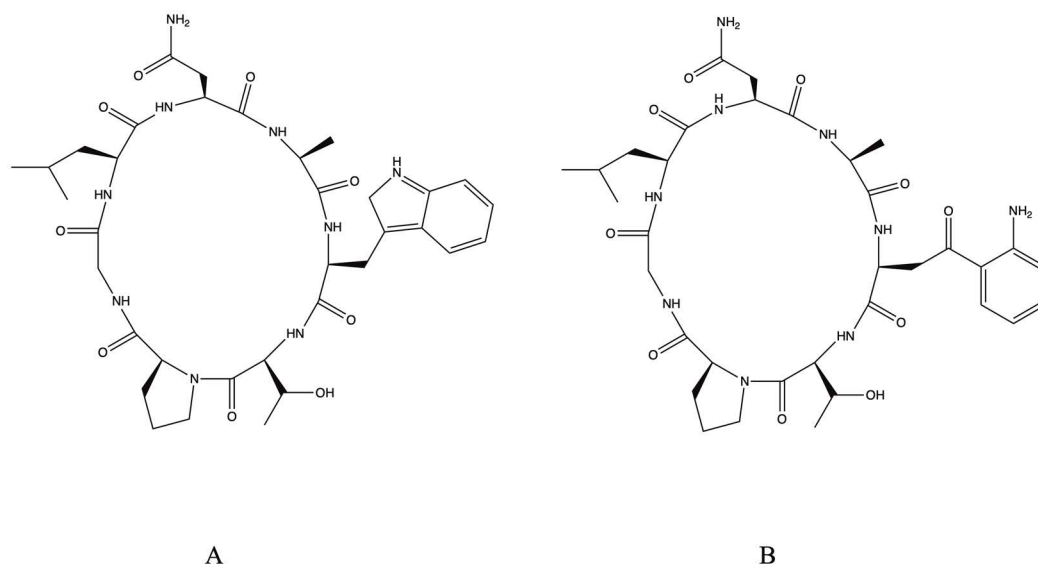


Figure 1. Structures of (A) cherimolacyclopeptide A and (B) cherimolacyclopeptide B.

The acetogenins (ACGs) are recognized as some of the most potent antitumor compounds, particularly effective against drug-resistant cancer cells. These compounds inhibit ATP synthesis and glucose uptake, which is significant given the heightened ATP demands of tumor cells. The ACG molvizarin, cherimolin-1, motrilin, annonacin, and anonisin, isolated from the deciduous leaves of *Annona cherimola*, demonstrate cytotoxic activity at a concentration of 100 μM for 24 hours against the ovarian cancer cell line IGROV-1 and the cervical cancer cell line HeLa. Notably, cherimolin-1 and annonacin exhibited the highest levels of cytotoxicity, with cell viability dropping below 10 %. These results indicate that they are more effective than the anticancer drug control, etoposide (Gutiérrez *et al.* 2020).

Despite the positive results, acetogenins are poorly soluble in aqueous media, which limits their usefulness *in vivo* models; further research in this area is therefore needed.

Annona glabra L. The anticancer effects of alcoholic extracts from the leaves, pulp, and seeds of *A. glabra* have been studied on human drug-sensitive (CEM) and multidrug-resistant (CEM/VLB) leukemia cell lines. The extracts demonstrated cytotoxic activity against both cell types; however, CEM cells were more sensitive to the treatment. Importantly, normal human lymphocytes treated under the same conditions showed no adverse effects. The seed extract specifically caused a concentration-dependent increase in the percentage of cells in the sub-G0/G1 and the G0/G1 phases, which contributed to the observed cytotoxicity. Additionally, it elevated the expression of the cyclin-dependent kinase inhibitor WAF1/p21, a protein associated with cell cycle regulation. (Cochrane *et al.* 2008).

The alcoholic leaf extract, at concentrations of 20, 40, 60, 80, and 100 $\mu\text{g/mL}$, in the mitochondria of MC-1010 human monocytic leukemia cells, increased cell apoptosis by 2.5, 3.9, 4.8, 10.7, and 22.4 %, respectively, after 72 h of incubation. Nuclear and mitochondrial morphology remained normal in untreated cells; on the contrary were markedly altered, fragmented and condensed in treated cells (Liu *et al.* 2018). Other extracts, such as hexane leaves, were active against colon cancer cell lines CACO-2 and lung A-549 (IC_{50} : 47.7 and 56.82 $\mu\text{g/mL}$) compared to the control drug doxorubicin (IC_{50} : 31.91 and 23.39 $\mu\text{g/mL}$). The three main compounds identified in the extract were tritriacontane (30.23 %), 13, 17-dimethyl-tritriacontane (22.44 %), and limonene (18.97 %) (Soleman *et al.* 2023).

Among the chemical components of *A. glabra* extracts are the diterpenoids cunabic acid (Figure 2A) and the ent-kauran-19-al-17-oic acid. These compounds exhibit cytotoxic activity against SMMC-772 human liver cancer cells. Specifically, the effect of cunabic acid is observed at concentrations greater than 5 μM , while ent-kauran-19-al-17-oic acid demonstrates activity at concentrations greater than 10 μM (Zhang *et al.* 2004). Additionally, the glycoside fruit known as icaricide D2 (Figure 2B) has shown significant effects on the human promyelocytic leukemia cell line HL-60, with an IC_{50} value of 9.0 μM . However, it does not affect the normal fibroblast line Hel-299, which indicates its specificity for tumor cells. This compound induces cell death by altering the expression of apoptosis-related proteins, leading to increased Bax levels and decreased Bcl-2 levels, as well as the cleavage of caspase-3 and PARP. It also reduces the phosphorylation of the regulatory protein AKT (Hien *et al.* 2015).

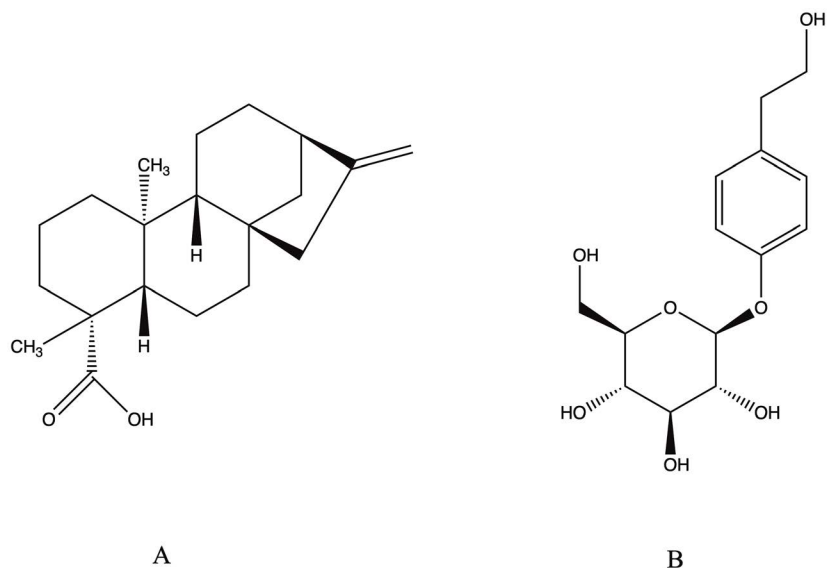


Figure 2. Chemical structures of the (A) cunabic acid and (B) glycoside icaricide D2.

Annona globiflora Schltdl. Also known as monkey's custard, it grows on designated livestock land and is therefore a vulnerable species (Hernández *et al.* 2014). Despite being an undervalued or underutilized species, knowledge of its phytochemical potential can promote its sustainable cultivation and conservation.

For example, from the ethyl acetate fractions of seeds, four cyclic octapeptides that share the amino acid sequence Pro-Met (O)-Tyr-Gly-Thr-, called squamins C to F, have been isolated. When analyzed on cultures of the DU-145 (human prostate cancer) and HeLa (human cervical carcinoma) showed strong cytotoxicity in the range of 8-25 μM . Other inhibitory activities reported for these compounds have been on trophozoites of *Acanthamoeba castellanii*, *A. polyphaga*, *A. quina*, and *A. griffini*, inducing programmed cell death mechanisms with chromatin condensation, mitochondrial damage, and inhibition of ATP production, compared to the negative control (Sosa-Rueda *et al.* 2022a, 2022b).

Annona reticulata L. The white anona, wild anona, or anonillo is characteristic of low- to medium-altitude tropical regions from southern Mexico and Central America.

Phytochemical and biological studies with extracts and components of *A. reticulata* suggest potential ingredients in cancer therapy. For example, three aporphine alkaloids were isolated from root extracts: liriodenine, norushin-sunine, reticuline, and the neoannonine acetogenin. In the cancer cell lines A-549 (human lung carcinoma), K-562 (myelogenous leukemia), HeLa (human cervix), and MDA-MB-435 (Human melanoma), the neoannonin exhibited significant dose-dependent inhibitory activity, with IC_{50} values ranging from 5.8 to 6.9 $\mu\text{g/mL}$ (Suresh *et al.* 2012).

Apoptosis induction via the mitochondrial pathway has been observed in T-47D human breast cancer cells treated with leaves methanolic extracts. The IC_{50} of 5 $\mu\text{g/mL}$ induces morphological changes including phosphatidylserine outside the cell membrane (normally inward), cell cycle arrest in the G2/M phase, as well as upregulation of the proapoptotic genes Bax and Bak and downregulation of Bcl-2 (Roham *et al.* 2016).

The antioxidant and anticancer potential of the alcoholic extract of leaves and administered orally at doses of 5 mg/kg and 10 mg/kg daily for 21 days, was studied in 1, 2-dimethylhydrazine (DMH)-induced colorectal cancer Wistar rats. Colon histopathological studies showed that DMH caused severe inflammation, hyperproliferative cells, and damage to the muscularis layer compared to the healthy control group. However, in the tissues of animals treated with alcoholic extract, DMH-induced inflammation and aberrant crypts were significantly reduced (Khan *et al.* 2021).

The acetogenin (Bis-Tetrahydrofuran) squamocin (Figure 3) has been isolated from *A. reticulata* seeds and exhibits cytotoxic activity against bladder (T24), ovarian (PA-1 and SKOV3), cervical (HeLa and HeLa S3), breast (MCF-7), and skin (BCC-1) cancer lines with an IC_{50} ranging from 233 to 434 $\mu\text{g/mL}$. The ACG showed selective cytotoxicity on T24 bladder cancer cells at the S-phase via Bax, Bad-, and caspase-3-related pathways. Results suggest that squamocin is a potentially promising anticancer compound (Yuan *et al.* 2003).

Annona scleroderma Saff. It is native to the Atlantic coast of Central America, and although studies on its activity against cancer cells are limited, they emphasize its potential as a source of active metabolites.

The cyclopeptide, sclerin (Figure 4), was isolated from the seeds' methanol extract together with the metabolite, cyclosenegaline A. The compounds showed moderate cytotoxic activity against the DU-145 human prostate carcinoma cell line, with sclerin having an IC_{50} of $27.3 \pm 4.19 \mu\text{M}$ and cyclosenegaline $54.9 \pm 2.35 \mu\text{M}$; the IC_{50} of the anticancer drug doxorubicin, used as a control, was 1.1 μM (Cen-Pacheco *et al.* 2019).

Kupchan fractions (hexane, dichloromethane, ethyl acetate, and methanol: water) from seeds tested against five cancer cell lines: HBL-100 (breast epithelial cells); HeLa (cervical cancer); SW1573 (non-small cell lung cancer); T-47D (breast cancer cells); and WiDr (colon cancer cells) indicated that the hexane and dichloromethane fractions exhibited between 92 and 78 % inhibition of cell proliferation respectively. Chemical study of these fractions led to the isolation of the acetogenins rolliniastatin 2 and bullatacinone, which could explain this activity (Sosa-Rueda *et al.* 2022b).

Annona spraguei Saff. This species is listed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (De la Cruz-Chacón *et al.* 2016).

The guanaconetins -1 to -4 acetogenins were isolated from the seeds and exhibited cytotoxic activity against a panel of human cancer cell lines. Guanaconetin-3 exhibited greater inhibitory activity on cell growth in human liver (HepG2, IC_{50} : 16.6 μM), breast (MCF-7, IC_{50} : 33.0 μM), ovarian (MES-SA, IC_{50} : 30.0 μM), doxorubicin-resistant ovarian (MES-SA/Dx5, IC_{50} : 77 μM), lung (A-549, IC_{50} : 102 μM), and colon (HT-29, IC_{50} : 129 μM) carcinoma cell lines. In the enzyme inhibition assay, acetogenins without acetyl moieties showed IC_{50} values lower than those with one, two, and three acetyl groups along the alkyl chain (Chahboune *et al.* 2006).

Annona squamosa L. It is found in the southeastern areas of Mexico, in the states of Campeche, Chiapas, and Yucatan; however, its cultivation has spread to Southeast Asia due to the acceptance of its fruits in those countries.

Ethyl acetate seed extract was tested on male Kunming mice implanted with H22 murine hepatoma cells. The extract was dissolved in olive oil and administered orally at concentrations of 18.0, 9.0, and 4.5 mg/kg/day for 10

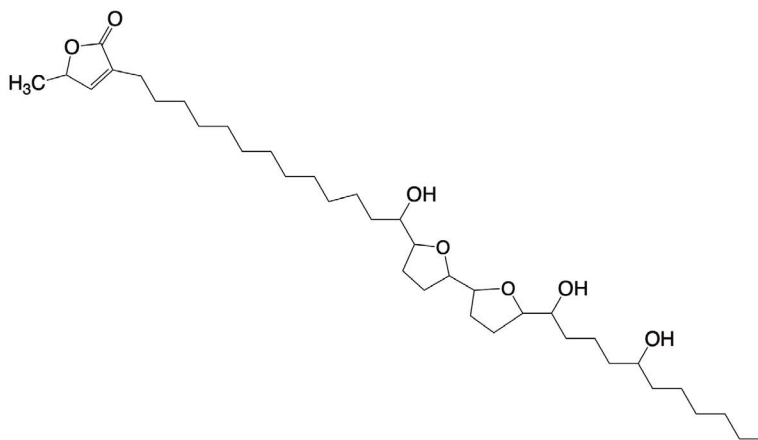


Figure 3. Chemical structure of the acetogenin squamocin with adjacent bis-THF configuration.

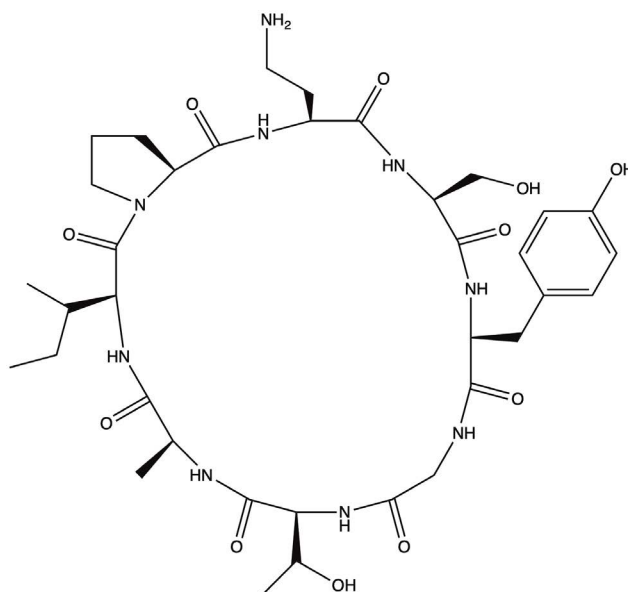


Figure 4. Chemical structure of the cyclic peptide, sclerin.

days. The drug control was CTX (cyclophosphamide), intraperitoneally administered at 30 mg/kg/day. The tumor reduction reached 69.55 % at a dose of 18 mg/kg/day, with no side effects in the treated animals. Its antitumor activity was superior to CTX, which was 63.75 % (Chen *et al.* 2012b).

Seed oil contains a high percentage of fatty acids, including palmitic acid (9.92 %), linoleic acid (20.49 %), oleic acid (56.50 %), and stearic acid (9.14 %). In a study, the oil was administered intragastrically at a dosage of 1.0 mL/kg per day during seven days to Kunming male mice implanted with H22 murine hepatoma cells. This treatment resulted in a tumor growth inhibition of up to 53.54 % compared to untreated animals. Immunohistochemistry on tumor sections of treated animals shows a decrease in interleukin-6 (IL-6), janus kinase (Jak), and phosphorylated signal transducers and activators of transcription (p-Stat3) expression. Therefore, *A. squamosa* could be a potential candidate for cancer treatment (Chen *et al.* 2016).

Various authors' observations suggest that the inhibitory activity of ACGs is accentuated when calcium is present in the culture media. Therefore, annonacin and uvariamicin-I interactions were measured by isothermal titration calorimetry (ITC). The results suggest that the Ca^{2+} ion binds to the hydroxyl groups adjacent to THF, forming complexes and allowing ACGs to act as ionophores (Liaw *et al.* 2008).

Based on the number and location of the tetrahydrofuran (THF) ring along the hydrocarbon chain, ACGs can be classified into three main types: mono-THF, adjacent bis-THF, and non-adjacent bis-THF (Figure 5). To determine which type of ACG has the greatest effect on solid tumors *in vivo*, two non-adjacent bis-THF ACGs (squamosatin A and squamosatin E), one mono-THF (4-deoxy annoneticin), and two adjacent bis-THFs (desacetyl uvaricin and bullatacin) were isolated from seeds. The compounds were administered to mice bearing HepS (human hepatoma) and S180 (murine sarcoma) at doses of 15 and 60 µg/kg/day for seven days; tumors were excised on the tenth day. Bis-THF ACGs showed the greatest antitumor effect, with bullatacin being the most potent compound (65.8 and 63.4 % relative to untreated animals). In general, non-adjacent bis-THF ACGs were superior to mono-THF, though not always. These findings align with *in vitro* observations where bis-THF ACGs demonstrate the highest cytotoxicity (Chen *et al.* 2012a).

Importantly, ACGs isolated from *A. squamosa* seeds exhibit activity against cells with multiple drug resistance (MDR) mechanisms. Thus, squamocin P and annosquatin III inhibited the proliferation of the human papillomavirus-associated cervical adenocarcinoma cell line (SMMC 7721/T) with an IC_{50} of 0.435 and 1.79 µM, as well as the adriamycin-resistant breast adenocarcinoma cell line (MCF-7/ADR) with an IC_{50} of 3.34 and 4.04 µM, respectively (Ma *et al.* 2017).

Alkaloids and acetogenins are poorly soluble in water. To enhance their bioavailability, nanoparticles were assembled with chitosan-coated leaf extracts. The nanoparticles were tested in cervical cancer cell cultures (HeLa), and the results indicate that they can inhibit cell proliferation with an IC_{50} value of 344.48 µg/mL. Furthermore, nanoparticles trigger apoptosis via the mitochondrial pathway by enhancing the activation of the effector protein caspase-3 (Fadholly *et al.* 2020).

Annona mucosa (Jacq.) Baill. The *Rollinea* genus has been incorporated into the *Annona* genus (iNaturalist 2023), so the bioactive compounds with anticancer activity are described in this section.

In this species, formerly called *Rollinia mucosa*, more than 17 Annonaceae acetogenins have been identified with antitumor activity, such as rolliniastatin-2, also known as bullatacin. Other isolated ACGs include rollicosine (an atypical ACG that has two lactone rings, one at each end of the aliphatic chain) (Figure 6) (Liaw *et al.* 2003), and epomusenins, rollinecins, rolliniastatin-1, rollidecins, rollinacin, mucosin, and muconin (Neske *et al.* 2020).

The acetogenins rollitacin and rollinacin were isolated from an ethanolic extract of *R. mucosa* leaves and exhibited selective inhibitory effects on the proliferation of human solid tumor cell lines. Rollitacin appears to be selective against MCF-7 breast cancer cells (IC_{50} : 2.5×10^{-4} mg/mL) and HT-29 human colon cancer cells (IC_{50} : 4.6×10^{-3} mg/mL), while rollinacin showed moderate selectivity against A-549 lung tumor cells (IC_{50} : 4.6×10^{-2} mg/mL) (Shi *et al.* 1997).

Rolliniastatin-1, isolated from seeds, exhibits potent cytotoxic activity against HepG2 liver cancer cells at concentrations from 10 to 50 µM; apoptosis and necrosis (12-24 hours post-treatment) were similar to those induced by rotenone. However, unlike rotenone, the induction of cell death is not clearly linked to the inhibition of mitochondrial complex I (De Pedro *et al.* 2013).

Rolliniastatin-2 (Bullatacin) is one of the most potent mitochondrial complex I inhibitors and can inhibit the growth of adriamycin-resistant human mammary adenocarcinoma cells (MCF-7/Adr) at concentrations of 1.08×10^{-2} (Oberlies *et al.* 1997), making it a molecule of great biotechnological interest. Furthermore, in a mouse implanted A2780 human ovarian carcinoma, the ACG at a dose of 50 µg/kg/day for 10 days, inhibited tumor cell growth by up to 67 % (Ahammadsahib *et al.* 1993), inhibition was also seen in S180 murine carcinoma cells and HepS human liver tumors, up to 65.8 and 63.4 % respectively (Chen *et al.* 2012b). Studies with this ACG point to the induction of apoptosis as well as the decrease in cAMP and cGMP levels (Chiu *et al.* 2003).

Annona macrophyllata Donn. Sm. Several secondary metabolites with significant antitumor activity have been identified in this species, such as the acetogenins (ACGs): laherradurin (Figure 7A), rollinastatin-2 (bullatacin), and cherimolin-2 (Figure 7B), as well as the alkaloid: liriodenine (Figure 7C).

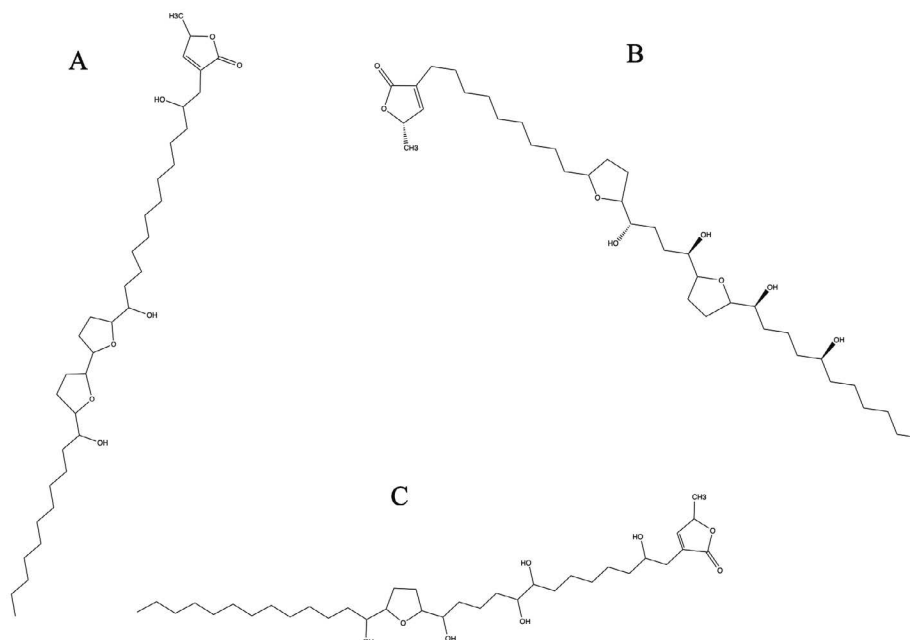


Figure 5. Classification of acetogenins based on their chemical structure: (A) bullatacin, an ACG with adjacent bis-THF rings, (B) squamostatin A, an ACG with non-adjacent bis-THF rings and (C) annonacin, an ACG with a mono-THF ring.

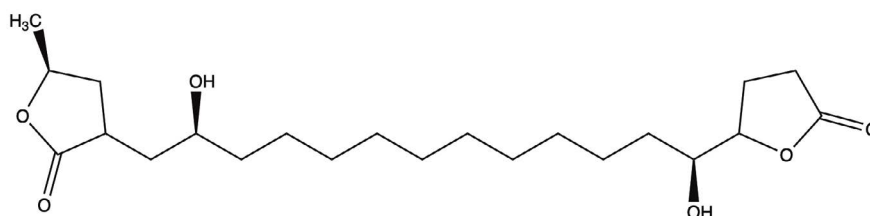


Figure 6. Structure of the atypical ACG rollicosine with two lactone rings.

In vitro, laherradurin had shown cytotoxic and antiproliferative activity against HeLa cervical and SW-480 colon human cancer cell lines, with an IC_{50} of 0.015 $\mu\text{g/mL}$. *In vivo*, it showed potent antitumor activity in nude xenograft mice with these same line cells (HeLa 64 %; SW-480 60 %) (Schlie-Guzmán *et al.* 2009). In a murine model of colon cancer induced by azoxymethane and sodium dextran sulfate, the group treated with the ACG, exhibited a reduction in both the size and number of tumors, demonstrating lower toxicity compared to the control drug, cisplatin. This study also found that laherradurin was effective in inducing apoptosis and inhibiting cell migration (Rendón-Barrón *et al.* 2024).

Annona macrophyllata roots are rich in liriodenine alkaloid (De la Cruz-Chacón & González-Esquinca 2012). In *in vitro* studies, the alkaloid inhibits cell proliferation and induces apoptosis in various cancer cell lines, such as breast cancer MCF-7, with an IC_{50} of 30 μM . Additionally, liriodenine leads to a reduction in VEGF, Bcl-2, and cyclin D1 levels, while increasing p53 and caspase 3 activity (Li *et al.* 2015, Nordin *et al.* 2015, Li *et al.* 2017). Furthermore, it has been characterized that this alkaloid inhibits topoisomerase II and induces cross-linking of proteins with the DNA helix, resulting in DNA lesions. (Woo *et al.* 1997).

Annona purpurea Moc. & Sessé ex Dunal. Commonly known as soncoya, sincuya, or chincuya, it is distributed along the Mexican Pacific coast and is widely used in folk medicine.

Several acetogenins have been isolated from this species, such as purpureacin-2, purpuracenin, purpurenin, and rullitacin (Bermejo *et al.* 2005), and annopurpuricins A-E. The latter ACGs were also identified from a dichloromethane extract of roots (Hernández-Fuentes *et al.* 2019).

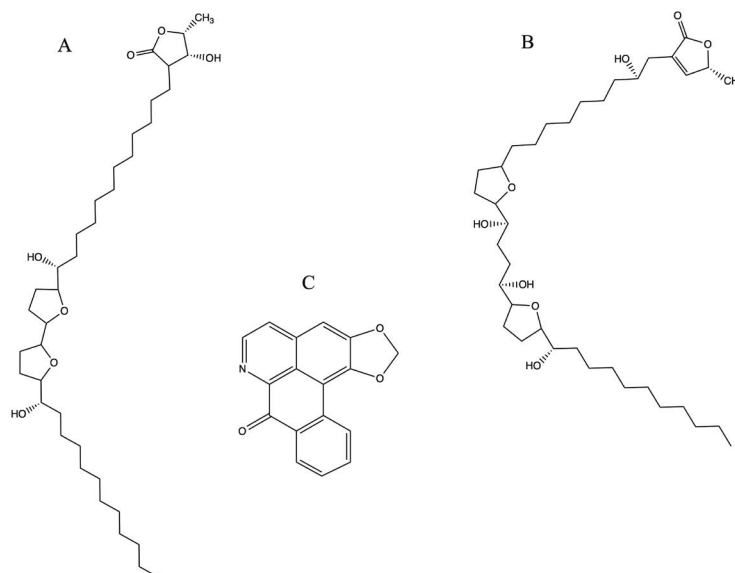


Figure 7. Chemical structure of the (A) bis-THF adjacent acetogenins laherradurin, the (B) non-bis-THF adjacent cherimoline-2 and the (C) alkaloid lirioidenine.

Annopurpuricins A, B, C, and D significantly inhibited the growth of HeLa cervical cancer cells and HepG2 liver cancer cells, with CI_{50} values ranging from 0.06 to 25.9 μ M. In particular, annopurpuricin A was the most active compound in both cell lines, being 9.0 and 6.6 times, respectively, more potent than the chemotherapeutic control camptothecin. In a murine model using the tumorigenic TC-1 mouse lung cell line, annopurpuricin A at a dose of 125 μ g/kg during 18 days inhibited tumor growth by 60 %. Annopurpuricin A was well tolerated and did not cause kidney damage in the animals (Hernández-Fuentes *et al.* 2023).

Several alkaloids have been found in this species, among them, the oxoaporphinic acid oxopurpureine and O-methyl ateroline (oxoglaucine) exhibited *in vitro* inhibitory activity against human 9-KB squamous carcinoma cells, with an IC_{50} of 15.2 and 14.5 μ M, respectively (Sonnet & Jacobson 1971).

The ACG purpuracenin has been evaluated in several cancer cell lines: A-549, MCF-7, HT-29, A-498, PC-3, and PACA-2 with an excellent IC_{50} range of 0.001 to 10 μ g/mL (Chávez & Mata 1999).

Annona muricata Linn. It is widely distributed, and it is perhaps the most studied of the Annonaceae species. Its extracts possess antibacterial, antitumor, antiprotozoal, insecticidal, immunomodulatory, hypoglycemic, and antiviral activity (Datiles & Acevedo-Rodríguez 2015).

Ethyl acetate extract of *A. muricata* leaves induces apoptosis in A549 lung cancer cells through the mitochondrial-mediated pathway, with involvement of the NF- κ B signaling pathway. This process involves several key steps: increased reactive oxygen species (ROS) production, mitochondrial membrane potential loss, cytochrome c release, and caspase activation. The extract also triggers cell cycle arrest at the G1 phase and suppresses NF- κ B translocation (Zorofchian-Moghadamtousi *et al.* 2014).

Several reports indicate that *A. muricata* extracts exhibit antitumor activity in pancreatic xenograft models using colon cancer cells (CD18/HPAF). In these studies, a dose of 50 mg/kg of the “Graviola extract” (a mixture of leaf and bark powder) inhibits tumor growth by 50 %. Additionally, the treatment caused cell death through necrosis and led to tumor inhibition by downregulating the HIF-1 α , ERK, and PI3K/Akt pathways, which are involved in cell survival and proliferation. (Torres *et al.* 2012).

In breast cancer cell lines, such as MCF-7, a methanolic leaf extract has been shown to induce apoptosis and cell cycle arrest, with an IC_{50} value of 100 μ g/mL (Naik & Sellappan 2020). When this extract is incorporated into

nanoparticles, the IC_{50} value can decrease to 12 $\mu\text{g/mL}$ or lower (Sabapati *et al.* 2019). In AMJ13 cells, the IC_{50} was found to be 17.34 $\mu\text{g/mL}$; additionally, the extract promoted autophagy and exerted an anti-inflammatory effect by downregulating IL-1 β and NLRP3, which are key components of the inflammasome (Jabir *et al.* 2021). In prostate cancer cells (PC3), the leaf extract exhibits an IC_{50} of 80 $\mu\text{g/mL}$ (Datiles & Acevedo-Rodríguez 2015), while the same extract but into silver nanoparticles, enhances its activity (IC_{50} : 47 $\mu\text{g/mL}$) (Gavamukulya *et al.* 2021).

Various extracts from *A. muricata* leaves have shown cytotoxic activity against cervical cancer cells, specifically HeLa cells. For instance, the hexane extract exhibits an IC_{50} of 8.39 $\mu\text{g/mL}$, the ethyl acetate extract has an IC_{50} of 7.56 $\mu\text{g/mL}$, and the ethanol extract shows an IC_{50} of 6 $\mu\text{g/mL}$ (Qorina *et al.* 2020). In the chronic myelogenous leukemia cell line K-562, both methanolic and acetone leaf extracts induce cell cycle arrest, with IC_{50} values ranging from 28 to 32 $\mu\text{g/mL}$ (De Castro-Nascimento *et al.* 2018). The dichloromethane extract has an IC_{50} of 4.7 $\mu\text{g/mL}$ in Raji cells (Md Roduan *et al.* 2019). However, when the extracts are converted into nanoparticles, the IC_{50} increases to 17 $\mu\text{g/mL}$ in human acute monocytic leukemia THP-1 cells (Jabir *et al.* 2021).

A wide variety of secondary metabolites, such as alkaloids, acetogenins, terpenes, phenols, and flavonoids with potent anticancer activity, have been identified in extracts from various parts of *A. muricata*. Some of the most representative results are described below.

Several alkaloids with cytotoxic activity against cancer cells have been identified in the roots of this species, including reticuline, xylopine, coclaurine, argentinine, and atherosperminine (Figure 8). These compounds demonstrated cytotoxicity with IC_{50} values ranging from 20 to 80 μM across various cancer cell lines, including HepG2 (human liver carcinoma), HL-60 (human leukemia), and A549 (human lung adenocarcinoma). Notably, xylopine is the most active molecule, exhibiting significant cytotoxicity against the K-562 human myelogenous leukemia cell line (Nugraha *et al.* 2019). Additionally, alkaloid liriodenine has also been identified (Fofana *et al.* 2012) and quercetin, which is a type of phenolic compound that shows cytotoxic activity in cancer (HeLa) cells at 25 and 50 ppm, while leaving normal (3T3 fibroblast) cells unaffected. (Valdez-Guerrero *et al.* 2021).

As *A. muricata* is one of the species with the greatest number of phytochemical studies, it is not surprising that acetogenins (emblematic compounds of the Annonaceae family) have been isolated. Thus, more than hundred ACGs have been identified in extracts from seeds, leaves, stems, roots, and ripe fruit, such as annomutacin, annonacin, annonamuricins, annomuricins, muricins, gigantetrocin-B, and muricatatins (Zorofchian-Moghadamtousi *et al.* 2015, Neske *et al.* 2020).

In leaf extracts, the ACG annonacin (cis-annonacin) is highly abundant and possesses potent cytotoxic activity against several cancer cell lines. It had a cytotoxic IC_{50} of 1×10^{-8} $\mu\text{g/mL}$ against the colon adenocarcinoma cell line HT-29 (Rieser *et al.* 1996) and human Burkitt lymphoma (Raji cells) IC_{50} of 3 μM (Md Roduan *et al.* 2019).

The annonacin can induce both cell cycle-dependent growth arrest and apoptosis in estrogen receptor- α (ER α)-positive MCF-7 breast cancer cells. The median effective dose (ED_{50}) is 0.31 μM (Ko *et al.* 2011). Another study estimated an IC_{50} of 10 $\mu\text{g/mL}$ (Naik & Sellappan 2021). Interestingly, this acetogenin demonstrates anti-angiogenic activity, inhibits tumor growth, and downregulates the expression of VEGF and HIF-1 α genes, as well as genes associated with multidrug resistance, including those from the ATP-dependent transmembrane transporter (ABC) system (Coothankandaswamy *et al.* 2010, Manoharan *et al.* 2024). The ACG annomuricin E isolated from ethyl acetate extract leaves, inhibited 61 % the aberrant polyp formation in a colon cancer murine model at a dose of 250 mg/kg/week for two weeks. *In vitro* assays, it had an IC_{50} of 1.62 ± 0.24 $\mu\text{g/mL}$ in HT-29 cells, with induction of apoptosis and cell proliferation inhibition (Zorofchian-Moghadamtousi *et al.* 2015).

Muricins are mono-THF acetogenins isolated from leaves and exhibit an IC_{50} range of 5 to 9 $\mu\text{g/mL}$ in human hepatoma HepG2 cells (Liaw *et al.* 2002). These acetogenins also induce apoptosis in human hepatocarcinoma (HCC) cells. Due to their synergistic activity with sorafenib, muricins are being considered as a potential adjuvant in the treatment of advanced liver cancers (Li *et al.* 2024).

Canaga Hook. f. & Thomson. These species thrive in humid environments but can also be cultivated in other areas if sufficient water is provided. The flowers of *Cananga odorata* are utilized in the cosmetics and perfume industries.

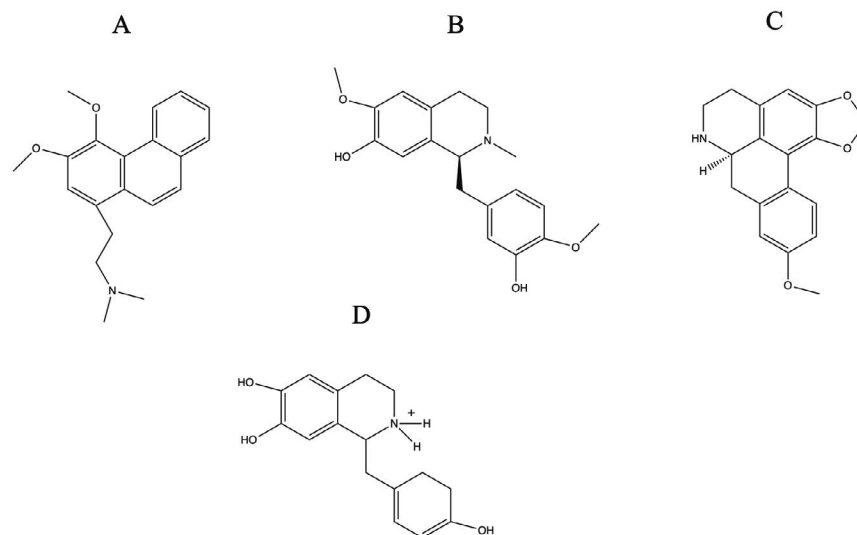


Figure 8. Chemical structures of alkaloids with antitumor activity: (A) atherosperminine, (B) reticuline, (C) xylopine, and (D) norcoclaurine (Higenamine).

Cananga odorata (Lam.) Hook.f. & Thomson. Native to Madagascar, but now it is found in various tropical regions, including parts of Asia, Malaysia, and tropical America. This plant produces a diverse range of alkaloids, several of which exhibit significant antitumor properties. Notably, liriodenine and sampangine are known for their pro-apoptotic effects through oxidative stress, and they are effective against trypanosomatids as well as the human leukemia cell line HL-60 (Kluza *et al.* 2005).

The essential oil extracted from *C. odorata* demonstrates cytotoxic effects on triple-negative breast cancer cells (MDA-MB-231), exhibiting an IC_{50} value of 29.01 $\mu\text{g/mL}$. When delivered using niosomes-nanovesicles made from a surfactant and cholesterol, this value decreases to 18.39 $\mu\text{g/mL}$ (Sedky *et al.* 2023). Additionally, a methanolic leaf extract of the plant has shown antiviral activity against the hepatitis B virus, with an IC_{50} of 56.5 $\mu\text{g/mL}$ (Indrasetiawan *et al.* 2019).

Nine acetogenins called cananginones have been identified from *C. latifolia* (Neske *et al.* 2020), and they exhibited significant activity against cancer cells, particularly on MCF-7 breast carcinoma cells. Additionally, they can inhibit cancer stem cell renewal, interfering with the epithelial-mesenchymal transition (EMT), and disrupting the metastatic activity by downregulating the expression of the Sonic hedgehog (SHH) protein (Bose *et al.* 2022).

Cymbopetalum Benth. There are several species of the genus *Cymbopetalum* in Mexico; however, no chemical profile of these species has yet been recorded. A phytochemical study of the *Cymbopetalum brasiliense* showed a large number of quaternary alkaloids, such as magnoflorine, tembetarine, and colletine. Aqueous extract administration enriched with norcoclaurine (higenamine) alkaloid (Figure 8D) at 5-10 mg/kg induces a potent inotropic response, lowering heart rate and blood pressure; the extract positively reacted to the Kedde reagent, suggesting the presence of other types of metabolites, such as ACGs (Cavé *et al.* 1984).

Desmopsis Saff. Although the anticancer activity of secondary metabolites in *Desmopsis* species found in Mexico has not been specifically reported, studies on other American species may provide useful insights. For instance, the leaf oils of *D. bibracteata* demonstrated cytotoxic effects on human breast tumor cells MDA-MB-231 and Hs578T, achieving a $\geq 99\%$ kill rate at a concentration of 100 $\mu\text{g/mL}$. These oils are rich in germacrene D (29.9 %), (E)-caryophyllene (11.5 %), and δ -cadinene (9.2 %). Notably, both germacrene D and (E)-caryophyllene also exhibited strong cytotoxicity against the MDA-MB-231 tumor cell line (Palazzo *et al.* 2009).

Free radicals can cause oxidative damage to biomolecules, leading to serious health issues such as atherosclerosis, cancer, aging, diabetes, inflammation, and degenerative diseases. This has prompted a search for molecules with antioxidant properties that could serve as potential therapeutic agents. For instance, using the DPPH (2, 2-diphenyl-1-picryl hydrazyl) method, it was found that 41.4 $\mu\text{g/mL}$ of *D. panamensis* methanolic extracts were required to decolorize 50 % of the DPPH radical (Jiménez *et al.* 2005).

Guatteria Ruiz & Pav. In Mexico, there are eight species of the *Guatteria* genus, but only *G. amplifolia* has been studied at the phytochemical level. Reports indicate that other *Guatteria* species have extracts with trypanocidal and antitumor properties. For instance, the essential oil of *Guatteria friesiana* contains sesquiterpenoids, specifically α , β , and γ -eudesmol, which have demonstrated antitumor activity in mice, reducing tumor weight by up to 43.4 to 54.2 % (Britto *et al.* 2012). Additionally, alkaloids with cytotoxic activity, such as liriodenine and palmatine. Palmatine has shown cytostatic activity against MCF-7 (breast cancer) and U-251 (glioma) cells, with an IC_{50} ranging from 10 to 16 $\mu\text{g/mL}$ (Costa *et al.* 2013).

The essential oil derived from *G. olivacea* leaves, administered at a dose of 40 mg/kg/day for 21 days, exhibited antitumor activity of up to 58 % in a mouse model with human liver cancer cells (HepG2). The oil's chemical profile showed a predominance of sesquiterpenes (germacrene and spathulenol) and fatty acids (epi-cubenol and caryophyllene epoxide) (Galvão *et al.* 2022). Moreover, several alkaloids with cytotoxic properties, such as lysicamine and melosmine, have been identified in a methanolic stem bark extract. Lysicamine inhibits cell proliferation with an IC_{50} of 6.64 $\mu\text{g/mL}$, while melosmine has an IC_{50} of 16.7 $\mu\text{g/mL}$ in colon HCT116 cancer cells (Costa *et al.* 2024). *Guatteria amplifolia* Triana & Planch. There is currently no specific anticancer research conducted on this species. However, the presence of aporphine alkaloids, such as xylopine, nornociferine, lysicamine, and laudanosine, has been reported. These compounds demonstrate strong trypanocidal activity, with xylopine being identified as the alkaloid with the highest level of activity (Montenegro *et al.* 2003).

Oxandra A. Rich in Sagra. The *Oxandra* genus includes approximately 25 species found across Mexico, Nicaragua, South America, and the Antilles. While the activity of secondary metabolites in the *Oxandra* genus has been examined for their potential as bioinsecticides (Rojano *et al.* 2009), research on their effects against tumor cell lines is limited. However, the findings suggest the presence of compounds that warrant further investigation.

One notable compound is isospintanol (2-Isopropyl-3, 6-dimethoxy-5-methylphenol), a monophenol isolated from an ether extract. It has been shown to affect the proliferation of various tumor cell lines, including MDA-MB-231 (breast cancer), A-549 (lung cancer), DU-145 (prostate cancer), A-2780 (cis-resistant ovarian cancer), and A-2780 (ovarian cancer), with IC_{50} values of 52.39, 59.70, 47.84, 60.35, and 42.15 μM , respectively. Isospintanol also induces nuclear compaction and the formation of membrane-bound cell fragments, both of which are indicative of apoptotic cell death.

In experiments involving MDA-MB-231 cell spheroids (3D cultures), exposure to isospintanol resulted in cell detachment after eight days and complete spheroid dissolution by ten days, particularly at concentrations four and eight times the IC_{50} . This indicates its potential efficacy as an anti-cancer agent (Contreras-Martínez *et al.* 2024).

Sapranthus Seem. The *Sapranthus* genus includes eight species distributed primarily in Mesoamerica; many of them are endemic to Mexico, unfortunately there are no studies on extracts or compounds with potential cytotoxic activity in cancer cells.

Sapranthus campechianus (Kunth) Standl. In this species, only antifungal activity of stem and root alkaloidal extracts have been investigated against the phytopathogens *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, and *Moniliophthora roreri*. The growth inhibition ranged from 14 to 45 %. The main component in these extracts was the oxoaporphine alkaloid liriodenine (Chong-Rodríguez 2015), which could be largely responsible for the effect shown, as it is a potent inhibitor of topoisomerase II (Woo *et al.* 1997).

Xylopia L. The essential oil extracted from the leaves of *Xylopia frutescens* Aubl. exhibits cytotoxic activity against lung carcinoma (NCI-H358M) and prostate carcinoma (PC-3) cell lines, showing an IC_{50} range of 24.6 to 40 $\mu\text{g/mL}$. Additionally, it can inhibit tumor growth by 31-37.5 %. The primary components of this oil are sesquiterpenes, including (E)-caryophyllene (31.5 %), bicyclogermacrene (15.13 %), germacrene D (9.66 %), δ -cadinene (5.44 %), viridiflorene (5.09 %), and α -copaene (4.35 %) (Ferraz *et al.* 2013).

The *Xylopi*a genus also produces a unique type of sesquiterpenoid dimer known as “guaianes,” such as vielanine, which demonstrates bioactivity against doxorubicin-resistant MCF-7 cells at doses of 10 μ M (Gao *et al.* 2019). Although there are no records of acetogenins (ACGs) isolated from *Xylopi*a species in Mexico, several acetogenins, including xylopiacin, xylopianin, xylomaticin, aromine, aromycin, and xymarginatin have been identified in *X. aromatica* and *X. emarginata* species (Neske *et al.* 2020). These compounds show strong cytotoxic activity in various cancer cell lines, such as MCF-7, A-549, HT-29, and BST, with an IC_{50} range from 1.36×10^{-6} to 0.25 μ g/mL. The most sensitive line was A-549, exhibiting an IC_{50} of 0.3 to 6.6 μ g/mL (Colman-Saizarbitoria *et al.* 1994).

Perspectives

Research on the anticancer properties of the Annonaceae family primarily focuses on two main areas: a) identifying extracts or compounds that effectively inhibit cancer cells, while remaining non-toxic to normal cells, and b) evaluating their effectiveness against drug-resistant tumor cells and their ability to induce programmed cell death. While the results obtained thus far are promising, several challenges remain before these compounds can be employed in clinical studies. Key concerns include the specificity of these substances toward cancer cells, their potential toxicity, and the optimal methods for administration, especially considering that many of these molecules and extracts have poor water solubility.

To address these issues, some researchers are developing chemical alternatives, such as semi-synthetic derivatives that are less toxic but still effective against cancer cells. One approach involves incorporating sugar units into acetogenins to enhance their solubility and overall physicochemical properties (Bachan *et al.* 2013). Another strategy is to modify the chemical structure by adding or removing functional groups to improve specificity. For example, the molecule “AA005,” a derivative of bullatacin acetogenin, has had its THF rings removed, resulting in enhanced antitumor activity and promoting cell death through both necrosis and apoptosis compared to its precursor (Huang *et al.* 2003, Han *et al.* 2015). In colon cancer cells, “AA005” activates the AMPK (AMP-activated protein kinase) pathway and inhibits the mTORC1 complex, inducing autophagy (Liu *et al.* 2012). It also negatively regulates several oncogenic proteins, such as myeloid leukemia factor 1 (Mcl-1) (Han *et al.* 2019), as well as key signaling pathways related to cell survival and proliferation, such as the PI3K/AKT pathway and aerobic glycolysis (Yu *et al.* 2022, Han *et al.* 2015). Another derivative, “AA0092,” with a linear structure, exhibits anti-angiogenic properties by negatively regulating growth factors such as VEGF, b-FGF, TGF β 1, and EGF (Zhang *et al.* 2015).

Another clear example of this strategy involves hybrid-acetogenins created by replacing the solamin lactone ring with 1-methylpyrazole. From the hybrid ACG-1-methylpyrazole, a series of 11 molecules was synthesized, with modifications to various functional groups typical of ACG. These included variations in the THF rings, hydroxyl groups, the length of the hydrophobic chain, and the addition of new functional groups to that chain. A structure-activity relationship study was conducted using a panel of 39 cancer cell lines (JFCR39). The results showed that replacing the lactone ring with 1-methylpyrazole increases the inhibition of cancer cell lines. The nature of the connecting group significantly affects both the potency and selectivity of the compound, especially the nitrogen atom at the 2'-position of the linker, which plays a crucial role in growth inhibition. *In vivo* studies demonstrated that the 1-methylpyrazole-5-sulfonamide analog effectively inhibits tumor growth in the xenograft mouse model using NCI-H23 human lung cancer cells, without causing systemic toxicity (Ohta *et al.* 2022).

Recently, another approach has emerged to address the challenges that have impeded clinical studies of Annonaceae metabolites: the use of nanosystems as vehicles for drug delivery targeting cancer cells. Two methods have gained particular attention in the application of these nanosystems: polymeric nanoparticles (NPs) and micelle synthesis (SMPMs). Both methods have shown promising results in improving the delivery, stability, and water solubility of ACG.

The first method involves utilizing polymeric nanoparticles with core/shell structures made from polyethylene glycol (PEG) or its derivatives, such as monomethoxycaprylate (mPEG) and ϵ -caprolactone (PCL). When combined

with cholesterol, these nanoparticles are effective at encapsulating hydrophobic compounds like acetogenins (Dong *et al.* 2008). This type of nanosystem was used to encapsulate bullatacin in poly ϵ -caprolactone (PCL-PEG) nanoparticles and tested in mice with 4T1 breast cancer tumors. The results demonstrated a 70 % reduction in tumor volume and up to a two-fold increase in potency compared to free bullatacin (Hong *et al.* 2016).

The second method uses cyclodextrins (ring-linked monosaccharides) due to their ability to form inclusion complexes with molecules of limited aqueous solubility. Micelles formed with α -Cyclodextrin and urea to encapsulate annonacin, were tested against drug-resistant ovarian carcinoma cells IGROV-1, obtaining promising results. The encapsulated annonacin increased up to twenty times the cytotoxic activity compared with the free annonacin in water (Gutiérrez *et al.* 2020).

Based on the aforementioned studies and others not addressed in this chapter, it has been established that many Annonaceae metabolites possess cytotoxic and/or antitumor activity due to their ability to modulate multiple cellular processes that influence tumor progression and metastasis, such as DNA replication, cell cycle, cell death, angiogenesis, tumor inflammation, cellular senescence, extracellular drug efflux, epithelial-mesenchymal transition, and cell migration. Therefore, compounds from the Annonaceae family have enormous potential for treating cancers with chemoresistance or those that lack effective therapies.

It is noteworthy that the genes influenced by Annonaceae secondary metabolites in cancer cells, as summarized in [Table 1](#), indicate a connection to cellular processes that are critical for cancer development. Consequently, we decided to perform a Gene Ontology term enrichment analysis (Pomaznoy *et al.* 2018) on this list, to identify the biological processes involved.

The results highlighted the formation of gene networks related to growth, proliferation, and cell death, as well as the development and functionality of the vascular system. These findings underscore the significance of the molecular targets identified for secondary metabolites from Annonaceae species, therefore their toxicity against cancer cells and anti-tumoral activity ([Figure 9](#)).

The Annonaceae plant family is an important source of molecules with potential pharmacological applications for various diseases, including cancer. Our country is home to over 70 species of Annonaceae, many of which are endemic. However, less than half of these species have undergone phytochemical studies, and there are currently no records evaluating their biological activity. As a result, these Annonaceae species represent a valuable phytochemical resource that could be explored and utilized sustainably.

Table 1. Gene cells modulated by Annonaceae secondary metabolites.

Symbol	Gene	Name	UniProt ID	Reference
Ciclina DA	CCNA2	Cyclin A2	P20248	(Xue <i>et al.</i> 2014)
Ciclina D1	CCND1	Cyclin D1	P24385	(Qian <i>et al.</i> 2015)
Cdk1	CDK1	Cyclin Dependent Kinase 1	P06493	(Lu <i>et al.</i> 2006)
Cdc25C	CDC25C	Cell Division Cycle 25C	P3030	(Lu <i>et al.</i> 2006)
mTOR	MTOR	Mechanistic Target Of Rapamycin Kinase	P42345	(Liu <i>et al.</i> 2012)
PCNA	PCNA	Proliferating Cell Nuclear Antigen	P12004	(Moghadamtousi <i>et al.</i> 2015)
MEK	MAP2K1	Mitogen-Activated Protein Kinase Kinase 1	Q02750	(Xue <i>et al.</i> 2014)
ERK	MAPK1	Mitogen-Activated Protein Kinase 1	P2848	(Xue <i>et al.</i> 2014)
JNK	MAPK8	Mitogen-Activated Protein Kinase 8	P45983	(Yuan <i>et al.</i> 2016, 2003)
Akt	AKT1	AKT Serine/Threonine Kinase 1	P31749	(Periyasamy <i>et al.</i> 2021)
PTEN	PTEN	Phosphatase And Tensin Homolog	P60484	(Bao-Bao <i>et al.</i> 2022)
VEGFA	VEGFA	Vascular Endothelial Growth Factor A	P15692	(Coothankandaswamy <i>et al.</i> 2010)

Symbol	Gene	Name	UniProt ID	Reference
Caspase 3	CASP3	Caspase 3	P42574	(Moghadamtousi <i>et al.</i> 2015)
Caspase 8	CASP8	Caspase 8	Q14790	(Qian <i>et al.</i> 2015)
Caspase 9	CASP9	Caspase 9	P55211	(Moghadamtousi <i>et al.</i> 2015)
Caspase 7	CASP7	Caspase 7	P55210	(Moghadamtousi <i>et al.</i> 2015)
Caspase 6	CASP6	Caspase 6	P55212	(Mondal <i>et al.</i> 2007)
Bcl-2	BCL2	BCL2 Apoptosis Regulator	P10415	(Moghadamtousi <i>et al.</i> 2015)
PARP1	PARP1	Poly(ADP-Ribose) Polymerase 1	P09874	(Yuan <i>et al.</i> 2006)
p21	CDKN1A	Cyclin Dependent Kinase Inhibitor 1A	P38936	(Yuan <i>et al.</i> 2016, 2003)
BAX	BAX	BCL2 Associated X, Apoptosis Regulator	Q07812	(Yuan <i>et al.</i> 2003, 2006)
BAD	BAD	BCL2 Associated Agonist of Cell Death	Q92934	(Yuan <i>et al.</i> 2003, 2006)
Survivin	BIRC5	Baculoviral IAP Repeat Containing 5	O15392	(Qian <i>et al.</i> , 2015)
HIF-1 α	HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	Q16665	(Coothankandaswam <i>et al.</i> 2010, Deep <i>et al.</i> 2016)
COX-2	PTGS2	Prostaglandin-Endoperoxide Synthase 2	P35354	(Wu <i>et al.</i> 2012)
NF- κ B	NFKB1	Nuclear Factor Kappa B Subunit 1	P19838	(Alshaeri <i>et al.</i> , 2020, Qian <i>et al.</i> 2015)
Hedgehog	SHH	Sonic Hedgehog Signaling Molecule	Q15465	(Bose <i>et al.</i> 2022)
Notch2	NOTCH2	Notch Receptor 2	Q04721	(Li <i>et al.</i> 2017)
Aurora B	AURKB	Aurora Kinase B	Q96GD4	(Lee <i>et al.</i> 2011)
MSK1	RPS6KA5	Ribosomal Protein S6 Kinase A5	O75582	(Lee <i>et al.</i> 2011)

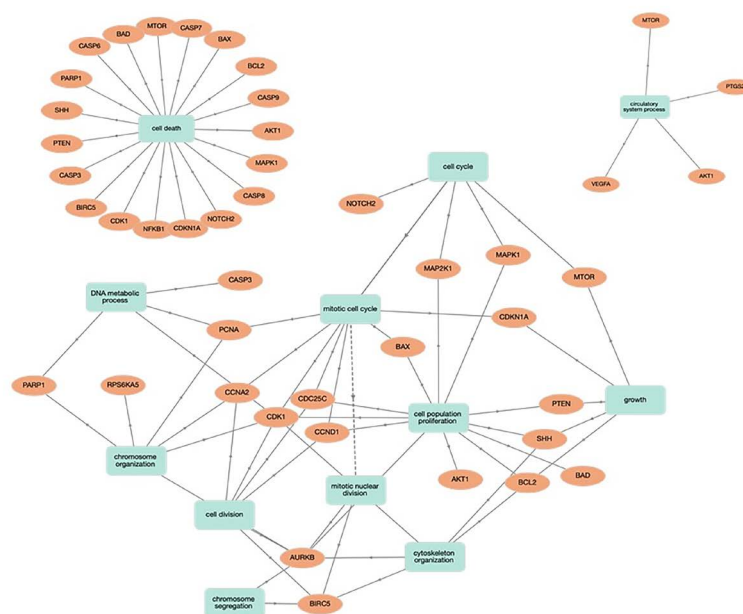


Figure 9. Gene networks modulated by Annonaceae secondary metabolites and associated with cell proliferation, cell death, and vascular changes in cancer cells, as well as the tumoral progression.

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