



# A review of the effects of *Calophyllum* spp. on cancer cells

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## Abstract

**Background:** The genus *Calophyllum* and its species has received great research interest for their phytochemical content and therapeutic potential. Said interest was sparked by the discovery of compounds with anti-HIV activities in one of its extracts and the genus has since been studied for potential in treating other morbidities. Generally, species under the genus contain various coumarins, xanthenes, triterpenoids, steroids, and chromanones. Extracts with said bioactive compounds can be obtained from all plant parts. This review aims to elucidate the anti-cancer activities of *Calophyllum* extracts and their potential in cancer treatment. **Results:** Independent in-vitro studies of the extracts on various cell lines have revealed the chemotherapeutic potential of the genus as shown by their cytotoxic, anti-cancer, and antitumor-promoting activities. Leukemic cancer cell lines, the most studied cell lines, have been shown to be the most sensitive to perturbations by *Calophyllum* extracts and compounds. **Conclusion:** *Calophyllum*-derived extracts and compounds have shown promising activities against cancer cell lines, particularly leukemic cancers. The presence of prenyl moiety at C-6 and the position of the hydroxy group and hydrophobic prenyl in the compounds have been attributed to their cytotoxicity. These findings are useful in providing leads

in producing naturally derived anti-cancer medication and developing potent analogs for the same purpose.

**Keywords:** Anticancer; benjaminin; *Calophyllum*, in- vitro, natural products

## 1. Introduction

*Calophyllum* is a genus of evergreen plants in the *Guttiferae* (*Clusiaceae*) family. Their occurrence covers a wide geographical area and a range of altitudes. Over 180 known species are distributed around tropical regions globally, mainly in Asia, Africa, America, Australasia, and the Pacific Islands. Generally, most species have opposite and decussate leaves, while others may have opposite, truly whorled, alternate, or adjacent leaf arrangements. Their trunk is mostly clean and straight. Their size varies from shrubs to trees. The species also vary in the pattern of their branch; some are erect, while others are pendulous. The type of modified roots present for support or breathing depends on and is consistent within their species and the nature of the vegetation region. Diamond- to boat-shaped fissures are often observed on the barks and may increase in depth and confluency with time; some possess smooth bark with lenticels lined vertically; others have flakes or scales (Stevens, 1980). Many parts of the plant have long been used traditionally to treat chronic diseases such as leprosy, inflammation, ulcers, and skin infections. For instance, the decoction of the root and leaf of *C. inophyllum* were used to treat eye and skin infections, respectively (Stevens, 1980). The seed oil of *C. apetalum* and seed latex of *C. inophyllum* was used to treat leprosy (Stevens, 1980; Watt, 2014). The seed oil of *C. soulattri* and

**Significance** | This review is shown the anti-cancer therapeutics effect of plant *Calophyllum*.

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and *C. tomentosum* were used to remedy skin infections (Stevens, 1980; Watt, 2014). Such applications of natural products in ethnomedicine have driven the phytochemical analysis of plant extracts. Phytochemical content of plants has long served as lead for drug discovery and development. Many of the currently used therapeutic drugs originated from plants, chemotherapy included. Relevant examples of anti-cancer drugs of plant origin include vincristin and vinblastine, isolated from *Catharanthus roseus*; and paclitaxel from *Taxus spp* (Cragg & Newman, 2013; Yuan et al. 2016). *Calophyllum* joined the long list of plants studied for their medicinal potential after the discovery of potent HIV-1 reverse transcriptase inhibitor (+)-calanolide A from *C. lanigerum* in the forests of Malaysia (Ito et al. 2006). Further studies found that *C. lanigerum*, *C. brasiliense*, and *C. teysmanii* contain calanolide A-D and calanolide E1-E2. Calanolide A and B isolated from Malaysian *C. lanigerum* demonstrates potent anti-HIV-1 activities via reverse transcriptase inhibition. Calanolide A has now reached clinical trials and passed phase 1 in 2016 (Kashman et al. 1992; McKee et al. 1996; Huerta-Reyes et al. 2004; Nahar et al. 2020). This article outlines the potential of *Calophyllum* spp. as reservoir for new anti-cancer drugs or drug leads with attributes to their chemopreventive phytochemical contents.

#### Effects of the different species of *Calophyllum* on cancer cell lines *in-vitro*

Many compounds have been extracted from different species of *Calophyllum* and their behavior toward various cancer cell lines studied over the decades. This review will highlight both the extract and isolated compounds from *Calophyllum* spp. that demonstrated potent or promising anti-proliferative potential against cancer cells as indicated by their half-maximal inhibitory concentration (IC<sub>50</sub>) that is comparative to the American National Cancer Institute (NCI) criteria of cytotoxicity and to that of standard drugs. The NCI states that crude extract is deemed cytotoxic if it has IC<sub>50</sub> value of < 30 µg/ml in the preliminary assay (Itharat et al. 2004).

#### *C. inophyllum*.

Caloxanthone N and gerontoxanthone C from the ethanolic leaf extract of *C. inophyllum* showed cytotoxicity against K562 (lymphoblast cell line) cells with IC<sub>50</sub> values of 7.2 and 6.3 µg ml<sup>-1</sup> respectively (Xiao et al. 2008). K562 cells are also shown to be inhibited by pyranojacareubin and macluraxanthone with IC<sub>50</sub> values of 8.62 and 5.28 µM respectively. The compounds showed only moderate activity when tested against HepG2 (hepatoblastoma cell line) cells (14.59 and 11.12 µM IC<sub>50</sub> values). However, SNU-1 (gastric epithelial cancer cell line) cells showed good response to macluraxanthone with 50% inhibition at 4.95 (Mah et al. 2014). On two separate *in-vitro* studies, the fruit extract and ethanolic leaf extract showed cytotoxic activity against MCF-7

(breast cancer cell line) cells with IC<sub>50</sub> value of 19.63 µg/mL and 120µg/mL respectively (Shanmugapriya et al. 2016; Jaikumar et al. 2016). Yellow and green pigments of *C. inophyllum* seed oil are shown to inhibit both the viability of DLD-1 (colorectal adenocarcinoma cell line) cells and survival of non-small-cell lung cancer (NSCLC) cells. The yellow pigment demonstrated higher toxicity than green pigment with 30% decrease in viability of DLD-1 cells (compared to control) at 1.5x10<sup>-3</sup>% concentration and 40% decrease at 12.4x10<sup>-3</sup>% concentration respectively (Hsieh et al. 2018). Synergistic evaluation on the green pigment and gefinitib observed increased cell death of A549 (lung carcinoma cell line) and H1975 (NSCLC) cells. Another coumarin, benjaminin, showed concentration-dependent cytotoxicity against SNU-1 (IC<sub>50</sub> = 70 µM), HepG2 (IC<sub>50</sub> = 109 µM), K562 (IC<sub>50</sub> = 150 µM) and NCI-H23 (lung cancer cell line) cells (IC<sub>50</sub> = 160 µM), indicating its potential to be candidate for drug development for treatment of stomach cancer (Mah et al. 2020). More recently, various xanthenes, especially caloxanthone A and macluraxanthone from the root of Thai *C. inophyllum* demonstrated cytotoxic activity against HCT-116 (colorectal carcinoma cell line) and HepG2 cells. However, the compounds only outperformed doxorubicin (standard drug, IC<sub>50</sub>=3.15µM) on HCT-116 cells with both compounds having IC<sub>50</sub> value of 3.04 µM. Other compounds from the extract, namely caloxanthone B, 7-O-dimethyl-mangostanin, and 7-prenyljacareubin also showed cytotoxic activity with IC<sub>50</sub> values 4.98 – 12.11 µM for HCT-116 cells and 5.94 – 24.50 µM for HepG2 cells (Haerani, Raksat & Pudhom 2021).

#### *C. brasiliense*.

Kimura et al. (2014) reported that a tricyclic coumarin named GUT-70 (5-methoxy-2,2-dimethyl-6-(2-methyl-1-oxobut-2-enyl)-10-propyl-2H,8H-benzo[1,2-b;3,4-b']dipyran-8-one) from *C. brasiliense* showed anti-cancer potential through growth inhibitory activities against a panel of leukemic cells; BV173 (chronic myelogenous leukemia cell line), K562, NALM6 (B cell precursor leukemia cell line), HL60 (promyelocytic leukemia cell line), and SEM (acute lymphoblastic leukaemia cell line) in a concentration and time-dependant manner (IC<sub>50</sub> 2 – 5 µM). Mammae type coumarins from *C. brasiliense* also exhibit cytotoxic activity against human tumor cell lines *in vitro*. Mammae A/BA achieved highest inhibition with IC<sub>50</sub> value of 0.04-0.59µM when tested against K562, PC3 (prostate cancer cell line), and U251 (malignant glioblastoma cell line) cells (Reyes-Chilpa et al. 2004). Ito et al. (2016) also reported observations of antitumor activities of mammae B/BB (and calophyllolide) against HL60 cells. Consistent with these results, Gomez-Verjan and colleagues also reported that mammae A/BA, mammae A/BB and B/BB from *C. brasiliense* demonstrated cytotoxic antitumor activity against K562 cell line (Gómez-Verjan et al. 2018).

***C. depressinervosum.***

Hexane extract of *C. depressinervosum* inhibited the proliferation of SNU-1 cells (IC<sub>50</sub> value of 9.50 µg/mL). Ananixanthine and caloxanthone B from the extract also demonstrated significantly higher cytotoxicity against K562 compared to control (cis-diammineplatinum (II) chloride) as suggested by the IC<sub>50</sub> values of 2.96 µg/mL and 1.23 µg/mL for ananixanthine and caloxanthone B respectively, being 27.45% and 69.85% lower than the standard drug which has IC<sub>50</sub> value of 4.08 µg/mL (Zamakshshari et al. 2019).

***C. buxifolium.***

Hexane extract of *C. buxifolium* exhibit anti-proliferative activity against LS-174T (colon adenocarcinoma cell line) with IC<sub>50</sub> value of 7.88 µg/mL, and K562 cells with IC<sub>50</sub> value of 16.72 µg/mL (Zamakshshari et al. 2019).

***C. soulattri.***

Calosubellinone from bark of *C. soulattri* showed cytotoxicity against HeLa (cervical cancer cell line) cells with IC<sub>50</sub> value of 19.3 µM. Garsubellin B from the same extract exhibit cytotoxic activity against HeLa cells and growth inhibitory activities against MDA-MB-231 (triple negative breast cancer cell line) cells with IC<sub>50</sub> values of 16.5 µM and 17.7 µM respectively. More importantly, these compounds demonstrated selectivity toward cancer cells as shown by the relatively significantly higher IC<sub>50</sub> values obtained for noncancerous HEK293 (human embryonic kidney cell line) cells (Lim et al. 2017). Phylatrin showed cytotoxic activity against SNU-1 (IC<sub>50</sub> = 9.79 µM), HeLa (IC<sub>50</sub> = 9.20 µM), and NCI-H23 (IC<sub>50</sub> = 10.45 µM) cells. Caloxanthone C is cytotoxic to HepG2 (IC<sub>50</sub> = 6.22 µM) and HeLa cells (IC<sub>50</sub> 6.88 µM). Soulattrin inhibited SNU-1, NCI-H23 and K562 cells with IC<sub>50</sub> value of 1.98, 2.64 and 2.23 µM respectively (Mah et al. 2012; Rumgay et al. 2022).

***C. sclerophyllum.***

Isodispar B isolated from *C. sclerophyllum* showed cancer-specific cytotoxicity via induction of apoptosis against SUNE1 (poorly differentiated nasopharyngeal carcinoma cell line), TW01 (Epstein-Barr Virus-negative human NPC cell line), CNE1 (nasopharyngeal carcinoma epithelioid cell line), and HK1 (differentiated squamous nasopharynx carcinoma cell line) cells with IC<sub>50</sub> values of 3.84, 11.49, 9.74, and 5.58 µM respectively. The IC<sub>50</sub> value of the compound against noncancerous NP460 (nasopharyngeal epithelial cell line) was 44.36 µM, demonstrating good selectivity of the compounds toward cancer cells (Lim et al. 2016).

***C. castaneum.***

Isoblancoic acid yielded from *C. castaneum* is reported for the first time to exhibit cytotoxic activity against HK1, TW01, and SUNE1 nasopharyngeal cancer cell lines (IC<sub>50</sub> < 30 µM)<sup>24</sup>. More recently, *C.*

*castaneum* isoblancoic acid also demonstrated potent cytotoxic activity against C6 (rat glioma cell line) and HCT-116 cells (IC<sub>50</sub> < 27 µM) while its friedelinol exhibited inhibitory activity against C6 cells (Lim et al. 2019).

***C. incrasaptum.***

Phenylcoumarins from *C. incrasaptum* was found to have significant anti-proliferative activity on human breast cancer cell MCF7 with IC<sub>50</sub> value of 2.23 µg/mL (Abbas, Z Udin & Hanafi, 2018).

***C. canum.***

A new xanthone dimer extracted from *C. canum*, along with known compounds vis trapezilofolixanthone and trapezilofolixanthone A, exhibited weak cytotoxic activity against A-549 (adenocarcinoma human alveolar basal epithelial cell line), MCF-7, and C33A (human cervical cancer cell line) cell lines (Taher et al. 2020).

***Calophyllum* as source of chemopreventive agents**

***C. inophyllum.*** *In-vitro* analysis on calocoumarin-A from *C. inophyllum* revealed that it exhibits no cytotoxicity to Raji (human B lymphoblastoid cell line) cells but potent inhibitory activity against Epstein-Bar virus – a pathogen associated with Hodgkin's lymphoma, Burkitt's lymphoma, gastric carcinoma, and nasopharyngeal carcinoma. *In-vivo* study of the same compound observed delayed carcinogenesis on mouse skin suggesting its potential as chemopreventive agent (Itoigawa et al. 2001).

***C. elatum.*** Isogarciniaxanthone E from *C. elatum* showed inhibitory activity comparable to curcumin (Ito et al., 2018). The compound was found to inhibit the activation of 12-Otetradecanoylphorbol-13-acetate (TPA) induced Epstein-Barr virus early antigen (EBV-EA) in Raji cells at 35.96% lower IC<sub>50</sub> value compared to curcumin, a well-known regulator of several cell signalling pathways in cancer including p53, PI3K-Akt, JAK-STAT, Wnt/β-catenin, MAPK, and NF-κB (Ito et al. 201; Wang et al. 2021). Carcinogenesis assay in mouse skin revealed the compound's ability in delaying papilloma development thus further indicating its chemopreventive potential (Ito et al. 2018).

***C. brasiliense.*** Brasixanthone-B, -C, -D, and 8-desoxygartinin isolated from *C. brasiliense* showed superiority to the well-known anti-tumor promoter, β-carotene, in inhibiting TPA-induced EBV-EA activation in Raji cells. The compounds scored IC<sub>50</sub> values of 120-310 mol ratio/TPA. These values are at least 22% lower than that of β-carotene which has IC<sub>50</sub> value of 400 mol ratio/TPA (Ito et al. 2002). Meanwhile, the plant's methanol extract is reported to display immunostimulatory activity and cytoprotective activity on normal cells (Philippi et al. 2010).

***C. polyanthum*.** Apetalic acid from *C. polyanthum* was found to have concentration and time-dependent anti-proliferative activity against LOVO (human colorectal carcinoma cell line) and SW480 (human colorectal adenocarcinoma cell line) human colon cancer cells via induction of apoptosis. Treatment with the compounds saw an increase in the levels of Bax, cleaved-caspase-9 and 3 while that of Bcl-2 and p-AKT are decreased. The compound is also able to inhibit invasion and migration of both cell lines in concentration-dependent manner (Liu et al. 2022).

***C. benjaminum*.** Benjaminin was extracted for the first time from chloroform extract of *C. benjaminum* ground stem bark along with other known xanthenes i.e. fuscaxanthone C,  $\beta$ -mangostin, thwaitesixanthone, dombakinaxanthone, and caloxanthone A, triterpenes i.e. friedelin,  $\beta$ -sitosterol, lupeol, and stigmaterol (Sahimi et al. 2015). However, there is no record of study done on the effects of *C. benjaminum* extracts on any cancer cell lines.

#### ***Calophyllum* spp. and apoptosis**

Apoptosis onset is characterized by cell and nucleus shrinkage, condensation of nuclear chromatin and eventually, karyorrhexis. Without leaking of cellular organelles, the cell will start to break into apoptotic bodies (blebbing) which will be phagocytosed by neighbouring cells i.e. macrophages and parenchymal cells. This distinguishes it from necrosis, as no inflammation is involved (Saraste 2000; Henry, Hollville & Martin 2013). Various genes and proteins are responsible for regulating apoptosis, and the ability to avoid it is an important hallmark in cancer. Cancer cells achieve this by downregulating pro-apoptotic genes and upregulating anti-apoptotic genes. Expression of pro-apoptotic gene, p53, is often lost in cancer cells. Therefore, apoptosis signalling pathway is a great target in anti-cancer therapy. There are three pathways through which apoptosis is induced: the extrinsic, intrinsic, and granzyme B (Vasanth Rupasinghe, Nair & Robinson 2014). All three pathways eventually wind up in activation of caspase-3, which is known as the effector protein in apoptosis. Activation of caspase-3 is achieved via interaction with caspase-8 (extrinsic pathway) or caspase-9 (intrinsic pathway) (Saraste 2000; Elmore 2007).. Studies have observed that *Calophyllum* spp. extract and compounds exert the anti-proliferative and cytotoxicity effects through induction of apoptosis via the intrinsic pathway. For example, in *C. brasiliense*, mammea-type coumarins are shown to be potent candidate for chemotherapy drug development in treating leukemic cancers due to their low IC<sub>50</sub> values *in-vitro* and ability to interact with multiple pathways including cell death, PI3K/AKT, MAPK, and Ras pathways (Ito et al. 2006; Gómez-Verjan et al. 2018). Caspase activation is a common mechanism of action of these compounds. Its mammea A/BB inhibits the autophagic flux in K562 cells and suppresses the expression of Bcl-2 while promoting over-expression of pro apoptotic Bax and Bak

proteins, which are known to permeate the mitochondria leading to release of cytochrome C, ultimately triggering activation of caspase-9 and later, caspase-3 (Gómez-Verjan et al. 2018). Its mammea B/BB and calophyllolide of are also reported to activate caspase-9 and -3 in HL-60 cells (Ito et al. 2006). Mammea-type coumarins are not the only compounds to exhibit such mechanistic. Similarly, apetalic acid from *C. polyanthum* induce apoptosis in SW480 and LOVO cells via interaction with caspase-9 and 3 whereby their levels are seen to be increased. Apetalic acid was also found to reduce tumour suppressor Bcl-2 expression levels and p-AKT (Liu et al. 2022). As for *C. inophyllum*, its fruit extract induces apoptosis in MCF-7 breast cancer cell via upregulation of Bax (and p53 pro apoptotic proteins) and down regulation of Bcl-2 (anti-apoptotic) proteins. As a result, mitochondrial membrane potential become decompromised and cytochrome C is released, prompting a sequence of downstream protein activations that lead to cell cycle arrest at G<sub>0</sub>/G<sub>1</sub> and G<sub>2</sub>/M phases and ultimately, commencement of apoptosis via caspase-3 cascade (Shanmugapriya et al. 2017). Yellow and green pigments extracted from *C. inophyllum* seed oil is reported to induce apoptosis and cell cycle arrest at G<sub>2</sub>/M phase in DLD-1 cells (Hsieh et al. 2018). Another potent coumarin from *C. brasiliense*, GUT70, inhibit growth of BV173, K562, NALM6, HL60, and SEM cells via interaction with caspase-mediated apoptosis pathway (Kimura et al. 2004). Interestingly, the induction of apoptosis by GUT-70 is independent of p53, as shown by the unaltered p21<sup>WAF1/CIP1</sup> expression – unlike most chemotherapeutic drugs – making it potentially useful against tumour with impaired p53-dependant pathway (Kimura et al. 2004; Avramis et al. 1998). Structural analyses found a link between presence of prenyl moiety in C-6 of the compounds and their anti-proliferative activity. This is seen where compounds of an extract that has a prenyl moiety located at C-6 has significantly higher cytotoxic activities compared to other compounds of the same extract where prenyl moiety is absent or located differently (Mah et al. 2020; Haerani, Raksat & Pudhom 2021; Itoigawa et al. 2001). Furthermore, in the context of antitumor-promoting effect, other than the presence of prenyl moiety in the molecule, the relative position of hydroxy group and hydrophobic prenyl moiety in the compound is also seen to influence the compound activity (Ito et al. 2002). It has also been reported that presence of prenyl at the xanthone nucleus (i.e. in soulatrin) contributed to comparatively higher activity against SNU-1 cells than other compounds (phylatrin and inophinnin) demonstrating moderate cytotoxic effect. Compounds with prenyl substituents exhibited slight activity while non-prenylated xanthenes saw a fall in cytotoxicity. Additionally, the position of hydroxyl group and the ultimate size of compound are also crucial influencers in the inhibitory effects of the compounds where the former contributes to cytotoxicity and the latter causes a total loss to the inhibitory effects of compounds (Mah et al. 2014).

## Conclusion

To date, the most studied variety of cancer cell line against action of *Calophyllum spp.* extract/compound is leukemic cell line (five varieties) followed by lung and nasopharyngeal carcinoma (four varieties respectively), colon (three varieties), breast and brain cancer cell lines (two varieties respectively), and liver, stomach, prostate, and cervical cancer cell lines (1 variety respectively). Early studies suggest the mechanism of action adopted by the extracts/compounds is the induction of apoptosis. Cytotoxicity of compounds is suggested to be greatly influenced by the presence of prenyl moiety at C-6 and the position of the hydroxy group and hydrophobic prenyl in the compounds.

## Author Contributions

Melissa Kilus wrote the manuscript; Shaari Daud reviewed the manuscript and supervised the study; Nozlina Abdul Samad reviewed, edited, supervised and studied the design.

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## Competing financial interests

The authors have no conflict of interest.

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