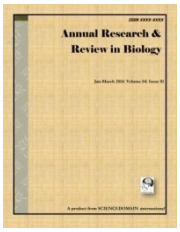

*Annual Research & Review in Biology*
*4(10): 1502-1517, 2014*

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# Biological Importance of Phytochemicals from *Calophyllum brasiliense* Cambess

**A. Bernabé-Antonio[1], L. P. Álvarez-Berber[1] and F. Cruz-Sosa[2*]**

*[1]Chemical Research Center, Autonomous University of the State of Morelos, 1001 University Avenue, Col. Chamilpa C.P. 62209, Cuernavaca, Morelos, México.*
*[2]Department of Biotechnology, Division of Biological and Health Sciences, Metropolitan Autonomous University-Iztapalapa Campus, 186 San Rafael Atlixco, CP. 09340 México D.F, México.*

***Authors' contributions***

*Authors ABA, LPAB and FCS collected the literature searches. Author ABA wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

***Review Article***

***Received 22nd December 2013***
***Accepted 23rd January 2014***
***Published 30th January 2014***

## ABSTRACT

A great diversity of medicinal plants have been traditionally used against gastropathy, infections and inflammatory pathological ailments. *C. brasiliense* (Clusiaceae) is a large tree native to the tropical forest which thrives from Brazil to Mexico. It is a plant commonly used in traditional medicine against several diseases, infections and other pathological disorders. Phytochemical isolations of heartwood, leaves, stem bark, and seed oil have confirmed the existence of several coumarins, xanthones and triterpenes which have a wide biological activity against bacteria, protozoa, fungus, virus and cancer. *C. brasiliense* is highlighted as an important resource of calanolides, a dipyranocoumarins that inhibit reverse transcriptase of human immunodeficiency virus type 1 (HIV-1 RT). Despite having wide medicinal importance, the fact that in Mexico it is poorly known is causing a reduction of this species. In this regard, studies on preservation and production of chemical compounds by plant cell culture need to be developed. The aim of this review is to provide the general characteristics of *C. brasiliense*, the most common traditional uses, and its phytochemical constituents. In particular, we discussed extracts and phytochemical components that have displayed anticancer, antiviral, antimicrobial and antiparasitic activity reported in the current literature. In addition, we intended to inspire new studies on phytotherapy, bioproduction and conservation to be developed.

**Corresponding author: Email: cuhp@xanum.uam.mx;*

*Keywords: C. brasiliense; Phytochemicals; Bioactivity; Calanolides, VIH-1.*

## 1. INTRODUCTION

Plants have been used since ancient times by human civilization to treat and cure diseases. They have made significant contribution to the great expansion of the pharmaceutical industry, with the isolation of bioactive substances, which owing to their complex structure, are very difficult to obtain by organic synthesis. However, its irrational use has resulted in a decline and disappearance of many tree species in the world. Therefore, a sustainable use of natural products is becoming more important [1]. In fact, the importance of some genus as *Calophyllum* has increased. *Calophyllum* is a large group of tropical trees consisting of ca. 180-200 different species. Among the eight found in the American Continent, *C. brasiliense* is the widest distributed species among the eight found in the American Continent and grows in the tropical rain forests from Brazil to Mexico [2,3].

Traditionally this species has been used in many different ways, such as wood, forages, dye extracts, making of soaps, biofuels and medicinal uses [4]. For instance, the oil has been used for rheumatic problems, skin diseases, hemorrhages and pain [5,6]. In Mexico, only one species of *Calophyllum* exists, namely *C. brasiliense* Cambes [7] which contains a wide variety of phytochemicals with antibacterial, anticancer, antiparasitic and antiviral activity [7,8,9,10,11,12]. This tree shows two chemotypes, i.e., two different chemical compositions in the leaves have been characterized according to its natural distribution [7]. The first chemotype (CTP 1) grows in Sierra de Santa Marta, State of Veracruz, Mexico, and produces mammea type coumarins with high in vitro cytotoxic activity against human tumor cells [8]. The second chemotype (CTP 2) grows in San Andres Tuxtla, State of Veracruz, Mexico, and produces tetracyclic dipyranocoumarins, which provide complete protection against human immunodeficiency virus type 1 (HIV-1) replication and cytopathicity [7,9].

Due to the limited knowledge we have about the general aspects of *C. brasiliense* and its phytopharmacological importance, the aim of this review is to examine the different researches that have been carried out in Mexico, as well as in Central and South American countries, including the general aspects of the species, emphasizing extracts or active individual phytochemicals against cancer, viral, microbial, parasital and other disorders reported in the current literature. Finally, we discuss the option of using plant biotechnology to produce relevant phytochemical compounds, and further studies to be developed.

## 2. GENERALITIES AND TRADITIONAL USES OF *C. brasiliense*

*C. brasiliense* (Clusiaceae) is a medium-sized, evergreen tree 12-20 m tall and 0.5 m or more in diameter. The bark is light grey and smooth or slightly fissured, with numerous protuberances on large trunks. Flowers are numerous, small, fragrant and white .The fruits are drupes 2.5 to 3 cm long, ovoid or spherical, yellowish green when ripe. Seeds are spherical from 1.7 to 2.2 cm long and wide, yellowish white without endosperm [3,13]. *C. brasiliense* is distributed mainly in the rain forests of Latin America, from Brazil to Mexico [4]. In Mexico it is referred by various names depending on location. For example, in Veracruz, Tabasco, Chiapas and Oaxaca is named *ocú*, *bari* or *leche amarilla* (yellow milk), while in the north of Chiapas and Tabasco is named *cedro de borrego* (sheep cedar) or *guaya* [13]. In Veracruz, México, two populations (CTP 1 and CTP 2) have been reported differing in their leaf chemistry [8-9]. In fact, these populations exhibit anatomical differences in their leaves, supporting a speciation process in this taxon, associated with a specific leaf chemistry [14].

For its great versatility, *C. brasiliense* is used as forage, timber, utensils, furniture manufacturing, and as reforestation of degraded tropical areas, since it tolerates poor soils [4,15]. In some places it is planted to provide shade for coffee and cacao crops and as windbreak curtains without any problems of interaction [16]. The oil extracted from the seed is used for lighting purposes (biofuel). Among their medicinal uses, the stem bark produces a brown tincture, and the resin excreted from the trunk is used to decrease itchiness, heal ulcers, as diuretic, for swollen eyes and sunburn. Leaf extracts are frequently used to treat skin diseases, pain, asthma, and to make laxatives. It is also used to control diabetes, hypertension, diarrhea and herpes [17,18]. In some rural communities from Mexico, oil has also been used to heal skin diseases [13,18].

## 3. BIOLOGICAL ACTIVITY OF PHYTOCHEMICAL COMPOUNDS

In the world there is great diversity of plants (over 300,000 species), which remains a large reservoir of phytochemicals compounds, including secondary metabolites [1]. In contrast to primary compounds (lipids, carbohydrates, proteins, etc.), the secondary metabolites are characterized because they are stored in low concentration in plant tissues and play an important role in the survival of the plant in the environment [19]. Secondary metabolites are a rich source of bioactive compounds that are biodegradable into nontoxic products [20]. Phenolics, terpenes and alkaloids are considered the main groups of secondary metabolites and they are classified according to their biosynthetic pathways [21].

It has been shown that the biological activities of *Calophyllum* genus contain a wide variety of phytochemical compounds against several affections (Table 1); however, *C. brasiliense* has been highlighted for producing potent compounds against cancer and HIV-1.

**Table 1. Main species of genus *Calophyllum* containing compounds with biological activity**

| Species | Plant portion | Compounds | Biological activity | References |
|---|---|---|---|---|
| *C. blancoi* | Seeds | Chromanones, xanthones | Antitumor, antiviral | [22,23] |
| *C. caledonicum* | Leaves, stem bark, trunk bark | Coumarins, xanthones | Aniviral, antifungal, antimalarial | [24,25,26] |
| *C. cordato-oblogum* | Twigs, buds | Coumarins, xanthones, biflavonoids, triterpenes | Antiviral | [27] |
| *C. dispar* | Stem bark | Coumarins | Antileukemia | [28,29] |
| *C. enervosum* | Stem bark | Xanthones, ketones | Antibacterial, antioxidant, cytotoxicity | [30,31] |
| *C. inophyllum* | Stem bark, leaves | Coumarins, xanthones, triterpenes | Antitumor, antiviral, cytotoxic, antibacterial, analgesic | [32,33,34] |
| *C. mucigerum* | Stem bark | Xanthones, coumarins | Antileukemia, insecticidal | [35] |
| *C. panciflorum* | Stem bark | Xanthones, biflavonoids | Antitumor | [36] |
| *C. teysmannii* | Wood | Xanthones | Immunomodulatory | [37,38] |

## 3.1 Anticancer

Plant-derived compounds have played an important role in the development of several clinically useful anticancer agents [39,40]. For instance, some xanthones such as the brasixanthones A (Fig. 1A), B (Fig. 1B), C (Fig. 1C), D (Fig. 1D), E (Fig. 1E), F (Fig. 1F), and G (Fig. 1G) isolated from *C. brasiliense* stem bark have demonstrated anticancer activity. However, only four compounds (Fig. 1B, Fig. 1C, Fig. 1D and Fig. 1G) have displayed high significant inhibitory activity (100%) against 12-*O*tetradecanoylphorbol-13-acetate induced by Epstein-Barr virus (a common form of herpes virus) early antigen activation in Raji cells, which causes infectious mononucleosis. This may be used as cancer chemopreventive agent [41].

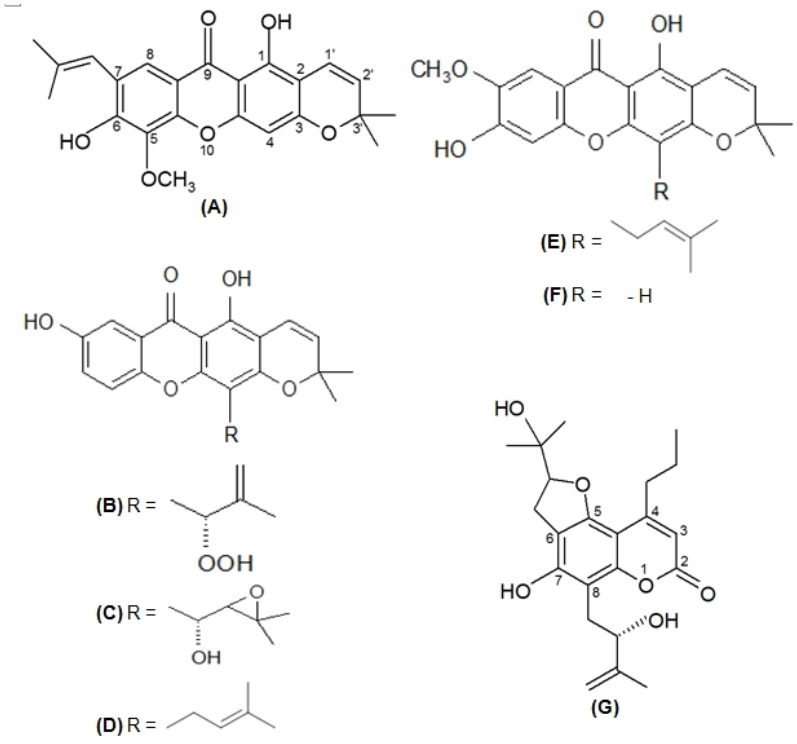



**Fig. 1. Xanthones isolated from *C. brasiliense* with anticancer activity.**

Another group of compounds known as Mammea type coumarins isolated from leaves hexane extracts have been investigated since they have favorable effects against certain cancers. For instance, coumarin mammea A/BA (Fig. 2A) has displayed the highest cytotoxic activity $IC_{50}$ = 0.04 to 0.59 µM) against K562 (lymphoma), U251 (central nervous system), and PC3 (prostate) human tumor cell lines, whereas mixtures of mammea A/BA (Fig. 2A) + A/BB (Fig. 2B), mammea B/BA (Fig. 2C) + B/BB (Fig. 1D), and mammea C/OA (12) + C/OB (13) were also highly active ($IC_{50}$ < 4.05 µM). When a mixture of 31µM of each compound (Fig. 2A + 2B or Fig. 2E + Fig. 2F) was tested, mammeas showed inhibition values of 88 to 100% on the three human tumor cells. Triterpenoid friedelin (Fig. 2G) isolated from the same extract also showed to be cytotoxic against PC3 (61.9%) and U251 (25.8%) lines [8].

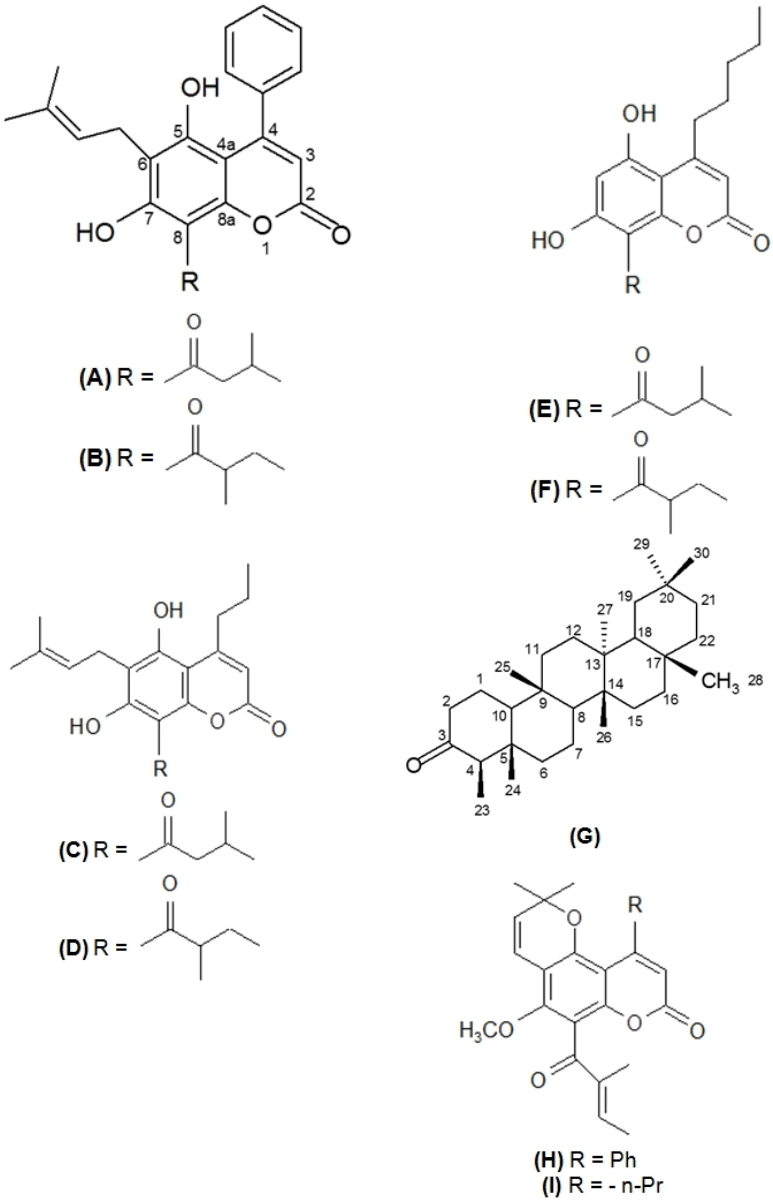



**Fig. 2. Coumarins and other compounds isolated from *C. brasiliense* with anticancer activity.**

Coumarins also have been evaluated on the survival cell cycle and apoptosis of cells in-vitro and their antitumor effects in mice. These studies showed that mammea A/BA (Fig. 2A) and mammea A/BB (Fig. 2B) caused a decrease greater than 50% in the survival of BMK (baby mouse kidney) cells by inducing apoptosis and, to a lesser degree, necrosis when concentrations higher than 20 μg $mL^{-1}$ were used. Further, the cell cycle in S-phase was arrested and the division of BMK cells was inhibited [42]. Calophyllolide (Fig. 2H) and mammea B/BB (Fig. 2D) coumarins have showed significant cytotoxicity against human leukemia cell line HL-60, suggesting that both compounds also induced apoptosis in HL-60

cells through activation of the caspase (cysteine-aspartic protease)-9/caspase-3 pathway, which is triggered by mitochondrial dysfunction [43]. The tricyclic coumarin GUT-70 (5-methoxy-2,2-dimethyl-6-(2-methyl-1-oxo-2-butenyl)-10-propyl-2H,8H-benzo[1,2-b,3,4-b0]dipyran-8-one) (Fig. 2I) isolated from the stem bark has shown significant inhibition ($IC_{50}$ 2–5 μM) against growth of human leukemic cell lines BV173, K562, NALM6, HL60, SEM and colorectal adenocarcinoma cell line HCT116, including P-glycoprotein overexpressing cell line. Additionally, GUT-70 (Fig. 2I) activated caspase 2, 3, 8 and 9, and induced apoptosis in leukemic cells, which was inhibited by caspase inhibitors [44]. Studies cited above suggest that *Calophyllum* produces a wide range of xanthones and coumarins that may be used against different types of cancer.

## 3.2 Antiviral

Viruses have been resistant to therapy or prophylaxis longer than any other form of life. For instance, several ailments, viral infections, mainly infections associated with human immunodeficiency virus type 1 (HIV-1) and 2 (HIV-2), and newly emerging infectious viruses have been difficult to eradicate [45]. Therefore, several plants have been studied and demonstrated promise to treat a number of viral infections [7,9,45,46]. Recently, it has been possible to isolate several agents plants yield showing anti-VIH-1 activity, and one of these, (+)-calanolide A (Fig. 3A), is in clinical development [39,47]. In fact, other organic plant extracts of 21 species of Clusiaceae from Mexico were screened for anti HIV-1 reverse transcriptase activity in a non-radioactive immune colorimetric assay. Only five species exhibited significant inhibition (≥ 70%) of HIV-1 activity, and *C. brasiliense* hexane extract showed high significant inhibition on viral replication ($ED_{50}$=37.1 mg $ml^{-1}$) [9]. Due to this, extracts were fractionated, and calanolide A (Fig. 3A), calanolide B (Fig. 3B), calanolide C (Fig. 3C) and soulattrolide (Fig. 3D) where isolated in low concentrations (≤ 0.009%). All compounds tested showed high inhibition (over 70%), mainly calanolide A (Fig. 3A) with 81.5% and showed no toxicity to MT2 human lymphocytes with $IC_{50}$ values from 0.34-0.66 μM $ml^{-1}$ [9].

Current research carried out in Mexico, have shown that populations of *C. brasiliense* collected in Soconusco, State of Chiapas, also have activity against HIV-1 [48]. They demonstrated that leaf extracts from *C. brasiliense* displayed potent anti-HIV-1 inhibition ($IC_{50}$=20.2 μg $ml^{-1}$), but was not toxic in mice with a lethal dose ($LD_{50}$=1.99 g $kg^{-1}$). In addition, the histological study of mice treated at the highest dose revealed no alteration on hepatocytes, and an increase in number of spleen megakaryocytes. It has been demonstrated that GUT-70 coumarin (Fig. 2I) is an anticancer compound [44]. However, it has also inhibited HIV-1 replication in both acutely and chronically infected cells through suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) strengthening the idea that NF-kB pathway is one of the potential targets to control HIV-1 replication and that GUT-70 (Fig. 2I) could serve as a lead compound to develop novel therapeutic agents against HIV-1 infection [49]. These results support the idea that *C. brasiliense* represent a valuable source of potential anti-HIV-1 compounds.

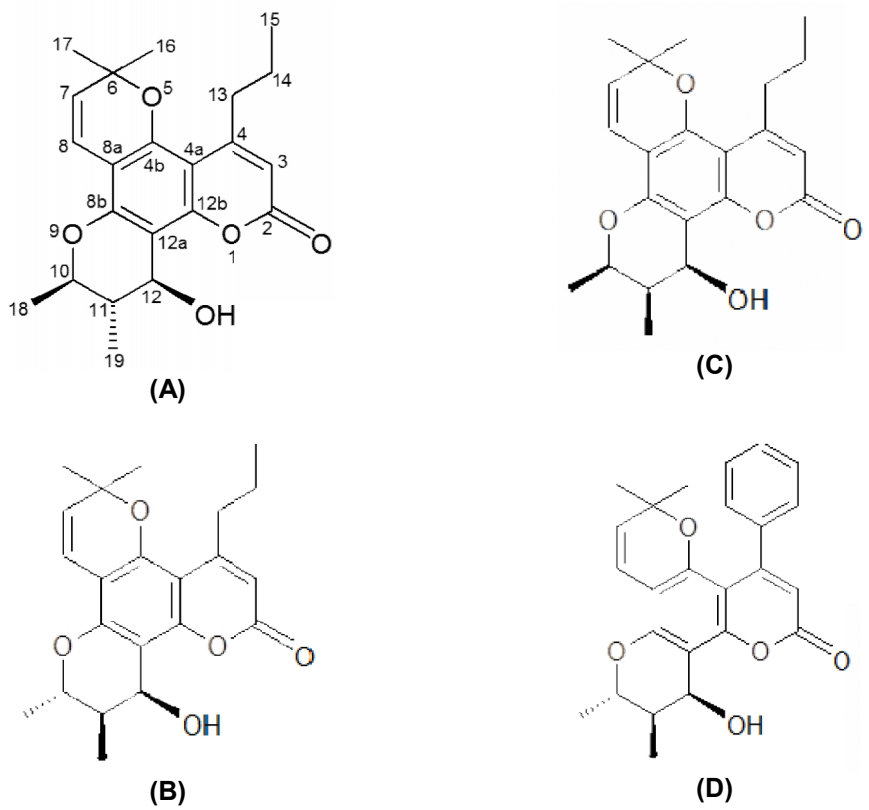


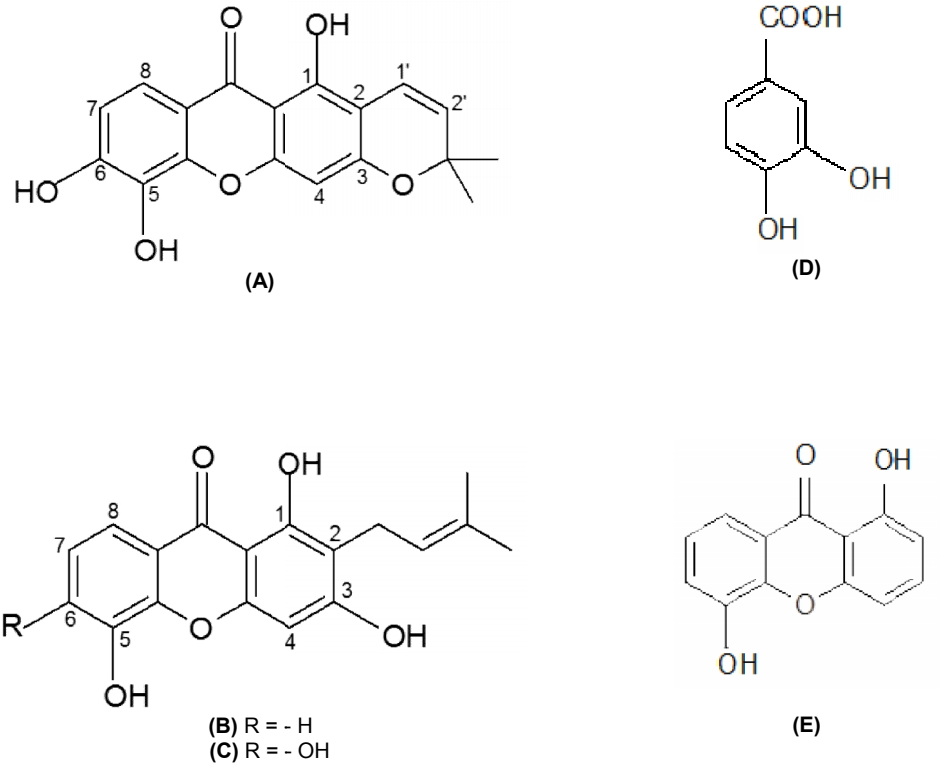

**Fig. 3. Dipyranocoumarins isolated from *C. brasiliense* with anti-VIH-1 activity.**

## 3.3 Antimicrobial

Numerous surveys have demonstrated the wide occurrence of active antimicrobial substances in plants. The array of compounds with unique structures which plants produce has served as a stimulus to a continual search for useful antimicrobial substances, especially from plant sources. In this regard, the heartwood of tropical tree *C. brasiliense* is known to be highly resistant to fungi and termites. Methanol, acetone, and water extracts (5 mg $ml^{-1}$ = 0.5%) from heartwood inhibit mycelial growth of the brown rot fungus *Postia placenta* by 83%, 59%, and 21%, respectively. Subsequently, isolation of individual compounds confirmed that jacareubin (Fig. 4A), 2-(3,3-dimethyllallyl)-1,3,5-trihydroxyxanthone (Fig. 4B) and 2-(3,3-dimethylallyl)-1,3,5,6-tetrahydroxyxanthone xanthones (Fig. 4C) inhibit mycelial growth *of P. placenta* at 0.25 mg/ml, ranged from 55.5 to 68.8% [50]. Crude methanolic extracts and two fractions from roots, stems, leaves, flowers and fruits were studied, and all extracts exhibited antimicrobial activity against Gram-positive bacteria. Authors demonstrated that protocatechuic acid (Fig. 4D) is responsible for antimicrobial activity against several Gram-negative bacteria, Gram-positive bacteria and yeasts, whereas compound 1,5-dihydroxyxanthone (Fig. 4E) exhibited activity only against Gram-positive bacteria [51]. Growth of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis* also was inhibited by using a mixture of mammea A/BA + A/BB (Fig. 2A and Fig. 2B) and mammea C/OA + C/OB (Fig. 2E and Fig. 2F) [8,10]. Xanthones, jacareubin

(Fig. 4A) and 2-(3,3-dimethylallyl)-l,3,5,6-tetrahydroxyxanthone (Fig. 4C), have displayed moderate activity against *Escherichia coli* [10].

On the other hand, *Helicobacter pylori* is considered the main etiological agent of human peptic ulcer, with a worldwide prevalence rate of about 40% in developed countries and over 80% in developing countries [52]. A few studies to heal gastric disorders have been carried out. Hydroethanolic and dichloromethanic fractions of bark extract evaluated in in vitro assays revealed to be potent against *Helicobacter pylori* growth with a minimum inhibitory concentration (MIC=31 µg $ml^{-1}$) and when treatments of hydroethanolic (50, 100 and 200 mg $kg^{-1}$) and dichloromethanic (100 and 200 mg $kg^{-1}$) were evaluated in ulcerated rats, wounds were reduced in all cases [53]. However, more detailed studies are required to reveal the compound that acts against *H. pylori* and on the healing of ulcers.




**(A)** **(D)**

**(B)** R = - H
**(C)** R = - OH

**(E)**

**Fig. 4. Xanthones and phenolics isolated from *C. brasiliense* with anti-microbial activity.**

## 3.4 Antiparasitic

It is known that more of than 30% of the human population is affected by endoparasites, including protozoa, nematodes, trematodes and cestodes. Parasites have become resistant to available synthetic therapeutics and sometimes the drugs of choice for treatment are harmful, showing renal and cardiac toxicity [11,54]. Medicinal plants are an alternative source of antiparasitic drugs. In this regard, chemical constituents of *C. brasiliense* have been highly active against certain parasites. For instance, the purified compound (-)-

mammea A/BB (Fig. 5A) from *C. brasiliense* was evaluated in in-vitro against leishmaniasis, and showed significant activity against promastigote and amastigote forms of *L. amazonensis*, with $IC_{50}$ =3.0 or 0.88 µg $ml^{-1}$, and $IC_{90}$ = 5.0 or 2.3 µg $ml^{-1}$, respectively [55]. However, no cytotoxicity against J774G8 macrophages cell line was showed when it was tested at high concentrations that inhibited promastigote forms. The same authors investigated the cytotoxicity activity of dichloromethane extract and (-)-mammea A/BB (Fig. 5A) on mouse peritoneal macrophages at concentrations of 60 and 23.2 µg $ml^{-1}$ respectively, emphasizing that these extracts and compounds exhibit significant activity against promastigote forms of *L. braziliensis*, being 50% a lethal dose ($LD_{50}$). Also, they reduced by 50% the infection index of parasites in macrophages at concentrations of 22 and 29 µg $ml^{-1}$, respectively, while doses of dichloromethane extract (up to 1000 µg $ml^{-1}$) did not affected the mouse peritoneal macrophages [56].

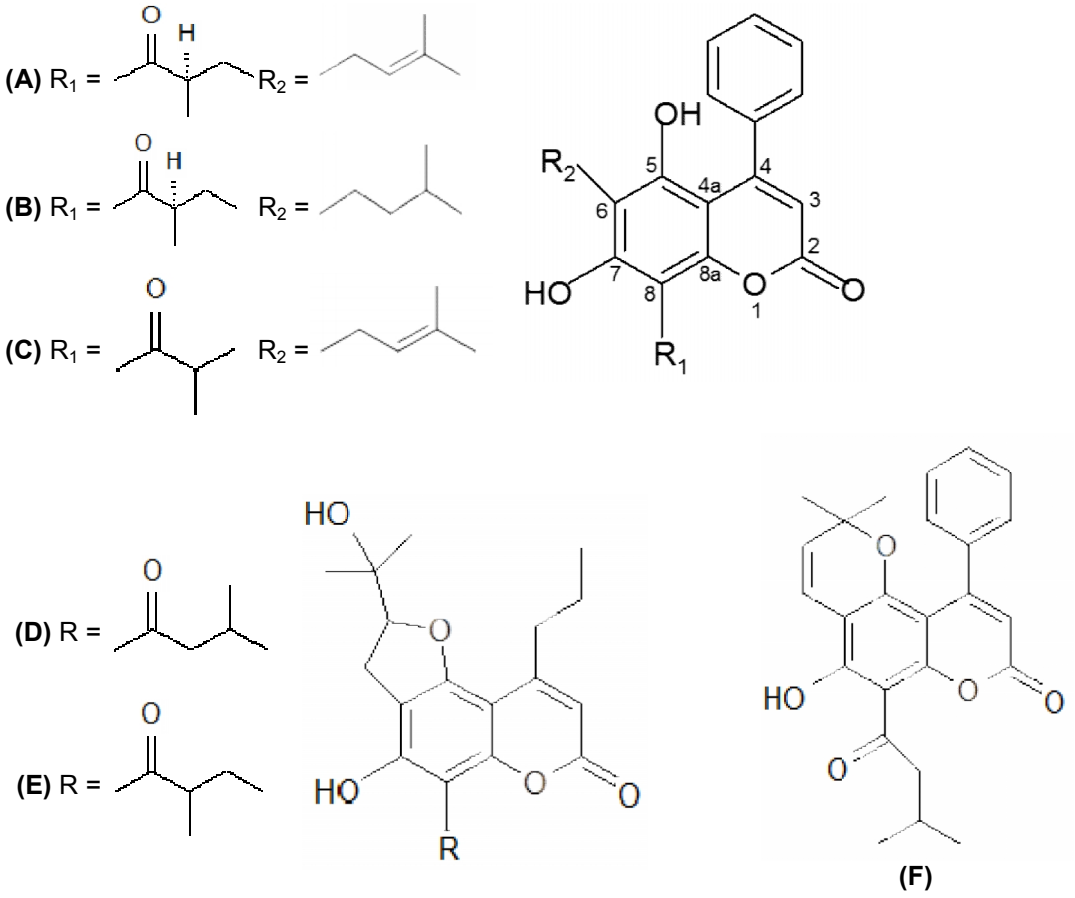



**Fig. 5. Coumarins isolated from *C. brasiliense* with antiparasitic activity.**

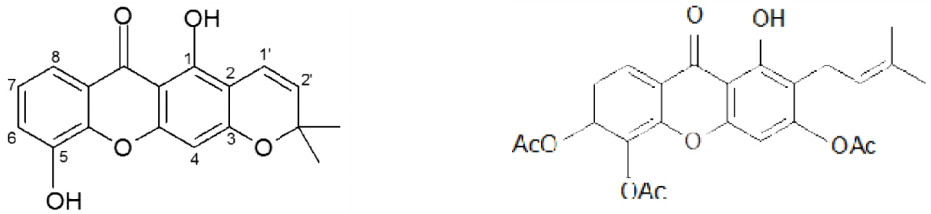
Similar works carried out in mouse peritoneal macrophages infected with *Leishmania* amastigotes showed that by using dichloromethane extract at 80 mg $ml^{-1}$ and 40 µg $ml^{-1}$ concentrations, infection index decreased 100% and 84.2%, respectively; whereas with hexane fraction, infection index decreased by 98.7% and 91.3%, respectively, when using the same concentrations [57]. A derivative of (-)-mammea A/BB (5,7-dihydroxy-8-(2-

methylbutanoyl)-6-(3-methylbutyl)-4-phenyl-chroman-2-one) (Fig. 5B), isolated from dichloromethane from leaf extract displayed the most antileishmanial activity with $LD_{50}$ of 0.9 µM, inducing cell shrinkage and a rounded appearance of cells. All parasites treated with the same compound showed ultrastructural changes in the appearance of mitochondrial swelling with reduction in density of the mitochondrial matrix and the presence of vesicles inside the mitochondrion, indicating damage and significant change in this organelle; furthermore, abnormal chromatin condensation, alterations in the nuclear envelope, intense atypical cytoplasmic vacuolization, and the appearance of autophagic vacuoles were also observed [55].

It has also been shown that mammea A/BA (Fig. 2A), A/BB (Fig. 2B), A/BD (Fig. 5C) and B/BA (Fig. 2C), have been effective with minimum concentration values ($MC_{100}$ = 15 to 90 µg $ml^{-1}$) displaying high trypanocidal activity in vitro against epimastigotes and trypomastigotes of *Trypanosoma cruzi*, the etiologic agent of Chagas disease [12]. Same authors demonstrated that mammea B/BA cyclo F (Fig. 5D) + B/BB cyclo F (Fig. 5E), and isomammeigin (Fig. 5F) coumarins, also showed $MC_{100}$ values > 200 µg $ml^{-1}$ for this etiologic agent. Additionally, several active coumarins were tested against normal human lymphocytes in vitro showing that mammea A/BA (Fig. 2A) is not toxic [12]. Other studies using leaf and stem extracts have revealed molluscicidal properties. The main bioactive compound was identified as (-)-mammea A/BB (Fig. 5A) coumarin, demonstrating after 24 h a $LD_{50}$ value of 0.67 ppm and a $LD_{90}$ value of 1.47 ppm against *Biomphalaria glabrata*, a mollusk vector of schistosomiasis, also named snail fever [58].

## 3.5 Other Disorders

Extracts and isolated compounds from *C. brasiliense*, have been evaluated on gastrointestinal disorders, diabetes, as antioxidants and antinociceptives. Gastrointestinal affections induced by ethanol, indomethacin and hypothermic restraint have been evaluated in mice and rats. Oral administration of dichloromethane fraction, obtained from the hexane extract of bark at doses ranging from 12.5 to 250 mg $kg^{-1}$, have shown significant inhibition on the development of gastric lesions in all three induced affections. Furthermore, extracts caused significant decreases of pyloric-ligation and bethanechol-stimulated gastric secretion, and of total acidities [59]. A similar work reported high inhibition of gastric ulceration (96.9% and 95.4%) caused by ethanol and indomethacin treatments when 100 mg $kg^{-1}$ of fraction from stem bark hexane extract or a mixture containing chromanone acids was applied to in-vitro and in-vivo models of rats [60]. Xanthones 6-desoxyjacareubin (Fig. 6A), jacareubin (21), 2-(3,3-dimethylallyl)-l,3,5,6-tetrahydroxyxanthone (Fig. 4C), and 1-hydroxy-3,5,6-tri-*O*-acetyl-2(3,3-dimethylallyl)-xanthone (Fig. 6B) have also shown inhibition of the gastric H+,K+-ATPase with $IC_{50}$ values ranging from 47 µM to 1.6 mM. Similarly, mammea A/BA (8) and mammea C/OA (Fig. 2E) inhibit H+,K+-ATPase with $IC_{50}$ values of 110 and 638 µM [61].




**(A)** **(B)**

**Fig. 6. Xanthones isolated from *C. brasiliense* against gastrointestinal affections**

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both, which result in abnormal levels of glucose in the blood stream [62]. Diabetes-induced in rats by intraperitoneal administration of 100 mg $kg^{-1}$ streptozotocin and then treated with 1000 mg $kg^{-1}$ of atomized extracts of *C. brasiliense*, displayed a good hypoglycemic effect (70 to 100%) compared with glibenclamide at 10 mg $kg^{-1}$ (65 - 70%), suggesting that presence of flavonoids in the extract spray could be the cause of the hypoglycemic effect [63]. On the other hand, current research has reported that mammea A/BB (Fig. 2B) extracted by supercritical fluid method have displayed higher antioxidant activity (12.4-79.7%) at extract concentrations of 25 - 350 µg $ml^{-1}$ and $IC_{50}$ = 131.73, 149.13 and 149.35 µg $ml^{-1}$ [64].

Other fractions obtained from different parts of *C. brasiliense* (roots, flowers and fruits) were evaluated as antinociceptive agents, where writhing and formalin induced-pain models in mice were used [65]. They found that all extracts, mainly friedelin (Fig. 2G) ($DI_{50}$ 12 µmol $kg^{-1}$) and 1,5-dihydroxyxanthone (Fig. 4E) ($DI_{50}$ 30 µmol $kg^{-1}$) exhibited considerable antinociceptive properties (≥ 70%), particularly against the writhing test, emphasizing that these are more potent than acetyl salicylic acid and acetaminophen (35 and 38%, respectively), used as references.

## 4. PHYTOCHEMICAL PRODUCTION OF *C. brasiliense* BY USING PLANT BIOTECHNOLOGY

The isolation of parts of plant tissue culture maintained in an aseptic, artificial nutritive medium, under controlled environmental conditions is a powerful tool from plant biotechnology and has had a tremendous impact in the genetic and biochemical study of natural products biosynthesis [66]. In several cases, tissue culture can be used for large-scale production of plant natural products. Importantly, cell culture provides a renewable source of natural products and can be produced and harvested year round without damage to the environment. In addition, it is an attractive alternative source to whole plants for production of high-value secondary metabolites. However the use of plant cell or organ cultures has had limited commercial success as often it does not produce sufficient amounts of the required secondary metabolites. Nevertheless, a number of bioactive substances have been produced by plant cell culture [67].

On the other hand, despite the importance of *C. brasiliense*, there have been no researches on in vitro propagation. However, efforts have been made for the purpose of identifying and quantifying calanolides production (anti-VIH-1) and other compounds of interest in plant cell culture. For instance, calanolide B (309.25 mg $kg^{-1}$) and calanolide C (117.7 mg $kg^{-1}$) were produced in calluses culture from seed explants [68]. Other works have also been developed on plant cell culture of *C. inophyllum* for dipyranocoumarins production and have found positive results [69]. These studies have demonstrated that plant tissue culture is a potential tool, which can and should be used for production of compounds of the greatest interest, especially those that are difficult or very expensive to synthetize.

## 5. CONCLUSIONS

*C. brasiliense* is a species containing large amounts of phytochemicals with a wide variety of biological activities such as antimicrobial, antiparasitic, antiviral, and against other disorders in favor of public health. This species is of great relevance due to its high potential for producing calanolides, which are highly potent phytochemical compounds against HIV-1.

Phytochemicals with new effects have been reported recently; for instance, mammea A/BA coumarin, which have a wide biological activity. However, phytochemical compounds, specifically secondary metabolites are found in very low concentrations (<1%) in plants, which is a disadvantage. Furthermore, there are only a few studies aimed at increasing production of high-value phytochemicals such as calanolides. Therefore, it is necessary to explore new options of plants, efficient methods for in-vitro propagation of medicinal plants, and use of biotechnological tools such as plant cell cultures for efficient production of phytochemicals in a sustainable way. For now, we are developing a cell line of suspension culture from *C. brasiliense* to identify and quantify calanolides and other compounds production which will be reported in further publications.

## ACKNOWLEDGEMENTS

The review reported here was carried out during a postdoctoral stay supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT) (project 99963: 2012-2013).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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