Review

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Review

Phytochemistry and Biological Activities of *Cissus trifoliata* against Carcinomas

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Abstract: There is an urgent need to develop new therapies for cancer treatment due to high rates of resistance and tumor relapse. Moreover, chemotherapy and radiation weaken the immune system and leave patients susceptible to sudden death from infections and organ failure since antineoplastic drugs lack specificity. Hence, scientists worldwide continue the search for natural alternatives with anticancer activities and fewer side effects. In this regard, traditional medicine offers strategies for preventing and treating numerous diseases and nowadays scientific studies worldwide highlight some of the mechanisms underlying their potential as an effective alternative for the management of cancer. In Mexico, Mayan healers have been prescribed mainly medicinal plants for more than 2,000 years. Furthermore, their ethnomedical knowledge has led to drug discovery or the development of herbal remedies. Medicinal plants from the genus of *Cissus* remain an important element of all indigenous medical systems in Mexico including the Mayan tribes in Yucatan, particularly for the management of gastrointestinal illnesses, sores, cutaneous diseases, and tumors. In this review, we present the reported bioactivities against cancer cells of the extract and compounds from the species of *Cissus* with a particular focus on recent studies on the phytochemistry of *C. trifoliata* which is widely distributed in Mexican territory and used for tumor management in traditional medicine.

Keywords: *Cissus*; phytochemicals; apoptosis; proliferation; EMT; anticancer

Introduction

Cancer is the second leading cause of morbidity and mortality worldwide, with approximately 19 million new cases per year, which are expected to rise by about 70% over the next two decades. Moreover, cancer is responsible for 10 million deaths annually, representing 1 of 6 deaths [1]. In Mexico, cancer is the third leading cause of death, and 45% of deceases occur in the economically active population. Malignant tumors from the lung, liver, prostate, breast, and cervix account for approximately half of all cancer deaths [2]. In this respect, neoplasias that arise from epithelial tissues are classified as carcinomas and account for 90% of human cancers [3]. Hence, developing successful therapies for them may result in dramatic changes in the mortality associated with malignant neoplasias [3]. Cancer is a disease characterized by the uncontrolled growth of abnormal cells that impair the biological process of tissues. In addition, malignant cells spread by invasion of nearby

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tissues and metastasize to distant organs, compromising their function and ultimately resulting in death. Importantly, metastasis is the leading cause of cancer-associated deceases since they usually display increased resistance to radio and chemotherapy [3]. The malignant neoplasias from the epithelia possess an enhanced tolerance to cell death along with the sustained proliferative signaling and the evasion of growth suppression signals that lead to tumorigenesis [4], whereas the process of epithelial to mesenchymal transition (EMT) promotes the generation of metastasis and the emergence of drug resistance [5]. Therefore, compounds with the ability to trigger apoptosis, cell cycle arrest, or prevent the process of EMT may represent promising therapeutic strategies against carcinomas [4, 5].

Cancer management includes surgery, radiation, and systemic drugs such as the conventional cytotoxic and endocrine agents. More recently, the knowledge of the molecular biology of tumors has given rise to molecules designed to target specific oncogenes hoping to reduce the side effects of common drugs [6]. However, most employed therapeutic regimens for cancer include at least one type of cytotoxic drug; whose mechanisms of action may include the generation of DNA damage, the inhibition of essential enzymes, microtubules, and topoisomerases, which ultimately generates unspecific cell death [6]. Hence, antineoplasic treatment causes toxicity in normal tissues leading to side effects that include vomit, alopecia, diarrhea, constipation, and life-threatening consequences such as myelosuppression, cystitis, gastric ulcers, lung fibrosis, cardiotoxicity, hepatotoxicity, mucositis, and nephrotoxicity. Additionally, second malignant neoplasms can develop as a result of chemotherapy regimens or radiation within the first year after treatment, being acute leukemias the more common [7]. Moreover, treatment is expensive and reduces the quality of life during and after their administration [8]. Importantly, over half of patients are diagnosed in advanced stages in which therapies are mostly ineffective [9]. Therefore, despite advances in cancer care tumor resistance and toxicity derived from therapy remain the big challenges limiting the survival of patients [10].

Plants as a potential source of new anticancer drugs

Drug discovery from medicinal plants has played an important role in the treatment of cancer [11]. Antineoplastic agents from plants in clinical use include the vinca alkaloids (vinblastine, vincristine and vinorelbine), epipodophyllotoxin lignans (etoposide and teniposide), taxane diterpenoids (paclitaxel and docetaxel) and camptothecin alkaloids (topotecan and irinotecan) [12]. According to the National Cancer Institute (NCI), the initial screenings for plants with potential anticancer activity should be performed in established cell lines, in which the toxic effects of extracts or isolated compounds are measured. Following that guideline, a plant is considered anticancer if its extracts or isolated compounds show a half maximal inhibitory concentration (IC_{50}) value of ≤30 μg/ml or ≤4 μg/ml, respectively [13]. In general, the MTT method is selected to evaluate the cytotoxic activity of both in different concentrations. The assay relies on mitochondrial metabolism since living cells reduce the tetrazolium salts to formazan products. Then, this reaction is quantified by optical density in an ELISA reader providing information on the amount of living cells. Hence, the MTT assay uses the IC_{50} value as a parameter to measure cytotoxic activity, indicating the amount of extract or compound concentration needed to inhibit half of the proliferation of cancer cells [14]. In this contribution is reviewed the reported phytochemical composition of *C. trifoliata* and the bioactivity of their extracts or compounds against cancer cells along with the underlying mechanism of action. Emphasis was made on recovering the IC_{50} against the cell models of lung cancer (A549), liver cancer (HepG2), breast cancer (MCF7), cervix cancer (HeLa), and prostate cancer (PC3) since they represent the most studied cultures of the most common types of carcinomas diagnosed in Mexican population [15]. In addition, evidence from those phytochemicals against the process of proliferation and EMT was also discussed due to their importance in tumorigenesis and for the emergence of drug resistance and metastasis in carcinomas.

Anticancer effects of *C. trifoliata*

Cissus plants are 350 species of lianas widely distributed in the tropical and subtropical regions in Asia, the Americas, Africa, and Australia [16]. They belong to the family Vitaceae and have been used in traditional medicine for the management of several diseases, such as diabetes, infections, arthritis,

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menopause, obesity, pain, and cancer [17]. *Cissus trifoliata* is widely distributed in tropical America and Mexican territory, being native to Baja California, Chihuahua, Coahuila, Durango, Nuevo León, San Luis Potosí, Sinaloa, Sonora, Tamaulipas, Puebla, Michoacán, Veracruz, Oaxaca, Quintana Roo and Yucatán. Is also present in the United States, Venezuela, Colombia, and Ecuador [18]. Moreover is also dispersed among some islands in the Caribbean such as Aruba, Bahamas, Cuba, Haiti, Jamaica, and Puerto Rico [19]. *Cissus trifoliata* mexican specimens have also been referred to as *C. incisa* (Nutt.) Des MouL [19], but recent genetic studies confirm the lack of differences between both species [20]. Although *C. trifoliata* (L.) is the accepted botanical name; is also referred to as *C. acida*, *C. carnifolia*, *Vitis incisa*, *V. trifoliata,* possum-grape, sorrelvine, vine-sorrel, *Hierba del buey* in spanish and called *Xbolontibi* in Maya [19]. *Cissus trifoliata* has been an important medicinal plant for the Mayan tribes in Yucatan, particularly for the management of gastrointestinal illnesses [21], sores [22], cutaneous diseases, and tumors [23, 24]. Likewise, in Northeastern Mexico, is used by the native population to treat skin infections, inflammation, abscesses, and tumors [25]. In other parts of America ethnobotanical uses for *C. trifoliata* include burns, sore, and tumors [19]. Modern studies of its biological activities against cancer include the evaluation of the cytotoxic activity of the stem extracts against the human cancer cell lines A549, Hep3B, MCF7, HeLa, and PC3 obtained from the ATCC. Notably, the *in vitro* evaluation was consistent with the antitumor properties suggested by traditional medicine since the hexane extract showed exceptional activity against the cancer cell lines Hep3B with an IC_{50} value of 26 μg/ml and MCF7 with an IC_{50} value of 30 μg/ml whereas the aqueous extract also displayed antiproliferative properties on MCF7 cells with an IC_{50} value of 30 μg/ml. Although the $CHCl_3$-MeOH extract lacks activity at the desired range of $IC_{50} \leq 30$ μg/ml, all tested cells were sensitive to all extracts at doses below 100 μg/ml [15] (Table 1). In this regard, based on other authors, plant extracts in the range of IC_{50} from 20-100 μg/ml are classified as moderately active against cancer cells [26].

Table 1. Activity of *C. trifoliata* stem extracts against cancer cell lines (IC50 μg/ml).

Cell line	Hexane	$CHCl_3$-MeOH	Aqueous
Lung cancer A549	51	85	94
Liver cancer Hep3B	24	81	81
Breast cancer MCF7	30	78	30
Cervical cancer HeLa	35	82	90
Prostate cancer PC3	62	61	58

Although scarce, similar studies have been performed for other species of the genus *Cissus*. Namely, the hexane extract of *C. quadrangularis* stems displayed low activity against KB (keratin-forming tumor cell line HeLa) and A431 (epidermoid carcinoma) with an estimated IC_{50} value of 200 μg/ml for both [27]. In addition, the acetonic extract was more active against HepG2 with an estimated IC_{50} value of 43 μg/ml and IC_{50} value of 48 μg/ml for KB cells [28]. Likewise, the ethyl acetate extract from the stems of *C. sicyoides* showed an IC_{50} value of 43 μg/ml against the HepG2 [29] while the methanolic extract from the stems of *C. debilis* inhibits the colon carcinoma cell line CaCo-2 with an estimated IC_{50} value of 50 μg/ml [30]. Finally, the ethyl acetate and ethanolic extracts of *C. verticillata* exhibit an IC_{50} value of 43 μg/ml and 50 μg/ml against the HepG2 and NCI-H292 cells respectively [30]. Therefore, most *Cissus* plant extracts can be considered moderately active against all carcinoma cells tested according to their calculated IC_{50} value. The presence of active extracts suggests that tested plants possess bioactive compounds with potential anticancer activities. Therefore, following the NCI guideline further studies are desirable in those specimens [13]. In the next sections, current work on the phytochemistry of *C. trifoliata* is provided, along with the role of compounds in plant physiology and their cytotoxic, antiproliferative, and anti-EMT activities against the carcinoma cell lines of interest.

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Cissus trifoliata compounds and their role in plant physiology

Metabolic profiling has been previously useful to understand the chemical diversity of a medicinal plant. Chromatography coupled with mass spectrometry is the most widely applied technology used for the analysis of samples in very complex matrices such as those of plant extracts [15]. Accordingly, the metabolic profile of the hexane, $CHCl_3$-MeOH, and aqueous extracts of *C. trifoliata* stems was assessed by Gas chromatography-mass spectrometry (GC-MS) and Ultraperformance Liquid Chromatography-Quadrupole Time of Fly-Mass Spectrometry (UPLC-QTOF-MS) analysis. Broadly, the analysis showed a metabolic profile composed of flavonoids, stilbenes, phenolics, fatty acids, sterols, alkanes, alcohols, and triterpenes [15]. Furthermore, results were consistent with chemical classes reported in previous studies in some other species of *Cissus* plants [31] and well-characterized specimens from *Vitaceae* [32]. The most abundant lipid compounds corresponded to alkanes, fatty acids, fatty alcohols, sterols, and terpenes. The alkanes comprise the hentriacontane, nonacosane, and octacosane, and the alcohol triacontanediol. The fatty acids include palmitic acid, stearic acid, and arachidic acid. Finally, identified sterols consist of campesterol, stigmasterol, and sitosterol, whereas the triterpenes involve squalene, betulinic acid, ursolic acid, and lupeol. Overall, the lipid composition seems consistent with typical plant waxes [33, 34], since major constituents are usually unsaturated linear hydrocarbons, long-chain saturated fatty acids [35], long-chain saturated esters [36], terpenes, and sterols [37]. Furthermore, the alkanes identified octacosane, nonacosane, and hentriacontane are predominant cuticular wax components in plants [38]. In the case of triacontanediol, is a common fatty alcohol present in the plan cuticular wax [38] that also plays a role as a growth regulator [39]. Palmitic and steric acids are the most abundant plant membrane fatty acids that play structural roles [40] and influence the fluidity of cellular membranes of higher plants [41]. In the case of eicosanoic acid in general is a minor constituent of plant cell membranes [42]. Likewise, sterols regulate the fluidity of membranes, and cellular developmental processes in plants, acting as precursors of plant hormones [43]. While a variety of sterols exist, campesterol, stigmasterol, and β-sitosterol are the most abundant in plants [43] playing structural roles in plant membrane fluidity [44]. However, when plants are infestated by insects changes in plant sterol structure confer them an antifeedant role [45]. The ursolic acid also exhibits antifeedant properties against insect larvae [46] whereas the biosynthesis of lupeol is induced by pathogens and exerts antimicrobial activities [47]. Squalene increases the rigidity and the size of the cell membrane, enhancing the polarity and hydrophobic interactions, contributing to membrane reconstitution, functional regulation of proteins, and movement of ions. Therefore, squalene plays an important role in electrochemical cell gradient [48]. Finally, betulinic acid has been detected in different plant species, and its common occurrence in exposed organs such as leaves, bark, or fruits, suggests a defensive role for this triterpenoid [49].

Most lipid compounds present in *C. trifoliata* have been previously identified as constituents in other *Cissus* plants (Table 2). For example, the alkane hentriacontane was reported in *C. quadrangularis* stems [50], while the nonacosane in *C. cornifolia* [51]. Similarly, the analysis of the hexane extracts of *C. quadrangularis* stems identified as the main components the hexadecanoic acid ethyl ester, the octadecanoic acid ethyl ester, and phytol [52]. The palmitic acid is also the principal component of the hexane extract of the stems of *C. quadrangularis* [52] and their aerial parts (aqueous alcoholic extract) [53]. Palmitic acid is also present in *C. vitiginea* [50], and its content in plant extracts is high in low-polarity solvents. The steric acid was found as a major constituent of the hexane extract of stems of *C. quadrangularis*, and in the methanolic extract of the entire plant [52, 54]. In the case of eicosanoic acid, was also a major constituent of hexane extract of roots in *C. quadrangularis* [52]. Regarding sterols, β-sitosterol and stigmasterol were previously isolated from the hexane extract of stems of *C. quadrangularis* [52] and the methanolic and ethyl acetate extracts of the aerial parts and roots of *C. assamica* [55], *C. polyantha* [56], *C. rheoifolia* [57, 58] and *C. pteroclada* [58]. Campesterol was isolated from hexane [52] and ethanolic extract of *C. quadrangularis* stems [59] and is also found in the methanolic extract of the roots of *C. rheifolia* [57]. Concerning terpenes, squalene was previously isolated from *Cissus quadrangularis* [60], lupeol was found as a major constituent of hexane extract of stems and roots in *C. quadrangularis* [52, 60], ursolic acid was previously isolated from *C. assamica* [55] and *C. repens* [61] and the ursolic and betulic acids in *C. assamica* [62]. Overall, the lipid composition reported for *C. trifoliata* is

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widely distributed in the plant kingdom [37] and corresponds mainly to constituents of plant cuticular waxes and membranes [63]. In contrast, the polar composition presented the chemical classes of phenolic acids, flavonoids, and stilbenes, with these last phytocompounds characterized by a narrow distribution within plants [15]. The phenolic compounds detected include isoferulic acid, protochatechuic acid, trans-p-coumaric acid, and trigallic acid. Flavonoids represent the most abundant class with glucosides of apigenin, chrysoeroil, cyanidin, delphinidin, dihydrokaempferol, kampferol, myricetin, naringenin, quercetin, and syringetin. Finally, the stilbenes identified comprise resveratrol, piceatannol, pallidol, piceid and viniferin.

Table 2. Lipid constituents in the stems of *Cissus trifoliata.*

Function in plants	**Compound**	**Presence in *Cissus* plants**
Components of cuticular wax	Triacontanediol	*C. trifoliata*
	Hentriacontane	*C. quadrangularis*
	Nonacosane	*C. cornifolia*
	Octacosane	*C. trifoliata*
Structural role in cellular membranes	Arachidic acid	*C. quadrangularis*
	Stearic acid	*C. quadrangularis*
	Palmitic acid	*C. quadrangularis, C. vitiginea*
	β-sitosterol	*C. quadrangularis, C. assamica, C. polyantha, C. rheoifolia, C. pteroclada*
	Campesterol	*C. quadrangularis, C. rheifolia*
	Stigmasterol	*C. quadrangularis, C. assamica, C. polyantha, C. rheoifolia, C. pteroclada*
	Squalene	*C. quadrangularis*
Defensive role, acting as antifeedant, and antimicrobial	Ursolic acid	*C. assamica, C. repens*
	Betulinic acid	*C. assamica*
	Lupeol	*C. quadrangularis, C. assamica, C. repens.*

Phenolic compounds are of considerable physiological and morphological importance in plants, contributing to growth and reproduction, protection against pathogens or predators, and to the color and sensory characteristics of fruits and vegetables. For example, gallic acid derivatives like the protocatechuic acid, and trigallic acid are secondary metabolites widely distributed in the plant kingdom that play a regulatory role in the induction of abiotic stress tolerance or to enhance the direct defense against insects [64]. Isoferulic acid is a structural component in the plant cell wall and serves to increase its rigidity and strength [65]. Trans-p-coumaric acid biosynthesis and storage in the plant cell play a vital role in response to pathogenic infections [66]. Concerning flavonoids, they have multiple functions including the response to environmental injuries, the regulation of cell growth, and the attraction of pollinators [67]. For example, flavonoids yield stress protection by acting as ROS scavengers or inducers of antioxidant enzymes. In this regard, apigenin and other flavones protect photosynthetic tissues from oxidative damage and confer tolerance to salinity [68]. Chrysoeriol provides strong antifeedant activity since it is toxic for insects [69]. In the case of the anthocyanins cyanidin and delphinidin, both are involved in responses to oxidative stress induced by heat

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conditions, water or nutrient deficit, and mechanical damage due to herbivore attack, insect infestation, or fungal infection [70]. Kaempferol and quercetin are the two main flavonoid species abundantly present in plants. Both were found to be associated with auxin-regulated cell division acting as signaling molecules. In addition, environmental and pathogenic stress induces kaempferol accumulation, whereas quercetin and anthocyanins, alter an interaction between jasmonic acid and gibberellic acid which results in the regulation of the defense system to cope with stress. Likewise, in some stressful conditions, plants convert kaempferol and quercetin glycosides into anthocyanins, which promote the plant defense system. Flavonoids accumulate in mesophyll cells, vacuole, and chloroplasts reducing ROS generation. For example, quercetin generated in the plant epidermal tissue protects from oxidative stress induced by an intense light [71]. Moreover, the concomitant increase in flavonoids with the concentration of heavy metals in plant tissue suggests a role in alleviating stress. Indeed, myricetin in conjunction with kaempferol was observed to increase the phytoremediation capacity of some plants due to the high accumulation of heavy metals [72]. Likewise, dihydrokaempferol and quercetin increased salt tolerance [73] and the naringenin suppressed the growth of annual plant species, acting as an allelochemical [74]. This inhibitory effect of naringenin was attributed at least to some extent, to impaired auxin transport. In addition, recent studies find that naringenin alleviates osmotic and salinity stresses by regulating photosynthetic machinery and chloroplastic antioxidant metabolism [74]. Hence, the induction of flavonoids correlates with mechanisms against different types of stress such as ozone, light, heat, and salinity but also with exposure to biotic aggressors. For instance, bacterial or fungal-mediated infection is inhibited by flavonols such as myricetin and anthocyanins like delphinidin [74]. Transcriptional upregulation of flavonols, anthocyanin, and proanthocyanidins biosynthesis was found to promote the accumulation of quercetin, kaempferol, and anthocyanins and enhance resistance to infections. Additionally, higher levels of flavonols such as kaempferol, isorhamnetin, and syringetin, reduce oxidative damage and susceptibility to fungal infections [75]. Finally, in contrast to phenolics and flavonoids, stilbenes show a narrow distribution in the plant kingdom. They have been identified in 72 plant species belonging to Pinaceae, Gnetaceae, Fabaceae, Polygonaceae, Moraceae, and Vitaceae. Stilbene compounds mostly derive from resveratrol although different structures can be found in specific plant families. In *C. trifoliata*, resveratrol was identified along to piceatannol, pallidol, piceid and viniferin. Stilbenes are mainly involved in constitutive and inducible protection of the plant against phytopathogens, hence displaying antibacterial, antifungal, nematocidal, and insecticidal properties. In addition, their levels increase in response to drought, heat, radiation, heavy metals, salts, air pollutants, and mechanical stress [76,77]. Broadly, the presence of phenolics in *C. trifoliata* includes gallic acid derivatives such as protocatechuic acid, trigallic acid, and methyl digallate which are secondary metabolites widely distributed in the plant kingdom [64]. Apigenin was previously isolated from *C. adnata* [78], *C. ibuensis* [79], *C. digitata* [80], *C. repens* [81], *C. verticillata* [82] and *C. quadrangularis* [83]. Kaempferol presence has been identified in *C. quadrangularis* [84], *C. ibuensis* [79], *C. repens* [81], and *C. sicyoides* [85] whereas dihydrokaempferol and myricetin in *C. quadrangularis* [86]. Quercetin has been reported on alcoholic extracts from *C. digitate* [80], *C. quadrangularis* [83], and *C. repens* [81]. The anthocyanidins, cyanidin and delphinidin were identified in the methanolic extract of *C. sicyoides* [87] and petroleum ether extract of the stems of *C. quadrangularis* [86]. Chrysoeriol was found in *C. aralioides*, *C. lageniflora* and *C. petiolata* [88] and naringenin in *C. rotundifolia* [89]. Syringetin is not previously reported in *Cissus* plants, however, is a common flavonol identified in the close relative *Vitis vinifera*, which is also rich in several glycosides with quercetin, myricetin, and kaempferol [90]. Finally, resveratrol, piceatannol, and pallidol were isolated and characterized in ethanolic extracts from the stems of *C. quadrangularis* [31], whereas pallidol in *C. pallida* [91]. Additionally, the glucosides of the stilbenes piceatanol and *ε*-viniferin were previously identified in *C. quadrangularis* [31] and *C. repens* [81] (Table 3).

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Table 3. Polar constituents in the stems of *Cissus trifoliata.*

Function in plants	Compound	Presence in *Cissus* plants
Structural component in the cell wall, and serves to enhance its rigidity and strength	Isoferulic acid	*C. trifoliata*
Regulatory role in inducing abiotic stress tolerance and enhancing the direct defense against insects	Protocatechuic acid	*C. trifoliata*
Antimicrobial properties and protection against oxidative stress	Trigallic acid	*C. trifoliata*
Vital role in response to pathogenic infections	Trans-p-coumaric acid	*C. trifoliata*
Protect photosynthetic tissues from oxidative damage, and confer enhanced salinity tolerance and growth	Apigenin	*C. adnata, C. digitata, C. verticillata and C.quadrangularis*
Protect from oxidative stress induced by heat, water, nutrients, or mechanical damage due to insects, or fungal infections	Cyanidin	*C. sicyoides, C. quadrangularis*
Promote plant defense systems to environmental and pathogenic stress	Delphinidin	*C. sicyoides, C. quadrangularis*
Inhibits bacterial or fungal infections	Myricetin	*C. quadrangularis*
Involved in auxin-regulated cell division as a signaling molecule. Increases grown in heavy metal-contaminated soils	Kaempferol	*C. ibuensis, C. sicyoides, C. quadrangularis*
Regulate response to salt stress tolerance	Dihydrokaempferol	*C. quadrangularis*
Allelochemical, impairing auxin transport and alleviates short-term osmotic and salinity stresses	Naringenin	*C. rotundifolia*
Antifeedant activity and toxicity against insects	Chrysoeriol	*C. aralioides, C. lageniflora and C. petiolata*
Protect epidermal tissue from oxidative stress in response to high light intensity. Regulates the antioxidant enzymes at the transcriptional level	Quercetin	*C. ibuensis, C. digitata and C.quadrangularis*
Reduce the oxidative damage and susceptibility to fungal infections	Syringetin	*C. trifoliata*

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Antimicrobial, nematocidal, and insecticidal activities, increase resistance to drought, thermal stress, ultraviolet radiation, mechanical stress, heavy metals, salts, and air pollutants	Resveratrol	*C. ibuensis, C. sicyoides, C. quadrangularis*
Contribute to resistance against fungal infection	ε-viniferin	*C. repens, C. sicyoides*
Display antifungal activities	Pallidol	*C. quadrangularis, C. pallida*
Produced in response to infection, has antimicrobial activity and increases the tolerance to stress	Piceatannol	*C. quadrangularis*

Mechanisms implicated in the cytotoxicity of *C. trifoliata* compounds

The process of apoptosis is characterized by several morphological changes including cell shrinkage, membrane blebbing, and nuclear DNA fragmentation. Broadly speaking, this form of cell death is the result of two mechanisms, one is the cytoplasmic pathway triggered by extracellular signals through death receptors located on the cell membrane of the target cell that belong to the TNF superfamily. On the other hand, the intrinsic pathway is activated by intracellular signals that induce the release of cytochrome-C to the cytoplasm from the intermembrane area of the mitochondria and leads to the activation of the apoptosome complex composed of cytosolic factor Apaf-1, ATP and active caspase 9. Caspases are inactive cysteine proteases until their proteolytic cleavage, caspases 2,8,9 and 10 act as initiators while 3,6 and 7 as effectors. On the other hand, members of the Bcl-2 family include proteins that prevent apoptosis such as Bcl-2, and Bcl-xL, and those that promote it such as Bax, Bad, Bid, Bak, and Bcl-xS [92]. Given that the apoptosis of cancer cells continues to be one of the main objectives in preliminary screenings in the search for antineoplastic drugs [93], the following section provides the *in vitro* experimental evidence of the compounds present in *C. trifoliata* that have a calculated IC_{50} against all the carcinoma cell lines of interest and the apoptosis-inducing mechanisms that have been described to date (Table 4).

Terpenes are derived from five-carbon isoprene units and classified based on the number of them. In nature, triterpenoids are biosynthesized from five isoprene units using the mevalonate pathway and are usually found as tetracyclic or pentacyclic structures [94]. Plant sterols belong to this subclass of terpenes and are composed of three 6-carbon rings and a 5-carbon ring with a double bond between carbons 5 and 6, a hydroxyl group on carbon 3, and a hydrocarbon side chain at the 17C position [94]. The more abundant sterols are the β-sitosterol, campesterol and stigmasterol. *In vitro* studies have revealed potential anticancer effects of phytosterols, particularly from β-sitosterol and stigmasterol [94]. In this regard, β-sitosterol shows cytotoxic activity against the cell lines A549 (231 μM) [95], Hep3B (60 μM) [96], MCF7 (603 μM) [95], PC3 (178 μM) [97], and HeLa (410 μM) [98]. Treatment of MCF7 cells with β-sitosterol result in increased caspase-8 activity [99] and defects in sphingolipid metabolism, causing apoptosis and cell growth inhibition in a dose-dependent manner [100]. Stigmasterol also display cytotoxic activity against A549 (51 μM), MCF7 (22 μM), PC3 (18 μM) [95], HeLa (412 μM) [98] and Hep3B (30 μM) cells [101]. Evidence from HepG2 cultures indicates that apoptosis is triggered by the upregulation of Bax and p53 expression and the downregulation of Bcl-2 [102]. The pentacyclic triterpenes also have anticancer activity, they are generally present in higher plants and contain the ursane, oleanane, lupane, and friedelane skeletons [103]. Lupeol is the form of lupan in which hydrogen at the 3β position is replaced by a hydroxy group. It shows cytotoxic activity against A549 (49 μM) [95], HepG2 (112 μM) [104], MCF7 (75 μM), PC3 (70 μM) [95], and HeLa (88 μM) cell lines [105]. In PC3 cells, lupeol induces apoptosis through the mitochondrial cell death pathway by downregulation of Bcl-2 expression and cell cycle arrest [106]. In addition, the exposition of liver cancer

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cells to lupeol suppresses STAT3 activation along with cyclin D1, Bcl-2, Bcl-xL, and survivin expression [107]. Betulinic acid is a lupane-type pentacyclic triterpenoid having a double bond at position 20, a 3 beta-hydroxy and 28-carboxy substituents. It has been shown to induce apoptotic cell death in A549 cells (33 μM) through the mitochondrial intrinsic pathway [108]. Betulinic acid produces cell rounding, chromatin condensation, nuclear fragmentation, membrane blebbing, and formation of apoptotic bodies in HepG2 cells (23 μM) [108]. The cytotoxic activity *in vitro* against MCF7 (20 μM) cells resulted in a dose-dependent inhibition of cell proliferation and apoptosis independent of the p53 pathway [109]. Betulinic acid also induces apoptosis on HeLa cells (23 μM) by the sequential activation of caspases 9, 3, and 7 and the cleavage of poly (ADP-ribose) polymerase (PARP), a nuclear enzyme fragmented during the programmed cell death [108]. Its study on PC3 cells (22 μM) reveals that apoptosis also resulted from NF-κB inhibition, associated with a decrease in the activity of IKKβ, the serine/threonine protein kinase that phosphorylates IκB*α*, which negatively regulates the activation of the transcription factor [110]. Ursolic acid derives from the ursane skeleton, hence is an alkene at C12-C13, but is also substituted by a beta-hydroxy group at position 3 and a carboxylic moiety at carbon 28. It triggers apoptosis of human lung cancer cell line A549 (40 μM) by upregulation of Fas/APO-1, a member of the TNF receptor superfamily, and downregulation of NF-κB, Bcl-2, and Bcl-xL [111]. Against Hep3B (50 μM), it inhibits cell viability by negative modulation of the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway and downregulation of Bcl-xL [112]. MCF7 cells exposed to ursolic acid (53 μM) undergo apoptosis through the intrinsic mitochondrial pathway by modulating the glucocorticoid receptor, Activator Protein-1 (AP-1), and decreasing Bcl-2 protein and PARP cleavage [113]. Ursolic acid also inhibited the cell viability of PC3 (32 μM) and HeLa (10 μM) cells by activation of the mitochondrial pathway of apoptosis [114].

Flavonoids are a large group of polyphenolic compounds characterized by a core structure of 15 carbons arranged as two benzene rings connected by a heterocyclic pyran ring [115]. Naringenin is a flavanon, a subclass of flavonoid that is characterized by a benzopyran bearing a ketone at the carbon C4. In MCF7 cells (1719 μM) it showed a dose-dependent response, reducing cell viability and inducing apoptosis [116]. Naringenin also induces apoptosis in HepG2 (200 μM) cells as evidenced by the exposure of phosphatidylserine in the outer layer of the cell membrane and the activities of caspases 9, 8, and 3. Furthermore, the protein expression levels of Bax and Bak were increased whereas the level of Bcl-xL was decreased [117]. Antiproliferative activities on naringenin treatment of PC3 (50 μM) were evidenced by the low abundance of PCNA, a marker of mitosis. In addition, naringenin induced apoptosis by DNA fragmentation and downregulation of Bcl-2 in a dose-dependent manner [118]. In A549 cells (800 μM) naringenin induces ROS production and Bax-mediated mitochondrial apoptotic cell death by formation of the apoptosome complex and activation of caspase-3. In addition, it enhances the expression of death receptor 5 and upregulates TRAIL-induced apoptosis [119]. The cytotoxic effect and apoptosis induction of naringenin in HeLa (195 μM) cells showed a similar effect with an increment in the expression of Bax and decreased expression of Bcl-2 [120]. The enzyme flavone synthase catalyzes the conversion of naringenin to apigenin by forming a single double bond between the C2-C3 atoms of the pyran ring. The treatment of MCF7 cells (30 μM) with this flavonoid increased apoptosis evidenced by p53 expression, PARP cleavage, and augmenting the release of cytochrome *c* into the cytosol. In the case of PC3 cells (40 μM), apigenin induced a significant decrease in Akt phosphorylation at Serine 473; inhibiting its kinase activity, which was confirmed by reduced phosphorylation of the proapoptotic proteins Bad and glycogen synthase kinase-3, their essential downstream targets. Exposure to apigenin induced caspase-9 activity and decreased the survival of PC3 cells in a dose-dependent manner [121]. The molecular mechanism and signaling pathway of apigenin in induced cytotoxicity in A549 (72 μM) was accompanied by morphological changes, DNA damage, reduction of cell viability, and apoptosis. In addition, it induces protein production of p53, Bid, and Bax while decreasing the levels of Bcl-2 [122]. Treatment of HepG2 cells (81 μM) with this flavone resulted in the induction of DNA fragmentation triggering apoptosis as evident from the morphology of cells [123]. Apigenin also exerted concentration-dependent cytotoxic effects on HeLa cells (10 μM) inducing pronounced morphological changes, retraction of cytoplasm, and detachment from the plate associated with apoptosis [124]. Concerning

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flavonols, they are flavonoids that have an unsaturated pyran ring at the C2-C3 position, oxidized at C4, and hydroxylated at C3. Quercetin, kaempferol, and myricetin are the main flavonols and exhibit a myriad of anticancer properties [115]. They differ in the number of hydroxyl groups at the B-ring, in which kaempferol is hydroxylated at C4′, quercetin at C3′and C4′, and myricetin at C3′, C4′, and C5′ positions. Quercetin exerts cytotoxic effects against many different types of cancer cells. In MCF7 (165 μM) cells exposed to quercetin, their nuclei exhibit chromatin condensation and changes in the expression of the proapoptotic proteins Bax, and caspase-3 [125]. Regarding PC3 cells (46 μM), their exposure to quercetin results in cell death via downregulation of NF-κB, mTOR and Bcl-2 while increasing the activity of caspase-3 [126, 127]. Quercetin in HepG2 cells (24 μM) induced cell death via caspase 3 and 9 activation, regulation of Bcl-2, and inhibition of PI-3-Kinase/Akt and ERK pathways [128]. Likewise, in HeLa cells (185 μM), it modulates the PI3K/Akt pathway and induces apoptosis by caspase-3 activation [129], mitochondrial dysfunction, and the generation of ROS [130]. Quercetin is also able to induce apoptosis through the regulation of Bcl-2 and Bax in A549 cells (74 μM) [131]. Kaempferol triggers apoptosis in a dose-dependent manner in MCF7 cells (168 μM) by increasing the generation of ROS, cell shrinkage, and loss of adhesion [132]. A study on A549 cells (35 μM) found that kaempferol leads to cell death via upregulation of caspase-7 and downregulation of Bcl-2 and Bcl-xL [133]. Similarly, the cytotoxicity against HeLa cells (48 μM) was accompanied by increased expressions of p21, p53, caspase-3, and decreased expression of Bcl-2 causing apoptosis [134]. Consistent with those results, the exposition of HepG2 cells (40 μM) with kaempferol induces apoptosis by the activation of caspase-3, and caspase-4 [135]. In the prostate cancer cells PC3 (58 μM), kaempferol also promotes apoptosis in a dose-dependent manner although the mechanisms behind this effect remain to be clarified [136]. Myricetin has been found to suppress cell viability of MCF7 cells (80 μM) by apoptosis through inhibition of the protein p21-activated kinase 1 and phosphorylated extracellular mitogen-activated protein kinase (ERK1/2) with negative modulation of β-catenin pathway, survivin, and activation of caspase-3 [137]. The treatment of PC3 (48 μM) with myricetin also induced apoptosis with upregulation of the expression levels of caspase-3 and caspase-9 and inhibition of the phosphorylation of ERK1/2 and AKT [138]. It has also been reported that myricetin exposition to Hela (60 μM) cells results in apoptosis via caspase-3 activation with loss of mitochondrial membrane potential [139]. Likewise, it triggers apoptosis in A549 cells (50 μM) by changes in the mitochondria, ROS generation, and p53 expression [140]. Myricetin treatment also leads to apoptosis in HepG2 cells (95 μM) through the intrinsic pathway increasing Bad expression [141, 142]. Finally, resveratrol is a non-flavonoid polyphenol of the stilbene class that shares a structure characterized by a 14-carbon skeleton composed of two benzene rings linked by an ethylene bridge. This phytoalexin displays a wide array of capabilities against numerous malignancies [143]. The exposition of HepG2 cells (52 μM) resulted in the induction of apoptosis via activation of caspase-9, and caspase-3, upregulation of p53 expression, and downregulation of Bcl-2 [144]. Resveratrol also induces apoptosis of MCF7 cells (196 μM) through a caspase-independent mechanism with downregulation of Bcl-2 and NF-κB [145]. Cell death by resveratrol against A549 cells (8 μM) was found to be mediated by apoptosis with upregulation of caspase-3, Bax and downregulation of the Bcl-2 [146, 147]. Studies on HeLa cells (90 μM) revealed that resveratrol also induces apoptosis by ROS overload and mitochondrial function impairment [148]. Similarly, PC3 cells (202 μM) exposed to resveratrol experience growth inhibition by interfering with glucose metabolism [149] and undergo p53-independent apoptosis by cytochrome c release and caspase activation [150]. Naturally-derived compounds are considered to have less toxic side effects when compared to cancer drugs [151]. For example, the IC_{50} value of paclitaxel against PC3 cells is 5 nM [152] whereas the lowest concentration reviewed against the same cancer cell line was for resveratrol, with an IC_{50} value of 8 μM. In other words compounds present in *C. trifoliata* harbor low cytotoxicity since the concentration to induce cell death by apoptosis needs to be one thousand times higher than molecules used in antineoplasic therapy. Thankfully, terpenes and phenolics exert anticancer activities through a variety of mechanisms different from apoptosis including the inhibition of proliferation [153] and metastasis [154]. Furthermore, cancer management has been progressing from the use of general cytotoxic agents to molecules able to attenuate signaling pathways that control other important aspects involved in the tumoral progression [151]. In this regard, since cancer cells develop a

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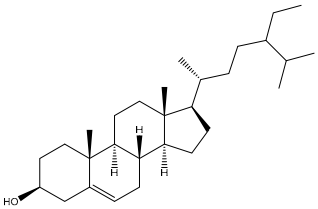
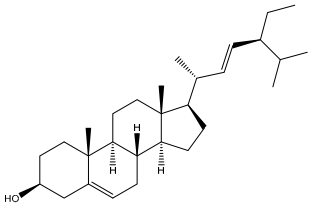
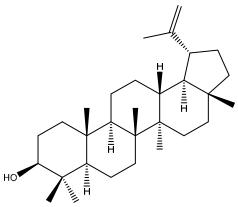
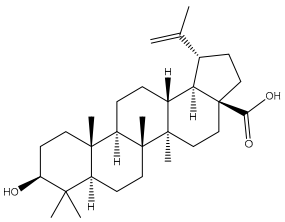
degree of autonomy from growth signals, they undergo uncontrolled growth and proliferation leading to tumorigenesis. Therefore compounds that block proliferation may hinder tumor mass growth and result in overall anticancer effects [155]. On the other hand, effectively targeting the process of EMT has the potential to improve carcinoma therapy due to its relevance for invasion, metastasis, and drug resistance [5]. Hence, in an attempt to further understand the potential anticancer effects of *C. trifoliata*, the antiproliferative and anti-EMT activities of compounds in Table 4 were also reviewed in the next section. In addition, when possible, the potential mechanisms underlying those activities were retrieved from findings in the carcinoma cell lines of interest.

Table 4. Cytotoxic activity (IC_{50} μg/ml)† of compounds present in *Cissus trifoliata* against carcinoma cells.

Compound	A549	MCF 7	HeLa	PC 3	Hep*	Mechanisms underlying apoptosis
 β-sitosterol	96	250	170	74	25	Increases caspase-8 activity and impairs sphingolipid metabolism
 Stigmasterol	21	9	170	8	12	Upregulation of Bax and p53 expression and downregulation of Bcl-2
 Lupeol	21	32	38	30	48	Triggers mitochondrial cell death pathway by downregulation of Bcl-2, Bcl-xL, and survivin expression with a negative modulation of STAT3 activities
 Betulinic acid	15	9	10	10	10	Cell death through intrinsic pathway promoting the activities of caspases 9, 3, and 7 and the cleavage PARP and inhibition of NF-κB pathway

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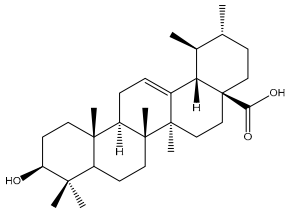
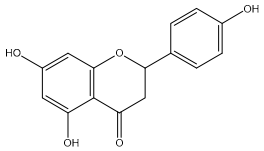
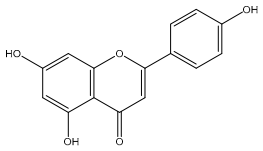
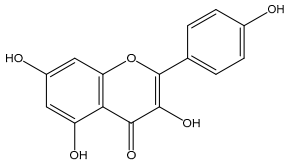
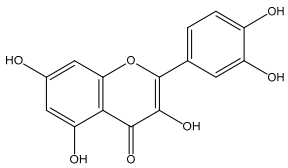
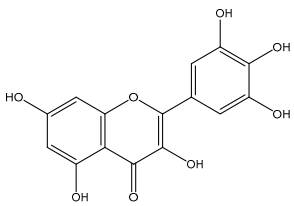
 Ursolic acid	18	24	5	15	22	Upregulation of Fas/APO-1 and downregulation of JAK2/STAT3 and NF-κB pathways, Bcl-2, and Bcl-xL expression
 Naringenin	218	468	53	13	54	Enhances TRAIL death receptor expression, the formation of the apoptosome complex, caspase-3 activation, and decreases Bcl-2 expression
 Apigenin	20	8	3	11	22	Induces protein production of p53, Bid, and Bax while decreasing the levels of Bcl-2. It also causes the release of cytochrome c, caspase activation, and suppresses the phosphorylation of Akt, Bad, and glycogen synthase kinase-3
 Kaempferol	10	48	14	17	11	Upregulates p21, p53, caspases 3, 4, 7, and downregulates of Bcl-2 and Bcl-xL
 Quercetin	22	50	56	14	24	Induces the expression of Bax, and caspase-3 with downregulation of NF-κB, mTOR, Bcl-2, and the PI3k/Akt and ERK pathways
 Myricetin	16	26	19	15	30	Inhibits survivin, protein p21-activated kinase 1, PI3k/Akt, ERK, and the β-catenin pathway, with activation of caspases 3, 9 and increasing the expression of Bad and p53

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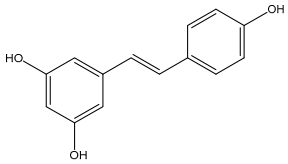 Resveratrol	2	45	20	46	12	Induces cytochrome c release and activation of caspases 3 and 9, while upregulates p53, Bax and downregulates Bcl-2 and NF-κB

† All IC_{50} values were subjected to unit conversion since according to NCI active compounds must show inhibitory activity at concentration ≤4 μg/ml. * Column groups data from HepG2 or Hep3B cells, both derived from liver carcinoma.

Antitumoral and antimetastatic activities of *C. trifoliata* compounds

Approved drugs for the treatment of cancer are highly toxic and induce resistance, which ultimately results in tumor recurrence and metastasis. In addition, the non-specific toxicities towards normal cells also limit their anticancer activities [156]. In contrast, natural products have been observed to influence multiple oncogenic signaling pathways simultaneously by modulating the activity or expression of their molecular targets. Moreover, phytocompounds have chemical diversity, low toxicity, safety, and availability, which make them an attractive and affordable alternative to synthetic products [151]. The relationship between cancer and the cell cycle seems obvious since neoplasms are considered to be a disease largely caused by the lack of regulation in cell proliferation. Furthermore, for many years it has been considered that such conditions lead to an increase in errors during DNA replication, thereby increasing the chances of acquiring favorable mutations for tumor progression [4]. The cell cycle has four sequential stages where the S phase produces DNA replication, and the M phase divides the cell into two daughter cells. Among them, there are two separation phases called G1 and G2. During G1 the cell is sensitive to positive and negative signals from growth signaling networks. In G2, which occurs after the S phase, the cell prepares to enter mitosis. On the other hand, when cells have reversibly withdrawn from the cell division cycle in response to high cell density or mitogen deprivation, they are considered to be in the G0 phase. The progression through the cell cycle is controlled by the family of cyclin-dependent serine/threonine kinases (CDKs) and their regulators, the cyclins. Specifically, cyclin D-CDK4, cyclin D-CDK6, and cyclin E-CDK2 drive G1 progression while S phase is initiated by cyclin A-CDK2 and cyclin B-CDK1 regulates progression through G2 and entry into mitosis. If sensing mechanisms detect aberrant events during such processes, an arrest in the cell cycle can be triggered until the problem is resolved. Those functions are driven by members of the Ink4 family, such as p16, p15, p18, and p19, which suppresses CDK4 and CDK6, and the Cip/Kip family of inhibitors, which block the activity of CDK2, such as the proteins p21, p27, and p57 that can reversibly arrest cell cycle progression [157]. Given that one of the main characteristics of tumor cells is their uncontrolled proliferation, it is not surprising that many of the compounds with anticancer potential have been evaluated in the context of cell cycle effectors [158].

The biological activities of phytocompounds against cancer cells other than apoptosis are usually observable when cells are exposed at sublethal doses and for longer periods. For example, the exposition of MCF7 cells to β-Sitosterol at low concentrations (19 μM) for 3 days decreased almost 90% the proliferation rate [100]. Similarly, stigmasterol (20 μM) impairs proliferation by G1 cell cycle arrest diminishing the activity of cyclin D-CDK6 in Ishikawa endometrial cancer cells [159]. Regarding the pentacyclic triterpenes, HeLa cells exposed to lupeol (25 μM) undergo S-phase cell cycle arrest after 24 hours of incubation along with decreased protein levels of Cyclin E, Cyclin A, and CDK2 [160]. Treatment of MCF7 cells with betulinic acid (20 μM) halts cell proliferation and induces G0/G1 phase cell cycle arrest in HepG2 cells at a relatively low concentration (11 μM) [108]. Ursolic acid (40 μM) inhibits the 85% proliferation of A549 cells by G1 phase cell arrest associated with a marked decrease in the protein expression of cyclins D1, D2, and E and CDK2, 4, and 6 with induction of p21 [111]. Furthermore even at a lower concentration (10 μM) can decrease the 80% of proliferation in MCF7

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cells [161]. Regarding the phenolic compounds, naringenin (100 μM) inhibits HepG2 proliferation inducing cell cycle arrest at G2/M phase [162]. In the same model, treatment for 48 hours with apigenin (30 μM) induces the expression of p53, p21, and the accumulation of cells arrested in G2/M phase [123]. Kaempferol (40 μM) also inhibits cell proliferation through G1 cell cycle arrest by activation of p53 gene expression and downregulation of cyclin D1, CDK4, and CDK6 in HepG2 cells [163]. The exposition of PC3 to quercetin (100 μM) for 24 hours led to cell cycle arrest and 80% diminution of cell proliferation by downregulation NF-κB and mTOR protein expression [126, 127]. Treatment for 24 h of HepG2 cells with myricetin (66 μM) also resulted in G2/M phase arrest and inhibition of proliferation mediated by decreased protein levels of CDK2 and cyclin B-CDK1 [142]. On the other hand, the stilbene resveratrol (25 μM) was shown to induce cell cycle arrest in the G0/G1 phase on A549 cells through downregulation in the expression levels of cyclin D1, CDK4, and CDK6, and upregulation of p21 and p27 [164].

The process of metastasis involves the migration of cancer cells from the primary neoplasia to different locations in the body and the generation of new tumor colonies. One of the most important events in gaining metastatic qualities in cells derived from carcinomas is the induction of EMT. This process of transdifferentiation is characterized by the loss of adhesion to neighboring cells and the cellular matrix as well as the adoption of a mesenchymal phenotype. Broadly speaking, cells that undergo EMT exhibit increased resistance to anticancer therapy and tend to produce more aggressive lesions with an overall worse prognosis. Markers linked to this event include N-cadherin, vimentin, and fibronectin, while the expression of epithelial junctional proteins such as E-cadherins, claudins, and occludins are downregulated. Among the transcription factors capable of inducing this process are the proteins Snail, Slug, Zeb, and Twist, which in turn can be activated by the increase in the production of cytokines such as IL-6, TNF-α and TGF-β in the tumoral microenvironment [5].

β-sitosterol (32 μM) prevents EMT, migration, and invasion of HepG2 cells by downregulation of N-cadherin, Twist, vimentin, and Snail expression while inducing upregulation of E-cadherin [154]. In the Ishikawa endometrial carcinoma cells, stigmasterol (20 μM) was found to suppress Zeb, Slug, and N-cadherin while increasing the expression of E-cadherin [159]. Lupeol (50 μM) also exhibited anti-EMT properties against A549 cells as shown by the downregulation of N-cadherin and vimentin with repression of migration [165]. The potential of betulinic acid in preventing EMT was assessed by the exposition (20 μM) of the human gastric carcinoma cell line SNU-16 imparing migration and invasion through the downregulation of N-cadherin [166]. Similar results were found with ursolic acid (25 μM) treatment against the migration capabilities of the human gastric carcinoma cells BGC823 associated with a negative modulation of the NF-κB pathway and vimentin, Snail, and Twist gene expression [167]. Concerning polyphenols, naringenin (50 μM) prevents TGF-β induction of EMT in the pancreatic carcinoma cells panc-1 by inhibiting the Smad3 pathway, impairing migration, invasion, and the mesenchymal phenotype as noted by the repression of vimentin and N-cadherin [168]. Interestingly, apigenin (20 μM) reverses EMT in the human hepatocarcinoma cells Bel-7402 as indicated by changes in cell morphology and reexpression of E-cadherin and claudin-3 linked to repression of the NF-κB and Snail axis [169]. Kaempferol (25 μM) was also able to suppress EMT induced by TGF-β in A549 cells along with the prevention of their invasive potential through downmodulation of Akt1 and Smad pathways and inactivation of MMP-2 [170]. In PC3 cells, quercetin (25 μM) treatment prevented the invasiveness via down-regulation of N-cadherin and vimentin and reexpression of E-cadherin [171]. Dual effects were shown on MCF7 exposed to quercetin (37 μM) since cells undergo G1 phase arrest with cyclinD1, p21, and Twist gene expression suppression suggesting anti-proliferative and anti-EMT activities [172]. The flavonol myricetin (37 μM) also inhibited migration, invasion, and EMT in PC3 cells by downregulation of vimentin [138]. The effects of resveratrol (37 μM) in preventing EMT on HeLa cells were shown to suppress migration and invasion by downregulation of IL-6 and STAT signaling, along with a reduction in the protein levels of N-cadherin and vimentin [173].

Conclusion and perspectives

The development of antineoplastic therapy based on medicinal plants begins with the evaluation of total extracts against a diversity of cell lines for a possible anticancer biological activity followed

by the purification of active phytochemicals based on the fractionation guided by *in vitro* bioassays. Then, candidate molecules can be tested *in vivo* using preclinical models of cancer to establish the preliminary efficacy, toxicity, pharmacokinetic, and safety profile and decide whether a compound should be taken further for clinical trials [174]. The bioassay-guided study of *C. trifoliata* allowed us to find the most active fraction against PC3 cells, composed of ursolic acid, betulinic acid, naringenin, apigenin, kaempferol, and resveratrol [175]. That is, triterpenes, flavonoids, and stylbenes, this resembles results from the bioassay-guided study performed in *Vitis vinifera* extracts in which the fraction that reduces most of the proliferation in MCF7 cells was composed of β-sitosterol, oleanolic acid, betulinic acid, resveratrol and ε-viniferin [176]. Furthermore, the bioassay-guided study of *C. quadrangularis* performed on MCF7 cells showed a highly antiproliferative fraction characterized by the presence of the flavonoids quercetin and rutin [83]. Furthermore, ursolic acid [177], betulinic acid [178], naringenin [179], apigenin [121], kaempferol [180], and resveratrol [181] have been shown antitumoral activities in different preclinical models of carcinomas. However the medicinal use of natural products is now considering mixtures rather than purified compounds as a more effective strategy due to potential synergies [182]. In addition, cancer is a disease regulated by multiple pathways and numerous studies suggest that resistance is less likely to occur when a combination of compounds is provided instead of single active constituents. In addition, plants evolved to fight adversity through the combined action of structurally and functionally diverse constituents [182]. As such, the evaluation of complex mixtures might be valuable in improving the therapy against cancer, but few studies have been performed to test this possibility. In this regard, the extracts from leaves of *C. sicyoides* suppressed the Ehrlich carcinoma in mice and the bioactivity was related to the content of β-sitosterol and resveratrol [183]. Hence, seems plausible that *C. trifoliata* extracts may exhibit antitumoral activities *in vivo* as suggested by their chemical composition and ethnomedical uses. Furthermore, the study of the molecular mechanisms implicated in the synergies resulting from fractions, extracts, or from the entire plant may help to clarify their potential uses against carcinomas.

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