Anticancer Secondary Metabolites Found in Native Ecuadorian Plant Species *Uncaria tomentosa* DC. (Rubiaceae), *Croton lechleri* Müll. Arg. (Euphorbiaceae), and *Equisetum giganteum* L. (Equisetaceae)

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1 Introduction

Ecuador is a country that is located northeast of the South American continent, it limits the north with Colombia, the south and east with Peru, and the west with the Pacific Ocean. It is one of the smallest countries in South America with an area of approximately 283,560 km^2, or 1.5% of the total surface of the continent (Sierra et al., 2002). Despite this, Ecuador is recognized as one of the countries with the highest biodiversity worldwide (Bailon-Moscoso et al., 2015a). In terms of vegetation, diversity is extraordinary, containing an immense number of native and endemic species (Malagón et al., 2003). The diversity of species in Ecuador is due to many factors such as the variety of climates and its distinguished regional separation, mainly due to the presence of the Andes Mountains (Sierra et al., 2002). It has four geographic regions: Amazonia, Sierra, Costa, and Insular, in which the composition of the soil, temperature, humidity, latitude, and altitude change, thus giving a great variety of ecosystems and therefore an immense diversity of animal and plant species.

For a long time, indigenous cultures around the world, such as the Ecuadorian population nowadays, maintain their ancestral traditions regarding the use of native and endemic plants as natural remedies to combat different diseases (Seca & Pinto, 2018; Tene et al., 2007). This knowledge in ethnobotany is orally transmitted from generation to generation (Tene et al., 2007). There is a wide variety of native plants known in Ecuador thanks to their great diversity with great medicinal potential, specifically with an anticancer effect (Bailon-Moscoso et al., 2015a). There are reports that around 3000 plants worldwide have these anticancer properties and

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many of them can be found in Ecuador. These plants are of great interest since cancer nowadays is the disease with the highest mortality and incidence rates worldwide (Sung et al., 2021). According to the GLOBOCAN 2020, there are proximally 19.3 million new cancer case diagnosed in 2020 and around 10.0 million cancer deaths were reported (Sung et al., 2021). Moreover, it is estimated 28.4 million new cancer cases for 2040. It represents an increase of 47% in comparison with 2020 (Sung et al., 2021). In Ecuador in 2020 there was 29,273 new cancer cases, and 15,123 deaths in which breast, prostate, colorectal, stomach, and thyroid cancers predominate (World Health Organization, 2020). This is an alarming number, for this reason the search for a treatment that helps fight this disease is of utmost importance (Seca & Pinto, 2018).

The compounds that behave as the most important anticancer agents and are found in these plants are called secondary metabolites (Demain & Fang, 2000). In this chapter, the general characteristics of secondary metabolites will be described and the effect of this metabolites found in different plants of the world finally focuses on three Ecuadorian species, *Uncaria tomentosa* DC., *Croton lechleri* Müll. Arg., and *Equisetum giganteum* L. against different types of cancer.

2 Secondary Metabolites

Secondary metabolites are organic molecules produced within the plant, which are not necessary for its development, growth, and reproduction (Seca & Pinto, 2018). They have unique structures and are found in specific cells and organs where they accumulate in vacuoles. The production of secondary metabolites depends mainly on the adaptations and interactions of plants with their environment (Baikar & Malpathak, 2010). Furthermore, it has been proved that some secondary metabolites have anticancer activity. Observe Table 1 about plants around the world with secondary metabolites that were used for studies to demonstrate their anticancer properties. Secondary metabolites are classified according to the route by which they are synthesized, so they have three main groups: alkaloids, terpenoids, and phenols (Seca & Pinto, 2018).

2.1 Alkaloids

Alkaloids are considered among the most active secondary metabolites and widely distributed in the plant kingdom, mainly in angiosperms (Coqueiro & Verpoorte, 2019; Wink, 2015). Their structures consist of one to several nitrogen atoms in a ring structure (true alkaloids) or in a side chain (pseudoalkaloids). In addition to carbon, hydrogen, and nitrogen, this group may also contain oxygen, sulfur, and rarely elements like chlorine, bromine, and phosphorus (Kabera, 2018). The alkaloids are synthesized by various organisms, such as bacteria, fungi, animals and plants,

Table 1 Plant species around the world with secondary metabolites and anticancer properties

Species	Distribution[a]	Compounds used for cytotoxicity study	Type of cancer	Line cell	IC_{50} values	References
Ormenis eriolepis Coss	Moroccan	Dichloromethane Fraction (Oe-DF) Hexanic Extract (Oe-HE)	T cell leukemia Mantle Cell Lymphoma Glioblastoma Caucasian prostate adenocarcinoma	Jurkat Jeko-1 LN229 PC-3	11.63 ± 5.37 μg/ml 13.33 ± 1.67 μg/ml 41.67 ± 1.98 μg/ml 19.31 ± 4.88 μg/ml	(Belayachi et al., 2017)
Podophyllum peltatum L.	Canada, United States	Podophyllotoxin	Esophageal squamous cell carcinoma (ESCC)	KYSE 30 KYSE 70 KYSE 410 KYSE 450 KYSE 510	0.27 μM 0.30 μM 0.17 μM 0.30 μM 0.30 μM	(Yoon et al., 2020)
Camptotheca acuminata Decne	China	Camptothecin derivative, CPT211	Breast cáncer	MCF-7 MDA-MB-231	191.2 nM 478.4 nM	(Chiu et al., 2020)
Linum narbonense L.	Portugal, Switzerland, Corsica	Arylnaphthalene lignan Justicidin B	Chronic myeloid leukemia Hodgkin lymphoma Human urinary bladder carcinoma	K-562 LAMA-84 HD-MY-Z EJ cells	0.597 ± 0.019 μg/ml 0.029 ± 0.05 μg/ml 0.620 ± 0.022 μg/ml 0.812 ± 0.031 μg/ml	(Ionkova et al., 2013)
Cotula cinerea Delile	Algeria, Morocco, Red Sea region, Sinai, Qattara Depression, and Mali	Hexane	Human embryonal rhabdomyosarcoma Monkey kidney cancer	RD Vero	57.21 ± 3.43 μg/mL 142.27 ± 11.33 μg/mL	(Guaouguaou et al., 2018; Lakhdar, 2018)
Cotula cinerea Delile	Algeria, Morocco, Red Sea region, Sinai, Qattara Depression, and Mali	Ethyl acetate	Human embryonal rhabdomyosarcoma Monkey kidney cancer	RD Vero	187.52±6.27 μg/mL 212.83±9.02 μg/mL	(Guaouguaou et al., 2018; Lakhdar, 2018)

(continued)

Table 1 (continued)

Species	Distribution[a]	Compounds used for cytotoxicity study	Type of cancer	Line cell	IC_{50} values	References
Cotula cinerea Delile	Algeria, Morocco, Red Sea region, Sinai, Qattara Depression, and Mali	n-butanol	Human embryonal rhabdomyosarcoma Monkey kidney cancer	RD Vero	>500 μg/mL 447.38 ± 6.52 μg/mL	(Guaouguaou et al., 2018; Lakhdar, 2018)
Cotula cinerea Delile	Algeria, Morocco, Red Sea region, Sinai, Qattara Depression, and Mali	Essential oil	Human embryonal rhabdomyosarcoma Monkey kidney cancer	RD Vero	173.05 ± 4.46 μg/mL 72.72 ± 2.18 μg/mL	(Guaouguaou et al., 2018; Lakhdar, 2018)
Berberis vulgaris L.	Canada, United States, most of Europe	Ethanol extract Ethyl acetate extract	Human breast adenocarcinoma	MCF-7	3.54 μg/mL 596.71 μg/mL	(El Khalki et al., 2018)
Solanum aculeastrum Dunal	Tropical to South Africa	Solamargine	Human neuroblastoma Breast cancer	SH-SY5Y SK-Br3	13.54 μg/mL 16.12 μg/mL	(Burger et al., 2018)
Leonotis leonurus (L.) R.Br.	South Africa	Marrubiin	Human colorectal adenocarcinoma	HT-29	50 μg/mL	(Kee et al., 2008)
Hypoxis hemerocallidea Fisch. & C.A. Mey.	South Africa	Hypoxoside	Cervical cancer Colorectal Adenocarcinoma Breast adenocarcinoma	HeLa HT-29 MCF-7	26.46 μg/mL 82.80 μg/mL 45.62 μg/mL	(Boukes, 2010)
Nelumbo nucifera Gaertn.	Australia, Bhutan, Burma, China, Guyana, India, Japan, Nepal, Pakistan, Philippines, Russian, South Korea, Thailand, United States	Neferine	Hepatocellular carcinoma (HCC)	HepG2	10 μM	(Poornima et al., 2013)

Podophyllum peltatum L.	Canada, United States	Glucoside derivatives of podophyllotoxin	Leukemia hepatoma Lung cancer Breast cancer Colon cancer	HL-60 SMMC-7721 A-549 MCF-7 SW480	11.37 ± 0.52 μM 8.41 ± 0.48 μM 10.74 ± 0.37 μM 9.18 ± 0.49 μM 3.27 ± 0.21 μM	(Zi et al., 2019)
Zingiber officinale Roscoe	Australia, Belize, Bhutan, Bolivia, Burma, Cambodia, China, Colombia, Costa Rica, Ecuador, Gabon, Guatemala, Honduras, India, Japan, Laos, Madagascar, Mexico, Nicaragua, Panama, Philippines, Thailand, Vietnam	[10]-gingerol	Triple negative breast cancer (TNBC)	4T1Br4 MDA-MB-231BrM b	29.9 ± 4.8 μM 65.2 ± 5.1 μM	(Martin et al., 2017)
Inula viscosa (L.) Aiton	Mediterranean Basin	Hexane extract	Acute T cell leukemia Non-Hodgkin lymphomas Glioblastoma Colorectal adenocarcinoma Prostate adenocarcinoma	Jurkat Jeko-1 LN229 SW620 SW480 PC-3	7.52 ± 4.57 μg/mL <1 μg/mL 5.9 ± 3.57 μg/mL 8.40 ± 3.31 μg/mL 10.36 ± 6.59 μg/mL 6.63 ± 1.42 μg/mL	(Belayachi, 2013; Talib et al., 2012)
Azima tetracantha Lam.	Madagascar, South Africa	Friedelin	Breast cancer	MCF-7	1.2 μM	(Subash-Babu et al., 2017)
Aristolochia longa L.	Europe	Hexanic extract	Rhabdomyosarcoma Kidney adenocarcinoma of hamster	RD BSR	30 ± 0.70 μg/mL 18 ± 3.53 μg/mL	(Aneb et al., 2016)

(continued)

Table 1 (continued)

Species	Distribution[a]	Compounds used for cytotoxicity study	Type of cancer	Line cell	IC_{50} values	References
Aristolochia longa L.	Europe	Dichloromethanic extract	Rhabdomyosarcoma Kidney adenocarcinoma of hamster	RD BSR	15 ± 1.06 μg/mL 60 ± 2.47 μg/mL	(Aneb et al., 2016)
Carica Papaya L.	America	Lyophilized papaya leaf juice extract (LJP)	Prostate cancer (PCa)	RWPE-1 BPH-1 PC-3 LNCaP	0.22 mg/mL 0.79 mg/mL 0.95 mg/mL 0.96 mg/mL	(Pandey et al., 2017)
Pistacia lentiscus L.	Spain Algeria, Egypt, Libya, Morocco, Tunisia, Cyprus, Israel, Jordan, Lebanon, Syria, Turkey, Albania, Greece, Italy, France, and Portugal	*P. lentiscus* essential oils obtained from fruits (PLFEO)	Rhabdomyosarcoma	RD	26.43 ± 2.18 μg/mL	(Bouyahya et al., 2019)
Trollius chinensis Bunge	China	Orientin Vitexin	Esophageal cancer	EC-109	5–8 μM	(An et al., 2015)
Cajanus cajan (L.) Huth	America, Asia, Africa	Cajanstilbenoids A	Hepatocarcinoma Breast adenocarcinoma Lung cancer	Hep2 MCF-7 A549	2.50 ± 0.2 μM 2.56 ± 0.3 μM 2.14 ± 0.2 μM	(Zhang et al., 2018)
Cajanus cajan (L.) Huth	America, Asia, Africa	Cajanstilbenoids B	Hepatocarcinoma Human breast adenocarcinoma Human lung cancer	Hep2 MCF-7 A549	5.99 ± 0.3 μM 22.63 ± 0.4 μM 6.18 ± 0.3 μM	(Zhang et al., 2018)
Celtis Africana Burm. f.	Angola, Madagascar, Mozambique, Nigeria, South Africa, Zambia, Zimbabwe	Ethyl acetate extract	Mouse lymphoma	L5178Y	8.3 μg/mL	(Perveen et al., 2015)

Elephantopus mollis Kunth	America, Fiji, Gabon, Micronesia Federated S tates, Tanzania, Uganda	Methanol extract	Lung cancer Mesothelioma Hepatocarcinoma Colorectal adeno-carcinoma Breast adenocarcinoma	A549 SPC212 HepG2 DLD-1 MCF-7	24.38 ± 1.86 μg/mL 4.05 ± 0.69 μg/mL 3.74 ± 0.07 μg/mL 3.29 ± 0.04 μg/mL 3.97 ± 0.48 μg/mL	(Kuete et al., 2017)
Enantia chlorantha Oliv.	Gabon	Methanol extract	Lung cancer Mesothelioma Hepatocarcinoma Colorectal adeno-carcinoma Breast adenocarcinoma	A549 SPC212 HepG2 DLD-1 MCF-7	>40 μg/mL 25.16 ± 1.30 μg/mL 17.32 ± 0.13 μg/mL >40 μg/mL >40 μg/mL	(Kuete et al., 2017)
Kalanchoe crenata (Andrews) Haw.	Belize, Brazil, Ecuador, El Salvador, Guatemala, Guyana, Honduras, Madagascar, Sierra Leone, South Africa, United States	Methanol extract	Lung cancer Mesothelioma Hepatocarcinoma Colorectal Adenocarcinoma Breast adenocarcinoma	A549 SPC212 HepG2 DLD-1 MCF-7	8.23 ± 0.15 μg/mL 2.33 ± 0.23 μg/mL 28.96 ± 3.51 μg/mL 23.87 ± 1.69 μg/mL 19.31 ± 0.79 μg/mL	(Kuete et al., 2017)
Lophira alata Banks ex C.F. Gaertn.	Gabon	Methanol extract	Lung cancer Mesothelioma Hepatocarcinoma Colorectal adeno-carcinoma Breast adenocarcinoma	A549 SPC212 HepG2 DLD-1 MCF-7	>40 μg/mL >40 μg/mL 32.98 ± 4.20 μg/mL >40 μg/mL >40 μg/mL	(Kuete et al., 2017)

(continued)

Table 1 (continued)

Species	Distribution[a]	Compounds used for cytotoxicity study	Type of cancer	Line cell	IC_{50} values	References
Millettia macrophylla Benth.	Austral Africa	Methanol extract	Lung cancer Mesothelioma Hepatocarcinoma Colorectal adeno-carcinoma Breast adenocarcinoma	A549 SPC212 HepG2 DLD-1 MCF-7	>40 μg/mL 7.54 ± 0.26 μg/mL 2.01 ± 0.04 μg/mL 31.02 ± 2.86 μg/mL 25.99 ± 1.68 μg/mL	(Kuete et al., 2017)
Phragmanthera capitata (Spreng.) Balle	Gabon	Methanol extract	Lung cancer Mesothelioma Hepatocarcinoma Colorectal adeno-carcinoma Breast adenocarcinoma	A549 SPC212 HepG2 DLD-1 MCF-7	>40 μg/mL >40 μg/mL >40 μg/mL >40 μg/mL >40 μg/mL	(Kuete et al., 2017)
Withania adpressa Cors.	North Africa, Mediterranean basin, and India	Withanolide F	Multiple myeloma cancer stem cells Plasmacytoma	MM-CSCs RPMI 8226	5.3 ± 0.9 μM 0.1 ± 0.01 μM	(Ben Bakrim et al., 2018)
Withania adpressa Cors.	North Africa, Mediterranean basin, and India	Withafein A	Multiple myeloma cancer stem cells Plasmacytoma	MM-CSCs RPMI 8226	0.33 ± 0.02 μM 0.17 ± 0.03 μM	(Ben Bakrim et al., 2018)
Cedrus deodara Cors.	Afghanistan, Bolivia, China, India, Nepal, and Pakistan	*Cedrus deodara* total lignans CTL	Lung carcinoma	A549	39.82 ± 1074 μg/mL	(Shi et al., 2019)
Dysoxylum nutans Cors.	India, China, Malaysia, Indonesia, Australia, and New Zealand	3α-Hydroxystigmast-5 (6), 22-Dien-7-en	Breast adenocarcinoma	MCF-7	20.13 ± 0.06 μM	(Mayanti et al., 2020)

Peganum Harmala L.	Afghanistan, China, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russian Federation, South Africa, Tajikistan, United States, Uzbekistan	Harmine	Breast Lung Colon Cervic Colon	HBL-100 A549 HT-29 HEeLAa HCT-116	32.56 ± 0.65 μM 106-71 ± 2.49 μM 45.55 ± 0.87 μM 61.81 ± 0.65 μM 33.97 ± 0.21 μM	(Ayoob et al., 2017)
Allium wallichii Kunth	Bhutan, Burma, China, India, Nepal	Crude extract of *Allium wallichii*	Prostate cancer Breast cancer Cervical cancer	PC3 MCF-7 HeLa	69.69 μg/mL 55.29 μg/mL 46.51 μg/mL	(Bhandari et al., 2017)
Mitracarpus frigidus (Willd. ex Roem. & Schult.) K. Shum	Bolivia, Brazil, Guyana, Peru, Venezuela	Psychorobrin (14)	Breast cancer Human promyelocytic leukemia T lymphocyte	MCF-7 HL60 Jurkat	1.1 μM 4.5 μM 5.6 μM	(Fabri et al., 2012)
Sphagneticola trilobata (L.) J.F. Pruski	Australia, Belize, Bolivia, Brazil, Caribbean, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guinea, Guyana, Honduras, Malaysia, Mexico, Micronesia Federated States, Nicaragua, Panama, Peru, Philippines, United States, Venezuela	Ethyl acetate extract of *S. trilobata*	Breast cancer	MCF-7	58.143 μg/mL	(Mardina et al., 2020)

[a]The distribution information was obtained by accessing the database of the web pages: Tropicos.org (2021) and GBIF.org (2021)

especially by the latter. Unlike the other secondary metabolites, alkaloids have a great structural diversity and are very heterogeneous, so they do not have a very precise classification (Coqueiro & Verpoorte, 2019). Most are toxic to other organisms and are obtained using the acid-base extraction technique (Kabera, 2018). They have pharmacological effects and a great use as medicines. Some alkaloids are being used in cancer therapies such as chemotherapeutics. Similarly, there are alkaloids that act as neuroreceptors, or modulate the transduction of neuronal signals. Others interfere with DNA, telomeres, telomerase, or apoptosis-inducing protein biosynthesis (Tiwari, 2015; Wink, 2015).

2.2 Terpenoids

Also known as isoprenoids, they are the most numerous and structurally diverse natural products, with more than 23,000 known structures. They are derived from polymeric isoprene and are synthesized from the mevalonic acid pathway (Kabera, 2018). They are classified according to the number and structural organization of the isoprene units (Tiwari, 2015; Wink, 2015). For this reason, isoprene can be considered as the basic component of terpenoids, it is 2-methylbuta-1,3-diene (C_5H_8). The most basic class of terpenoids is hemiterpenoids which consists of only one isoprene unit (Ludwiczuk et al., 2017). Monoterpenoids are major components of essential oils and are known for their aromatic properties. These are the main components of spruce (38%), pine (30%), Angelica species (73%), rose (54%), among more species. Flower and seed essential oils have more specialized monoterpenoids. Diterpenoids are found mainly in the Lamiaceae family and have antimicrobial and antiviral properties. And in the same way, each of the other divisions can be found in specific plant families (Ludwiczuk et al., 2017; Wink, 2015). Terpenoids have great pharmacological activity and are used in the treatment of various diseases. According to researchers they have anticancer activity and their mechanism of action is the prevention of tumor cell proliferation through necrosis or apoptosis induction (Kabera, 2018; Ludwiczuk et al., 2017; Wink, 2015).

2.3 Phenols

Phenolic compounds are secondary metabolites that are universally distributed in all plants (Ghasemzadeh & Ghasemzadeh, 2014; Kabera, 2018). They have various structures, but all have hydroxylated aromatic rings. Most phenolic compounds polymerize into larger molecules, such as proanthocyanidins and lignins. Likewise, they can be found in food plants such as glycosides or esters (Kabera, 2018; Tiwari, 2015). In tea, coffee, berries, and fruits, phenolic compounds could be found up to a total of 103 mg/100 g of fresh weight (Kabera, 2018). These play an important role in the development and reproduction of plants. They also are produced in response to

different environmental factors (light, drought, cold, etc.) and to defend the plant from pathogens or herbivores. Phenolic compounds provide odor, taste, and antioxidant, anti-inflammatory, anticancer properties among many more found in different species of the plant kingdom (Ghasemzadeh & Ghasemzadeh, 2014; Kabera, 2018).

3 Plant Families and their Native Ecuadorian Plant Species that have Secondary Metabolites for Anticancer Activity: Three Case Studies

3.1 Rubiaceae

Rubiaceae is a member of the Gentianales and represents one of the four most species-rich families of angiosperms (flowering plants), with 13,143 species classified in 611 genera (Davis et al., 2009), more than 40 tribes, and 3 subfamilies (Bremer & Eriksson, 2009; Goevarts et al., 2006). Rubiaceae have a cosmopolitan distribution, so they are widespread and are located in all major regions of the world, including the Antarctic continent, but the diversity of species and biomass is found in tropical and subtropical areas. Species belonging to this family occupy many types of habitat in different biogeographic regions (Bremer & Eriksson, 2009; Davis et al., 2009). The diversity of the family is enormous, with life forms ranging from small grasses to large trees, with flowers adapted to different types of pollinators, with fruits that have many types of dispersal mechanisms, and with a wide variety of different chemicals that accumulate in the plants (Bremer & Eriksson, 2009). And according to Ulloa (2006), in Ecuador we have 658 species distributed in 88 genera, positioning itself as the third richest family in species, after Orchidaceae and Asteraceae, in the country.

To the present, several species of the Rubiaceae family have been reported that have a wide range of medicinal uses and pharmacological activities such as antioxidant, antifungal, antidiabetic, antibacterial, antidiarrheal, anti-inflammatory, anticancer, and more (Ekalu, 2021). All this is due to the bioactive secondary metabolites that have been detected in plants, thus allowing the development of new drugs for the treatment of diseases such as cancer (Singh et al., 2020). Because of this, various plants of this family have been studied for possible cancer treatments, some examples are: (1) *Mitracarpus* species where bioactive secondary metabolites with anticancer activity have been detected such as psychorubin, ursolic acid, rutin, kaempferol-3-O-rutinoside, kaempferol, stigmasterol, and quercetin (Ekalu, 2021); (2) *Gardenia jasminoides* Elli with the bioactive compound genipin, extracted from desiccative ripe fruits, as a chemopreventive agent to prevent cancer (Chen et al., 2020); (3) *Hedyotis corymbosa* from which the secondary metabolite asperuloside is isolated (Manzione et al., 2020); (4) *Mitragyna speciosa* Korth, recognized as a rich source of alkaloids, exhibits the bioactive compound mitragynine with cytotoxic activity against breast cancer cells (Firmansyah et al., 2021); and (5) *Uncaria*

species, which are an important source of bioactive indole alkaloids (Liang et al., 2020; Qin et al., 2021); in the last 20 years around 100 new secondary metabolites have been elucidated in which they include alkaloids, triterpenes, and flavonoids that provide anticancer biological functions among many more (Liang et al., 2020). *Uncaria tomentosa* native to Ecuador is the most studied species among the numerous species of genus *Uncaria* because it contains bioactive compounds with anticancer properties (Martins & Nunez, 2015) and will be described in more detail below.

3.1.1 *Uncaria tomentosa* DC

It belongs to the family Rubiaceae (Bacher et al., 2006; Bailon-Moscoso et al., 2015a; De la Torre et al., 2008; Farias et al., 2013; Rinner et al., 2009), is a native liana to Ecuador and is distributed in Asia (Bailon-Moscoso et al., 2015a) and in different countries in South America (Amazon of Brazil, Colombia, Peru, Bolivia, Venezuela, and Guyana) and Central America (El Salvador, Guatemala, Belize, Hondura, El Salvador, Nicaragua, Costa Rica, and Panama) (Farias et al., 2013). It is known by its spanish common name "uña de gato" and translated as cat's claw (Bailon-Moscoso et al., 2015a; De la Torre et al., 2008). In Ecuador, it is distributed in the provinces of Napo, Orellana, Sucumbíos, and Zamora-Chinchipe at altitudes of 0 to 900 meters above sea level (masl) (Bailon-Moscoso et al., 2015a). Throughout history, it has been used as traditional medicine by indigenous peoples of Ecuador, Peru, and Bolivia (Bacher et al., 2006; Bailon-Moscoso et al., 2015a) and the method of administration of this plant is by taking the extract of the bark or root boiled with water or by maceration in alcohol (Pilarski et al., 2013; Rinner et al., 2009). The extracts of this plant are used for treatments of inflammation, rheumatism, arthritis, infections, and cancer, due to its antiproliferative activity, which induces apoptosis in cancer cells (Bacher et al., 2006; Bailon-Moscoso et al., 2015a; Farias et al., 2013; Rinner et al., 2009).

Uncaria tomentosa contains several active secondary metabolites with pharmacological activity, as well as sterols, tannins, procyanidins, flavonoids, triterpene derivatives (polyhydroxylated triterpenes, oleanolic and ursolic acid), quinovic acid glycosides and oxindole alkaloids (including speciophylline, mitraphylline, uncarine pteropodine, isomitraphylline, and isopteropodine) (García Prado et al., 2007; Pilarski et al., 2013).

The mechanism of *U. tomentosa* cytotoxicity is involved in the inhibition of proliferation and inactivation of NF-$_k$B and the synthesis and release of pro-inflammatory cytokines such as TNF-α (Fig. 1) (Pilarski et al., 2013). In normal cells, NF-$_k$B proteins regulate cell proliferation and cell survival. However, in tumor cells there is a failure in the NF-$_k$B pathway. More studies demonstrated that NF-$_k$B inhibition of TNF secretion in monocyte-like THP-1 cells exposed by *U. tomentosa* extracts is associated with the deactivation of the c-Jun, JunB, p65, RelB, and p50 subunits (Allen-Hall et al., 2010). Therefore, the development of anticancer therapies that include the inhibition of NF-$_k$B that can stop the proliferation of cancer

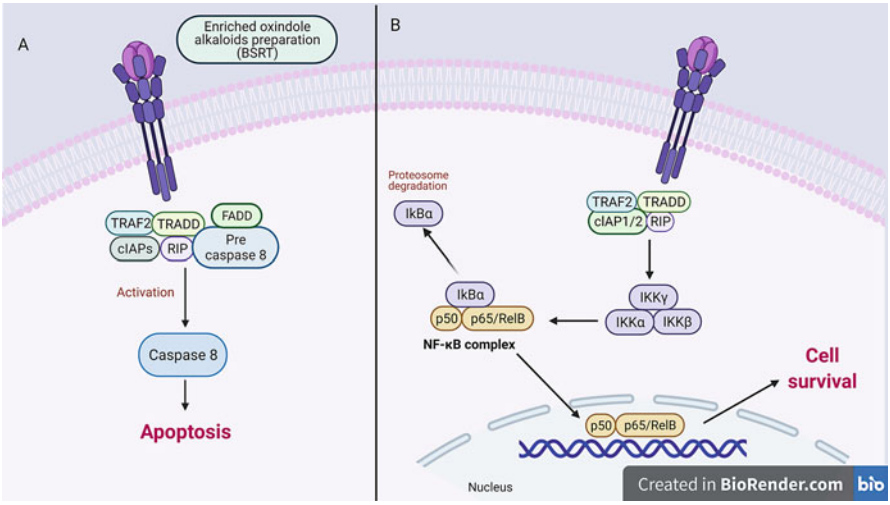

Fig. 1 Apoptosis induced by mechanism BSRT + TNF-α vs cell survival by TNF-α/NF-$_k$B pathway. (**a**) Tumor cell pretreated with BSRT inhibits the NF-$_k$B complex and promote the action of TNF-α to activate proapoptotic activity. (**b**) Tumor cell without BSRT ha*s* a misregulation of NF-$_k$B complex that causes the tumor cell survival (Pilarski et al., 2013)

cells, produce cell death, or improve the response of antitumor drugs, is one of the greatest challenges in today's pharmaceutical industry.

In a study from 2013 researchers used 10 g of the bark of *U. tomentosa* originating in Peru to obtain an enriched preparation of oxindole total alkaloids named BSRT. According to the high-performance liquid chromatography (HPLC) analysis, more than 50% of pentacyclic oxindole alkaloids (POA) in dry weight of BSRT was obtained with predominance of pteropodine, speciophylline, and isopteropodine. Besides, POA show greatest pharmacological activity, as opposed tetracyclic oxindole alkaloids (TOA) which were found in very low concentrations (García Prado et al., 2007; Pilarski et al., 2013). Cytotoxicity tests indicate that the BSRT concentration required to inhibit the 50% growth (IC_{50}) of promyelocytic leukemia HL-60 cells is 60 μg/mL. And analysis by flow cytometry confirms that the alkaloid enriched extract induces apoptosis in promyelocytic leukemia HL-60 cells and the highest result was obtained with the BSRT treatment combined with TNF-∞ with a 39.9% apoptotic increase (Pilarski et al., 2013).

Medullary thyroid carcinoma (MTC) is a calcitonin producing tumor of the parafollicular C-cells (Rinner et al., 2009). MTC is insensitive to radiation therapy as well as chemotherapy. Furthermore, it has been shown that resistance to chemotherapy is due to the expression of the mdrl gene that causes resistance to multiple drugs. Also, studies indicate that different MTC cell lines have an increase in the anti-apoptotic protein Bcl-2, which allows tumor cells to survive (Yang et al., 1991). Rinner and colleagues reported that extracts from *U. tomentosa* affected the growth, viability, and apoptosis of MTC-SK cells. The antiproliferative effect in MTC-SK

cells is due to the alkaloids stopping the cell cycle in G2 while specific alkaloids such as isopteropodine at 100 μM and pteropodine between 50 and 100 μM significantly inhibited cell growth and viability of MTC-SK cells (Rinner et al., 2009).

Other researchers worked with the following specific alkaloids: isopteropodine (A1), pteropodine (A2), isomitraphylline (A3), uncarine F (A4), and mitraphylline (A5) from extracts of *U. tomentosa,* where the highest cytotoxicity effect in different leukemia cell lines was obtained with alkaloids A2 and A4, with more than 95% inhibition of proliferation of CEM-C7H2 cells at a concentration of 100 μmol/L. Furthermore, treatment with these alkaloids was found to activate the intrinsic mitochondrial pathway of apoptosis. In this way, apoptosis occurs by proapoptotic members of the Bcl-2 family of proteins, causing activation of the caspase-9 primer. Likewise, experiments indicated that Bcl-2 overexpression could not prevent alkaloid-induced apoptosis in CEM cells, although it delayed induced cell death (Bacher et al., 2006).

In other study, five different extracts of 1 g of *U. tomentosa* bark originating in Peru were prepared, which differed in the use of water or different concentrations of ethanol with or without boiling (B/W_{37}, B/W_b, $B/50E_{37}$, B/E_b, $B/96E_{37}$). They also obtained a bark preparation rich in alkaloids with another extraction technique (BSRT), using 10 g of the same raw material as the rest. The highest alkaloid content was obtained with the B/SRT preparation where just over 50% of the dry weight was pure oxindole alkaloids. Following $B/96E_{37}$, B/E_b, $B/50E_{37}$, B/W_b, and B/W_{37} with a 3581, 3408, 1897, 509, 430 mg/100 g of pure oxindole alkaloids content, respectively. Cytotoxicity test results indicated that the highest rate of antiproliferative activity was by $B/96E_{37}$ with growth inhibition in Lewis lung carcinoma (LL/2) (IC_{50} = 25.06 μg/ml), cervical carcinoma (KB) (IC_{50} = 49.06 μg/ml), and colon adenocarcinoma (SW707) (IC_{50} = 49.06 μg/ml). Preparation B/SRT obtained an inhibition of proliferation in cervical carcinoma (KB) (IC_{50} = 23.57 μg/ml), breast carcinoma (MCF-7) (IC_{50} = 29.86 μg/ml), and lung carcinoma (A-549) (IC_{50} = 40.03 μg/ml). However, the authors conclude that the idea that the carcinogenic activity of *U. tomentosa* is only by the quantity of alkaloids is wrong because the BSRT preparation did not have the highest antiproliferative activity among the rest of the extracts. Therefore, it is concluded that there are other phytochemicals responsible for the pharmacological activity that *U. tomentosa* possesses (Pilarski et al., 2010).

3.2 Euphorbiaceae

The Euphorbiaceae family is one of the largest of the flowering plants, having 300 genera and approximately 10,000 species (Aleksandrov et al., 2019). It is widely distributed in the Indo-Malayan region, tropical America, tropical Africa, Mediterranean Basin, Middle East, South Africa, and southern USA (Mahbubur Rahman & Iffat Ara Gulshana, 2014). This family contains plants of all kinds such as large woody trees, climbing lianas to simple weeds. They can survive different hot and dry

conditions such as tropical climates or even hot and humid such as the rainforest (Mwine & van Damme, 2011). The Euphorbiaceae family has been used frequently with medicinal uses, thanks to its active components such as alkanes, triterpenes, phytosterols, tannins, polyphenols, and flavonoids (Aleksandrov et al., 2019). Some plants have been tested as cancer treatments thanks to these secondary metabolites, some examples are: *Euphorbia hirta* L., *Euphorbia tirucalli* L., *Euphorbia helioscopia* L. (Aleksandrov et al., 2019), *Croton caudatus* (Shantabi et al., 2020), *Croton oblongifolius* (Sajon, 2019). In Ecuador, there are 244 registered species, of which 46 are endemic (Cerón et al., 2011). These species are mainly found in foothill forests and inter-Andean vegetation, littoral forests, and Galapagos. The genus Croton has the highest number of endemic species in the country (Cerón et al., 2011). Next, the anticancer effect of *Croton lechleri* will be described in detail which is native to Ecuador.

3.2.1 *Croton lechleri* Müll. Arg.

The genus Croton of the Euphorbiaceae family that contains around 1300 species is generally found in tropical and subtropical areas of the world (De Lima et al., 2018). Specifically, *Croton lechleri* a small-size Amazonian tree is distributed on the slopes of the eastern Andes of Peru, Colombia, Ecuador, Bolivia, Venezuela, Brazil, and Mexico (Alonso-Castro et al., 2012; Rossi et al., 2011). In Ecuador, *Croton lechleri* is native and it is found at elevations of 0 to 2000 masl, in the Andean and Amazon region of the country in the provinces of Loja Esmeraldas, Carchi, Morona-Santiago, Napo, Pastaza, Sucumbíos, Tungurahua, and Zamora-Chinchipe (Bailon-Moscoso et al., 2015b). It is better known by its Spanish name "Sangre de Drago" due to the red thick latex that is obtained from it (Alonso-Castro et al., 2012). In Latin America, the red latex of this species has been used as a traditional medicine by indigenous Amazonians for some purposes like heal wounds, to treat gastrointestinal diseases and also as a treatment for cancer due to its cytotoxic and antiproliferative activity (Alonso-Castro et al., 2012; Bailon-Moscoso et al., 2015b; Rossi et al., 2013).

Polyphenolic compounds are the main components of the sap of the *C. lechleri* in Ecuador since it contains 90% dry weight of proanthocyanidin oligomers (Gonzales & Valerio, 2008). Among them catechin-(4α-8)-epigallocatechin, gallocatechin-(4-α-8)-epicatechin, gallocatechin-(4α-6)-epigallocatechin, catechin-(4α-8)-gallocatechin-(4α-8)-gallocatechin and gallocatechin-(4α-8)-gallocatechin-(4α-8)-epi-gallocatechin mixtures of proanthocyanidins, flavon-3-ols (Gonzales & Valerio, 2008). Other elements present in *Croton lechleri* are steroids like β-sitosterol and β-sitostenine, aromatic compounds including 1,3,5-trimethoxybenzene, 2,4,6-trimethoxyphenol, 3,4-dimethoxyphenol, 3,4-dimethoxy benzylalcohol, and 4-hydroxyphenethylalcohol; diterpenoids and a very important compound taspine alkaloid (Gonzales & Valerio, 2008).

In recent years, there has been a growth in interest in the *C. lechleri* sap and its taspine alkaloid. Since proanthocyanidins and other compounds in this plant are known to protect against various pathologies like cancer, taspine is the distinctive

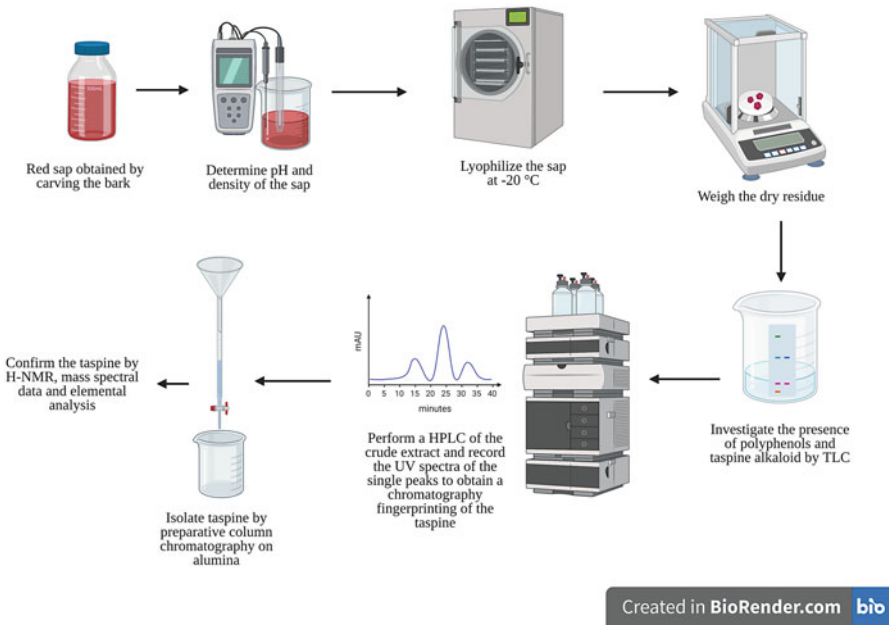

Fig. 2 Representation of the extraction, isolation, and determination of the taspine, secondary metabolite of the *Croton lechleri* (Froldi et al., 2009)

alkaloid of the *C. lechleri* sap and is an active compound with wound healing properties and anticancer activity.

In a study published in 2012 in the Journal of Ethnopharmacology, *Croton lechleri* was evaluated in cell lines such as melanoma (SK-23) and colorectal carcinoma (LoVo and HT29) to determine its anticancer activity (Montopoli et al., 2012). The study was based on cell viability and cell cycle of melanoma and colon cancer cell lines in culture by applying the sap of *C. lechleri* and isolated taspine (Montopoli et al., 2012). This is because the incidence rate of cutaneous malignant melanoma and colorectal cancer has been increasing and the known treatments are not sufficient. For example, malignant melanoma is the most serious skin cancer and does not respond to chemotherapy or radiotherapy, therefore, in vitro studies have been carried out with the use of *C. lechleri* and taspine on epithelial cancer as its traditional use, which is by topical application (Montopoli et al., 2012).

To carry out the research about melanoma and colorectal carcinoma, the red sap of *C. lechleri* was collected from trees in the province of Napo, Ecuador (Montopoli et al., 2012). The sap was lyophilized and stored at 20 °C, and taspine was isolated according to the previously reported (Fig. 2) (Froldi et al., 2009). In this study, the activity of *C. lechleri* sap and taspine in cell proliferation was measured using the MTT test. For this, melanoma and colorectal carcinoma cells were treated for 24 h with the sap in concentrations from 0.01 μg/ml to 100 μg/ml and as a result it was obtained that at 0.5 μg/ml, the sap decreased cell proliferation in 67.57 ± 3.4% in

melanoma cells, and there were no significant results in colorectal carcinoma cells. While inhibition colorectal carcinoma cell proliferation started with 10 μg/ml of sap. The case of taspine was evaluated from 1 ng/ml to 1 μg/ml, showing significant inhibition activity at 0.1 μg/ml in the melanoma and colorectal carcinoma cell lines (Montopoli et al., 2012).

To assess the sensitivity of cells during the cell cycle, the melanoma cell line was exposed to *C. lechleri* sap and taspine for 24 h. Sap at 1.0 μg/ml had no influence on the cell cycle, but at 10.0 μg/ml the cells increased in the G1/G0 phase from 17.77 ± 3.5%, while the S and G2/M phases decreased from 7.97 ± 3.1% and 11.77 ≠ 2.8%, respectively. When the sap was used at 50.0 μg/mL, we observed a sharp decrease in the G1/G0 and S phases with a surprising increase in subG0 cells, which proves the cytotoxic action of the *C. lechleri* sap. In the case of the alkaloid taspine (0.1 and 0.5 μg/mL), there were no changes in the cell cycle (Montopoli et al., 2012). With this result there is a probe of the anticancer activity of the secondary metabolites present in *C. lechleri.*

3.3 Equisetaceae

The Equisetaceae family is one of the oldest found among vascular plants (Christenhusz et al., 2019). It has fossil remains dating from the Carboniferous (Christenhusz et al., 2019). It is known as the horsetail family, because it contains its only surviving genus Equisetum, which has approximately 15–30 species (Xu & Deng, 2017). This family is widely distributed around the world, mainly in the northern hemisphere, South America, Africa, and Asia (Christenhusz et al., 2019). However, it is absent in Australasia and Antarctic (Boeing et al., 2021). They are generally small plants that rarely measure up to one meter in height (Mello & Budel, 2013), rhizomatous perennial plants that have a characteristic jointed-looking stem, containing small leaves fused at a node (Hauke, 1990). They can be adapted to high temperatures, tropical and cold regions (Boeing et al., 2021). In the same way, they can live in both terrestrial and aquatic environments (Xu & Deng, 2017). The genus Equisetum is known to contain a wide variety of secondary metabolites such as saponins, flavonoids, tannis, and alkaloids (Mello & Budel, 2013). By containing these compounds, the genus has several medicinal properties such as antitumor, antimicrobial, antioxidant, anti-inflammatory, and diuretic (Boeing et al., 2021). Speaking of anticancer properties, some species have been shown to have it, such as *Equisetum arvense* (Bhat et al., 2020) and *Equisetum ramosissimum* (Li et al., 2016). In Ecuador, this family is widely distributed in the provinces of Bolívar, Carchi, Chimborazo, Cotopaxi, El Oro, Galapagos, Imbabura, Loja, Morona-Santiago, Napo, Pastaza, Pichincha, Sucumbíos, Tungurahua, Azuay, and Zamora-Chinchipe. The *Equisetum giganteum* native to Ecuador contains these anticancer properties that will be discussed in more detail below.

3.3.1 *Equisetum giganteum* L.

The Equisetaceae family is currently made up of 15 species, *Equisetum giganteum* belonging to it and is better known by its spanish name "cola de caballo" (Ricco et al., 2011). It is a plant endemic to South America and Central America (Alavarce et al., 2015). In Ecuador, *E. giganteum* is native and is found at altitudes ranging from 0 to 3000 masl, in the Coastal, Sierra, and Amazonian regions. Specifically, it is found in the provinces of Azuay, Chimborazo, Cotopaxi, Imbabura, Loja, Morona-Santiago, Napo, Pastaza, Pichincha, Tungurahua, and Zamora-Chinchipe. It is used in traditional medicine as a diuretic and hemostatic in urinary disorders and inflammatory conditions, among other applications such as cancer treatment (Alavarce et al., 2015).

The composition of *E. giganteum* has shown a great abundance of phenolic compounds derived from caffeic and ferulic acids and flavonoid heterosides derived from quercetin and kaempferol, in addition to styrylpyrones (Francescato et al., 2013). Phenolic compounds are among the most studied secondary metabolites due to their beneficial effect for good health. Several in vitro and in vivo studies have reported the important role of phenolic compounds in the fight against cancer and among them are ferulic acid, caffeic acid, and quercetin present in *E. giganteum*, among others. These secondary metabolites have mechanisms of antitumor activity, although they are not fully understood (Jabeur, 2016).

In a 2017 study by the Journal Food & Function, cytotoxicity of *E. giganteum* was evaluated using three tumor cell lines, HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and MCF-7 (breast adenocarcinoma) (Jabeur et al., 2017). It was shown that a low concentration of the *E. giganteum* extract can give 50% inhibition of growth in human tumor cell lines. In general, total phenolic compounds, phenolic acids, and flavonoids are highly related to antioxidant, anti-inflammatory, and antitumor activities, presenting correlation factors for the human tumor cell lines HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and MCF-7 (breast adenocarcinoma) (Inés Jabeur et al., 2017). *E. giganteum* gave a total flavonoid content of 21.7 ± 0.4 mg/g and phenolic acids 4.98 ± 0.03 mg/g, giving a very interesting biological potential, thus confirming that it is an option for the treatment of these types of cancer (Inés Jabeur et al., 2017).

4 Conclusions

It is clear that Ecuador has a great diversity of plant species, resulting in turn in a wide variety of compounds, that we may not know in its entirety, with potential pharmacological use. As presented in this review, *Uncaria tomentosa*, *Croton lechleri,* and *Equisetum giganteum* have a considerable content of secondary metabolites, among which oxindole alkaloids, taspine alkaloids, proanthocyanidin oligomers, flavonoids, and phenolic acids are the most prominent. Furthermore, in vivo

and in vitro tests indicate that the secondary metabolites present in these three plants present a great pharmacological activity for the treatment of different cancer cell lines. The mechanism of action is summarized in that they possess antiproliferative activity and are also capable of inducing apoptosis in different tumor cell lines. In addition, it is important to emphasize the importance of starting to do studies with samples of species from Ecuador because the content of metabolites can vary according to the ecological interactions between the plant and its environment. Furthermore, it is necessary to start doing more in vivo clinical trials in order to understand in more specific detail the mechanisms of action that are involved in the inhibition of cancer cell proliferation and induced apoptosis.

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