Medicinal Plants from Peru: A Review of Plants as Potential Agents Against Cancer

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Abstract: Natural products have played a significant role in drug discovery and development especially for agents against cancer and infectious disease. An analysis of new and approved drugs for cancer by the United States Food and Drug Administration over the period of 1981-2002 showed that 62% of these cancer drugs were of natural origin. Natural compounds possess highly diverse and complex molecular structures compared to small molecule synthetic drugs and often provide highly specific biological activities likely derived from the rigidity and high number of chiral centers. Ethnotraditional use of plant-derived natural products has been a major source for discovery of potential medicinal agents. A number of native Andean and Amazonian medicines of plant origin are used as traditional medicine in Peru to treat different diseases. Of particular interest in this mini-review are three plant materials endemic to Peru with the common names of Cat's claw (*Uncaria tomentosa*), Maca (*Lepidium meyenii*), and Dragon's blood (*Croton lechleri*) each having been scientifically investigated for a wide range of therapeutic uses including as specific anti-cancer agents as originally discovered from the long history of traditional usage and anecdotal information by local population groups in South America. Against this background, we present an evidence-based analysis of the chemistry, biological properties, and anti-tumor activities for these three plant materials. In addition, this review will discuss areas requiring future study and the inherent limitations in their experimental use as anti-cancer agents.

Key Words: Peruvian Medicinal Plants, *Lepidium meyenii*, *Uncaria tomentosa*, *Croton lechleri*, anti-tumor, Cancer.

INTRODUCTION

Peru is characterized as a country with one of the highest biodiversities in the world. Populations from the Andes and the Amazonian have a long history of plant use for the treatment of diseases and this practical knowledge has been transmitted in various ways from generation to generation [1-4]. In a recent survey of a Peruvian Northeastern Andean region well known for its traditional practice of herbal medicine, 33 medicinal plant species were identified and nine were described as anti-cancer agents based on traditional usage [1]. These plants alone or in conjunction are used for the treatment of uterine, prostate, and stomach cancer [1,5]. This is an example of the importance of the Peruvian biodiversity, and the need for basic and clinical research.

At this time it is difficult to say if information on the use of plants for treatment or prevention against cancer in Peru has been traditionally transmitted. Confounding this observation is the lack of evidence about the diagnosis of cancer from historical records of the Andean population. Although it is likely that natives were aware of tumors as a health problem, it is not clear as to their knowledge of malignant tumors. Therefore the crudeness of health care in this population group presupposes an inability to detect carcinogenic tumors over benign ones. This observation has been described by other authors, suggesting that "cancer" was undefined, only described as "hard swellings", abscesses, calluses, corns, warts, polyps, or tumors [6]. Another observation potentially limiting use of herbs as specific treatments against cancer in historical Andean days is that cancer diseases are usually observed in populations where life expectancy is higher than others. The life expectancy in Andean and Amazonian populations, however, is reported to be low [7].

However there are anecdotic references about aborigine groups in Peru with a diagnosis of cancer who have been cured using Peruvian medicinal plants [8,9]. This is the case with Cat's claw (*Uncaria tomentosa* Willd), the best known plant from the Amazon used in traditional and cultural practices in South America for centuries, especially in Peru [10]. Keplinger *et al.* [8,9] started to study properties of cat´s claw in the 1970s. Keplinger reported that in Peru, two individuals, one from Germany, Arturo Brell and the second, a Tiroles farmer living in Pozuzo, Oscar Schuler-Egg, reported that the Asháninkas use cat´s claw for the treatment of cancer [9]. This plant is used in many parts of the world as a natural remedy for cancer, but a careful review about the scientific evidence is clearly required. Another Amazonian plant with a claimed use as an anti-tumor agent, very popular in Peru in a traditional sense and in modern times, is *Croton lechleri* (common name; English: Dragon's blood, Spanish: Sangre de grado) [11]. The resin is the medicinal part used of the plant and is dark red. For such reason its common name was given. Dragon's blood comes from a large tree that

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grows in the upper Amazon region from Colombia, Ecuador and especially Peru. In these regions because Spanish is the primary language, it is more commonly known by its popular name, Sangre de grado [11].

More recently an Andean plant that grows at over 4000 meters altitude has become popular. This plant named *Lepidium meyenii* (Spanish and English common name; Maca) is a brassicaceae (*cruciferous*) and is used and proposed as a nutritional, energizer, and substance with fertility-enhancing properties [12-14]. Although there is no reference in traditional herbal medicine to the effect of Maca as an anti tumor agent, its use is widespread in modern Peru and expanding outside of South America. A recent finding has emerged implicating Red Maca, as an agent which reduces prostate weight in prostatic benign hyperplasia induced by testosterone enanthate (TE) [15].

The search for plants to treat cancer is not illusory since it is well known that over 60% of currently used anti-cancer agents are derived from natural sources including plants [6]. In Peru several plants have been described with cytotoxic properties [16], however, *Uncaria tomentosa*, *Croton lechleri* and *Lepidium meyenii* [8,10,11] are the most popular medicinal plants from the Amazon and the Andean region respectively**.** In such sense, we present an evidence-based analysis of the chemistry, biological properties, and anti-tumor activities for these three plant materials. In addition, this review will discuss areas requiring future study and the inherent limitations in their experimental use as anti-cancer agents.

Although discovered from historical use and traditional medicinal practices, cat's claw (*Uncaria tomentosa*), maca (*Lepidium meyenii*), and dragon's blood (*Croton lechleri*) represent in modern day terms, three botanical mixtures in current use as supplements to the diet or as conventional food (maca). Because the amount and nature of the experimental evidence published on the anti-tumor activity of these plants is limited and contains a high degree of variability (*e.g.* different types of preparations), the focus of this review will be to provide a critical evaluation of published literature which are related to anti-cancer effects using a weight of evidence-based approach and attention to relationship(s) between the molecular structure of known chemical constituents derived from the plant and biological activity for anti-cancer effects whenever possible.

CHEMISTRY

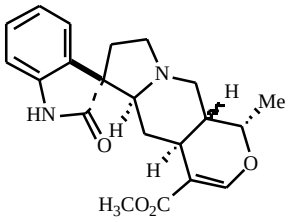
Uncaria tomentosa (Cat's Claw)

A number of chemical classes and individual chemical entities have been identified directly from the whole plant and various extracts of *Uncaria tomentosa.* Major classes of compounds that have been described include oxindole and indole alkaloids, pyroquinovic acid glycosides, organic acids, proanthocyanidins, sterols, and polyoxygenated triterpenes [10, 17] (Table **1**).

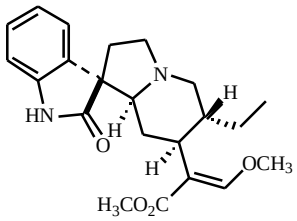
Studies isolating individual chemical entities from the plant have yielded approximately 47 compounds representing high structural and stereochemical diversity of molecular structures [17-18]. Some of the compounds that have been reported have generated interest in their potential pharmacological activity, toxicity, and cellular protective effects. Fig. (**1**) illustrates the molecular structures of a some representative chemicals found in *Uncaria tomentosa*. These compounds were selected to demonstrate the structural diversity of constituents found in the plant, and some are novel such as tomentoside A and a quinovic acid glycoside derivative. The other compounds are known to be prevalent across the *Uncaria* genus such as formosanine and rhynchophylline, or are found in other plants outside the genus such as cinchonain Ia and ursolic acid and have been tested for bioactivity and cytotoxicity [17]. However, the majority of investigations addressing potential biological activities have been conducted with whole extract preparations of *Uncaria tomentosa* instead of individual constituents derived from the plant. The sections on biological properties and anti-tumor activities will review these effects documented in the scientific literature for *Uncaria tomentosa.*

Table 1. Some Chemical Classes and Constituents Identified from Cat's Claw (*Uncaria tomentosa*)

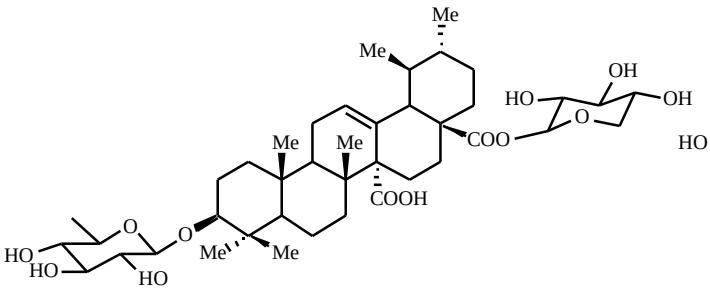
Chemical class	Constituent
Alkaloid Oxindoles	Pentacyclic: Formosanine (uncarine B), Pteropodine (uncarine C), Isopteropodine (uncarine E), Speciophylline (uncarine D), Speciophylline N-oxide, Uncarine F N-oxide, Mitraphylline, Isomitraphylline. Tetracyclic: Rhynchophylline, Rhynchophylline N-oxide, Isorhynchophylline, Isorhynchophylline N-oxide, Rotundifoline, Isorotundifoline, Corynoxeine, Isocorynoxeine
Alkaloid Indoles	Pentacyclic: Akuammigine, Tetrahydroalstonine, Isoajimalicine. Tetracyclic: Hirsutine, Hirsutine N-oxide, Dihydrocorynantheine, Hirsuteine, Corynantheine
Glycosides	Quinovic acid glycosides approximately 9 compounds (*e.g.* quinovic acid (28-1)-β-D-glucopyranosyl ester Pyroquinovic acid glycosides: Tomentoside A, Tomentoside B
Organic Acids	Oleanolic acid
Proanthocyanidines	(-)-Epicatechin, cinchonain 1a, cinchonain 1b
Sterols	β-Sitosterol, stigmasterol, campesterol
Triterpenes	Ursolic acid and nine derivatives (*e.g.* 3β,19α-dihydroxy-6-oxo-urs-12-en-23-ol-28-oic acid)



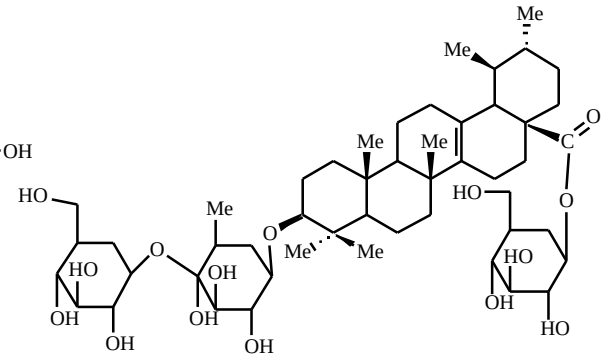
Formosanine (Uncarine B)



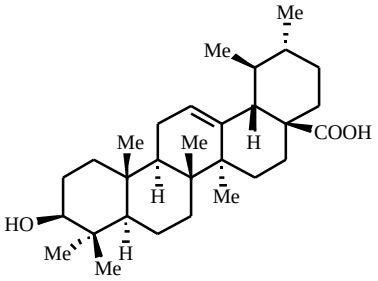
Rhynchophylline



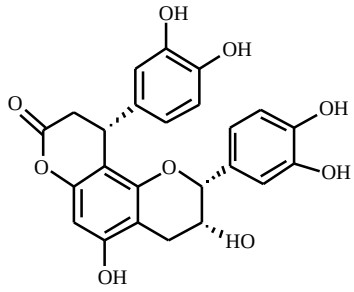
3 β-O-(β-D-Quinovopyranosyl)-(27-1)-β-D-glucopyranosyl ester



Tomentoside A



Ursolic acid



Cinchonain Ia

Fig. (1). Chemical structures of some alkaloid oxindole, triterpene, proanthocyanidine, and glycoside constituents from *Uncaria tomentosa*. Tomentoside A and 3β-O-(βD-Quinovopyranosyl)-(27-1)- βD-glucopyranosyl ester are novel constituents. Formosanine and rhynchophylline are prevalent within the *Uncaria* genus. Ursolic acid and cinchonain Ia can be found in other medicinal plants.

Lepidium meyenii (Maca)

Maca has several known constituents, some of which have been described in the scientific literature due to their existence in other plants whereas other principles are novel [19]. It has been suggested that the identification of novel constituents may be useful in the standardization of Maca extracts or as markers of the bio-availability of Maca [20,21].

Among secondary metabolites present in Maca but also in other plants, are glucosinolates [19, 22-24], tannins, saponins [25], sterols [21], polyunsaturated fatty acids [21, 26], beta carbolines, uridine and malic acid [19], prostaglandins [27], flavonoids [28] and anthocyanins [29]. Among the major flavonoids found is quercetin [30]. Anthocyanines are responsible for the external color of the hypocotyls. Alkaloids have also been described in Maca [31].

Novel compounds present in *Lepidium meyenii* are two new imidazole alkaloids (Lepidiline A and Lepidiline B) isolated from a hypocotyls-root extract of the plant and chemically identified as 1) 1,3-dibenzyl-4,5-dimethylimidazolium chloride and 2) 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride [31] (Fig. **2**). Recently, a series of new alkamides have also been isolated and identified recently from Maca with interest in their use for quality assessment as markers for authentication and standardization [20, 26, 32].

Maca contains glucosinolates as its major secondary metabolite [21]. The absolute content of glucosinolates in fresh Maca hypocotyls is relatively higher than that reported in other cruciferous crops [33]. The richest sources of glucosinolates were seeds, fresh hypocotyls and sprouts, in that order [33]. The most abundant glucosinolates detected in fresh and dry hypocotyls and leaves of Maca were the aromatic glucosinolates, benzylglucosinolate (glucotropaeolin) Fig. (**2**) [27, 33], and *m*-methoxybenzylglucosinolate [19, 24]. In fact, benzylglucosinolate is thought to be the main (non-protein) component of Maca and many studies have reported

anti-tumor effects from this constituent as will be discussed later [24,34,35]. Glucosinolates have been proposed to be natural pesticides protecting plants from various pests [36]. Similarly, the adverse conditions in which Maca grows would suggest their appearance as a natural defense mechanism for the plant. Phenylacetonitrile, a degradable product of benzylglucosinolate is another constituent that has also been found in the aerial part of the *Lepidium meyenii* [37].

Maca also contains sterols: beta-sitosterol, campesterol, and stigmasterol [21]. These compounds may have little biological importance since phytosterols are poorly absorbed when consumed orally [38]. Plant sterols including sitosterol, campesterol, and stigmasterol represent a large proportion of dietary sterols, however the levels of plant sterols in the blood and tissues of normal mammals is generally low. When phytosterols are administered by parenteral route, effects of reducing cholesterol have been observed [39].

Maca contains a series of novel benzylated alkamides called macaenes and macamides [20]. These compounds are illustrated in Fig. (**2**) and include: *N*-Benzyl-5-oxo-6E,8E-octadecadienamide, *N*-Benzylhexadecanamide, *N*-Benzyl-9-oxo-12Z-octadecenamide, *N*-Benzyl-9-oxo-12Z,15Z-octade-

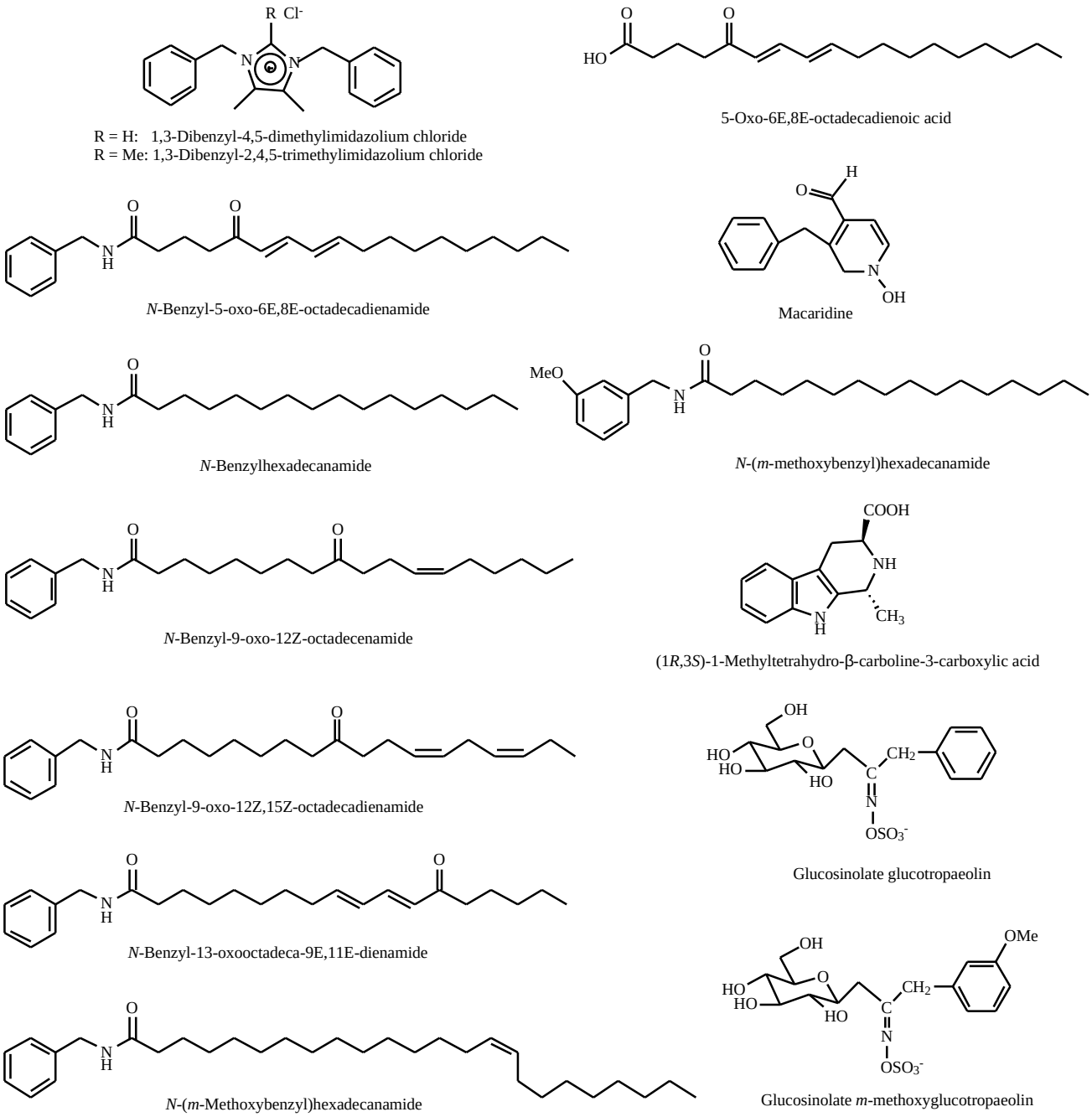


Fig. (2). Chemical structures of glucosinolates and novel constituents recently found in *Lepidium meyenii* (Maca).

cadienamide, *N*-Benzyl-13-oxooctadeca-9E,11E-dienamide, and *N*-(*m*-Methoxybenzyl)hexadecanamide [20]. A benzylated derivative of 1,2-dihydro-N-hydroxypyridine, named macaridine has also been reported as a novel natural product from Maca [26], and the fatty acid 5-oxo-6E,8E-octadecadienoic acid has been described (Fig. **2**).

Maca reportedly contains carbolines. A methanol extract of Maca was reported to contain (1R,3S)-1-methyltetrahydro-β-carboline-3-carboxylic acid [19] Fig. (**2**).

One particular compound found in the Maca plant as well as Cat's claw is the alkaloid isopteropodin [30], which has also been described in *Hamelia patens* [40].

Croton lechleri (Dragon's Blood)

The sap from the bark of *Croton lechleri* is the part of this large Amazonian tree that has been utilized in its long history of ethnotraditional practices in Peru. It is also of scientific and medical interest to medicinal chemists, pharmacologists, and clinical practitioners who have tested it in topical applications for wound-healing, and for internal uses for the relief of diarrhea and for cancer [41-45]. *Croton lechleri* is endemic to Peru but the tree can also found in the Amazonian region of neighboring Ecuador and Columbia [11]. Chemists have investigated the chemical composition of the sap from the bark of *Croton lechleri* with significant variation in the results for its main constituents [41-43, 46-49]. Variation has been reported in samples of the sap that have been collected from Ecuador and Peru [50]. In samples collected from Ecuador, the main components of the sap from the bark of *Croton lechleri* contained 90% dry wt. of proanthocyanidin oligomers, ranging from single monomer units to heptamers [46]. In addition, several novel catechin dimers and trimers were identified by this investigator, including catechin-(4α→8)-epigallocatechin, gallocatechin-(4 α→8)-epicatechin, gallocatechin-(4 α→6)-epigallocatechin, catechin-(4 α→8)gallocatechin-(4 α→8)-gallocatechin and gallocatechin-(4 α→8)-gallocatechin-(4 α→8)-epi-gallocatechin mixtures of proanthocyanidins, flavon-3-ols [50]. This investigator thus found that polyphenolic compounds are the main constituents of the sap from the tree bark of *Croton lechleri* collected from Ecuador. Other investigations have found that the phenanthrene alkaloid, taspine is as an important principal in the sap of *Croton lechleri* collected from Peru [41-43,49], whereas this constituent was found to be present only in trace amounts in samples from Ecuador [44-50]. The concentrations of taspine from the Peruvian sap of *Croton lechleri* have been reported to be > 1% of the dried sap [43,49]. In sap material obtained from a related tree, also of Peru, *Croton draconoides*, taspine was determined to be of an even higher concentration > 2% [51]. Although *Croton lechleri* may also be found in Columbia, a comparison study of its resin composition to samples collected from Peru or Ecuador has not yet been reported in the scientific literature. However, it is likely that most observed differences in composition depends greatly on extrinsic factors such as the influence of soil and climate conditions, and variability in process of preparation, rather than intrinsic causes inherent to the plant. Other constituents found to be present in *Croton lechleri* include steroids (common to many plants) such as β-sitosterol and β-sitostenine which are also found in Maca [21]; several low-molecular weight aromatic compounds including 1,3,5-trimethoxybenzene, 2,4,6-trimethoxyphenol, 3,4-dimethoxyphenol, 3,4-dimethoxybenzylalcohol, and 4-hydroxyphenethylalcohol; and diterpenoids many of which are novel and at low levels, Fig. (**3**) [47,48].

BIOLOGICAL ACTIVITIES

Uncaria tomentosa, Lepidium meyenii, and Croton Lechleri all have historical and current usage in ethnotraditional practice as medicinal plant remedies against a variety of human illnesses. This history of use has stimulated a diversity of experimental investigations aimed to address their potential therapeutic effects learned from ethnotraditional practice. These three plants display a remarkable spectrum of biological activities in experimental studies, including those that may influence processes that are dysregulated during the development of cancer. Examples include anti-inflammatory, anti-oxidant, and anti-mutagenic activity. In addition to these effects, non-anti-cancer biological activities reported on these plants are briefly summarized below to provide background on these potential effects. Caution, however, should be taken with this information since these effects have not been completely substantiated scientifically based on the totality of the available evidence. For example some biological activities have not been repeated across different laboratories using the same test material, and other investigations have reported an absence of the biological activity under their test system conditions [52]. As the focus of this paper is to review the chemical constituent composition and anti-cancer studies conducted with these three medicinal plants, a critical evaluation of all biological properties listed below is out of its scope. The usefulness of the information, however, will aid in building understanding and perspective on the diversity of potential biological properties reported on these plants, some of which may relate to possible chemopreventive or therapeutic effects against cancer.

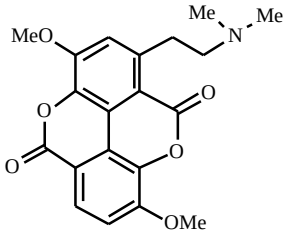
Uncaria tomentosa

Ethnotraditional practice has defined Cat´s claw known as Uña de gato, scientifically named *Uncaria tomentosa* and *Uncaria gianensis*, to be an effective treatment for several health disorders including chronic inflammation, arthritis, gastrointestinal dysfunction such as gastritis, ulcers, tumors and infections [53,54]. The medicinal efficacy of Cat´s claw was originally believed to be due to the presence of oxindole alkaloids. However, water-soluble Cat´s claw extract which does not contain significant amounts of alkaloids (<0.05%) still were shown to be very efficacious [53]. A summary of the biological properties described in the scientific literature for *Uncaria tomentosa* (Cat´s claw) is listed in Table **2**.

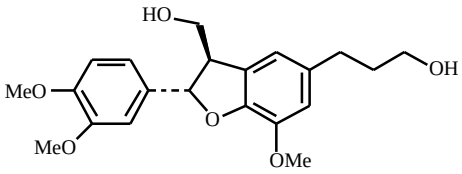
Lepidium meyenii

Maca a plant of the Brassicaceae family, that is cultivated and grows exclusively between 4000 and 4500 m altitude in the Peruvian central Andes is used as a food supplement and is also recognized in Peru to have supposed medicinal properties [74-81]. The product is exported from Peru in many forms as powder, capsules, pills, flour, liquor, tonic and mayonnaise [81].

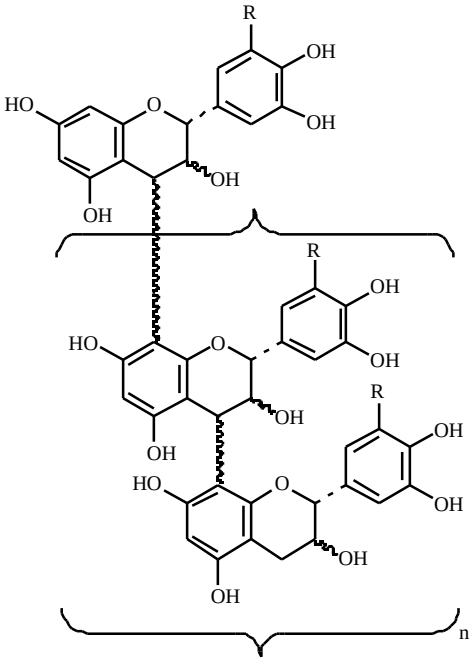
There are several studies in the scientific literature on the biological properties of Maca. Of these, one was performed in humans, but the majority have been conducted using ex-



Taspine

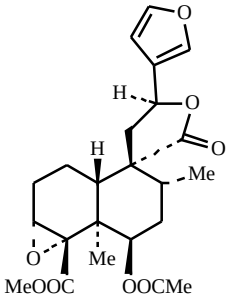


3,4-*O*-dimethylcedrusin

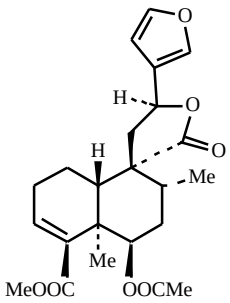


Proanthocyanidin oligomers

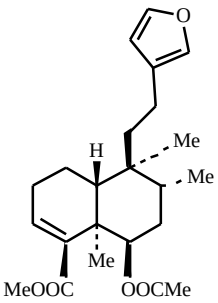
n = 1, 2, 3, 4 oligomer units R = H or Me



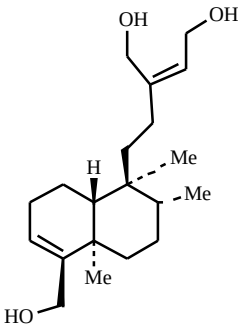
Korberin A



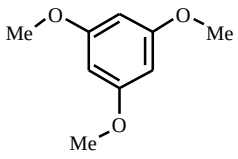
Korberin B



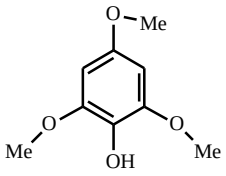
Crolechinic acid



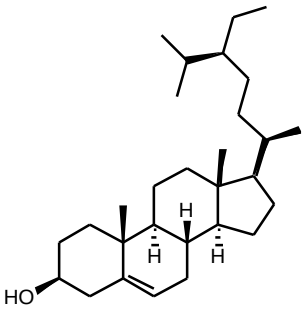
Crolechinol



1,3,5-Trimethoxybenzene



2,4,6-Trimethoxyphenol



β-Sitosterol

Fig. (3). Chemical structures of representative constituents present in the sap resin from *Croton lechleri* (Dragon's blood).

perimental animals such as rats, mice, and fish. In general, the biological properties of Maca relate to its nutritional value by the high protein content [22, 27, 30, 74-76] and the demonstration of fertility-enhancing properties in male humans and rodents [13-14, 77-80]. Furthermore, other studies have described its usefulness as an energizer [81,82]. These and other properties of Maca are summarized in Table **3** [82-87].

Table 2. Biological Properties Reported for *Uncaria tomentosa* (Cat´s Claw)

Species	Property	Source
Humans	Increases lymphocyte counts	[8]
	Osteoarthritis	[55]
	Rheumatoid Arthritis	[56]
Rats	Increases white blood cells	[53, 57, 58]
	Repairs DNA	[58]
	Reduced paw edema	[59]
	Against chemically induced inflammation	[60]
Mice	Anti-inflammatory	[61, 62]
In vitro	Anti-oxidant activities	[63-67]
	Immunomodulation	[68-71]
	Antiviral	[72,73]

Despite several studies identifying secondary metabolites in Maca, there are few reports investigating the biological properties of these novel metabolites. In fact, there is only one study with macaenes and macamides in which it was demonstrated to affect the sexual behavior in male rats and mice [21]. Some of the studies have focused on effects of methanol, hexane, chlorophormic, dichloromethane and ethyl acetate extracts [76, 85]. The hexane extract has been related to sexual behavior in rodents [85], and compounds with high polarity derived from methanol extracts have been found to have growth enhancing effects [76], and posses an anti-stress in experimental animal models [82]. Effects on reproductive function have also been observed using an aqueous extract of the hypocotyls of maca after a period of boiling as is used traditionally for centuries in Peru [12-14, 77, 79-81].

Table 3. Biological Properties Described in the Scientific Literature for *Lepidium meyenii* (Maca)

Species	Property	Source
Humans	Increase sperm count and sperm motility	[77]
	Increase sexual desire	[83]
	Anti-stress	[81]
	Decrease score for anxiety and depression	[81]
	Energizer	[81]
Rats	Increase sperm count	[79]
	Increase male sexual behavior	[21,84,85]
	Nutritional	[75]
	Anti-stress	[82,86]
	Prevent testosterone-induced prostatic hyperplasia	[15]
	Improve immunity	[82]
	Positive result in the forced-swimming test	[82]
Mice	Increase male sexual behavior	[21]
	Increase embryo survival	[12]
	No effect on serum estradiol	[87]
Fish	Nutritional	[30, 76]
	Increase embryo survival	[30]
	Improves immunity	[30]

The anti-oxidant potential of Maca has been documented from *in vitro* and *in vivo* studies. For example an aqueous extract of Maca (0.3-3 mg) was demonstrated to have the capacity to scavenge free radicals and protect cells against oxidative stress [88]. This anti-oxidant activity has also been demonstrated in fish [76]. The compound(s) with anti-oxidant capacity has not been identified as of yet, however, it is known to have high polarity and can be extracted by methanol [76].

Several chemical compounds present in Maca are also found in other plants, and it is possible to build a profile for proposed biological effects of these chemical constituents based on existing studies of such compounds (*e.g.* flavonoids). However, in the case of the chemical compounds described for the first time, it is not possible to determine their biological activities until well conducted experimental studies have been performed.

Croton lechleri

Although several plant species are described by common name as Dragon's blood, the most popular certainly is *Croton lechleri*. As mentioned previously, *Croton lechleri* can be found in three South American countries, but is mainly acquired from the upper Amazon region of Peru. The majority of biological studies conducted with the sap resin of *Croton lechleri* have tested this material as it was collected from Peru.

An important principal that has been identified from the bark of *Croton lechleri* is the low-molecular-weight alkaloid taspine (Fig. **3**). This compound is well known by its wound healing property [42]. Experiments with taspine hydrochloride in order to study its mechanism of action in cell culture systems showed that the alkaloid was non-toxic to human foreskin fibroblasts at concentrations below 150 ng/ml and that it had no effect on cell proliferation. On the other hand, taspine hydrochloride was found to increase the migration of human foreskin fibroblasts. This effect on the migration of fibroblasts is probably the mechanism by which Sangre de Grado and taspine hydrochloride accelerate the wound healing process. Using the two-stage mouse skin carcinogenesis system, we have been able to show that neither Sangre de Grado nor taspine hydrochloride had carcinogenic or tumour promoter activity after 17 months of treatment [42]. Further study showed, that taspine stimulated chemotaxis for fibroblasts. Taspine did not have an effect on specific assays for macrophage chemotaxis, neutrophil activation, fibroblast proliferation, or matrix assembly. These authors suggest that taspine promotes early phases of wound healing in a dose-dependent manner with no substantial modification thereafter. Its mechanism of action is probably related to its chemotactic properties on fibroblasts and is not mediated by changes in extracellular matrix [59]. However, in a single study, isolated taspine did not demonstrate immunomodulatory properties or anti-inflammatory activity despite evidence of a strong anti-inflammatory property when it was administered *via* intraperitoneally route [90]. Another compound, an oligomeric proanthocyanidin extracted from the

bark latex of *Croton lechleri*, has been reported to be a potent inhibitor of cholera toxin-induced fluid accumulation and chloride secretion [91]. This property might be useful for the treatment of fluid loss in watery diarrhea [91].

Both *in vitro* and *in vivo studies* have focused on investigating the scientific basis for known ethnomedical uses of *Croton lechleri* for health conditions such as a treatment for diarrhea, wounds, tumors, stomach ulcers, herpes infections, the itching, pain and swelling of insect bites [92]. Studies related to these properties are summarized in Table **4**.

Although beneficial properties have been described, there are also reports of deleterious effects in experimental toxicology testing. For example, the sap of *Croton lechleri* Muell-Arg showed mutagenic activity in strain TA1535 of *Salmonella typhimurium* in the presence of metabolic activation and a weak mutagenic activity in strain TA98. These strains detect base pair substitutions and frameshift mutations, respectively. Mutagenicity was also observed in a haploid *Saccharomyces cerevisiae* [93]. Furthermore, it should be pointed out that *Croton lechleri* at low concentrations may have pro-oxidant activities whereas at higher concentrations may behave more as an anti-oxidant [90].

The data obtained thus far suggest the need for further studies aimed to determine biological effects of *Croton lechleri* under standardized conditions. It is necessary to know if the activity is observed only in the latex or also in another part of the plant which will be useful for the conservation of this wild plant of the Amazonian.

Table 4. Biological Properties Described in the Scientific Literature for *Croton lechleri* (Dragon's Blood or Sangre de Grado)

Species	Property	Source
Humans	Relief of itching, pain, discomfort, edema, and redness in response to insects	[94]
	Anti-diarrheal effects	[45]
Rats	Reduced paw edema	[90,94]
	Treatment of gastric ulcers	[95]
	Anti-bacterial effects	[95]
Mice	Cicatrizant effect	[42]
	Anti-viral effects	[96]
	Anti-diarrheal effects	[97]
In vitro	Antibacterial activity against *Bacillus subtilis* and *Escherichia coli*	[98]
	Cytotoxic on cancer cell lines	[99,100]
	Anti-oxidant activities	[90,93,101]
	Pro-oxidant activities at low concentrations	[90,101]

ANTI-TUMOR ACTIVITIES

Cancer is an important cause of death in the world. In the USA it is the second leading cause of death [102]. Available treatments are costly, and they can cause other health problems, and are of varying effectiveness. For such reason the search for medicinal plants particularly on those having anti-oxidant properties has been increasing in recent years [103]. There are several experimental studies showing that *Uncaria tomentosa* has potential anti-inflammatory and anti-oxidant activities, as well as immunomodulator and DNA repair properties [58,60,61,63-66,68,71], *Lepidium meyenii* may have anti-oxidant and immunomodulator activities [76,82, 88], and *Croton lechleri* may have potential anti-inflammatory, anti-oxidant and cytotoxic activities [99,100]. It seems that phytochemicals from these plants may affect the above processes through several mechanisms. Free radical damage generated from enhanced oxidative stress may cause DNA damage, which in turn can lead to base mutation, DNA cross-linking, and chromosomal breakage and rearrangement. The oxidative damage may be suppressed by naturally occurring dietary antioxidants in plants through modulation of detoxification enzymes, scavenging of reactive oxygen species and oxidative agents, stimulation of anti-inflammatory processes in immune system, hormone metabolism, and regulation of gene expression in cell proliferation and apoptosis [104,105]. Collectively, the modulation of these properties by plant phytochemicals may have influence on the treatment of tumors. Importantly, the three plants subject of this review may be potential sources of such compounds, thus influencing the treatment of neoplastic cells. However, there are many limitations for such use as discussed previously, and it is clear that further research will be necessary in order to establish their potential usefulness in chemoprevention and as anti-cancer agents.

Uncaria tomentosa

It is well known from traditional medicine in Latin America that *Uncaria tomentosa*, known locally as Uña de gato or Cat´s claw, is one of the most commonly used herbal preparations in the region for the treatment of tumors [53]. Herbal remedies from Peru in particular have served in supplying the ethnotraditional basis that has helped spawn public interest and scientific experimentation for their use in industrialized countries where alternative therapies are being sought. Currently, in industrialized parts of Peru, and in more than 30 other countries, *Uncaria tomentosa* is commonly available as a tea, and in tablet or capsule form. However, according to traditional usage, *Uncaria tomentosa* preparation requires hot water extraction of the bark [53]. An impediment then in studying potential anti-cancer effects of *Uncaria tomentosa* described in ethnotraditional use is the integration of the chemical uniformity of the medicinal plant material used in ethnotraditional practice into experimental studies designed to assess anti-cancer effects. Aside from this variation in the chemical composition between herbal concoctions made in ethnotraditional practices and laboratory test material, there can also be substantial differences in bioequivalency, thereby affecting the outcome from *in vivo* testing [106]. These factors and others, especially the absence or presence of the bioactive constituents in the prepared plant extract material, can dramatically affect results obtained from experimental and clinical efficacy testing of these medicinal plants.

Complicating the issue further is that many of the biological studies with *Uncaria tomentosa* indicated that the anti-oxidant and anti-inflammatory effects observed were due to the presence of oxindole alkaloids [8, 66]. However,

several recent studies have reported that *Uncaria tomentosa* water (aqueous) extracts have good anti-inflammatory activity despite that these extracts were devoid of any alkaloid content [53,58,60,63]. Still reports show that a hydroalcoholic [62], and ethanolic extract of *Uncaria tomentosa* [66], have higher anti-inflammatory and anti-oxidant activity, respectively, than aqueous extracts. These inconsistencies in reporting with different types of preparations (e.g. aqueous, ethanolic, hydroalcoholic) pose great challenges to scientists weighing the evidence needed for determining mechanistic aspects, safety parameters, and potential efficacy for anti-cancer effects.

One possible explanation with a chemical basis is that the variable results between aqueous and ethanolic extracts of *Uncaria tomentosa* in producing anti-inflammatory and anti-oxidant activity could be attributed to higher amounts of total phenolic compounds in the ethanolic extract compared to the aqueous extract of the plant [66]. In support of this notion, the ethanolic extract of *Uncaria tomentosa* is reported to contain an even higher content of phenolics than that found in cereals, broccoli, garlic, pepper and fruits [66]. By the same token, other compounds prevalent in *Uncaria tomentosa* such as proanthocyanidins and triterpenes are also considered to be potent anti-oxidants, and therefore, have potential contribution to this activity [66,67].

Thus far in the scientific literature, studies conducted using aqueous, hydroalcoholic and ethanolic extracts of *Uncaria tomentosa* suggest that these extract preparations have potential to possess anti-inflammatory and anti-oxidant activities in their respective *in vitro* systems tested, albeit at different levels of effectiveness [53,58,60,62-67]. Therefore, it is likely that more than one active principle in the mixture is responsible for producing these activities. It is also important to observe active principles from other fractions of the bark, like the alkaloids. This is supported by a recent report in which, the antiproliferative and apoptotic effects on human lymphoblastic leukaemia T cells (CCRF-CEM-C7H2) of highly purified oxindole alkaloids, namely isopteropodine (A1), pteropodine (A2), isomitraphylline (A3), uncarine F (A4) and mitraphylline (A5) obtained from *Uncaria tomentosa* has been assessed [107].

The functional relationship between inflammation and cancer is multifactorial and complex. However, because many cancers arise from tissue sites of chronic infection, irritation, and inflammation, the concept that the inflammatory response portends to the initiation and progression stages of cancer remains a viable hypothesis with mounting evidence [108]. The association between oxidative stress and anti-oxidant status in the prevention of disease including cancer is still controversial. Thus far the information gathered suggests paradoxical effects for the role of dietary anti-oxidants in the prevention of cancer [109]. For example, individuals with diets loaded with naturally occurring anti-oxidants from fruits and vegetables are at decreased risk from cancer [109], however, supplements with β-carotene do not have an anti-cancer effect, rather the opposite in smokers [110]. Therefore, the accumulated studies testing a diversity of *Uncaria tomentosa* extracts which suggest anti-inflammatory activity and antioxidant potential are important, but have many limitations. The main limitation from all of these studies is the absence of direct evidence for anti-cancer efficacy. The other major limitation is that the above anti-inflammatory and anti-oxidant activities documented from various *Uncaria tomentosa* test extracts have been conducted using *in vitro* modeling systems, and therefore, further research is required to validate that these observations occur *in vivo*.

There are other experimental data using various types of *Uncaria tomentosa* preparations that relate to potential pathways for anti-cancer effects and describe a variety of potential mechanisms of action. These mechanisms of action studied include cytotoxicity [111], anti-proliferation [54,63, 112], suppression of apoptosis [70], anti-mutagenicity [125], and enhancement of DNA repair and mitogenic responses [113].

A single cytotoxicity study of several compounds isolated from *Uncaria tomentosa* demonstrated only weak toxicity against human cancer cell lines [111]. Uncarine D (Table **1**) showed weak cytotoxic activity against SK-MEL, KB, BT-549 and SK-OV-3 cell lines with IC50 values between 30 and 40 ug/ml, while uncarine C exhibited weak cytotoxicity only against ovarian carcinoma with IC50 at 37 ug/ml [111]. Clearly, additional testing is required such as, use of other cancer cell lines especially those derived from target organs, analysis of dose-response curves, and defining the mechanism(s) for observed toxicity, in order to demonstrate sufficient evidence for direct toxic effects against cancer cells as a probable mode of action. However, the available literature, thus far, on the chemical constituents identified in various types of cat's claw extracts are not suggestive, by the presence of structural alerts, to be of high toxic potential.

A series of water and methanolic *Uncaria tomentosa* extracts and fractions showed a direct anti-proliferative activity without cytoxicity in the human breast cancer cell line MCF-7 [54]. Depending on the extract and part of the plant used, 10-20 mg/ml produced 50% inhibition (IC50), and an approximate 90% inhibition of cell growth when the concentration was increased to 100 mg/ml, and extracted leaves showed a higher antiproliferative activity than the corresponding extract from bark. Anti-proliferative activity was also observed when HT29 and RAW 264.7 macrophages cells were treated with peroxynitrites and exposed to oxindole alkaloids isolated from *Uncaria tomentosa* [63]. The active ingredients characterized in the water extract were chemically defined as quinic acid esters and were also shown to be bioactive *in vivo* as quinic acid [53, 114]. In another study, the proliferative capacity of leukemic HL60s and EBV-transformed B lymphoma cell lines (Raji) were strongly suppressed in the presence of water extracts of *Uncaria tomentosa*, but the leukaemic K562 cell line was resistant to inhibition [115]. In contrast to the above data, an extract of *Uncaria tomentosa* did not exhibit anti-proliferative activity but rather stimulated survival of leukemic cells in 96% of 53 children with acute leukemias [116]. This study, however, did not describe which kind of extract was used or how the *Uncaria tomentosa* test material was prepared.

In testing with individual chemical constituents isolated from *Uncaria tomentosa* extracts for anti-proliferative activity, the pentacyclic oxindole alkaloid class of compounds was demonstrated to suppress growth of leukaemic HL60 and U-937 cells at concentrations ranging from $1x10^{-5}$ to 1 x

10^{-4} mol/L [112]. Similarly, a separate study demonstrated that the pentacyclic oxindole alkaloids inhibited the proliferation of normal human lymphoblasts, the human lymphoblastoid B cell line Rajii, and the human lymphoblastoid T cell line Jurkat without affecting cell viability [70]. In this study, the tetracyclic oxindole alkaloids were shown to dose-dependently enhance proliferation of normal human resting or weakly activated B and T lymphocytes. More recently, five alkaloids from *Uncaria tomentosa* were tested for their anti-proliferative potential against lymphoblastic leukaemia cells. Four of the alkaloids inhibited proliferation of the acute lymphoblastic leukaemia cells, and the anti-proliferative effect of the most potent alkaloids, pteropodine and uncarine F, correlated with the induction of apoptosis [107].

Neither of these studies either individually or collectively demonstrated obvious structure-activity relationships in the antiproliferative effects on the basis of kind or position of substituents within class, although, the pentacyclic oxindole alkaloid class was the most commonly tested group across studies and provided limited experimental evidence demonstrating anti-proliferative effects. However, since there is a plethora of chemical constituents present in aqueous and ethanolic extract preparations of *Uncaria tomentosa*, further testing with individual constituents would aid in identification of the chemical(s) or fraction responsible for observed *in vitro* anti-proliferative activity. Such an effort could include a deductive approach of treatments beginning with chemical classes (*e.g.* alkaloid oxindole), subclass (*e.g.* pentacyclic), and individual compounds (e.g. mitraphylline). In addition, further testing on the effectiveness of different whole extracts (aqueous vs. methanolic vs ethanolic) from *Uncaria tomentosa* to inhibit the proliferation of tumor cells should include using additional cancer cell lines, and normal human cells. If anti-proliferation is a viable mode of action for *Uncaria tomentosa,* then the observed anti-oxidant activity described in the previous section could reasonably be anticipated to have an influential effect on cellular hyperproliferation. One existing hypothesis in cancer prevention is that dietary constituents and micronutrients possess potent anti-oxidant activity and as a result of this activity, may contribute to the inhibition of tumor cell proliferation through blocking excess pro-oxidant processes (*e.g.* cellular membrane lipid peroxidation) that may act as catalysts for tumor promotion and progression [63]. Consistent with this notion, some dietary anti-oxidants such as the flavonoids are proposed to protect DNA from oxidative damage that leads to abnormal cell proliferation [103]. They also may inhibit cancer promoters and activate carcinogen-detoxification system in animal models [117].

Numerous naturally occurring substances have been demonstrated to have potential as cancer preventive agents through induction of apoptosis [118-121]. It has been suggested that the suppressive effect of *Uncaria tomentosa* on tumor cell growth operates through apoptotic mechanisms [107,115, 122,123]. One study provided evidence for induction of apoptosis in a dose-dependent manner when human leukemic HL-60s and EBV-transformed B lymphoma cell lines were treated with a water extract of *Uncaria tomentosa* [115]. Suppression of cell growth activity was demonstrated in association with nucleosomal DNA fragmentation after agarose gel electrophoresis and DNA fragmentation quantification. In another investigation, a 48 hr treatment of 100 micromol/L pteropodine or uncarine F to lymphoblastic leukaemia cells significantly increased apoptosis by 57% [107]. CEM-C7H2 sublines with tetracycline-regulated expression of bcl-2, p16ink4A or constitutively expressing the cowpox virus protein crm-A were used for further studies of the apoptosis-inducing properties of these alkaloids. Neither overexpression of bcl-2 or crm-A nor cell-cycle arrest in G0/G1 phase by tetracycline-regulated expression of p16INK4A could prevent alkaloid-induced apoptosis. These results showed the strong apoptotic effects of pteropodine and uncarine F on acute leukaemic lymphoblasts and suggested these alkaloids be further studied in xenograft models [107]. However, some inconsistencies have been reported in the literature with respect to the induction of apoptosis by various preparations of *Uncaria tomentosa*. Some authors showed that aqueous extracts of *Uncaria tomentosa* are anti-apoptotic in normal cells [52,124], whereas others showed that *Uncaria tomentosa* water extracts inhibited proliferation of tumoral cells *in vitro*, through induction of apoptosis which was demonstrated by characteristic morphological changes and internucleosomal DNA fragmentation [115, 122]. Because of these controversial data reported in literature, De Martino *et al.* [123] investigated the possible apoptotic effects of root bark extracts of *Uncaria tomentosa* on three tumoral cell lines, HeLa, MCF7 and SAOS, by a possible mechanism *via* the activation of caspase-3. The data obtained clearly showed an induction of apoptosis, by the *n*-BuOH soluble fraction of *Uncaria tomentosa*, *via* the activation of caspase3 [123]. Given the available literature reports, the molecular mechanisms by which *Uncaria tomentosa* or it chemical constituents may induce apoptosis are not yet been well established. Other areas of research in the field of apoptosis that would help clarify pathways by which *Uncaria tomentosa* may be envolved in inducing this active pathway of cell death include inhibition of DNA topoisomerase I/II activity, and regulation of expression of heat shock proteins.

The antimutagenic potential of five extracts and six different fractions from a chloroform/methanol extraction of the bark of *Uncaria tomentosa* was assessed *in vitro* and *in vivo* [125]. Toxicity of eleven extracts and fractions at different concentrations of *S. typhimurium* TA 98 and TA 100 strains was evaluated and slight toxicity was found when microsomes were present, but no mutagenic effect with or without metabolic activation in all strains tested was observed. *In vitro* antimutagenic potential of the *Uncaria tomentosa* extracts and fractions was tested by photomutagenesis induced by 8-methoxy-psoralen plus UVA irradiation in *S. typhimurium* TA 102. All plant extracts and fractions exerted a protective effect against the induced photomutagenesis. Methanol extracts of *Uncaria tomentosa* had a more potent inhibition of mutagenesis (59%) than the chloroform extracts (27%) of the plant. β-Carotene as a control was more potent at inhibiting mutagenesis (68%) compared to all *Uncaria tomentosa* samples. *In vivo* evaluation of antimutagenic potential was evaluated in *S. typhimurium* TA 98 and TA 100 using urine of only two subjects; one a smoker and the other non-smoker, before, during and after supplementation of a decoction of *Uncaria tomentosa.* This limited study found that the non-smoker's urine did not show any mutagenic acitivy before, during, and after oral treatment with *Uncaria tomentosa*. The smoker's urine did show mutagenic activity

before treatment and a dramatic decrease of mutagenic potential at the end of the *Uncaria tomentosa* treatment [125].

In a human clinical trial of 12 healthy adults, hydrogen peroxide was used to induce DNA damage and DNA repair was measured 3 weeks before and the last three 3 weeks of a 8 week oral treatment of 250 mg and 350 mg of a proprietary water extract of *Uncaria tomentosa* [113]. A statistically significant decrease in DNA damage concomitant with an increase in DNA repair was observed in the 250 and 350 mg treatment groups when compared to non-supplemented control subjects. An earlier study by these investigators using the same proprietary water extract of *Uncaria tomentosa* was administered *via* gavage at 40 mg/kg bw and 80 mg/kg bw for 8 weeks, and demonstrated a statistically significant improvement compared to controls in DNA single strand breaks (SSB) at both doses and double strand breaks (DSB) only at the highest dose (80 mg/kg bw) in rats allowed 3 hr repair time after 12 Gy of whole body irradiation [126]. DNA damage and repair were measured by alkaline elution for SSB and neutral elution for DSB of splenic single cell suspensions from 10 female W/Fu rats each group. Although the number of animals and study design seems appropriate, testing both males and females, in two different animal species would provide stronger evidence for the study results. Moreover a further understanding of the composition of the proprietary water extract blend would be advantageous.

An inherent limitation in experimental and clinical use of this complex plant extract mixture and others such as dragon's blood is the difficulty in developing a standardized mixture of sufficient quality, reliable specifications and consistency in composition of constituents so as to be able to conduct appropriate safety and controlled efficacy testing. Complete chemical composition of the mixture including components which are not thought to be bioactive constituents is desirable because of their potential influence on safety parameters and untoward pharmacological effects and interactions. The challenge in obtaining a high degree (>95%) of analytical characterization of natural product mixtures such as botanical extracts remains a formidable task in product development. In addition, further pharmacognosy studies and taxonomic determination of morpho-anatomical and micrographic features for identification would assist in quality control efforts [127].

There are other important areas in cancer research that to our knowledge have not been hypothesis tested with *Uncaria tomentosa*. These areas include prevention of carcinogen metabolic activation, effect on cell cycle arrest, promotion of differentiation, inhibition of the angiogenic process, and modulation of multidrug resistance. On this topic, inhibition of cytochrome P450 3A4 (CYP3A4) seems to be an interesting field for chemoprevention research, since this enzyme can metabolically activate a large number of environmental and dietary procarcinogens to toxic reactive intermediates that can modify cellular nucleophiles located on critical macromolecules such as DNA to initiate chemical carcinogenesis [128,129]. If a test agent produces a lasting inhibitory effect on CYP3A4 or other phase I metabolic enzyme activities at low K_i, then the pathway for metabolic activation of presenting procarcinogens is blocked conferring a protective effect. In a preliminary *in vitro* study, it was shown that an aqueous extract of *Uncaria tomentosa* inhibited cytochrome P450 3A4 (CYP3A4), however, the strength of the inhibition was only <1% (IC_{50} value), a questionably significant effect compared to typical CYP3A4 inhibitors [130].

Lepidium meyenii

There is an absence of reports from traditional usage about effects of *Lepidium meyenii* to treat tumors. However, in experimental studies *Lepidium meyenii* has shown antioxidant and immunomodulator activities in *in vivo* and *in vitro* models [76,82,88].

However two studies have studied anti-tumor effects *in vivo* [15] and *in vitro* [131]. The effect of *Lepidium meyenii* against testosterone-induced prostatic hyperplasia has been studied in rats [15]. Testosterone enanthate treatment alone was shown to induce prostatic hyperplasia in rats, however when red Maca was co-administered with testosterone enanthate, prostatic hyperplasia was not observed. Only red Maca significantly reduced ventral prostate size in rats treated with TE. Other varieties, such as black Maca or yellow Maca were not effective in reducing prostate size. Serum T or E_2 levels were not affected by any of the ecotypes of Maca. Histological assessment of rat prostates treated with the red Maca plus TE were similar to controls. The infrared (IR) spectra of the three ecotypes of Maca in the 3800-650 cm (-1) region had 7 peaks representing 7 functional chemical groups. The highest peak values were observed for red Maca, intermediate values were recorded for yellow Maca and low values for black Maca. These functional groups seem to correspond to benzylglucosinolate, although, further confirmation is needed [15].

The effectiveness of other plant species from the *Lepidium* genus to reduce prostate size has been assessed in other experimental studies. For example one study employed a chronic (6-month) oral treatment of an integral suspension of *Lepidium latifolium* (0.86 mg/Kg/Day) to determine the effect of the plant on experimental induced prostate hyperplasia in rats [132]. This species significantly reduced prostate size and volume in castrated rats where the hyperplasia was induced by steroid treatment [132].

Taken all together these data suggest that the *Lepidium* genus may be useful for experimental studies in treating prostatic hyperplasia. However, thus far it seems in the case of *Lepidium meyenii*, that not all varieties (yellow Maca or black Maca) are as effective compared to red Maca.

The effect of Maca does not seem to be related to androgens, since a recent study showed that Maca does not affect the androgen receptor [133] suggesting that the effect may be located in a step beyond the androgen receptor. Further studies are required to determine active principles responsible for this potential anti-tumor effect as well as the mechanisms of action.

In vitro studies have been done to assess the effect on human breast cancer MCF-7 cells [131]. Both methanolic and aqueous extracts have shown estrogenic activity comparable to silymarin in MCF-7 cell line. This activity was associated with a stimulation in cell proliferation. Maca extract estrogenicity was exhibited in the range of 100 to 200 ug/ml. This effect was not observed at dose of 0.01 to 10 ug/ml or 300 ug/ml [131]. Most of the *in vivo* studies with maca did not show changes in serum estradiol levels [10,81]. For such

reason, it is very important to perform controlled studies related to the biological impact of maca on estrogen function.

There is no published experimental evidence demonstrating a direct anti-cancer effect from Maca treatment. However some of its secondary metabolites have been studied as a class of compounds for anti-cancer effects. These classes of compounds are the glucosinolates and anthocyanines. Although novel compounds from Maca have been described, no studies at this time have been performed to demonstrate any anti-cancer properties for these novel compounds, only non-cancer biological effects from the macaenes and macamides in which one study demonstrated a favorable effect on sexual behavior in mice and rats [21].

Benzylglucosinolate appears to be the most plausible candidate as an anti-tumor agent among the glucosinolate constituents present in Maca due to its high concentration in the plant, and numerous studies demonstrating its inhibition of neoplasic effects induced by chemical carcinogens in animal models producing tumors in lung, bladder, esophagus, and intestine [134-139]. Further supporting experimental evidence for an anti-carcinogenic effect by benzylglucosinolate are the *in vitro* anti-proliferative effects demonstrated against various human cancer cells [140-142]. However, tumor promoting and carcinogenic activities in the rat urinary bladder have also been detected in several animal models with this compound. It may play a role in the early stage of rat urinary bladder carcinogenesis through continuous urinary epithelial cell proliferation due to its cytotoxicity [143-145].

Benzyl isothiocyanate is derived from benzylglucosinolate and has been identified as an anti-mitotic agent, which is consistent with its proposed role as an anti-carcinogenic compound [34]. The formation of benzyl isothiocyanate results from the catalytic activity of the enzyme myrosinase, which is activated in damaged plant tissue and also present in the gut microflora of the human digestive tract. Myrosinase converts glucosinolates to a number of compounds including isothiocyanates, which are compounds that have been implicated to have a preventative role against cancer [34,35]. Glucosinolates themselves are not thought to have potent biological activity, rather it is because of their enzymatic conversion to isothiocyanates that forms the proposed basis for their pro-apoptotic and anti-proliferative activities [34]. In the hydrolysis reaction of glucosinolates catalyzed by myrosinase, glucose and sulfate are formed along with an unstable aglycone that undergoes rearrangement to a thiocyanate, isothiocyanate, and nitrile depending on hydrolytic conditions and prior treatment of the plant material. The glucosinolate-myrosinase system is typical to the Brassicaceae (cruciferous) family to which Maca belongs.

Once formed, aromatic isothiocyanates are further metabolized in mammalian systems primarily to mercapturic acid conjugates. In humans and rats, these mercapturic acid conjugates, are subsequently hydrolyzed to the corresponding cysteine conjugate, and are excreted as the major urinary metabolite [146]. However, in rabbits, mice, and guinea pigs, the pathway of metabolism is different. In these animals the cysteine conjugate is hydrolyzed, then undergoes transamination and cyclization to form a substituted thiazolidine-2-thione as the principal urinary metabolite [147]. It is noteworthy that allylglucosinolate (sinigrin), a major aliphatic glucosinolate, has also been reported to be present in Maca [20], and has been well studied for its potential chemopreventive activity through induction of phase II detoxification enzyme systems such as NADP(H)-oxidoquinone reductase and the glutathione-*s*-transferases [148]. The aromatic isothiocyanates such as benzyl isothiocyanate also retain this potential property to modulate major enzymatic detoxification systems which is thought to be a chemo-protective strategy by cells encountering chemical carcinogens with electrophilic properties capable of forming Michael addition products with macromolecules such as the thiol functionalities on critical proteins or DNA [148].

Although isothiocyanates which are derived from glucosinolates could be important in the anti-carcinogenic properties of Maca, it is possible that other compounds may also demonstrate such activity. This is based on other studies in which *Lepidium sativum* as garden and water cress juices were highly protective against B(a)P-induced DNA damage in human derived cells independent of their isothiocyanate contents [149].

Croton lechleri

Sangre de grado is a member of the family Euphorbiaceae and it is one of the most widely used plants for treatment of wound-healing, gastrointestinal ulcers and cancer in the ethnomedicinal practices of the Amazonian regions of Peru and Ecuador. A number of plants of the Euphorbiaceae family have been used to treat cancers and tumors [150], and there are widespread reports that Sangre de grado may or may not be useful for the treatment of these diseases. For example, the latex from Sangre de grado has been claimed to be used as a treatment for certain forms of gastrointestinal cancers [150]. In support of this claim, Sangre de grado at a concentration of 100 ug/ml induced apoptosis and microtubule damage in several human cancer cell lines such as AGS (stomach), HT29 (colon) and T84 cells (colon) suggesting that this material of the plant may be a potential source of anti-cancer agents [150].

Similarly, sap from *Croton lechleri* showed an inhibitory effect against the mutagenic activity of the indirectly acting mutagen 2-aminoanthracene in the presence of S9 and a moderate protective activity against directly acting mutagens sodium azide and 2-nitrofluorene. Therefore, it was suggested that the sap of *Croton lechleri* interacts with the enzymes of the S9 mix, thereby inhibiting metabolic activation of 2-aminoantracene into its toxic forms [100].

The small molecule taspine (Fig. **3**) has been a recognized constituent of Sangre de grado in terms of experimental potential as an anti-cancer agent. This phenanthrene alkaloid was demonstrated to be highly cytotoxic to KB and V-79 cells with IC_{50} values of 0.39 μg/ml and 0.17 μg taspine/ml, respectively [43]. However, neither an aqueous solution of the sap nor a crude extract of *Croton lechleri* were cytotoxic to KB cells in a separate investigation [44]. Other investigators have reported taspine to be a highly cytotoxic principal in the taxonomically related plant, *Croton draconoides* of Peru, and is present in the sap at concentrations of 1-2% dry wt [50]. Early studies showed that the acute oral toxicity of taspine hydrochloride in male Wistar

rats was moderately toxic with LD_{50} values of 518 mg taspine/kg bw for a single dose and as low as 100 mg/kg bw under repeated dose testing for 7 days. In this study, it was also demonstrated that taspine hydrochloride had significant anti-inflammatory activity at doses well below acute oral LD_{50} values. The compound induced a dose-response effect against inflammation in the rat carrageenan-induced pedal edema model with an ED_{50} value of 58 mg/kg bw [41]. This observation is relevant because the inflammatory response has been implicated as a critical component of tumor progression [108], associated in epidemiological studies with the pathophysiology of cancer as well as many other chronic diseases [151]. In long term toxicology testing of taspine for carcinogenicity and tumor promotion, a two-stage mouse skin carcinogenesis system using 7,12-dimethylben[*a*]anthracene (DMBA) exposure for 12 months demonstrated that 0.2 mg taspine hydrochloride given twice weekly was not tumor promoting or carcinogenic [42].

Bioassay-guided fractionation of Sangre de grado, using an *in vitro* test system for the stimulation of human umbilical vein endothelial cells, has resulted in the isolation of a dihydrobenzofuran lignan, 3',4-O-dimethylcedrusin or 4-O-methyldihydrodehydrodiconiferyl alcohol [2-(3',4'-dimethoxyphenyl) - 3 - hydroxymethyl - 2, 3 - dihydro - 7 - methoxybenzo furan-5-propan-1-ol] [**1**] as the biologically active principle. A cell proliferation assay, measuring the incorporation of tritiated thymidine in endothelial cells, showed that compound **1** did not stimulate cell proliferation, but rather inhibited thymidine incorporation, while protecting cells against degradation in a starvation medium [152]. These dihydrobenzofuran lignans (2-phenyl-dihydrobenzofuran derivatives) constitute a group of anti-mitotic agents that inhibit tubulin polymerization *in vitro*, suggesting their usefulness for further experimentation as potential anti-tumor agents [153].

Other *Croton* species have also exhibited anti-tumor activities. A new furoclerodane, croblongifolin, together with one known clerodane, crovatin and one known labdane, nidorellol, were isolated from the stem bark of *Croton oblongifolius*. Croblongifolin showed significant cytotoxicity against various human tumor cell lines including HEP-G2, SW620, CHAGO, KATO3 and BT474 [154]. The effects of two nor-diterpenes, *trans*-dehydrocrotonin (DCTN) and *trans*-crotonin (CTN) from *Croton cajucara* (Euphorbiaceae), were tested on the survival of mice bearing Sarcoma 180 and Ehrlich carcinoma ascitic tumors, and on the proliferation of cultured Ehrlich cells and TNF alpha activity. When the mice were treated with 80 and 120 mg/kg of DCTN or 38 mg/kg of 5-FU, a significant anti-tumour activity was obtained (%T/C of 128-140). The cytotoxicity against Ehrlich carcinoma was 16 μM for DCTN and CTN, whereas the flavonoid quercetin was cytotoxic at 44 μM in 48 h cell culture. No apoptosis was observed in *in vitro* electrophoresis of DNA extracted from the tumor cells treated with DCTN and CTN. A significant TNF alpha activity was detected in Ehrlich tumor-bearing mice treated with DCTN suggesting an enhanced immune function [155].

FINAL REMARKS

In recent years, there has been increased interest in naturally occurring phytochemical compounds with anti-cancer potential. Throughout the history of medicine, secondary metabolites from plants have been shown to be valuable sources of novel anti-cancer drugs. Examples are the vinca alkaloids, the taxanes, and the camptothecins, derived from the Madagscan periwinkle plant *Catharantus roseus*, the Pacific yew *Taxus brevifolia*, and the Chinese tree *Camptotheca acuminata*, respectively [156-158].

The three plants presented in this review, two from the Amazonian region (*Uncaria tomentosa and Croton lechleri*), and one from the high altitude of the Central Andes of Peru (*Lepidium meyenii*) are promising plants for the experimental study of anti-tumor activities. They share some secondary metabolites known for their anti-tumor properties [159-168]. For example, proanthocyanidins were observed in *Uncaria tomentosa* and *Croton lechleri*. The catechins which are metabolic products of proanthocyanidins, have been described as anti-tumorigenic agents in animal models of chemical carcinogenesis [159-162]. β-sitosterol, is present in *Uncaria tomentosa*, *Croton lechleri* and *Lepidium meyenii*, and this phytosterol compound also has been described to possess an anti-tumorigenic property [167,168]. However its intestinal absorption is very limited [38], which reduces its potential utility as such, unless administered parenterally [39].

Lepidium meyenii is a cruciferous plant with a chemotype called red Maca and is only found in Peru. It has shown the greatest potential thus far to experimentally reduce prostate size in rats with hyperplasia induced by testosterone enanthate. Black and yellow Maca, however, did not produce the effect of reducing prostate size as red Maca was observed to do when tested at the same dose [15]. It is possible that differences between red Maca and black Maca could be in the content of anthocyanins. For example, in berries, delphinidin- 3-rutinoside is the dominant component in the reddish colored berries (onset of ripening), and cyanidin-3-rutinoside is a major pigment in the black ones (ripe berries). Cyanidin-3-rutinoside is the most thermally stable anthocyanin [169]. Moreover, since the use of Maca involves preparing an aqueous extract from boiling it in water, it is reasonable to expect differences in the content of anthocyanins between red and black Maca. This is of particular importance since anthocyanins as a class of compounds have been described as having anti-proliferative and apoptotic activities against cancer cells both *in vitro* and *in vivo* [163-166].

The scientific discovery of these three Peruvian medicinal plants from traditional knowledge has lead to a notable amount of scientific effort to assess the potential of these plants as agents against various types of cancer. Much of the existing experimental evidence, however, is *in vitro* data with some *in vivo* studies using animal models. Clearly, further preclinical research will be required in order to appropriately characterize potential mechanisms, pharmacokinetic parameters, adverse effects, dosing, and other safety considerations before testing in human clinical applications for specific neoplastic conditions.

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