REVALUATION OF TRADITIONAL HERBAL KNOWLEDGE, EXTRACTION OF BIOACTIVE PRINCIPLES WITH PHARMACOLOGICAL ACTIVITY AND FOREST CONSERVATION IN THE SIERRA DE MANANTLAN (COLIMA, MEXICO).

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Presentation

On a global scale, the available evidence points to a direction of increasing relevance of Traditional Ethnobotanical Knowledge (TEK) as an invaluable, underutilized and underdocumented body of knowledge.

In 1992, the Convention on Biological Diversity (CBD) was the first to develop measures for the use and protection of traditional knowledge related to the conservation and sustainable use of biodiversity. Member countries were expected to promote the use of indigenous knowledge (IK) systems in natural resource management (NRM), to adopt and expand the use of such knowledge, and to promote equity and the sharing of benefits arising from the use of indigenous knowledge systems.

Chapter 26 of Agenda 21 reiterates the "participation of indigenous peoples and their communities at national and local levels in resource management and conservation strategies to support and review sustainable development strategies."

The United Nations Organization of Scientific Conferences (UNESCO) and the International Council for Science Union (ICSU) in their model documents appreciate the role played by IK and advocate its application in all forms of humanity's commitments[5]. In defining TEK, several authors focus on the attributes of perception, management and utilization of plant resources by local communities. In specific terms, TEK research focuses on "how people classify, identify and relate to plant resources, examining the interactions of plants and people, the taxonomic identification of selected plants, and the biological and chemical analysis of their ingredients".

Indigenous knowledge, defined by Masango [1990], as "the totality of all knowledge and practices established on past experiences and observations possessed and used by people", is the main reservoir of ethnobotanical research and is commonly referred to as TEK.

However, changes in lifestyle brought about by globalization have led to the negation of TEK in ongoing efforts to ensure sustainable resource management with a simultaneous loss of related knowledge. In particular, the transmission of this knowledge between the older and younger generation is no longer connected.

There is a general consensus in the NRM field that traditional ethnobotanical/ecological knowledge of indigenous communities can positively influence sustainable land management (SLM) practices. In addition, TEK can broaden the way communities conceptualize and address environmental challenges, thereby enhancing the resilience of a social-ecological system.

TEK has received much attention from various researchers, hence the plethora of definitions. Raimundo et al. defined TEK as "a subset of indigenous knowledge that includes knowledge and beliefs transmitted from generation to generation by cultural transmission and that is related to human environmental interactions".

Fernández-Giménez describes TEK as "a system of experiential knowledge obtained by continuous observation and transmitted among the members of a community". We can define it as: "a cumulative set of knowledge, practices and beliefs, which evolves through adaptive

processes and is transmitted from generation to generation by cultural transmission, about the relationship of living beings (including humans) with each other and with their environment."

TEK is an important component of a number of concepts within the field of community-based natural resource management (CBNRM) and related concepts, including resilience, community participation and stakeholder collaboration. Sustainable land management often requires sufficient collection, retention and transmission of knowledge gained from years of interaction with a landscape. TEK transmission is the transfer of traditional knowledge between individuals of a particular indigenous group. The main modes of transmission are dynamic, varying by place and time, although commonly occurring through direct interaction with the environment. TEK is also often conferred during normal social interaction and by oral transmission through storytelling.

Project location

The biosphere reserve of the Sierra de Manantlan (Colima, Jalisco), with a total area of 139,577.12 hectares, is located in the Sierra Madre del Sur physiographic region with a high biodiversity index: 3,000 plant species, 1,200 insect species, 16 fish species, 10 amphibian species, 85 reptile species, 324 bird species and 110 mammal species. There are 28 endemic plant species and 214 endangered plant species. Among these are: maple (Acer skutchii), tilia (Tilia mexicana), cucharo (Symplocos sousae), Mammillaria beneckei, poplar (Populus guzmanantlensis), milpilla (Zea diploperennis) and the orchids Epidendrum parkinsoniaum and Brassavola cucullata.

Its altitude varies from 400 to 2860 meters above sea level and it is located 50 kilometers from the Pacific Ocean, creating varied environmental conditions with a high diversity of habitats and species. Manantlán means "place of springs". The Sierra de Manantlán is part of the Transversal Neovolcanic Axis and the Sierra Madre Occidental. It is divided into two physiographic units: the western portion has the highest altitudes and at the same time the lowest. The area has a complex and rugged relief. Large riverbeds with steep slopes and cliffs. In the upper part, in the eastern portion, there are hills and plains of calcareous origin. It includes plateaus in the highest parts with a great karstic development, dolines and a system of caverns that includes one of the deepest in the entire American continent.

The types of vegetation present are varied: pine, oak and oyamel forest; alpine scrub; pine forest; oyamel forest; oak forest; pine-oak forest; mesophyll forest; subtropical scrub; gallery forest; tropical deciduous forest; tropical thorn forest; savannah vegetation; grasslands.

Justification

Throughout the world, millions of human beings resort to traditional medicine, mainly because it is the main pillar for health care or to complement it. According to the World Health Organization (WHO), traditional medicine has a long history and constitutes the sum of knowledge, skills and practices based on theories, beliefs and experiences of different cultures, whether explainable or not, used to maintain health and prevent, diagnose, improve or treat physical, mental or worldview diseases.

It is important to highlight that traditional medicine is an option that continues to be practiced in tens of thousands of indigenous communities, rural and urban areas. Therefore, its protection, conservation, revitalization and recognition are relevant, as well as the knowledge

and sustainable use of the biological wealth used. The use of alternative medicines such as medicinal plants and dietary supplements has been a traditional practice that has not fallen into disuse (Barthelson 2006).

It is estimated that 80% of the world's population relies on traditional herbal remedies and that at least 35,000 plant species have potential for medicinal use (Annan and Houghton, 2007). Mexico's great plant diversity and cultural richness have favored the use of plants for medicinal purposes since pre-Hispanic times (Martínez, 1996). This cultural heritage has been transmitted from generation to generation, so that some customs survive and are practiced on a daily basis, both in rural and urban areas (Bye and Linares, 1987, Campos, 1993; Yeh 2003). These medical practices remain in force because, among other things, traditional treatments are based on the disease as it is conceived within their culture, so it is pertinent to perceive traditional treatment as an integrated aspect of it (Ryesky, 1976).

In Mexico, the expressions used to designate traditional medicine are abundant, for example, terms such as traditional indigenous medicine, popular medicine, parallel medicine, indigenous medicine, natural medicine, herbal medicine or ethnomedicine, among others, are used. Mexican traditional medicine is a mosaic of pieces coming from different cultures that have historically determined the development of the culture of this country. In their medical aspects, these cultures, or parts of them, form a puzzle with elements that are often contradictory, which makes it difficult to find a single, generalizing and organic framework for their interpretation. The culture of present-day Mexico comes from the syncretism that took place between the ancient Spanish and African cultures, merged since the end of the 16th century.

The process of acculturation has not stopped and continues to the extent that three medicines and three interacting cultures survive. Throughout the centuries, a hybrid and syncretic popular medicine has been generated in Mexico where resources, practices or nosologies coming from different episodes of a forced interculturation can be found. In Mexico, mixed clinics have been established where traditional and allopathic medicine are integrated (Gheno-Heredia 2011).

It is estimated that 90% of the Mexican population resorts to medicinal plants for the empirical treatment of various diseases; among the most commonly used plants are Allium sativum, Citrus limon, Gnaphalium sp., Eucalyptus globulus, Mentha sp., Matricaria recutita and Opuntia ficus indica (Robles-Zepeda 2011).

The medicinal tradition is reflected in specialized therapists, e.g., midwives, herbalists, bonesetters, healers, as in the forms of healing or treatment, healings or rituals, to mention a few, are massages, sobadas, infusions, poultices, baths, sweepings, cleansings, as well as in plants or animals and finally in mineral elements or religious symbols.

In each region of our country the above components of traditional medicine converge to diagnose, cure or maintain physical, emotional or spiritual health, either individually, collectively or communally, taking into account that illness is generally the result of the relationships that human beings establish among themselves and with their environment, the world of nature and the world of divinities.

In Mexico there is an extensive variety of phytotherapeutic treatments that are part of the traditional Mexican herbal medicine. Supported by approximately 4500 species, it occupies

the second place worldwide in the number of registered medicinal plants (Martínez, 1996; Barragán, 2006). In both economically developed and developing countries, the use and commercialization of phytopharmaceuticals and natural products for medicinal purposes has grown rapidly in recent years, as evidenced by the significant increase in world demand for these products. It has also been proven that some plants used for medicinal purposes have active ingredients that are used for the production of commercial drugs (Estrada, 1989, Méndez, 2000).

Project strategy

It is necessary to develop mechanisms to compensate the community for their participation in the research, to recognize the intellectual property rights of traditional knowledge about medicinal plants and the conservation of source ecosystems and useful species. It is important that the benefits include other aspects in addition to direct economic compensation, although this may be an important component, among other reasons, because of the time contributed by the population (Cunningham, 1996).

According to the regulations in force in Latin America on access to genetic resources and associated knowledge (Convention on Biological Diversity of 1992 and Decision 391 of 1996), the access contracts, established with the Competent National Authorities of each country, must contemplate the mechanisms and modalities of compensation to the communities, as well as for the recognition of intellectual property rights over traditional knowledge.

Martín (2001), states that this situation responds to the fact that researchers have traditionally directed their efforts towards the discovery of natural products of economic value, often for the benefit of developed countries, and to the theoretical understanding of how human groups perceive and manage the environment. Since the late 1960s, however, many ethnobotanists have sought to modify these objectives and have turned their attention to the application of their research findings to the solution of conservation and community development problems (Toledo, 1982).

This translates into providing local communities with research results and conclusions, strengthening traditional agricultural production systems, promoting the rational use of plants in health care, and fostering traditional ecological knowledge. Although the scale and objectives of each project vary, they should all have in common that they are driven by the enthusiasm of the researchers and the people of the communities, who working together can improve local conditions (Martin, 2001).

There is some consensus about the need to apply the results obtained in ethnobotanical research on medicinal plants to community development projects, which contemplate the rescue of traditional knowledge, as well as the safe and rational use of plants in primary health care (Sheldon 1997; Martin, 2001). Such projects include educational programs for young people (Balick, 1994; Martín, 2001), creation of local herbaria (Martín, 2001), popular publications on medicinal plants (House, 1989; Delens, 1992), supervised application of traditional medicine programs in rural communities (Sheldon 1997) and training in medicinal plant cultivation techniques (Martínez 2000).

Finally, since in some countries the natural source of many medicinal plants has been depleted, either by habitat destruction or intensive and uncontrolled collection, the

development of conservation projects has received special attention in recent years, emphasizing the value of forests or other natural areas as sources of species for health care (Sheldon 1997). Among the proposals and projects considered to achieve the conservation of source ecosystems and the useful plants present in them are the creation of forest reserves for the controlled extraction of medicinal plants, such as the one developed in Belize, which has also served as an open classroom for trainees in traditional medical practices in a program initiated by local healers (Balick 1994); the creation of regional botanical gardens (Estrada, 1998; Martín, 2001); and the use of family gardens as a strategy to reduce pressure on natural ecosystems.

In the Plantas Provechosas bioregion, an ethnobotanical study conducted in 1989 in the communities of Cuzalapa, Ahuacapán, Telcruz and Ayotitlán (Jalisco) reported the use of 135 plants for medicinal purposes, of which 45% are used for digestive system problems and 18% for respiratory tract disorders (DeNiz1989).

Most of the local species of fungi, plants and lichens present in this bioregion are completely unknown about their medicinal, food and cosmetic uses for the inhabitants of the Project area. Unfortunately, only fragmented parts of the herbal knowledge remain, traditional and not very empirical (with lack of dosages and precise therapeutic applications).

Objective and methodology for the elaboration of the project

One of the main objectives of the project is precisely to revalue this fragmented knowledge, to complement it with pharmacological and phytotherapeutic contributions, and to make known more local species with medicinal and food uses that are unknown or forgotten for their uses.

During the exploratory phase of this Project, during January 2022, field research was conducted to determine the wild plant species with herbal/medicinal and pharmacological potential in the territories of the ejidos El Terrero, Minatitlán and Lagunitas de Comala, Colima, as well as in the lands of the small landowners of La Parota Herrada, Minatitlán, Comala and Villa de Álvarez, also in the State of Colima. Of the 98 botanically identified species, 46 were found with local and national medicinal ethnobotanical records, of which 35 have pharmacological potential due to their bioactive principles.

Of these 35, 5 species were selected for this project, under the criteria of being common, abundant and easy to collect a good part of the year, of not being obviously endemic, protected or endangered species, of being species located by local therapists and with contents of bioactive principles interesting for the pharmaceutical industry, of containing bioactive principles quite easy to extract locally and, in addition, with bioactive principles known in their majority and with molecular ecotypes of the bioregion. These are:

- Old Man's Beard Lichen (Usnea barbata, Usnea florida, Usnea longissima).

- Hediondilla (Cestrum tometosum and Cestrum commune)

- Venus' Hair Fern (Adiantum capillus veneris and Adiantum andicola)

- Chias (Salvia hispanica, Salvia elegans, Salvia Gravida and Salvia Sessei)

- Sheep's tail / milfoil (Castilleja arvensis and Castilleja lithospermum)

- Gaultheria

Stages of the Project

1. Eco-sustainable collection of the indicated species in the local laboratory, cleaning, storage and extraction of mixtures of bioactive principles (4 months of duration).

2. Storage and packaging of medicinal preparations (dried plant, tinctures and hydroalcoholic and acetone macerations, medicinal and cosmetic oils, purified pharmaceutical preparations, essential oils, pure bioactive principles-flavonoids, terpenes and specific tannins).

3. Distribution in warehouses and local health centers, for the national market and for export (5 months of duration).

4. Ongoing technical training and workshops on the extraction of bioactive principles for the local beneficiaries of the project throughout the duration of the project.

5. Creation of a local herbalist center as a space for information transfer, participatory learning, teaching and local sale of products (3 months duration).

6. Creation of a training center in conservation, valorization and sustainable herbalism (6 months).

Annex. Specificities of the selected plants

01. (Usnea ssp)

The Usnea species most frequently found in the Project area are Usnea barbata, Usnea longissima and Usnea florida.

At the bioregional level, and in general throughout Mexico, popular herbalism prescribes these lichens for respiratory problems, coughs and as a cicatrizant. These species are well known for their medicinal and pharmacological potential, used in the pharmacopoeias of many countries.

The first recorded use of Usnea species in traditional Chinese medicine dates back to 101 BC, when it was used as an antimicrobial agent under the name Song Lo. Song Lo tea or its decoction is also used for internal and external liver detoxification, treatment of malaria, wounds, snakebite and cough. Common doses used in traditional Chinese medicine are 6 to 9 g of dried lichen, which corresponds to approximately 60 to 120 mg of usnic acid per day (21). However, despite its long history, Song Lo is classified as a rarely used herb in traditional Chinese medicine (only 500 are commonly used among the more than 6000 herbs recorded in traditional Chinese medicine).

Usnea species have been used as antimicrobial agents in many countries and were being developed as a modern drug just before the advent of penicillin antibiotics (4). Crude extracts of usnic acid-rich lichens (e.g., Usnea species) have been used worldwide to treat various diseases and ailments, including pulmonary tuberculosis, pain, fever, wounds, athlete's foot, and other dermal lesions. They have also been used as expectorants, in antibiotic ointments, deodorants, and herbal tinctures (3, 4, 22, 23). Therefore, Usnea species, or purified usnic acid, are currently the basis of a variety of herbal and phytotherapeutic products worldwide. For example, lichen extract and purified usnic acid have been widely used in perfumery, cosmetics, sunscreens and personal hygiene products such as toothpaste, mouthwash, shampoo and deodorant. In Germany, pure usnic acid has been formulated and used in cosmetics and pharmaceuticals under the trade names “Omnigran a, Granobil and Usnagren A” (24). In Finland, “ramalina thrausta” was used internally to treat sore throat and toothache and externally to treat wounds and athlete's foot (3, 23). In Italy, usnic acid has been used in vaginal creams, foot creams, powders and shampoo (25). In Argentina, “stone beard” (Usnea densirostra) has been sold to treat many ailments (26). In these preparations, usnic acid is used as the active ingredient or has functioned as a preservative. In the United States, Usnea is available in bulk powder form or as dried lichen from several herbal supply companies. It is widely available in dietary supplement stores, either alone or in combination with other herbs such as echinacea as tinctures in alcoholic or non-alcoholic preparations. In traditional Unani medicine, the medicinal uses of Usnea species are astringent, antidote, analgesic, cardiotonic, resolving and stomachic.

The bioactive principles of Usnea species, many of which are unique to the genus, have been extensively investigated. Among the major ones are usnic, protocetraric, barbaric, notstic, salazinic and stictic acids. All the major secondary metabolites in Usnea belong to aromatic products formed from b-orcinol units, while ceparatic acid and protolichesterinic acid belong to aliphatic acids. Most of these metabolites are unique to Usnea. Significant research has

been conducted on these metabolites with their diverse biological activities, including antimicrobial, antioxidant, antitumor, antiviral, anti-inflammatory, cardiovascular and hepatic protective. Recent pharmacological studies have revealed important anticancer, antigenotoxic, proliferative and antineoplastic activities.

Among other compounds, 7-hydroxyl-5-methoxy-6-methylphthalide, 18R-hydroxy-dihydroaloprotolichesterinic acid, (-)-placodiolic acid, (+) and (-)-isousnic acids, (+)-usnic acid, 5-resorcinol, ambewelamides A and B, atranorin, bailesidone, bailexanthone, barbaric acid, caffeic acid, chlorogenic acid, cinnamic acid, consalazinic acid, difractinic acid, divaricatinic acid, ellagic acid, ergosterol peroxide, etheric acid, menegaziaic acid ethers, fumarprotocetraric acid, galbinic acid, gallic acid.

A phytochemical investigation of the methanol extract of Usnea longissima led to the isolation of nine (1-9) phenolic metabolites, including compounds 6 and 7, which were reported for the first time from this species. All isolated compounds were fully characterized by spectroscopic analysis, including 2D NMR and HREIMS data. Individually, the activity of each purified compound was evaluated against bacterial neuraminidase (BNA), and most of them showed dose-dependent inhibition. Among the purified compounds, compounds 2 and 3 exhibited significant inhibitory activities, with IC50 values of 7.8 and 8.2 μM, respectively, and had a threefold higher potency than the reference compound quercetin (IC50 = 21.4 μM). Furthermore, enzyme kinetics revealed that compounds 2 and 3 exhibited reversible and mixed type I inhibitory behaviors, with inhibitory constant (Ki) values of 6.8 and 7.2 μM, respectively. Our results revealed the potential of U. longissima to inhibit BNA enzyme and highlighted its additional medicinal properties (Ullah 2019).

The anti-inflammatory properties of a U. barbata extract containing 4 % usnic acid were investigated in an ultraviolet-B (UVB) model with HaCaT keratinocytes. UVB irradiation induced PGE2 production and COX-2 expression in a time- and dose-dependent manner. This lichen extract inhibited PGE2 production at a maximum mean concentration of 60 μg/ml (2.4 μg/ml usnic acid) that did not affect UVB-induced positive regulation of COX-2, suggesting an effect on enzymatic activity rather than protein expression. The inhibition of PGE2 production was not due to cytotoxicity. In addition to its known antimicrobial properties, UBE shows specific UVB protective effects that could be useful in the topical treatment of UVB-mediated inflammatory skin conditions (Engel 2007).

Its extract is known to contain monosubstituted phenyls, depsides, anthraquinones, dibenzofurans and terpenoids that have been shown to exhibit insecticidal and antioxidant activities. Various bacteria and fungi (E. coli, Candida albicans, Bacillus subtilis, Mycobacteriun smegmatis, Trichophyton rubrum and Aspergillus niger) have been used to investigate the in vitro activity of usnic acid derivatives. Usnic acid derivatives are cytotoxic and antimicrobial, they are also used as expectorants and in the treatment of ulcer. It has been shown by Nishitoba [1987] that all orcinol derivatives and depsides of U. longissima act as growth inhibitors in lettuce seedlings. S

The anti-ulcerogenic effect of aqueous extract of U. longissima against indomethacin-induced ulcer in rats has been investigated. The extract showed moderate antioxidant activity compared to trolox and ascorbic acid as a positive antioxidant.

Usnic acid is an abundant constituent of several lichen species (3, 4). It has a long history of use in traditional medicine as an antimicrobial agent. However, during the last decade, usnic

acid has been marketed as a dietary supplement for weight loss because of its ability to increase fat metabolism and increase basal metabolic rate. Usnic acid was first isolated as a prominent secondary metabolite from lichen by the German scientist Knop in 1844 (5). When extracted from lichen, it is yellow in color and crystalline in appearance. Usnic acid [full name, 2,6-diacetyl-7,9-dihydroxy-8, 9 b-dimethyl-dibenzofuran-1,3(2H,9bH)-dione] exists in two enantiomers; (+) D-usnic acid and (-) L-usnic acid, indicating an R- or S-projection of the angular-CH3 group at the 9b position (Figure 1a). The enantiomers have been identified as showing different biological activities (6). In addition, two other natural isomers-(+) and (-) isousnic acids [2,8-diacetyl-7,9-dihydroxy-6,9b-dimethyldibenzofuran-1,3(2H,9bH)-dione]-are also found in lichens.

Antimicrobial activity

During the 1980s, there was renewed interest in usnic acid as an antimicrobial agent due to the increasing multidrug-resistant bacterial species caused by the overuse of synthetic antibiotics (4). Both optical enantiomers of usnic acid have been shown to be active against both Gram-positive bacteria and mycobacteria (3), and several research studies and clinical trials have confirmed the antibacterial properties of usnic acid. For example, in preliminary clinical trials, a mouthwash containing 1 % (+)-usnic acid was administered to volunteers. Samples of oral bacterial flora were examined at regular intervals. It was reported that the growth of Streptococcus mutans implicated in the etiology of dental caries was selectively suppressed (27). Using standardized assays, the in vitro susceptibility of pathogenic anaerobic and gram-positive bacteria to usnic acid has been confirmed (3).

It has been shown that usnic acid suppresses the growth of Gram-positive microorganisms that are primarily responsible for body odor. Ethoxydiglycol extracts of lichens containing 10 % usnic acid by wet weight were found to have potential as a preservative in moisturizer (3), and usnic acid is effective against Mycobacteriurn aureurn (28).

In in vitro tests, usnic acid and its salt inhibited the growth of Mycobacterium tuberculosis at relatively low concentrations (29). Partially purified usnic acid from Song Lo has also been tested therapeutically in China for tuberculosis and chronic bronchitis (21). For tuberculosis, patients received usnic acid tablets of 90 mg/day (or 1.5 mg/kg/day). Thirty patients were treated for an average of 71 days. Side effects reported included stomach pain and elevated liver enzymes. Some required discontinuation of usnic acid treatment for one week after taking the usnic acid tablets for approximately 3 months. For bronchitis, 91 patients were treated with one of four preparations (2 × 100 ml decoction of 30 g Song Lo/day, Song Lo crude crystalline material, sodium usneate, and unspecified partially purified Song Lo extract, in equal doses of 30 mg usnic acid equivalent three times daily). Ten days was considered a treatment period. Side effects of dry mouth, dizziness, and nausea were reported (21).

Other recent studies have shown that usnic acid is active against methicillin-resistant Staphylococcus aureus (30, 31) and its possible use in the sterilization of surgical implants is under investigation (32).

Antifungal activity

During a short-term treatment with usnic acid salt, 65 patients with Tineapedis exhibited significant improvement in their clinical conditions (4).

Antiprotozoal activity

Usnic acid (-) exhibited a significant inhibitory effect against the pathogenic protozoan Trichornonas vaginalis at comparatively lower concentrations than metronidazole (33). The compound also showed leishmanicidal properties in both in vitro and in vivo studies; intralesional administration resulted in a reduction of lesion weight and parasite body burden (34).

Antiviral activity

In a cancer chemoprevention assay, (+)-Usnic acid isolated from Usnea longissima was found to be highly effective against tumor promoter-induced Epstein-Barr virus with an ED5O of 1.0 µg/mL (35). (+)-Usnic acid also inhibited the cytopathological effects of herpes simplex virus type I and poliovirus type 1 in infected African green monkey kidney cells (3). A clinical trial evaluated the effect of an intravaginal formulation based on usnic acid and zinc sulfate as adjuvant therapy to radiosurgical treatment in 100 women with genital human papillomavirus infections. The treatment significantly improved re-epithelialization time one month after radiosurgery (36).

Antiproliferative activity

(-)-Usnic acid caused moderate inhibition in the P388 murine leukemia assay and also exhibited cytotoxic activity against cultured Ll210 cells; the p-triketone moiety was inferred to be essential for optimal activity (37). Moreover, (+)-usnic acid (50 µg/mL) reduced leukemic cell counts (K - 562) and in endometrial carcinoma cell cultures (HEC-50) (10, 38).

Anti-inflammatory activity

In an acute rat paw edema and chronic rat cotton pellet assay at an oral dose level of 100 mg/kg, the anti-inflammatory action of (+)-usnic acid was comparable with ibuprofen at the same dose level (39).

Analgesic and Antipyretic Activity

In two mouse studies, the analgesic and antipyretic effects of usnic acid were evaluated (22). At an oral dose level of 100 mg/kg, usnic acid exhibited a significant analgesic effect as indicated by acetic acid-induced tail pressure and contortion tests. At oral dose levels up to 300 mg/kg, usnic acid also expressed significant antipyretic activity as determined through lipopolysaccharide-induced hyperthermia.

Although pure usnic acid has been used for weight loss in recent times, this use cannot be considered safe and has been associated with hepatic failure. Usnea has been used in various formulations in modern China, usually in conjunction with the seaweeds Laminaria or Sargassum (or both). For the treatment of thyroid cancer, and in the treatment of bronchitis with profuse sputum. Other current uses include tablets for oral inflammation and many different ointments and creams with antimicrobial and anti-inflammatory action.

Antioxidant activity

A polysaccharide, CSL-0.1, was isolated from the medicinal lichen Usnea longissimi, a neutral rhamnose-containing glycogalactomannan with a molecular weight of 7.86 × 104 Da. The effects of CSL-0.1 on intestinal immunity and antioxidant activity were evaluated. CSL-0.1 increased spleen and thymus indices in a dose-dependent manner and conferred immunomodulation by reversing the Th1/Th2-related cytokine imbalance in cyclophosphamide (CP)-induced immunosuppressed mice. CSL-0.1 mice also injected with CP. In addition, antioxidant levels in the liver and intestine of mice increased by 20-50% after intragastric injection of CSL-0.1. (Wang 2021)

Two phenols, longissiminone A and longissiminone B together with a known compound, glutinol were isolated from Usnea longissimi, finding that longissiminone A possesses potent anti-inflammatory activity in an in vitro assay . The cytotoxicity activity measured by cell viability assay showed 100% viability in the presence of 200 μg/mL conc. of these compounds.(2)

Mechanism of action of usnic acid.

Several studies using well-established methods have evaluated the effects of usnic acid on mitochondrial function in vitro. As early as 1950, Johnson and colleagues (45) studied the effect of usnic acid using homogenates of rat kidney and liver. They reported that usnic acid, at the 1 μM level, stimulated oxygen consumption in the presence of various substrates. At higher concentrations of usnic acid (8-30 μM), phosphate uptake was reduced without a corresponding drop in oxygen consumption. This suggested uncoupling of oxidative phosphorylation. Pramyothin et al. (15) used primary rat hepatocytes to evaluate the hepatotoxic effect of usnic acid and demonstrated that usnic acid-induced loss of cell membrane integrity is accompanied by elevated AST and ALT activities. In the same study, stimulated respiration was also evaluated in isolated rat liver mitochondria using various substrates such as glutamate plus malate or succinate. The results showed that usnic acid stimulated oxygen consumption by inducing maximal stimulation of respiration (in the absence of ADP, state 4) (depending on the substrates from 3 to 7-fold), suggesting an uncoupling of oxidative phosphorylation, which is in agreement with the observation. by Johnson et al. (45).

Uncoupling of oxidative phosphorylation can also be assessed by a decrease in ADP/O ratios; the ratio of phosphate consumed (phosphorylation of ADP to ATP) to oxygen consumed. In a study with mouse liver mitochondria, it was reported that usnic acid at a concentration as low as 0.75 μM inhibited the ADP/O ratio by 50 %, causing a maximal 100 % stimulation of state 4 respiration and a 300 % induction of Mg2+- ATPase activity. The uncoupling effect was dose-dependent, and a higher concentration of usnic acid exhibited a complete uncoupling of oxidative phosphorylation (13).

Han et al. (14) demonstrated in isolated rat liver mitochondria a similar uncoupling effect of usnic acid in the presence of buffered bovine serum albumin (BSA). However, usnic acid acted as an uncoupler and inhibitor of mitochondria in the absence of BSA in buffer in primary mouse hepatocytes and rat liver mitochondria. Inhibition of usnic acid in mitochondria further caused oxidative stress with an increase in hydrogen peroxide generation. These data suggest that the adverse hepatic effects of usnic acid on mitochondrial function may not be limited to uncoupling of oxidative phosphorylation.

The ability of usnic acid to interfere with transmembrane ion gradients and mitochondrial function may also contribute to its reported antimicrobial properties (30). Usnic acid concentrations of 0.1 mg/ml (approximately 300 μM) stimulated oxygen consumption by the mitochondria-containing fungus Saccharomyces cerevisiae, whereas concentrations above 100 mM inhibited oxygen consumption (10). This is strikingly similar to the biphasic concentration-response to usnic acid observed in homogenates of mammalian tissues and mitochondria.

In vivo toxicities of usnic acid have been reported in both animals and plants, although data are sparse. In several species of experimental or wild animals, such as guinea pigs, mice, rats, domestic sheep, elk, and mosquitoes, general toxicity or organ-specific toxicity, or both, have been reported. In 1950 (48) it was reported that in female guinea pigs with tuberculosis, subcutaneous injection of usnic acid (20 mg per animal for 6 days, followed by 10 mg per animal for 24 days) caused slight weight loss in the first week and significant inhibition of weight gain for the next 3 weeks. Even after discontinuation of usnic acid, weight gain was still significantly inhibited for at least 2 weeks. This is the first report showing that usnic acid could cause weight loss with the possibility of general toxicity, although this possibility was largely ignored in the following decades. Notably, no apparent organ-specific toxicities were observed in the liver, spleen, or lungs in this report (48). In healthy male Swiss mice, treatment with usnic acid intraperitoneally at 15 mg/kg for 15 days caused no apparent general toxicity, as evidenced by negative observations in clinical signs or changes in body weight (49, 50). However, strong hepatotoxicity, including elevated serum transaminase activity and extensive hepatic necrosis, was observed. Toxicity in other organs such as kidney and spleen was not detected in the study. The similar pattern of toxicity was also revealed in tumor-bearing mice (49, 50). In male Wistar albino Wistar rats, usnic acid (i.p., 50 or 200 mg/kg for 5 days) induced marked inflammation of liver mitochondria and endoplasmic reticulum assessed by electron microscopy, although no changes in serum transaminase activity were observed, indicating that mild hepatotoxicity occurred (15). With the same rat strain, another group reported that usnic acid at doses of 500 and 1000 mg/kg orally produced no toxic effects at 24 h, but higher doses of 2000 mg/kg showed some toxicity (51). Unfortunately, there was no further information on usnic acid-induced toxicity described in the report (51). Feeding of domestic sheep with usnic acid 323-776 mg/kg for up to 9 days induced various clinical signs such as lethargy and anorexia, or even death, with an estimated median toxic dose between 485 and 647 mg/kg (52). Other toxicity indices, such as serum lactate dehydrogenase, aspartate aminotransferase, and creatine kinase, were also increased. A complete postmortem examination revealed that the pathological changes occurred exclusively in skeletal muscle (52).

This contrasts sharply with mice, rats, and humans, in which the liver is considered to be the organ most vulnerable to usnic acid insult. Usnic acid is also the suspected toxicant and was associated with an estimated 400 to 500 elk deaths in Wyoming in 2004 (52, 53). Necropsy revealed extensive muscle damage, such as muscle pallor and striae, particularly in the semitendinosus, semimembranosus, and pelvic limbs. Histological examination showed necrosis, rupture, inflammation and myofiber degeneration. However, a causal relationship between muscle damage and usnic acid exposure has not yet been established. Usnic acid also serves as a strong toxicant for certain insects such as mosquitoes. It has been reported that usnic acid (5 and 10 ppm) killed all larvae of certain mosquito species at stages 3 and 4, suggesting that it could be developed as a new natural insecticide (54). In addition to toxicity to animals, usnic acid also shows phytotoxicity. It exerts toxic effects on onion and lettuce

growth, possibly through inhibition of plant p-hydroxyphenylpyruvate dioxygenase, indicating potential use as a herbicide (6).

The idea of using chemicals with mitochondrial uncoupling activity for weight loss originated in the early 1930s after it was noted that munitions workers exposed to 2,4-dinitrophenol lost weight (55). Subsequently, 2,4-dinitrophenol was formulated into an anti-obesity drug that was prescribed by some physicians or marketed directly to the public with some claims of efficacy. However, many serious side effects were also reported, including liver, heart, and muscle toxicity and cataract formation, so that in 1938, the FDA finally declared 2,4-dinitrophenol too toxic to be used under any circumstances (55).

After this, reports of misuse of 2,4-dinitrophenol became less frequent. Interest in decoupling chemicals has resurfaced primarily in the bodybuilding community with the advent of the Internet and the passage of DSHEA, resulting in the underground trade of 2,4-dinitrophenol (56, 57) and the open marketing of usnic acid and other natural products. products in dietary supplements formulated for weight loss (21). Such formulations generally contain relatively high concentrations of usnic acid, alone or in combination with other ingredients, and their use has been reported to be associated with hepatotoxicity.

In 2000, Favreau et al. (58) reported that seven previously healthy patients developed acute hepatitis after ingesting LipoKinetix (Syntrax, Cape Girardeau, MO) and recovered spontaneously after discontinuing its use. Subsequently, two more cases of acute hepatitis were reported after taking LipoKinetix, one of which resulted in liver transplantation (59). LipoKinetix was a multi-ingredient product; one capsule contained 25 mg norephedrine hydrochloride, 100 mg usnic acid, 100 µg 3,5-diiodothyronine, 3 mg yohimbine hydrochloride, and 100 mg caffeine. It was sold as a dietary supplement to promote weight loss. The manufacturer claimed that LipoKinetix "affects oxidative phosphorylation in such a way that an incredible amount of fatty acids are burned," which promotes weight loss. The recommended dosage of LipoKinetix was 1 to 2 capsules 3 times a day, which is 3 to 6 times higher than the doses of usnic acid used in TCM. The production and sale of LipoKinetix ended in 2001, but Syntrax continued to produce a product with similar ingredients but without usnic acid, which was called AdipoKinetix.

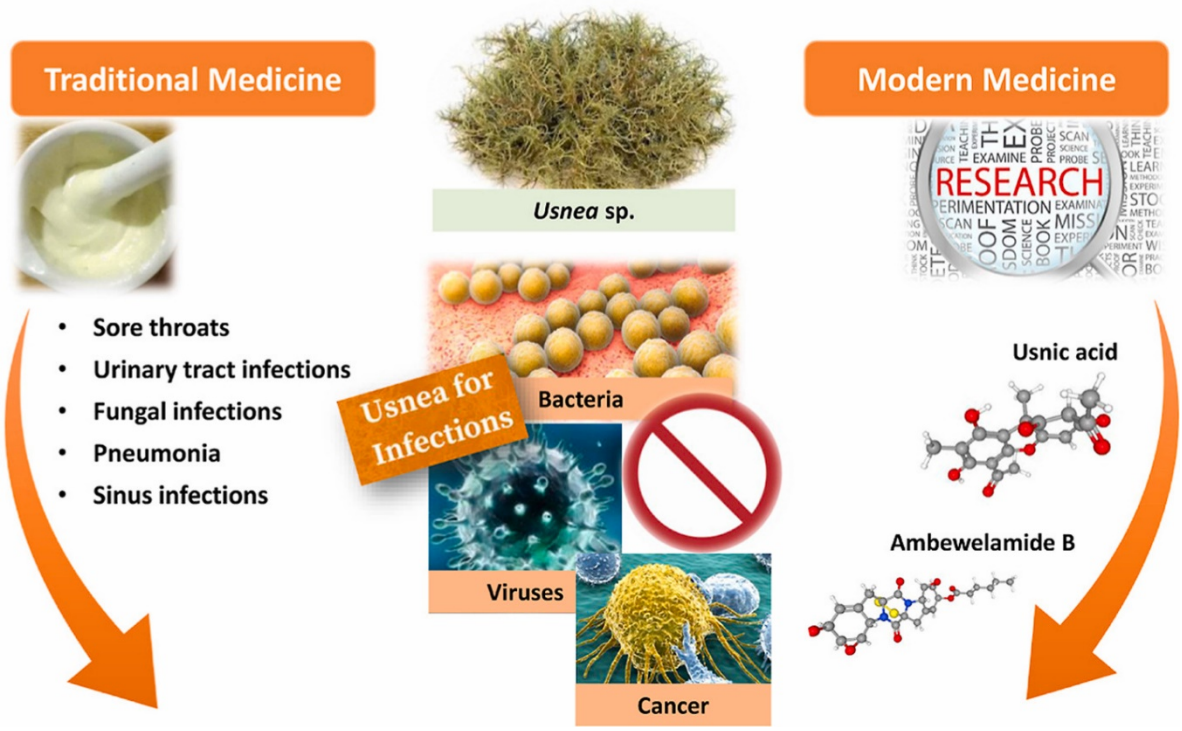
UCP-1 (BDC Nutrition, Richmond, KY) was marketed as a weight-loss product containing 150 mg usnic acid, 525 mg L-carnitine, and 1050 mg calcium pyruvate per capsule. The recommended dose of UCP-1 was 3 capsules 3 times daily. Sanchez et al. (60) reported the development of severe liver failure in two patients who were taking the recommended dose of UCP-1. One resulted in liver transplantation. Druazo et al. (61) also reported a case of a healthy woman who, after taking pure usnic acid (Industrial Strength AAA Services, Frazer Park, CA) for weight loss, developed liver failure requiring transplantation. The recommended dose of pure usnic acid from this manufacturer was 500 mg per day.

To date, FDA has received at least 21 adverse event reports, including 1 death attributed to the weight-loss dietary supplement containing usnic acid (LipoKinetix and UCP-1) or pure usnic acid. Twelve cases associated with hepatotoxicity appeared in the literature (60-62), and are summarized in Table 1. These included 8 women and 4 men. The median age of the patients was 31 years. Two patients required liver transplantation and the others eventually recovered. Although the total number of people who have experimented with weight-loss supplements containing usnic acid is unknown, the manufacturer of LipoKinetix claims to have sold more than 30 000 bottles of the supplement (63).

Based on the adverse events described above and first reported by Medwatch in November 2001, the Center for Food Safety and Applied Nutrition (CFSAN), the Food and Drug Administration (FDA) issued a warning letter (64, 64) on November 19, 2001, entitled “FDA warns consumers not to use the dietary supplement LipoKinetix” because it has been implicated in several serious liver injuries. After receiving additional reports of individuals who developed liver damage or liver failure while using LipoKinetix, the FDA issued a strong recommendation to the manufacturer, Syntrax Innovation Inc. to remove the product from the market (Letter to Distributor Regarding Dangerous LipoKinetix Dietary Supplement) .

In order to better understand the human health risk of usnic acid and Usnea preparations, the Division of Dietary Supplement Programs/CFSAN/FDA nominated usnic acid to the NTP for evaluation of short- and long-term toxicity in January 2005 (21). The secondary objectives of the nomination were to examine the dermal toxicity of usnic acid and the evaluation of usnic acid and Usnea lichens for genotoxic, developmental, and reproductive toxicity. The studies were initiated in 2006 and are being conducted in our laboratories at the National Center for Toxicological Research (NCTR). They currently involve acute and subchronic toxicity testing in rodents of usnic acid and Usnea lichen preparations and associated in vitro and mechanistic studies. Fourteen-day acute toxicity studies have shown that usnic acid is hepatotoxic to both rats and mice exposed via food at concentrations of 100 or 200 mg/kg/day, but that lower doses are well tolerated (Table 2). Usnea lichen preparations containing equivalent concentrations of usnic acid produced greater toxicity than pure usnic acid (66).

In addition, NTP has conducted and completed screening and testing of usnic acid for its mutagenic potential in bacteria. Three strains with different genetic backgrounds were used for the study, including one strain of E. coli and two strains of Salmonella typhimurium (TA98 and TA100). No significant mutagenicity was observed in the strains tested (67). Mechanistic studies performed at the NCTR have shown that in mice exposed to usnic acid for 14 days, genes encoding proteins involved in mitochondrial electron transport, fatty acid metabolism, and apoptosis are up-regulated, suggesting that the adaptive mechanism the response to usnic acid hepatotoxicity requires increased β-oxidation of fat and induction of mitochondrial respiratory complexes I-IV to increase proton flux to compensate for the uncoupling activity of usnic acid (68). Studies have also shown that usnic acid was strongly toxic to rat hepatocytes in primary culture, with an IC50 of 10 μM after 24-h exposure. Cell lethality was associated with mitochondrial permeability transition (MPT) and mitochondrial depolarization. Similar effects were also observed with human hepatoma HepG2 cells (69). Usnic acid showed no effect on the activity of respiratory complexes I or IV, but slightly inhibited the activity of respiratory complexes II+III at 150 μM.



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O2. (Castilleja arvensis/Castilleja lithospermum)

Both species of Castilleja are fairly common herbaceous plants found in the Project area. They are native to Mexico. They inhabit warm, semi-warm, semi-dry and temperate climates between 1000 and 2400 meters above sea level. They generally grow in rainfed cultivated land, tropical deciduous and sub-deciduous forests, xerophytic scrub, thorn forest, mountain mesophyll forest, oak, pine and mixed pine-oak forests.

This species is often used against coughs in Tlaxcala and in the State of Mexico, it is recommended to take the infusion of the flowers. Throughout Mexico it is widely used as a naturopathic treatment for various ailments, such as cancer, whooping cough, gastrointestinal, liver and kidney diseases. In the purepechas bioregions (Michoacan) it is known as Uitsapirhaticha ("little dogs"), Chongo de uarhi ("trensa de muchacha"). The flower and leaves are taken in infusion against stomach pains and bile. In Valle de Mexico the decoction of the plant is used as a diuretic, antitussive (Sta Caterina del Monte, IMSS 1990) digestive and to increase bile flow. On the other hand, in Michoacán the decoction of this herb is used to cure stomach pain and bile. In Puebla it is used to wash wounds and in Guanajuato the infusion of the flower is taken to cure the heart. It is also used to treat "lack of digestion" (dyspepsia), scorpion stings and to lose weight.

In several rural areas of Mexico the whole plant is taken in infusion against "rabadilla" pain (Guerrero), cough, diuretic, digestive, increases bile flow (edomex) to treat "lack of digestion" (dyspepsia), scorpion stings and to lose weight.

The Ojibwe in Canada used a hair wash made from Indian paintbrush to give shine and body to their hair, and as a treatment for rheumatism. The high selenium content of this plant has been cited as the reason for its effectiveness for these purposes.

Food use

The flowers are edible and were consumed sparingly by several Native American tribes as a condiment with other fresh vegetables. These plants tend to absorb and concentrate selenium in their tissues from the soils in which they grow, and can potentially be very toxic if the roots or green parts of the plant are consumed. Highly alkaline soils increase selenium levels in plants. Indian paintbrush has similar health benefits to garlic consumption, although only if the flowers are eaten in small quantities and in moderation. [Tilford 1997]

In the Coroico bioregion (Bolivia) the plant is used to treat kidney conditions. At the level of its phytochemistry, alkaloids[1,2]) and iridoid glycosides[3,4,5] were isolated (San Martin 2012).

Castilleja lithospermoides is known in the purepecha lacustrine bioregion as Chanquintsi kontsi ("crane's topknot"). The flower is taken in infusion as an antidysenteric, nerve calming, diuretic and urinary disinfectant.

Major compounds were identified in samples obtained from methanolic extracts in control plants and in those from seed with storage times: 30, 50 and 100 days. The control group presented iridoids (aucubin, epiloganin and bartsioside), phenylethanoids (verbascoside and isoverbascoside) and flavonoids (rutin and luteolin). On the other hand, the iridoid aucubin,

the phenylethanoid verbascoside and lignan were present in all seedlings from seed, while the presence of other compounds was variable (Leon Romero 2020).

Eight compounds were isolated from Castilleja tenuiflora, four were new furofuran-type lignans for the species magnolina, eudesmina, sesamin and kobusina. Magnoline (60.97%) was the most effective lignan in inhibiting the NF-κB / AP-1 pathway, followed by eudesmin (56.82%), tenuifloroside (52.91%), sesamin (52.63%) and kobusin (45.45%). Verbascoside, a major compound contained in wild C. tenuiflora, showed an inhibitory effect on NF-κB/AP-1 (Arango 2021). All isolated compounds (verbascoside, teniufloroside and geniposide / musseanoside mixture) showed gastroprotective effects and antidepressant activity at 1 or 2 mg / kg. The above results allow us to conclude that these polyphenols and iridoids of C. tenuiflora are responsible for the gastroprotective and antidepressant effects (Lopez Rodriguez 2019).

Castilleja tenuiflora has been used for the treatment of several diseases of the central nervous system (CNS), specifically for the antidepressant activity of the methanolic extract of the leaves . Oral administration of MeOH extract (500 mg / kg) induced a significant decrease ($p < 0.05$) of the immobility parameter in the forced swimming test (FST) and an increase in the latency and duration of hypnosis, induced by the administration of sodium pentobarbital (Pbi, 40 mg / kg, ip). Chemical analysis of this antidepressant extract allowed the isolation of (+)-piperitol-4-O-xylopyranosyl- (1 → 6)-O-glucopyranoside. This new furofuran lignan diglycoside was named tenuifloroside Tenuifloroside, caryoptoside and luteolin 5-methyl ether were isolated for the first time from the genus Castilleja. These findings demonstrate that the methanolic extract of C. Tenuiflora has a beneficial effect on depressive behaviors, and the knowledge of its chemical constitution allows us to propose a new standardized treatment for future investigations of this species in depressive illnesses (Herrera Cruz 2015)

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03. (Adiantum capillus veneris, Adiantum andicola)

These two ferns are quite common in the humid areas, ravines and riacheulos of the Project's bioregion; they are typical ferns of the pine-oak forests, cloud forests, high or medium sub evergreen forests, rocky edges.

In the area the infusion of the plant is still used against coughs. In Colima and Jalisco (states where the Manantlán reserve is located), traditional herbalism recommends it mainly to accelerate childbirth and regulate postpartum menstruation. It is also used as a contraceptive, for this purpose the infusion made with the leaves is used, mixed with seven other plants, and to reduce hemorrhages the cooking of the branches with royal sage, “pastito de popotito” and muicle is used. It is also used to treat kidney diseases.

In the bioregions of the Purepecha indigenous people (Michoacan), the plant is known as velo, Kulandu (allusion to coriander), Misituriaki, Kagui-arimbikua. It is prescribed against diseases of the kidneys (infusion to drink) and against chest pain, back pain, to stop menstruation, to accelerate childbirth (decoction/infusion taken in tea) and against the “empacho de agua de las madres” (when the woman is very thirsty and her milk becomes soggy) (Gioanetto 2003, 2005).

The antibacterial properties of A.capillus-veneris have been proved against the parasitic bacteria Streptococcus pneumoniae, Eschericia coli and Candida albicans with an action comparable to the chemotherapy antibiotics gentamicin and ketoconzole, while the related ferns A. peruvianum, A.venustrum and A.caudatum act against Micrococcus luteus, Aspergillus terreus, Streptococcus pneumoniae and Candida luteus (Singh & Singh 2002).

Already known in pre-Hispanic times, and in particular by classical Aztec medicine as a febrifuge and to disinfect wounds (Sahún 1577, Hernández 1578), it is still used throughout the country to alleviate the symptoms of dry cough, to purify the blood, as an abortifacient, cathartic, diuretic, contraceptive, hemostatic after an abortion, without forgetting its more common and generalized uses against bronchitis and to control alopecia (hair loss) (Herbario IMSS 1994, Cabrera and Martinez 1969).

In many North American, Asian, European and African pharmacopoeias and therapeutic traditions, this fern and the related species A. andicola and A. poiretii are used, in decoction and infusion, to control and heal respiratory diseases, bronchitis, chronic nasal congestion, asthma, cough, throat sores and to reduce mucosal excesses.

Already known in ancient Greek herbalism for relieving coughs and as a pectoral, according to the accounts of the classical antique physicians Dioscorides (100 BC) and Theophrastus (327-287 AD) this fern was already prescribed in the Sanskrit pharmacopoeia of the Hindu physicians Sushruta & Charaka (100 BC). It was used as a febrifuge, against coughs, respiratory disorders, as a stimulant, purgative, emollient and hair tonic; it also possesses antiviral, antibacterial, antitumor properties, and is also recommended for its aphrodisiac properties and to alleviate hypoglycemic and diabetic disorders (Kumar 2003, Parihar 2006). It is mainly recommended to accelerate childbirth and regulate postpartum menstruation. It is also used as a contraceptive, for this purpose it is used the infusion made with the leaves, mixed with seven other plants, and to reduce hemorrhages it is used the cooking of the

branches with royal sage, “pastito de popotito” and muicle. It is also used to treat kidney diseases.

In the traditional medicine of the Muina people (Colombia), the Adiantum fern (called Jokome) is of great importance since it is used in different treatments.

1. Headache and flu: for this, the new leaves are collected, then macerated by hand and then the curaca or traditional doctor “ikoriraima” must narrate the prayer, so that the patient can inhale the steam from the boiling for 30 minutes to an hour and for a week. In this sense, other species of the genus Adiantum (e.g. A. aethiopicum L., A. capillus-veneris L. and A. caudatum L.) were used by herbalists to cure a vast number of diseases and are useful in the treatment of fevers, influenza and related diseases (Lloyd, 1964; May, 1978).

2. Fever: new leaves are collected and placed in a container with boiled water, then prayed by the curaca and then a portion (200-250 mm) is given to the patient. This treatment should be done twice a day for a week. May (1978) comments that A. capillus-veneris is the main ingredient of the famous “Syrup of Capillaire”, widely used to calm fever in France.

3. Decrease of aging: the best and youngest leaves are collected, then deposited in a container with water to be subsequently prayed by the curaca. Then they proceed to take baths where the leaves of the Jokome should be distributed with pressure all over the body, causing the person to get rid of the negative energy they carry and thus maintain their youth. In this regard, Keller & Prance (2015) mention the existence of substances with antioxidant activity in various ferns, which are used in medicine to reduce stress caused by oxidation, among them, a relative of jokome, Adiantum capillus-veneris could be cited as an example (Kumar, 2009).

Ritual use for hunting

There is also a ritual use that the Muina people determine for the Adiantum fern, which is intended to increase success in an activity that is exclusive to men, hunting. For this, the circinate vernations (undeveloped young leaves of the fern) are collected, which are then macerated by hand, distributing them with pressure all over the body before going out to hunt. This allows the animals to not perceive the hunter, or to fail to register his scent, which can be explained by some kind of mimicry generated by the smell of the plant, and in turn indicates a legendary logic explored and used by the Muina that evokes an ancient European Christian idea described by Paracelsus (1493-1541) as “the doctrine of the subjects, theory of the sign, or of the subjects”, from which in the Middle Ages medicine and astrology were combined with botany and superstition, determining that the nature of the shapes, colors, flavors, smells and other particular attributes of plants (leaves and flowers, etc.), could indicate their usefulness, especially for disease control (Pearce, 2008). However, for ferns, given their complex foliar architectures, their degree of lamellar dissection, and above all, their enigmatic and inconspicuous reproduction by spores, which, due to their size (30 and 100 mμ) Tryon & Lugarton (1990), was imperceptible to the human eye of the time. In the light of this doctrine, it could be assumed that ferns represented unknown, enigmatic plants, and even related to magic and witchcraft. Thus a fixation was generated by the ignorance of the reproductive structures of ferns and by relating them to plants with seeds, it was assumed that their seeds were invisible (Moran, 2004), and therefore, anyone who had the privilege of possessing them, acquired this attribute (Duran, 1949). The Celtic and Germanic ethnic groups concluded that these plants granted this privilege, because these plants grew and

increased their populations without any apparent reproductive organ (Friend, 1884), considering them as a sacred source of power (May, 1978).

Based on the above-mentioned arguments, it is possible that the Murui-Muina people, due to the apparent absence of reproductive structures (fruits or seeds), the ecology of the plant - located mainly in open and intervened areas- and above all, the continuous contact with Adiantum pulverulentum, considered that the species provided advantages to the person who used it in hunting and fighting, since by never observing its fruits or seeds, it could be a plant that promoted invisibility.

This fact represents an interesting example of cultural convergence, where the human being beyond their cultural barriers, when relating to their natural environment, has been provided with ferns, to defend themselves, heal, dress, feed, and because they are so important in their evolution, they have coated them with a halo of magic.

The ritual dance of Yuaki or dance of the fruit, is currently practiced by the Muina as one of the four fundamental pillar dances in the process of formation of a cacique or maloka owner, which corresponds to the "pillar of abundance". In the realization of the dance of the fruit, the Jokome fern (Adiantum pulverulentum) is fundamental to mark the rhythm of the dance and as its air freshener. For its use, a bunch of tied and compacted dry leaves of Jokome is taken to be shaken according to the rhythm of the chant, generating a characteristic sound, which according to our narration, is used in a special point of the ceremony to stabilize and filter the energy. It is considered as a catalyst of the good energies, given that for the Muina, its aroma does not allow to identify the individual from the bad spirits, nor from the smell of the animals, being recognized as an aromatizer. In this regard, May (1978) comments that some species of ferns, including Adiantum capillus-veneris L., a species related to Jokome, have constituents that include tannic acid, gallic acid and traces of essential oils, which can produce a tonic placid odor.

Tannins, mucilages (Fontquer 1980), terpenoids, flavonoids, triterpenoids, phenylpropanoids, carotenoids (violaxanthin, zeaxanthin), caffeic acid, beta sitosterol and saponins were found in species of the genus Adiantum (Singh & Singh 2008, Banerjee & Datra 1991). Different analyses of A.capillus veneris confirmed the presence of the following bioactive principles: flavonoids, triterpenoids, aoleananes, phenylpropanoids, carbohydrates, carotenoids and allycyclics. Among the triterpenoids: 21-hydroxy adiantone, triterpenoid epoxide (adiantoxide), Fern-9(11)-en-12-one, isoadiantone, isoglaucanone, hdoxyhopane, isoadiantol, hydroxyadiantone, olean-12-en-3-one, olean-18-en-3-one, fern-9(11)-ene, ferna-7, 9(11)-diene, 7-fernene, hop- 22(29)-ene, filic-3-ene , neohop-12-ene , pteron-14-en-7a-ol, fern-9(11)-en- 3a-ol, fern-7-en-3a-ol, adian-5(10)-en-3a-ol, adian-5-en-3a-ol, fern-9(11)-en-28-O, fern-9(11)-en-12- beta-ol and 4-α-hydroxyfilican-3-one were isolated from the leaves of Adiantum capillus-veneris [9-21]. Four sulfate esters of hydroxycinnamic acid-sugar derivatives were isolated from the fronds of Adiantum capillus-veneris. L. These compounds have been shown to be 1-p-coumarylglucose 6-sulfate, 1-p-coumarylglucose 2-sulfate, 1-caffeoylgalactose 3-sulfate, and 1-caffeoylgalactose 6-sulfate [22]. Adiantum capillus-veneris leaves were reported to contain different flavonoids such as rutin, quercetin, quercetin-3-O-glucoside, querciturone, isoquercitrin, nicotiflorin, naringin, astragalin, populnin, procyanidin, prodelphinidin, and kaempferol-3-sulfate [23-26].

Total phenols and flavonoids in the leaves were 224.76 ± 9.75 and 49.62 ± 0.875 in the aqueous extract, 156.34 ±9.70 and 78.18 ± 1.741 in the methanolic extract and 36.53 ± 3.65 and 50.15 ± 4.79 mg / 100g in the ethanolic extract, respectively [27].

The leaves contained 8.3 % moisture, 11.44 % ethanol extractable matter and 24.00 % water extractable matter. The soxhlet extraction of Adiantum capillus-veneris showed the presence of phenolic compounds and terpenoids (2.73 %), fats and waxes (0.20 %), alkaloids (0.53 %), quaternaries and noxides (26.33 %) and fiber (67.23 %). Ten trace elements (Mg, Ca, K, Mn, Fe, Co, Na, Ni, Cu and Zn) were determined in leaf extracts, with Ca and K having the highest content [23].

Antimicrobial activities

Methanol extracts of the aerial parts Adiantum capillus- veneris indicate antimicrobial properties at concentrations between 0.5-2 mg/ml against Bacillus, E. coli, Staphylococcus, Proteus, Pseudomonas, and Candida [28]. The methanol extract of Adianthus capillus-veneris was also tested for its antimicrobial properties against 5 gram-positive bacteria (including multi-resistant strains of Staphylococcus aureus), six gram-negative bacteria and against 8 strains of human pathogenic fungi. The extract showed strong antibacterial activity also at very low concentrations (0.48 mμg/ml) against Escherichia coli [29]. Aqueous and alcoholic extracts of Adiantum capillus-veneris leaves were found effective against Agrobacterium tumefaciens, Escherichia coli, Salmonella arizonae, Salmonella typhi and Staphylococcus aureus [30]. Aqueous extracts and phenols extracted from gametophytes of Adiantum capillus-veneris showed antifungal activity against Aspergillus niger and Rhizopus stolonifer [31]. The ethanol extract of Adiantum capillus-veneris rhizome exerted in vitro antiviral activity against vesicular stomatitis virus [32].

Aqueous and alcoholic eaxtract of A. capillus-veneris leaves were found effective against Agrobacteriumtumefaciens, Escherichia coli, S. arizonae, S. typhi and S. aureus strains with maximum zone of inhibition against S. faecalis. A previous study by Medrar et al.,(2014) showed that P. aeruginosa was the most vulnerable bacteria. Aqueous and methanolic extracts showed comparatively superior competence against P. aeruginosa than amoxicillin. The antibacterial activity of against multidrug-resistant (MDR) strains of the methanolic extract of the leaves recorded the highest zone of clearance/inhibition against Providencia, Klebsiella pneumoniae, Shigella, Vibrio cholera, S. aureus, Proteus vulgaris and S. typhi. However, while methanolic stem extract inhibition was very high against E. coli, K. pneumoniae and S. typhi. In another study, the antibacterial activities of the methanol extract of the leaves was shown to be effective against S. aureus, E. coli and Helicobacter pylori has been tested (Shirazi et al.,2011).

Anti-inflammatory and analgesic effects

The alcoholic extract of Adiantum capillus-veneris and its hexane fraction exerted significant anti-inflammatory activity against formalin-induced inflammation. The hexane fraction showed topical anti-inflammatory activity after 6h and continued for 30h in croton oil-induced inflammation. The ethyl acetate fraction of the ethanolic extract of Adiantum capillus-veneris showed significant inhibition of carrageenan-induced hind paw edema.

The chronic anti-inflammatory activity of the ethanol extract was also evaluated by the carrageenan-induced hind paw edema method. The results, at the two dose levels tested in

rats, indicate significant anti-inflammatory activity. The maximum inhibition of inflammation (71.15 %) was recorded at 100 mg/kg of plant extract.

The analgesic activity of ethanolic extract of Adiantum capillus-veneris and its fraction carried out by tail flick method and writhing test, the result showed significant analgesic activity with negligible gastric ulceration as compared to standard anti-inflammatory analgesic antipyretic drugs [26, 33-34] The anti-inflammatory and antinociceptive activities of crude ethanolic extract of Adiantum capillus veneris and its various fractions were studied using carrageenan-induced hind paw edema, tail wagging method and wriggle test at a dose of 300 mg / kg po

Gastric ulceration studies have been carried out for the ethanolic extract and its various fractions at a dose of 900 mg / kg bw. Among the fractions tested, the ethyl acetate fraction exhibited a better anti-inflammatory effect (67.27%) at a dose of 300 mg / kg po compared to the standard drug, indomethacin (63.63%) after 3 h in carrageenan-induced hind paw edema. The anti-inflammatory activity of the ethanolic extract and its various fractions seem to be related to inhibition of NO release and decrease in TNF-α level. The ethanolic extract and all its fractions, especially ethyl acetate ($p<0.01$) showed significant analgesic activity with negligible ulceration compared to the standard drug, ibuprofen. Histopathological study of the effect of ethanolic extract and its fractions on the stomach revealed that none of them produced ulceration [33].

The anti-inflammatory effect of ethanolic extracts of Adiantum capillus-veneris and the involvement of NF-κB signaling in the regulation of inflammation were studied. Ethanolic plant extracts effectively suppressed the release of PGE2, IL-6 and TNFα with an IC50 below 50 μg/ml. Furthermore, luciferase expression could be specifically blocked in HepG2 cells, demonstrating that plant extracts show a cell-specific pattern in NF-κB gene transcription. The tested biological activity also depended on the order of addition of TNF-α and plant extracts because plant extracts could only block NF-κB activation if added before, but could not stop the signal when added after TNF-α. However, plant extracts exerted no effect on ubiquitination that regulates several steps in the NF-κB pathway. Furthermore, plant extracts down-regulated phosphorylation of IKKα/β at S176/180, p38 at T180/Y182 and p65 at S536, but not p65 at S276. This was confirmed by their ability to selectively abrogate the induction of IL-8 transcription, whereas the ICAM-1 gene, which is not selectively transcribed by an NF-κB complex containing a form of p65 phosphorylated at Ser536, did not change. Finally, plant extracts at 200 μg/mg could normalize LPS-induced elevation of spleen index as well as NF-κB and p38 activations in CD1 mice [35].

Hypoglycemic activity

When the total plant extract prepared by boiling the dried material with water was administered to mice (25 mg/kg) orally, it reduced glucose-induced hyperglycemia.

However, the plant extract prepared by maceration with 80% ethanol did not show hypoglycemic activity when administered to mice at doses of (25 mg/kg) orally [36]. The alcoholic extract of Adiantum capillus-veneris showed a significant hypoglycemic effect in the rabbit model, started after 30 min of administration of the extract and continued for 4 h [26]. El-Tantawy et al. recorded the antidiabetic and diuretic effects of alcoholic and aqueous extracts of Adiantum capillus-veneris, as well as its isolated mucilage [37]. Thus confirming the traditional herbal uses in the control of diabetes.

Antioxidant activity

The antioxidant potential of Adiantum capillus-veneris leaf extract was studied in vitro against H2O2-induced oxidative damage in peripheral blood lymphocytes. Pretreatment with plant leaf extract for 18 h inhibited lipid peroxidation and significantly enhanced antioxidant enzyme activities and glutathione content. The results were attributed to their direct potential to scavenge free radicals and modulate the antioxidant defense system. Total flavonoids from Adiantum capillus-veneris showed high hydroxyl radical scavenging activity [38-39].

The ethanol extract showed good antioxidant activity compared to ascorbic acid, presents a low IC50 value, 0.3986 mg/g for the DPPH assay and 0.695 mg/g for the ABTS assay. The results obtained indicated that the leaves of Adiantum capillus-veneris are endowed with free radical scavenging molecules and can be used as a potential source of antioxidants and natural nutrients [27].

Antilithiasic activity

The in vitro antilithiasic activity of the hydroalcoholic extract of Adiantum capillus-veneris was evaluated by crystallization, aggregation and nucleation assays. The result indicated that the extract significantly inhibited crystallization and aggregation, which was further confirmed by an in vivo study against ethylene glycol (0.75 %) and ammonium chloride (1 %) induced urolithiasis in male Sprague Dawley rats. Urine microscopy showed a significant reduction in the number of crystals in the test groups [40].

The antiurolithiasis effect of hydroalcoholic extract of Adiantum capillus-veneris was also evaluated in male rats. Urine microscopy showed a significant ($p<0.001$) reduction in the number of crystals. Serum blood levels of calcium, phosphorus and urea were found to be significantly reduced. Serum creatinine level was found to be similar to the single control. Animals treated with the test drug showed great improvement in body weight. Histopathology of the kidney showed almost normal renal architecture in the treated groups [41].

Hair growth promoting effect

This is one of the most common uses of this fern in traditional herbal medicine. The hair growth promoting activity of a preparation of Adiantum capillus-veneris was evaluated in albino mice using a model of testosterone-induced alopecia. The Adiantum capillus-veneris solution was topically applied to the skin of the dorsum of the animals and hair growth was assessed by visual observation and histological study of several skin sections through various parameters such as follicular density (number of follicles/mm) and anagen/telogen ratio. After 21 days, a patchy diffuse hair loss was observed in animals receiving testosterone, while animals treated with Adiantum capillus-veneris showed less hair loss compared to those treated with testosterone alone. The follicular density observed in the Adiantum capillus veneris-treated group was 1.92 ± 0.47, compared to 1.05 ± 0.21 in the testosterone group and 2.05 ± 0.49 (follicles/mm) in the finasteride-treated animals.

The anagen/telogen ratio was significantly affected by Adiantum capillus-veneris, which was 0.92 ± 0.06 compared to 0.23 ± 0.03 and 1.12 ± 0.06 for the testosterone- and finasteride-treated groups, respectively [42].

Antidiarrheal, antispasmodic and antiasthmatic effects.

In one study, the crude extract of dried fern leaves was evaluated for its antidiarrheal and antispasmodic activities. The antidiarrheal effect was tested through castor oil-induced diarrhea in mice model. In addition, the inhibitory effect on K+-induced contraction was observed in isolated rabbit jejunum preparation which established the antispasmodic activity of the plant (Swaroop et al., 2012). The ethanol extract of the leaves was experimented for its antiasthmatic effect through histamine aerosol-induced asthma in guinea pigs, thus confirming its traditional use as an antiasthmatic (Yousaf et al., 2016).

Other pharmacological activities.

Compounds isolated from the plant showed many pharmacological effects. Astragalin exerted antiproliferative activity, inhibiting human mesangial cell proliferation and matrix oversynthesis, possibly by decreasing the overexpression of the beta-1 integrin gene. These effects may prevent the progression of chronic kidney disease.

It also exerted anti-dermatitis activity, reducing the severity of pre-existing dermatitis and preventing the development of atopic dermatitis.

The shikimic acid contained in this fern acted as a neuroprotectant. Focal cerebral ischemic lesion induced by thrombosis of the middle cerebral artery was reduced.

Rutin exerts an anticholesterolemic effect. It reduced total cholesterol, LDL, VLDL and triglycerides without reducing HDL. It also exerted gastroprotective effects. It protected against reflux esophagitis by inhibiting gastric acid secretion, oxidative stress, inflammatory cytokine production and intracellular calcium mobilization.

Naringin showed antioxidant effects. It increased hepatic superoxide dismutase and catalase activity, decreased hepatic mitochondrial hydrogen peroxide and increased plasma vitamin E concentration. It also exerted an anticholesterolemic effect by reducing plasma cholesterol and triglyceride concentrations, as well as HMG-Co reductase activity. Naringin also had a DNA protective effect, protecting mouse bone marrow cells against gamma radiation-induced DNA damage [43-49].

Adverse reactions and contraindications

There are no known health risks or side effects associated with the proper administration of the designated therapeutic doses [49]. However, the plant was documented to reduce blood sugar levels in animal studies.

Toxicity study of crude ethanolic extract of fern in rat demonstrated behavioral reactions in experimental model at 300, 1000 and 2000mg/kg doses for varying time intervals without the cause of mortality (Swaroop et al., 2012; Vijayalakshmi and Kumar, 2013; Yuan et al., 2013). Acute oral toxicity of aqueous and methanolic extracts was recorded at 2000 mg/kg as a single dose with remarkable change in behavior without lethality after first 30 minutes, 4 hours and 24 hours (Ranjan et al., 2014). The plant should not be used in pregnant women during lactation period (Gruenwald et al., 2008).

People with diabetes and people with hypoglycemia should use this plant with caution and control their blood sugar levels accordingly. The plant has a long history of use in phytotherapeutic and traditional herbal medicine systems to stimulate the uterus and promote menstruation; therefore it is contraindicated in pregnancy. The plant has been shown to have an anti-implantation effect in animal studies and may prevent conception; therefore couples seeking fertility treatment or pregnancy should not take the plant. Because of its effect on fertility and menstruation, it may have estrogen-like effects and should probably be avoided by women with estrogen-positive cancers.

Dosage

The fresh or ground powdered plant is taken internally as an infusion (tea). The standard single dose is 1.5 g of plant in 1 cup of liquid [3,50]. It should be noted that the plant is edible and enters into various preparations such as jellies, soft drinks and smoothies.

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4. (Cestrum commune and Cestrum tomentosum).

It is a perennial woody herb/shrub that can reach 2-6 meters in height with one or more fragile green stems. It is typical of disturbed sites in mesophyll forest, pine-oak forest and medium rainforest. The leaves when crushed emit a strong unpleasant odor similar to gum when crushed.

The traditional Mexican herbalist prescribes it for its cathartic properties (purgative) to the wood is used to remedy fever, in Tamaulipas the leaves are macerated in water and bathed the patients against the rash and for rubbed or smoked cleanings against the "mal de aire". In Ecuador the leaves and flowers are taken in infusion to alleviate the symptoms of the flu.

It is common in several rural areas of Mexico to see the leaves used in chicken nests to keep away bugs and the black fruit was formerly used as a dye (Standley, 1920-1926).

A nearby species present in the project area but in smaller quantities, Cestrum nocturnum ("smells at night") , is very interesting for its medicinal properties. It is used as an anti-inflammatory, analgesic (Mazumder et al., 2010), antimicrobial (Khan et al., 2011), antiepileptic (Perez et al., 2008), anticancer (Zhong et al., 2008), insecticide (Savchenko et al., 2000), local anesthetic (Zeng et al., 2002) and CNS depressant (Zeng et al., 2003). Flavonoids, alkaloids and phenols have been reported in this species (Prasad et al., 2013). Most of the flavonoids have hepatoprotective activity (Ali et al., 2013). The plant has been shown to be antioxidant (Al-Reza et al., 2010). In the 20th century Maximino Martinez refers to huele de noche as an antiepileptic. Later the Pharmaceutical Society of Mexico notes its use also as an antiepileptic and as a sedative.

In Central and South America, local species of Cestrum have been used as master and medicinal plants for quite some time. The Lacandon Maya, who have preserved many of the original beliefs of the ancient Maya, say that Kisin, the Lord of Death, was born from a Cestrum nocturnum flower. Therefore, it has been suggested that this plant may have played a role in Maya necromantic ritual (Ratsch 1998). The plant is also used as a stupefying amulet medicine in the West Indies (Voogelbreinder 2009). In Kathmandu, C. nocturnum flowers are presented as offerings to Shiva and Ganesh. Nepalese shamans create a ritual incense from the fresh leaves and flowers, eat the fresh flowers, and smoke them when dried to enhance spiritual healing energies. The plant is occasionally added to liquor in Kalinchok, a region north of Kathmandu (Voogelbreinder 2009, 126). In Mexican folk medicine, an extract of the leaves is used to treat epilepsy and other convulsive disorders, as well as headaches and nervous imbalances (Argueta 1994).

This herb is considered a major problem because of its toxicity to livestock and poultry that eat it when there is a lack of other food. The whole plant is toxic, stems, leaves, berries and even partially buried roots are a serious risk to livestock. The plant even when dried maintains its toxicity.

In the leaf shoots of Cestrum dumetorum beta-sitosterol has been identified and the presence of tannins has been detected (Domiguez 1985).

Alkaloids, flavonoids, saponins, terpenes, phenolic compounds, carbohydrates and volatile oils were identified in the genus Cestrum. Most of the flavonoids have hepatoprotective activity. Therefore, the hepatoprotective activity of C. nocturnum may be due to the presence

of flavonoids and phenolic compounds. In a study, aqueous ethanolic extract of C. nocturnum leaves was shown to have hepatoprotective activity against acetaminophen-induced hepatotoxicity in albino mice.(Qadir 2014).

The absolute of Cestrum nocturnum L. (Jessamine, “Night Queen,” Solanaceae) was analyzed by GC and GC/MS. More than 130 compounds could be detected of which more than 100 were identified. The compounds of olfactory value that were found with a concentration higher than 1 % were: linalool (3.1 %), benzaldehyde (2.5 %), benzyl alcohol (2.4 %), phenylacetaldehyde (2.4 %), cis-jasmona (2.1 %), benzyl acetate (1.8 %), phenol (1, 6 %), methyl jasmonate (1.5 %), 1,8-cineole (1.4 %), borneol (1.3 %), eugenol (1.3 %), linalyl acetate (1.2 %) and citronellyl propionate (1.1 %). In addition, more than 70 other interesting volatiles, as well as more than 10 higher hydrocarbons (above C16) and more than 10 fatty acids and their esters were also identified in the absolute. (Buckbauer 2011)

In fresh flowers, The major constituents were β-phellandrene (12.1 %), linalool (11.3 %), α-phellandrene (9.2 %), and (E)-β-ocimene (9.1 %).(Nickavar 2008). An n-hexane extract of fresh, mature leaves of Cestrum nocturnum (Solanales: Solanaceae) containing thin-layer epicuticular waxes was analyzed by thin-layer chromatography, infrared chromatography, and liquid gas using standard hydrocarbons. Seventeen long-chain alkanes (n-C18 to n-C34) were identified and quantified. Hentriacontane (n-C31) was established as the major n-alkane, while nonadecane (n-C19) was the least abundant component of the extracted wax fraction (Chowdhury 2010).

The steroids sapogenin tigogenin, smilagenin, and yucagenin were found in the leaves. The leaves also contain traces of nicotine. The composition of the plant's amazing aroma is not well understood, but many find that simply inhaling the perfume deeply is enough to induce a psychoactive state. The berries and leaves are said to induce hallucinations when consumed (Argueta 1994). C. nocturnum can cause poisoning in both humans and animals. Symptoms of this are similar to those induced by atropine, the substance found in Belladonna and other members of the Solanaceae family. Effects include hallucinations, nervous irritability, tachycardia, increased temperature, increased salivation and paralysis. However, C. nocturnum has not shown toxicity in several laboratory animal tests (Voogelbreinder 200). Some people, especially those with asthma, have reported difficulty breathing, irritation of the nose and throat, headaches, nausea and other negative symptoms when exposed to the odor of C. nocturnum. This may be due to the fact that the plant contains chlorogenic acid, which is a potent sensitizer. Some reports have indicated that ingesting the fruit results in elevated temperature, rapid pulse, and increased salivation (Erowid n.d.). Descriptions of shamanic smoking of dried C. nocturnum flowers in Nepal include experiences of “hallucinatory” effects without unpleasant side effects. However, since very little is known about this plant, and since it is a member of the nightshade family, great care should be taken when working with it in any form (Voogelbreinder 2009).

05a. (Salvia hispanica)

Mexico is considered one of the areas with the greatest diversity of the genus Salvia in the world (Ramamoorthy, 1984; Walker 2004), with approximately 300 species, a significant percentage of which (85-88%) are endemic (Ramamoorthy, 1984; Dieringer et al., 1991; Ramamoorthy and Elliott, 1998). Villaseñor (2004) indicates that *Salvia* is the second most diverse genus in the Mexican Republic (292 species). The greatest diversity of species of the genus *Salvia* occurs in the mountainous areas of Mexico, mainly in the center-south of the country (Espejo and Ramamoorthy, 1993). Consequently, temperate forests, particularly coniferous and oak forests, are the vegetation types with the highest proportion of *Salvia* species (Ramamoorthy and Lorence, 1987). However, they are also found in tropical deciduous and sub-deciduous forests, arid and desert areas (Dávila et al., 1993; Fernández et al., 1998; Ramamoorthy and Elliot, 1998).

Wild chia is a wild annual herbaceous plant that is quite common in the project area. It is typical of the mountainous areas and oak-pine forests of west-central Mexico.

The word chia comes from the Nahualt Chian (Chyan) word meaning oily and the Mexica used this term to refer only to the edible species of the genus Salvia whose distinctive characteristic is their high oil content (Salvia hispanica L., Salvia polystachya O., Salvia tiliifolia V. and possibly Salvia columbariae B.).

The seeds soaked in water release the mucilage, producing a practically tasteless gelatinous liquid; in Mexico it is flavored with vegetable juices or essences and consumed as a refreshing drink. The seeds can also be dried and ground to prepare a fine, intensely flavored flour, called pinole, which is consumed mainly as a sweet. The tender shoots are consumed as a raw or cooked vegetable and can be used in salads (4). They do not require grinding to be consumed, and can be added to smoothies, sprinkled on salads, soups, cereals, oatmeal, or yogurt, and mixed into virtually any cooking recipe.(3)

Before the conquest of America, chia was a staple food for the civilizations of Mexico; its cultivation was probably the third in economic importance, surpassed only by corn (Zea mays) and beans (Phaseolus vulgaris). In ancient times this seed was used by the semi-nomadic ethnic group of the Tecuexes (Chichimeca group), located in the current municipalities of Guadalajara, Zapotlanejo, Acatic, Tepatitlán, San Miguel el Alto, Cuquio, Yahualica, etc. and with which they paid tribute to the Mexicas.

The history of chia (Salvia hispanica L.) in Mexico is full of contradictions, since after being used as food and medicine for almost 4,500 years, in only three centuries it became almost unknown. According to Ayerza and Coates (2006), the tradition of its use was lost, because the Spaniards considering it sacrilegious prohibited its cultivation; however, there is no evidence to support this (Hernández and León, 1994) just as actually happened with amaranth (Chenopodium hypochondriacus L.) (Mapes, 2015; Mapes, 2017). The causes that reduced the use of chia in Mexico are several, however, Sosa et al. (2017) point out that the main ones were (a) the decrease of the native population where chia was part of the diet (Gerhard, 1986); (b) the abolition of tribute to the Mexica1 dominated peoples (Jiménez, 2009); (c) the replacement of its sown area by plant and animal species introduced from Europe (Hernández and León, 1994); (d) the modification of the diet due to the availability of new food sources (Román et al., 2013); and (5) crossbreeding resulting from Mexican crossbreeding with other breeds (Salzano and Bortolini, 2002). Regardless of the causes, the damage caused by

Spanish rule was so great that from an estimated 30,000 ha of chia cultivated in 1520, it was reduced to a few in 1810 (Sosa et al., 2017a).

Initially this seed was collected from wild plants (not cultivated), until the Tlaxcalteca and Otomí, brought to the region by the conquering Spaniards, domesticated it, thus initiating the cultivation of this important seed. Contributions were up to 1500 tons per year; it was used as food, as an offering to the gods, and as an oilseed to produce an oil as a base for body and decorative paints.

Chia shoots were offered to Chicomecóatl, the goddess of corn, during the feast of the hueytozoztli twenty; during the ritual hueytecuílhulhuitl twenty, pinole was prepared from toasted chia seeds to fill a boat, which was floated among the attendees, who took a portion of it until it was emptied.

In turn, the Purépecha of Michoacán used pinole to make small tamales that they used as an offering on the altar of their dead. The Codex Florentinus, which transcribes Fray Bernardino de Sahagún's Historia general de las cosas de Nueva España, details the importance of chia in the pre-Columbian economy; it describes in detail the production, commercialization and uses of this product (12).

Displaced by the cereals brought by the Spanish, chia cultivation disappeared during the colonies; it survived only in isolated mountainous areas of Mexico, Guatemala, and El Salvador. The largest production center in Mexico is in Acatic, Jalisco (,13) from where increasing quantities are exported to Japan, the United States and Europe. (14) Since 2011 Bolivia has initiated a chia production program with highly satisfactory results, achieving a quality that is already beginning to distinguish itself in the world market by being marketed with 99.5 % purity, although Acatic and surrounding municipalities in the Altos de Jalisco continue to be the main producers and exporters of seed worldwide, with purity percentages of up to 99.99.

Chia seed contains many nutrients such as: proteins, calcium, boron (a mineral that helps to fix calcium in the bones), potassium, iron, fatty acids such as omega 3, antioxidants and also trace elements such as magnesium, manganese, copper, zinc and vitamins such as niacin among others. The seed contains about 40% carbohydrates; of these, 30% is insoluble fiber, 3% is soluble fiber and the rest are essential starches. Compared to other foods, it has twice as much protein as any seed, five times more calcium than whole milk, twice the amount of potassium in bananas, three times more antioxidants than blueberries, three times more iron than spinach and seven times more omega-3 than salmon (2).

Oil composition

Vegetable fats and oils are made up, among other less abundant components, of fatty acids (FA): saturated, monosaturated, polyunsaturated, etc., generally esterified to glycerol. The FA composition of vegetable oils is an important characteristic from a nutritional and industrial point of view. According to its composition, chia seed oil has a predominance of unsaturated FA (about 75 % of the total), the most abundant being oleic (18:1, 6.9 %), linoleic (18:2, 18.8 %) and linolenic (18:3, 58.7 %) acids, the latter belonging to the omega-3 series. Furthermore, several studies have shown that chia seed additionally contains compounds with potent antioxidant activity, such as caffeic acid, myricetin, quercetin and kaemperol.

In recent years, numerous studies have been conducted proving the various benefits of chia consumption.(5) Its concentration of soluble fiber causes it to absorb a large amount of water, delaying absorption during digestion and increasing the feeling of satiety, which contributes to a lower food intake, with positive studies conducted by the Autonomous University of Yucatan in Mexico(6)or studies conducted at the Faculty of Medicine of the University of Ankara, Turkey.(7). Benefits have also been studied and concluded for maintaining blood pressure and blood sugar levels, reducing cardiovascular risk factors in hypertensive patients or those with type 2 diabetes (2, 8). There are no known toxic components in it. (9,10) There is no evidence to date that consumption of the seeds has adverse effects or drug interactions. Traditionally the plant is used to lower blood pressure(11).

Ayerza[8] in a study published in 2009 shows the differences found in crop growth, protein and lipid percentages and fatty acid concentration in seeds of *S. hispanica* genotype Tzoltzol from five different ecosystems.

Vicente Murillo et [al9] found no significant differences in the organoleptic characteristics and total fatty acid contents of oils from seeds grown in Cuba and Ecuador in relation to those grown in other parts of the world. The same conclusion was reached by another study comparing the fatty acid content of seeds grown in four states of Mexico.[10]

In the study that compares the chemical composition of *S. hispanica* seeds with those of flaxseed and rosehip, it is evident that the fatty acids present in these seeds are mostly polyunsaturated, such as linolenic acid in chia. The results also show that these oils can be used as possible sources of functional ingredients rich in omega-3 fatty acids.[11]

To characterize the proteins present in the seed of *S. hispanica*, a study was conducted at the Autonomous University of Querétaro, Mexico, which concluded that the main protein fraction corresponds to globulins, in addition to a positive balance of essential amino acids in the part of the plant studied.[12]

Research on the phytochemical profile and nutraceutical potentialities of *S. hispanica* showed that the plant is a seed with high antioxidant capacity and a novel source of isoflavone and can be incorporated into the human diet.[13]

In a study on the water absorption capacity, water and oil retention capacity and viscosity of *S. hispanica*, it was found that from a functional point of view, *S. hispanica* gum is also an important food ingredient due to its emulsifying and stabilizing properties.[4]

Preclinical studies

The knowledge that there is a relationship between highly nutritious diets, health and the reduction of the risk of chronic diseases has motivated research aimed at studying the so-called functional foods, among which *S. hispanica*, recognized as one of the major natural sources of omega-3 fatty acids and several important nutrients such as proteins, fiber and phenolic compounds, stands out.

Sargi et al. in a research published in 2013, which compared the chemical composition and antioxidant capacity of *S. hispanica* seeds with those of *Perilla frutescens* and *Linum usitatissimun,* revealed that they were excellent sources of alpha linoleic acid, a precursor of

long-chain polyunsaturated fatty acids. *S. hispanica* seeds showed the highest fatty acid content and intermediate antioxidant capacity relative to the other seeds investigated.[14]

In a study with a murine model of breast adenocarcinoma, it was shown that the addition of *S. hispanica* to the diet inhibited the growth and metastasis of this tumor model.[15]

Synthetic angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of cardiovascular disease, but may have undesirable side effects, whereas natural inhibitors lack these effects and are potentially nutraceutical. Proteins hydrolyzed from *S. hispanica* with the sequential alcalase-flavourzyme enzyme system resulted in hydrolysates with ACE inhibitory activity making it a potential functional food ingredient.[16]

The effects of this well-known plant on lipid and carbohydrate metabolism have been studied in various experimental [models17-19] by a group of researchers from the Universidad del Litoral, Santa Fe, Argentina, who set out to evaluate the preventive effect on the onset of dyslipidemia and insulin resistance (IR) in Wistar rats fed for 3 weeks on a semisynthetic diet with sucrose as carbohydrate and corn or *S. hispanica* seed oil as a fat source. The results showed that the *S. hispanica* seed diet prevented the occurrence of dyslipidemia, IR and reduced the existing visceral adiposity in the rats studied.[17]

Using a similar experimental model, an investigation was conducted to explore the mechanisms underlying altered lipid metabolism in the heart of insulin-resistant rats with dyslipidemia and to investigate whether *S. hispanica* seeds ameliorate cardiac lipotoxicity. Normalization of dyslipidemia and insulin resistance reduces plasma fatty acid availability, suggesting a different environment that prevents translocation on the FAT/CD36 transporter responsible for the regulation of fatty acid transport in the myocardium; this could reduce their influx, decrease M- CPT1 activity and lipid storage, and improve glucose oxidation in cardiac muscle. The authors point out that despite the warranted caution before extrapolating the results obtained in rodents to humans, *S. hispanica* seeds may serve as a dietary alternative in the treatment of these metabolic alterations susceptible to dietary manipulation.[20]

During 2012, a study was conducted with Chilean pregnant women. The main objective was to compare the acceptance of canola oil and *S. hispanica* oil, to evaluate the knowledge of omega-3 polyunsaturated fatty acids and the tendency to consume them. There was no significant difference between the acceptance of the two oils evaluated. It is concluded that *S. hispanica* oil could be used as a dietary alternative for pregnant women due to its alpha linolenic acid (ALA) content. ALA is the precursor of docosahexaenoic acid (DHA), which is involved in brain and visual development of the fetus and infant.[21]

In a randomized clinical trial conducted between 2012 and 2013, the modification of the fatty acid profile of milk obtained from Chilean mothers who received *S. hispanica* oil during gestation and lactation was evaluated. It was demonstrated that the intake of *S. hispanica* oil during pregnancy leads to a significant increase of DHA in breast milk, although further research is needed to scientifically support the recommendation of consuming ALA to increase the DHA content of breast milk.[22]

A randomized, double-blind, single-dose study was conducted in 11 male and female patients to study the acute effect of increasing doses of *S. hispanica* on glycemia and satiety. Reduction of postprandial glycemia and prolongation of satiety, both dose-dependent

responses, were observed. The decrease in postprandial glycemia offers a possible explanation for the improvement in blood pressure, coagulation markers and inflammatory markers previously observed after 12 weeks of *S. hispanica* supplementation in type II diabetes.[23]

In order to evaluate the effect of supplementation with *S. hispanica* flour on body composition, lipid values and blood glucose in overweight and obese individuals, individuals of both sexes were included in a randomized, double-blind, placebo-controlled study. *S. hispanica* supplementation for 12 weeks was shown to slightly reduce body weight and abdominal circumference clinically, and was only observed in the within-group analysis. The results of the study and its comparison with similar studies conducted previously, suggest investigating whether protocols with longer periods of supplementation may result in greater weight loss. Although improvement in lipid profile was observed, these effects were dependent on baseline values; decreased cholesterol and increased amounts of HDL-c were only evident in patients with altered baseline values.[24]

In the study of a longitudinal cut-off design conducted prior to the experiment carried out in Peru with the objective of evaluating in a group of university students the effect of *S. hispanica* consumption on constipation, it was found that a significant decrease in symptoms such as excessive straining to defecate, hard stools, incomplete evacuation, and obstruction or blockage takes place. These physiological effects of *S. hispanica* on constipation are attributed to its soluble and insoluble fiber content compared to other sources of fiber such as soybean or corn.[25]

In order to evaluate the potential therapeutic benefits of *S. hispanica* on pruritus in patients with end-stage renal disease, a study was conducted in Korea, although objective evaluation was difficult due to the characteristics of the symptom studied, but along with subjective measurements, functional measurements of skin characteristics such as epidermal barrier function of permeability, which manifests as water loss through the epidermis, skin hydration (expressed by skin capacitance), and skin surface pH were performed. The researchers concluded that the topical use of *S. hispanica* seed oil is effective for the treatment of pruritus and xerosis, is also beneficial for moisturizing the skin of healthy volunteers with xerosis, as well as that of patients with chronic renal failure or diabetes, and recommended further studies with larger numbers of patients, as well as additional studies to evaluate the efficacy of topical application of *S. hispanica* seed oil as an adjuvant treatment for chronic eczematous dermatitis.[26]

The effect of *S. hispanica* supplementation on blood pressure and associated cardiometabolic factors was investigated in Brazil and showed that *S. hispanica* meal has the ability to reduce blood pressure in both treated and untreated hypertensive patients, and lipid peroxidation and plasma nitrite concentrations only in patients who received *S. hispanica* supplementation .[27]

In a study published in 2009, U.S. researchers set out to evaluate the effectiveness of *S. hispanica* seed in promoting weight loss and altering disease risk factors in overweight adults. Body measures before and after chia seed supplementation and placebo administration, inflammation, oxidative stress, blood pressure, and lipoproteins did not differ between the *S. hispanica* and placebo groups, and the authors concluded that ingestion of 50 g/d of *S. hispanica* seed for 12 weeks had no influence on body mass or risk factors for various diseases.[28]

More recently, a systematic review by Brazilian authors was published that aimed to summarize the findings of studies that evaluated the effect of *S. hispanica* seed consumption in preventing and controlling cardiovascular risk factors. Seven studies were included in this review, most of which did not demonstrate statistically significant results in relation to cardiovascular risk factors and presented numerous limitations.[29]

Regarding adverse reactions, an article was published in 2015 about a 54-year-old patient with a history of allergic rhinitis and asthma who consumed *S. hispanica* seeds to lower cholesterol values and suffered an anaphylactic reaction caused by the seeds. The researchers identified lectin, an elongation factor, 11S globulin, and 3 others not described at the time of the study as the allergens involved.[30]

Salvia hispanica (chia) is recognized as an important source of polyunsaturated fatty acids and natural antioxidants, and it also contains essential amino acids that determine its nutraceutical potential. Its effectiveness has been studied to reduce the risk of certain cardiovascular diseases, reduce postprandial glycemia, prevent dyslipidemia, promote weight loss, alleviate pruritus in the final stages of renal disease and the symptoms associated with constipation, but more research is needed to scientifically support the proposed indications.

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05b. (Salvia elegans)

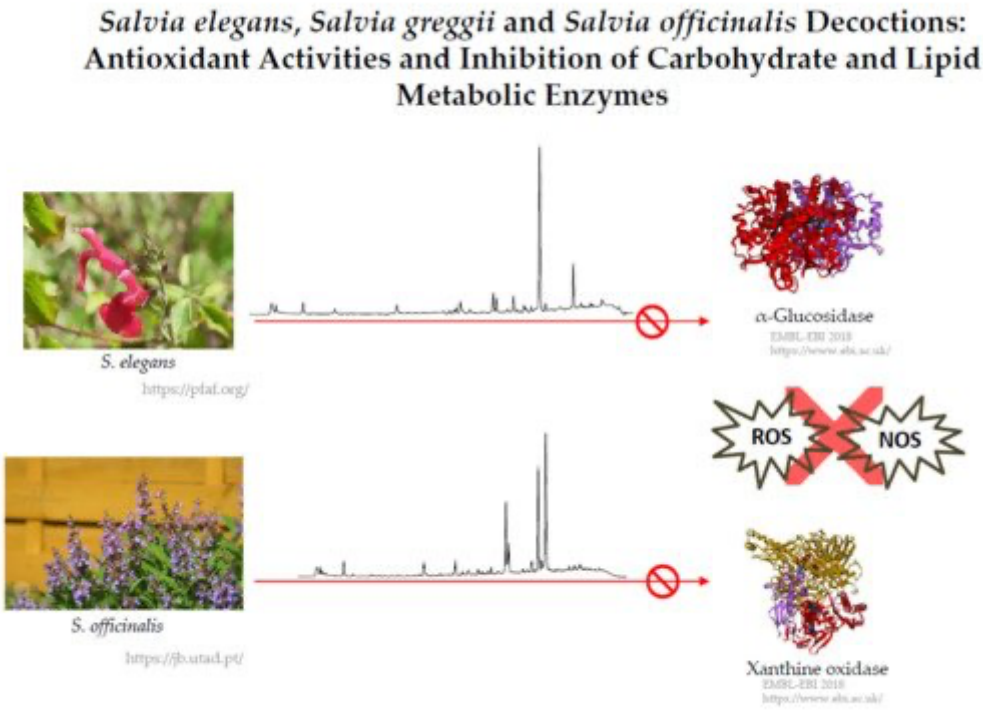
Salvia elegans is a perennial shrub native to Mexico and Guatemala. It inhabits the pine-oak forests of Mesoamerica between 1,800 and 2,700 meters and is quite common in the project area. It has tubular red flowers and an attractive pineapple-like leaf scent. It produces numerous, leafy erect stems and flowers in late fall. The red flowers are attractive to hummingbirds and butterflies. In the temperate highland forest in central Mexico, S. elegans was found to be one of the three species most visited by hummingbirds. It is a day plant. The flowering season in Mexico is from August onward; farther north, the flower may not bloom until late fall, and if there is no frost, it may bloom until spring.

The leaves and flowers of S. elegans are edible with a slight pine cone scent (1).

The plant is widely used in traditional Mexican medicine, especially for the treatment of anxiety, and also for the reduction of blood pressure. Although scientific information on these medicinal properties is scarce, a preliminary study on mice found support for the plant potentially having antidepressant and anxiolytic properties.(2) S. elegans has also been shown to have a dose-dependent, antihypertensive effect, attributed to its action as an angiotensin II receptor antagonist and angiotensin-converting enzyme inhibitor.(3)

Polar extracts of S. elegans have received some attention and, in particular, hydroalcoholic extracts have been shown to exhibit antihypertensive, antidepressant, and anxiolytic effects in in vitro models. However, to our knowledge, the bioactive components of S. elegans polar extracts and their ability to counteract oxidative stress-related events have not been previously elucidated.

In a pharmacological study, S. elegans decoctions were the most promising in terms of antioxidant activity and inhibitory potential against α-glucosidase, a fact that could be related to their richness in caffeic acid and its derivatives (4). In the essential oil, caffeic acid, rosmarinic acid, salvianolic acid B (which represents /0% of the total phenols of the plant), caffeoyl rosmarinic acid, cichoric acid, quinic acid and several flavones were found: 2,4 dimethyl Ba, luteolin, apigenin and scutellarin (4).



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05c. Salvia sessei Benth. (=Syn. Salvia calycinflata Sessé & Moc., S. dielytroides Roezl ex Van Houtte, S. fastuosa Sessé & Moc., S.roezlii Scheidw., S. semperflorens, S. souchetii Van Houtte) (Labiatae) (Labiatae)

Mex: sabanito, pipilolxóchitl" (from Nahuatl pipilōlxōchitl, 'flower of earring')

Aerial parts - decoction in washes - erysipelas (bacterial skin infection caused by Streptococcus pyogenes).

Pharmacological uses

A study has proven the anti-inflammatory, antibacterial (against Staphylococcus haemolyticus, Staphylococcus aureus, St.epidermis, Streptococcus pyogenes and antioxidant effect of methanolic, hexinic and dichloromethanic extracts (Gomez-Rivera 2018).

About Salvia sessei there are no reported uses in the data banks, but locally in Jalisco rural people use it as an anti-inflammatory on wounds and bruises.

06. Gaultheria procumbens L. (=Syn. Gaultheria humilis Salisb., Gaultheria repens Raf.) (Ericaceae)

Mexico: axocopaque, gaulteria

In traditional medicine, various tissues of G. procumbens and other Gaultheria species are used to treat inflammatory disorders, especially rheumatoid arthritis, swollen pain, chronic tracheitis, colds, and acute and chronic prostatitis [Middleton 1991). The analgesic and anti-inflammatory activities of these plants are believed to be mainly influenced by salicylic acid derivatives, especially methyl salicylate (essential oil), which acts through several mechanisms, including some antioxidant effects [Zhang 2011, Nikolic 2013].

The leaves were widely used by North American Native Indians in the treatment of aches and pains and to aid breathing while hunting or carrying heavy loads [Bown 1995]. An essential oil (known as 'wintergreen oil') obtained from the leaves contains methyl salicylate, which is closely related to aspirin and is an effective anti-inflammatory [Weiner 1980]. This species was once a major source of methyl salicylate, although it is now mainly synthesized [Bown 1995]. The leaves and oil are analgesic, anti-inflammatory, aromatic, astringent, carminative, diuretic, emmenagogue, stimulant and tonic [Grieve 1984, Mills 1990]. An infusion of the leaves is used to relieve flatulence and colic. The plant, especially in the form of essential oil, is most useful when applied externally in the treatment of acute cases of rheumatism, sciatica, myalgia, sprains, neuralgia and catarrh . The oil is sometimes used in the treatment of cellulitis, a bacterial infection that causes the skin to become inflamed. Some caution is advised, especially if the oil is used internally, as the essential oil is toxic in excess, causing liver and kidney damage. It should not be prescribed in patients hypersensitive to salicylates (aspirin). The leaves can be harvested anytime from spring to early fall, dried to make infusions or distilled to produce the oil [Bown 1995), the oil is also used in the treatment of cellulitis, a bacterial infection that causes the skin to become inflamed (Genders, 1977).

Pharmacological uses

Extracts of dried leaves of eastern teaberry (Gaultheria procumbens L.) were evaluated as a source of bioactive phytocompounds through systematic testing of activity and phytochemical profiles. Antioxidant efficacy was tested using five complementary in vitro models (DPPH; FRAP; linoleic acid (LA) peroxidation assay; O2-- and H2O2--scavenging tests) in parallel with standard antioxidants. The 75% methanol extract and its diethyl ether, ethyl acetate (EAF), n-butanol, and water fractions exhibited the dose--dependent responses in all assays, with the highest capacities found for EAF (DPPH EC50 = 2. 9 μg/mL; FRAP = 12.8 mmol Fe2+/g, IC50 for LA peroxidation = 123.9 μg/mL, O2-- SC50 = 3.9 μg/mL, H2O2 SC50 = 7.2 μg/mL). EAF also had the highest anti--inflammatory activity in the lipoxygenase and hyaluronidase inhibition tests (60.14% and 21.83% effects, respectively, at the concentration of 100 μg/mL). The activity parameters of the extracts correlated strongly with the levels of total phenols (72.4-270.7 mg GAE/g), procyanidins and phenolic acids, whereas only moderate effects were observed for flavonoids. Comprehensive UHPLC-PDA-ESI-MS3 and HPLC-PDA studies led to the identification of 35 polyphenols with a procyanidin A-type trimer, 3-O-glucuronide of quercetin, isomers of caffeoylquinic acids and (-)-epicatechin as dominant components . Significant activity levels, high phenolic contents and high extraction yields (39.4%-42.5% DW for defatted and crude methanol extracts, respectively) indicate the value of eastern teaberry leaves as bioactive products. (Michel 2013)Gaultheria procumbens and Boswellia carterii essential oils cause significant inhibition of acetylcholinesterase

enzyme activity and impairment of the antioxidative defense system of Sitophilus oryzae and Callosobruchus chinensis exposed to sublethal concentrations [Natural Bioactive Compounds, 2021).

The aim of this study (Nikolic 2013) was to examine the chemical composition and biological activity of the essential oil (EO) of Gaultheria procumbens L. against food spoilage and oral microorganisms. The components of EO were identified by GC-MS. Antimicrobial activity against food spoilage (five bacteria and six fungal species) and oral microorganisms (eight bacteria and thirty-two fungal species) was determined by microdilution and microplate biofilm assay, antioxidant activity was tested using the persistent free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), while antiradical activity was examined by fluorescence spectroscopy and electron paramagnetic resonance (EPR) spectroscopy. GC-MS analysis showed that methyl salicylate (96.90 %) was the major component of the oil. The essential oil inhibited the growth of all microorganisms. tested, i.e., food and oral bacteria and fungi, respectively (MIC 0.18-3.00 mg/ml and MBC 1.25-4.00 mg/ml; MIC 0.73-5.00 mg/ml and MFC 2.92-26.67 mg/ml); The oil effectively inhibited the biofilm formation of oral Streptococcus mutans and Candida albicans as well (MIC 25.00 MBC 50.00 mg/ml; MIC 12.50, MFC 50.00 mg/ml). In addition, the oil exhibited a dose-dependent DPPH radical scavenging activity with IC50 value of 30.61 mg/ml. The specific fluorescence probe 2-[6-(4-amino)phenoxy-3H-xanthen-3-on-9-yl]benzoic acid (APF) and the spin trap 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO), capable of simultaneous detection of different free radical species were used in the antiradical activity of the oil measurements. The oil showed moderate antiradical activity, reducing the amount of hydroxyl radicals produced to about 20% of the initial value.

Food use

Fruit - raw or cooked, palatable but tasteless. Fruit is not at all tasteless, has a very strong spicy germolene flavor, like being in a hospital waiting room[K]. Best after a frost, the fruit hangs on the plant until spring if not eaten by birds,]. Fruits can also be used in pies or in jams, Tender leaves - raw . A nice roadside snack if used when very young. Dry and powdery according to our taste buds[K]. A very pleasant tea is made from the fresh leaves . A stronger tea can be made by fermenting the bright red leaves first]. Wintergreen oil' can be distilled from this plant. It is used to flavor beer, candies, chewing gum, etc. [Facciola 1990).

The active components of the fruits were identified (UHPLC-PDA-ESI-MS3, isolation by preparative HPLC and NMR structural studies) and their biological capacity was evaluated in vitro in cellular and non-cellular models. It was revealed that fruits are the richest known dietary source of salicylates (38.5 mg per g dw fruit). They are also rich in procyanidins (28.5 mg per g dw fruit). Among the five solvents tested, acetone was the most efficient in concentrating the phenolic matrix (39 compounds identified; 191.3 mg g-1, 121.7 mg g-1 and 50.9 mg g-1 dry extract for total phenols, salicylates and procyanidins, respectively). Compared to the positive controls (dexamethasone, indomethacin and quercetin), the extract (AE) and pure salicylates showed strong inhibitory activity of proinflammatory enzymes (cyclooxygenase-2 and hyaluronidase). The analytes were found not to be cytotoxic (flow cytometry) to human neutrophils ex vivo. In addition, they significantly decreased, in a dose-dependent manner, the release of ROS, TNF-α, IL-1β and elastase-2 and slightly inhibited the secretion of IL-8 and metalloproteinase-9 in the cells. The observed effects could support the use of G. procumbens fruits as functional components of an anti-inflammatory diet and indicate the potential of EO for use in adjuvant treatment of inflammatory disorders cross-

linked with oxidative stress and associated with excessive production of TNF- α, IL-1β and elastase-2. (Michel 2020)

The aerial parts, leaves and stems of Gaultheria procumbens are herbal medicines rich in polyphenols with anti-inflammatory and antioxidant effects. The present study focused on identifying active markers of G. procumbens extracts in an integrated approach combining phytochemical and biological capacity tests. Target compounds, representing all classes of Gaultheria polyphenols, were pre-selected by LC-ESI-PDA-MS/MS. For unambiguous identification, key analytes, including a rare procyanidin trimer (cinnamtannin B-1), potassium salt of myquelianin and two new natural products: quercetin and kaempferol 3-O-β-d-xylpyranosyl-(1→2)-β -d-glucuronopyranosides, were isolated by preparative HPLC and investigated by spectroscopy (HR-ESI-MS, UV-vis, CD, 1D- and 2D-NMR), thiolysis, flame photometry, optical rotation experiments and absolute configuration studies. The significant contribution of the preselected compounds to the biological effects of the extracts was confirmed in vitro: the analytes significantly and dose-dependently decreased the prooxidant and proinflammatory functions of human neutrophils ex vivo (inhibited the release of reactive oxygen species, IL-1β, TNF-α and neutrophil elastase, ELA-2), inhibited two key proinflammatory enzymes (cyclooxygenase, COX-2 and hyaluronidase), and most of them, except gaultin, exerted potent direct antioxidant activity (ferric reducing antioxidant power and superoxide anion scavenging capacity). In addition, cell safety was confirmed for all compounds by flow cytometry. Finally, since these mechanisms have been related to the health benefits of G. procumbens, 11 polyphenols were accepted as active markers and a simple, accurate, reproducible and fully validated RP-HPLC-PDA method was proposed for the standardization of the target extracts.(Olszewska 2021)

Here we evaluated (Michel 2019) the phytochemical value and biological effects in vitro and ex vivo of the stems of one such plant, Gaultheria procumbens L.. The best extract for effective recovery of active parent molecules was established in comparative studies of five extracts. UHPLC-PDA-ESI-MS3, HPLC-PDA and UV-photometric assays revealed that the selected acetone extract (AE) accumulated a rich polyphenolic fraction (35 identified constituents; total content 427.2 mg/g dw), mainly flavanols (catechins and proanthocyanidins; 201.3 mg/g dw) and methyl salicylate glycosides (199.9 mg/g dw). The extract and its model components were effective inhibitors of cyclooxygenase-2, lipoxygenase and hyaluronidase; exhibited strong antioxidant capacity in six non-cellular in vitro models (AE and procyanidins); and also significantly and dose-dependently reduced reactive oxygen species (ROS) levels and cytokine (IL-1β, IL-8, TNF-α) and proteinase (elastase-2, metalloproteinase-9) release in human neutrophils stimulated ex vivo by lipopolysaccharide (LPS) and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP). The cell safety of AE was demonstrated by flow cytometry. The results support the application of the plant in traditional medicine and encourage the use of AE for the development of new therapeutic agents.

This study investigated (Verdi 2022) the chemical constituents of Gaultheria procumbens essential oil and is the first to relate cytogenotoxicity to oxidative metabolism and antimicrobial activity. Chromatographic analysis of the essential oil showed methyl salicylate (99.96 %) and linalool (0.04 %) as the major compounds. The essential oil showed no signs of cytogenotoxicity at different concentrations (1.82 to 58.34 mg mL-1). In addition, G. procumbens essential oil and methyl salicylate were used to evaluate the minimum inhibitory concentrations (MIC) and minimum microbicidal concentrations (MMC). The results showed efficacy against several microorganisms, including Aeromonas caviae, Candida albicans and

Mycobacterium fortuitum with MIC values ranging from 1.82 to 3.64 mg-ml-1 and MMC values ranging from 3.64 to 12.67 mg-ml-1, which were confirmed by time-kill kinetics. According to our results, the essential oil is a promising alternative for developing future formulations to treat infections caused by microorganisms.

The monotropitoside (gaulterin) is hydrolyzed by bacterial flora, giving rise to methyl salicylate, anti-inflammatory, analgesic, antipyretic, anticoagulant; The arbutoside has a urinary antiseptic effect. Diuretic emmenagogue. The essential oil, in topical use, has a rubefacient action. The tannins give it an astringent action (antidiarrheal, local hemostatic, healing). The essential oil called “wintergreen essence” (0.5-0.8%): it contains gaulterin, which can be split into methyl salicylate and primaveroside. Phenolic acids: salicylic acid, caffeic acid. Hydroquinonic heterosides: arbutoside. Catechin tannins.

In the essential oil were detected: α-Thujene, α-Pinene 2.24%, Camphene; Limonene 5.54, Fenchol 1.43, p-Mentha-1,3,8-triene 2.65, cis-Tagetone 6.16, Ethyl 3-octanoate 0.53, Methyl salicylate 61.14, n-Butanoic acid 7.06 , 7-Hydroxy-3,7-dimethyl octanal - 2.24, Eugenol 1. 73, 1-Ethenyl 3,5-dimethyl -benzene - 0.51, Ethyl cuminaldehyde 2.08, 1, 2-Dihydroxybutanyl 4-1′,2′,4′-trimethyl benzene - 0.06, o-Methylphenoxy-2,3-dimethyl benzene - 2.70 , 2-Butenyl isopulegol - 1.16, o-Methylphenoxy-2-propenyl toluene - 1.25 (Singh 2008).

Essential oils (EO) of Gaultheria procumbens were obtained by hydrodistillation with a yield of 1.30% ± 0.05 (v/dw) for leaves and 2.68% ± 0.08 (v/dw) for fruits. GC-FID/MS analysis led to the identification of 64 volatile components, among which 27 were found in leaf EC, 49 in fruit EC and 59 analytes were detected for the first time in G. procumbens. Methyl salicylate, which is known as a potent anti-inflammatory agent, was the dominant component accounting for up to 97.5 % and 99.8 % of the total essential oils from fruits and leaves, respectively. The remaining volatiles from leaves included mainly monoterpenes (α-pinene, β-pinene, limonene), methyl-o-anisate, masoilactone, spiro[4,5]decane-1-one and aliphatic alcohols (heptan-2-ol , octan-1-ol), while fruit EO contained, in addition to the dominant aliphatic and aromatic alcohols (benzyl alcohol, heptan-2-ol, p-cymen-7-ol), also carboxylic acids (3-hydroxyphenylacetic acid, heptanoic acid, octanoic acid) and aldehydes (hexanal, furfural, pent-4-enal). The antibacterial activity of EOs was evaluated by the broth microdilution method against twelve reference strains, as well as clinical and environmental isolates. The differences between the activity parameters of both ECs were not statistically significant ($p > 0.05$) for most of the bacteria tested. On the other hand, both ECs were significantly more effective against Gram-negative bacteria (MIC, 8.2-10.0 mg/mL) than against Gram-positive bacteria (MIC, 13.5-16.7 mg/mL). Leaves and especially fruits of G. procumbens grown in Poland proved to be valuable sources of methyl salicylate-rich essential oils of moderate antibacterial activity, which, therefore, could partly explain the traditional use of the plant materials and essential oils in the treatment of bacterial infections. related inflammatory disorders.(Magiera 2019)