PHYTOCHEMICAL PERSPECTIVE OF *EUPATORIUM* SPP.

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Summary

In the span of more than a century, seventy-three *Eupatorium* species inhabiting the geographical coordinate extremes: 20°24' to 17°55' North latitude, 100°04' to 103°44' West longitude have been collected and recorded. They are members of the diverse ecosystems located in tropical, subtropical and temperate regions, some of which are used in Mexican traditional medicine. The genus *Eupatorium* is the biggest of the tribe Eupatorieae, contains approximately 1,200 species largely distributed throughout the American continent, and has been subject to an intense and critical taxonomic revision. Given the scarce phytochemical and pharmacological information on the reported *Eupatorium* spp in this region of the world, the purpose of this work is to put into perspective the importance of the phytochemical study of *Eupatorium* spp as an opportunity to find novel molecules that can potentially contribute to the wellbeing of mankind in terms of healthcare and of the conservation of economic, environmental and cultural patrimonies.

Key words. *Eupatorium*, Michoacan, Mexico, secondary metabolites, biodiversity

Plants are sessile organisms that possessing physiological mechanism to detect and process information and then to discern and respond to environmental stimuli, they doing throughout their life cycles. In each step of the process of plant development (germination, growth, reproduction, and defense), the plant produces secondary metabolites that act as chemical messengers, which are the basis for the establishment of defense mechanisms and communication within and between species, forming a phytochemical arsenal that offers an excellent opportunity for the isolation, purification, and identification of chemical compounds for their potential application.

[1]**NOTE**. CBRL and EGS, they are sharing the first authorship of this manuscript.

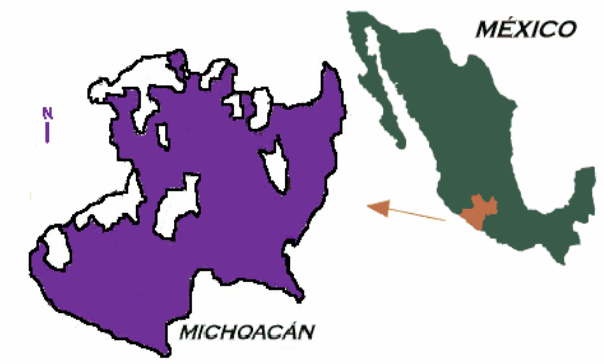

Figure 1. State of Michoacan de Ocampo. Extreme geographic coordinates: 20°24' to 17°55' North latitude, 100°04' to 103°44' West longitude. The shaded area corresponds to the territory in Michoacan where *Eupatorium spp* have been collected for over 100 years.

A possible approach is to use socio-cultural and botanical knowledge of native plants of the vast Mexican flora with or without biological or pharmacological relevance. It is common knowledge that government institutions and pharmaceutical industries around the world have had successful programs in the search for new drugs based on natural products and in particular on secondary metabolites of plants. In a general overview it is important to note that this potential source of new molecules and new sources of secondary vegetal metabolites is still unknown. Some observations supporting such lack of knowledge of the vegetal metabolome and that show the opportunity to find novel molecules that can potentially contribute to the wellbeing of mankind in terms of healthcare and of the conservation of economic, environmental and cultural patrimonies are described below.

The growing and increasingly reliable ethnobotanical and ethnopharmacological information on plants and on certain plant genera important in traditional medicine, as well as of related species and genera that can be sources of metabolites of pharmacological interest, has been compiled in a useful form, since there are regional floristic studies that represent an approximation of the plant biodiversity in the region, but not so in the case of ethnic or regional pharmacopeias. Therefore, in some region of Mexico, there are approximations of the floristic richness and thus the lack of knowledge of the potential phytochemicals, which could be of economic, cultural, and academic interest or in conservation manifests itself. It also enables the development of appropriate programs and plans for the protection of regional and national biodiversity.

The floristic studies show that Mexico is a country rich in biodiversity, its flora being 20 to 30 thousand species. Among these, it was found that the largest number of vegetal species is located in the Family Asteraceae. It was also noted that Michoacan is one of the Mexican states with a vast flora, location where we did this study (Figure 1) [1-3]. The ten families with the greatest diversity in the division Magnoliphyta [Format: Family (genus and species) in Mexico are: Asteraceae (402 and 3084), Poaceae (207 and 1,317), Fabaceae s. I. (164 and 2,028), Orchidaceae (156 and 1,062), Cactaceae (76 and 836), Euphorbiaceae (56 and 816), Rubiaceae (94 and 639), Lamiaceae (42 and 530), Solanaceae (34 and 458) and Cyperaceae (22 and 438), including non-native species [1].

The intrinsic central importance in the search of new drugs based on ethnomedical information on plants used in traditional medicine and its great value in the daily lives of native of any earth place is based on the fact that 65 % of the world's population makes use of medicinal plants [4,5]. In Mexico, the importance of using medicinal plants can be visualized by comparing the proportion of physicians using Western medicine with that of traditional physicians, healers, and shamans using medicinal plants for healing; the ratio is 1 to 4 [6]. It is also estimated that in Mexican traditional medicine there is a core group of approximately 3,000 medicinal plants used for the treatment of common diseases, of which approximately 1,000 have been used for over 400 years [7,8]. However, most of these plant species have not been subjected to phytochemical, toxicological, or pharmacological research.

The search for new active principles based on traditional use of plants, assumes that, both medicinal plants and those incorporated in the daily lives of the natives, provides evidence of biological activities with the likelihood of success in finding new active principles. It is known that plants have a great biological and chemical variability, which will directly modify the quantity of potential active principles. Such chemical heterogeneity in different groups of plants relocated and collected at different places and times of the year is this fact a problem in the process of identification, isolation and purification of plant components of interest [9,10]. Such salient fact leads to the pursuit of the same active principles in closely related vegetal species. This circumstance leading to another specific interest, which focuses on those plant species of same genus with no predation as it follows that they produce metabolites toxic to plants, microorganisms, insects, and vertebrates, of which there is scientific evidence that some of these metabolites are bioactive in humans.

The lack of full knowledge of the vegetal metabolome is demonstrated with the metabolomic analysis of *Arabidopsis thaliana,* the widely used biological model for the study of fundamental plant biology and whose genome is small and known, although its metabolome is unknown. Giavalisco et al. using the stable ^{13}C isotope in this plant showed the lack of knowledge of its secondary metabolism [11]. This research group obtained 1,024 chemical formulae, of which 12.5 % (128 metabolites) were registered and corresponded to metabolites of the primary metabolism but surprisingly 896 condensed formulae were not registered in databases such as: AraCyc, KNApSAcK, KEGG and NIST. All these databases are specific to plants, containing the information of metabolites reported in the literature with and without any biological or pharmacological significance including metabolic maps and information on the metabolic enzymes involved.

The central vision that the vegetal metabolome is composed of a large number of metabolites that remain to be discovered is strengthened adding to the possibility of finding new molecules, considering the conservative estimate that there are 250,000 terrestrial plants (angiosperms and gymnosperms), 15 % of which have been subject to phytochemical study and 6 % have been scrutinized for any biological activity [12,13]). Strengthening the observation with the fact of the persistence of medicinal plants in homes and popular markets is an indicator of the cultural value and an example of the effectiveness of this resource in healthcare. In Mexico, the State of Michoacan has more than 5,000 plant species [1,14]. Some of them are beginning to be subjected to studies of their pharmacological properties for example the determination of the antifungal and antioomycete properties of some medicinal plants used in traditional medicine in western Mexico [15,16]). While Morales Lopez et al. determined the effect of Bornyl-*O*-Deca-*2E,6Z,8E*-trienate obtained from *H. longipes*, a plant endemic to the central Mexican plateau, on the growth of pathogenic organisms [17]. Similarly, Raya Gonzalez et al. isolated a cyclitol from the *Enterolobium cyclocarpum* tree (common name, parota), pinitol—a molecule that induces biological effects of interest, specifically in glucose metabolism [10]. It is estimated that the phytochemistriy of 5 % of the plants that inhabiting in the State of Michoacan is known; although the medicinal plants used by natives of these lands have not been validated pharmacologically. In this area of the country many species of the genus *Eupatorium* grow and propagate and some of them have medicinal uses.

Eupatorium genus belongs to the tribe Eupatorieae, Subfamilly Asteroideae and Family Asteraceae. It is a genus complex with respect to its taxonomic classification. It 1,200 species have been described, suggesting that the center of origin of *Eupatorium* spp is the American continent

[18]. Therefore, the importance is greater in the case of a genus that groups important species in traditional medicine of several societies in five continents, in South America and Asia they are used to relieve maladies associated with signs and symptoms of microbial and parasitic infection.

Table 1. Use in traditional medicine, common name, organs used, and place of some species of the genus *Eupatorium* [19-30].

Species	Common Name	Use in traditional medicine	Part used	Country
E. buniifolum	Romerillo romerito	Antiprotozoal, disinfectant and to treat disorders of the Central Nervous System, digestive system, treatment of liver	Aerial	Argentina
E. candolleanum	Tabaco del monte	Laxative	Aerial	Argentina
E. lindleyanum	Ye Ma Zhui	Antihistamine and antibacterial	Whole	China
E. capillifolium	Dogfennel	Applied externally on insect and reptile bites	Aerial	Asia
E. inulaefolium	Sanalotodo	Washing bile and injuries	Aerial	Colombia
E. glandulosum	Mexican devil	Disinfecting injuries and to deparasitize	Leaves	India
E. ayapana	Snakeroot	Cardiac stimulant, laxative and anticoagulant	Leaves	India
E. glutinosum	Matico Serrano	Astringent, antirheumatic, antimicrobial, to treat ulcers, diarrhea and headaches	Leaves and stems	Ecuador and Perú
E. arnottianum	Clavel or uoué	Gastric pains, antimicrobial, antiviral, and contraceptive	Leaves	Argentina
E. hecatanthum	Uoué	Analgesic, antitusive and antispasmodic	Aerial	Argentina
E. salvia	Salvia macho, pagajosa, pega-pega	Antiseptic and treatment of insect bites	Whole	Chile

Another use is to treat disorders of the Central Nervous System and of the liver, as laxative and antihistaminic (Argentina), cardiac stimulants, laxative and anti-coagulants (India), astringent, antirheumatic (Peru) and as antiviral (Argentina) (Table 1).

The presence of species of *Eupatorium* in Michoacan territory dates from the late nineteenth century with the recollection and report in 1891 of a sample of *E. areolare*. Since then, plant specimens have been collected on near bodies of water or on the banks of streams and rivers, and all of them early have been taxonomically placed in the genus *Eupatorium* [31].

This territory has a range of 0 to 3,840 meters above sea level, has a great variety of climates, soils and vegetation types, which generate a variety of environmental conditions occurring in this region of the world, hosting an enormous biodiversity. In a conservative approximation of the presence of species of *Eupatorium* in this region, a group of 69 species of *Eupatorium* successfully thrive in 70 % of the territory. Also, it was detected a subgroup of ten species of the Mexican core group of *Eupatorium* with folk medicinal uses, see Table 2. Only, three of them are used in the region and another seven are used in other regions of the country.

Into Michoacan territory there are zones where various species of the genus *Eupatorium* are found, e.g. the Cuenca del Río Balsas, considered one of the regions with the greatest biodiversity and of economic importance in Mexico, is limited by the neovolcanic axis and the Sierra Madre del Sur.

Table 2. *Eupatorium* spp in Mexican traditional medicine [32-45].

Species	Common Name	Use	Chemistry
E. albicaule Schultz Bip. ex Klantt	Midracia	Pain	β-sitosterol, flavonoids, tannins
E. albicaule Schultz-Bip	Okiny, vishé, tokaban, tok'te', yaxal	Asthma, dissolution of renal calcium, diarrhea, fever	Flavonols, flavonol glycosides
E. areolare DC	Borreguillo	Digestive disorders, Diarrhea	Sesquiterpene lactones sharing unusual C_{10} diester side chain, 7-acetoxytinifoline, *cis*-hydroxy cinnamic acid, germacranolides, heliangolides, thymol derivative, areolal
E. aschenbornianum S. Schauer	Axihuitl	Skin problems, tumors, injuries and thrushes	5-acetyl-3β-angeloyloxy-2β-(1-hydroxy isopropyl)-2,3-dihydro benzofurane, 5-acetyl-3-β-angeloyl oxy-2β-(1-hydroxy isopropyl)-6-methoxy-2,3-dihydrobenzo furane, espeletone, encecalinol, O-methyl encecalinol, encecalin, sonorol, taraxerol, (+)-β-eudesmol, a mixture of β-sitosterol and stigamasterol, encecanescin
E. daleoides (D.C.) Hemsley	Palo de lodo	Dysentery, antiparasitic	Diterpenic acids, euparine derivatives, guaianolides, labdane derivatives, furano diterpenes.
E. glabratum Kunth	Chamisa, Hierba de la paloma, hierba verde, Jesús, sopilla, Jesús deni	Stomachache, postpartum bath	Acacetin, eupaglabric acid
E. morifolium Miller	Callotillo.	Rheumatic pain and itching	Morifoline, eudesmanolides, rinderine, 12-acethylrenderine,
E. odoratum L.	Tokaban, Cruz dulce grande, te kaban, tok, tok'aban, xtok'aban, tok'ja'aban, xtok'ja'aban; krus tok'te'	Puerperal fever, retention of urine, Pharmacology: Aqueous and methanol extract, spasmogenic action, antihepatotoxic, molluscicidal,	Eupatenol, sakuranetin, *iso*-sakuranetin, salvigenin, odoratin, acacetin, velutin, tamerixetin and mikanin
E. petiolare Moc.	Hierba del ángel or yolochíchitl, Amargocilla, coñesdá, co-ye-sa, coyes-da, cunisha, peshtó, ejutho, pechto, colochichi, yolochichi, huirapen	Digestive disorders, colics, gallstones, liver disease, purgative, dysmenorrheal postpartum baths, nervousness, cough, weight gain, diabetes	2α-*iso*-valeroyl oxyeperuic acid, kaurenoic acid, taraxasterol, 6-methoxy-benzyl salicylate, 11-13-dihydro-eupatoriopicrin and 2-hydroxy-6-methoxy-benzoic acid
E. pycnocephalum Less.	Hierba de la cruz, Hierba del burro, Cruz dulce chica	Postpartum baths, puerperal fever, inducing labor, erysipelas, cold	Caryophyllene derivative, deoxypetiolasides
E. quadrangulare D.C.	Carrizo, Tabaquillo	Body aches, swelling, postpartum recovery	Quadrangulin A, quadrangolide, sesquiterpene lactones
E. squarrosum Cav.	Prodigiosa	Stomach ailments, diarrhea, vermifuge	Atanasin, eupatolin, 5-hidroxy-4',6,7,8-trimethoxy flavone, gardenin B, glucoferide, santine, three derivatives of cativic acid and angeloyl-oxy-epoxy-brickelliol.

It has aprox. 58,643 km^2 that is shared with territories of several Mexican states, a fraction of land of 12,000 km^2 belong to Michoacan state and it has been explored for 30 years. During this period ten new species belonging to this genus and found only in this territory have been reported [46].

Eupatorium spp have been relocated to new genera and have given rise to synonyms such as *E. arsenei* [*Ageratina arsenei* (Rob.) King & Rob.] and *E. pulchellum* H.B.K. [*Chromolaena pulchella* (H.B.K.) King & Rob], since they have organs with similar morphological characteristics, mainly the flowers and leaves [47]. Hence, it is feasible to make a first approximation of the phylogenetic relationships of *Eupatorium* spp growing in Michoacan through the boarding molecular (ITS, RFLPs, microsatellite loci). Frequently the *Eupatorium* species having medicinal use are confused by the general population, for example *Eupatorium arsenei* known as the "angel herb" (hierba del angel; in Spanish language) used to relieve upset stomach and is commonly confused with *E. aschenbornianum*, *E. pazcuarense* and *E. petiolare.* While all these plants are used medicinally since prehispanic age, these uses do not coincide with current registries and none of their therapeutic applications have been confirmed experimentally. There is not mention of other *Eupatorium* sp from Michoacan of which there has not been detected a history of the medicinal use, nor chemical or pharmacological studies.

Isolated compounds from *Eupatorium* spp include monoterpenes, sesquiterpenes (guaianes, germacrenes, and cadinanes), diterpenes, triterpenes, flavanoids, and alkaloids of the pyrrolizidine type, specific examples of secondary metabolites purified from species of this genus have been integrated in the excellent revision by Zhang and colleagues, where the phytochemical potential of this genus is demonstrated [48].

However, with the information obtained relating to chemical, ethnobotanical, ethnopharmacological and traditional use of species in this genus it has become clear that the number of species of *Eupatorium* present in Michoacan is unknown and that the reported species are frequently re-classified. Likewise there is the exciting possibility of finding in species of the genus *Eupatorium* new molecules with biological activity, which serves as molecule prototypes for therapeutic candidates. Currently we give continuity to the phytochemical studies of *Eupatorium* spp that grow and thrive in Michoacan in the form of phytochemical studies biodirected in the search for secondary metabolites with biological activities of us interest such as; antimicrobial, cytotoxic and anticancer, antitermites, antioxidant, antiviral, vasodilatator and allelochemical.

Conclusion

The central idea of this work is that the metabolome of *Eupatorium* spp inhabiting in this region of the world is composed of a great number of metabolites that remain undiscovered is strengthened with the possibility of success in finding molecules that could potentially be therapeutic candidates in the near future.

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