

A revision of *Eupatorium* (Compositae: Eupatorieae) from Michoacan

Una revisión de *Eupatorium* (Compositae: Eupatorieae) de Michoacán

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Abstract. An important study in species of the genus *Eupatorium*, that inhabits other parts of the world, was conducted in the search for new molecules with pharmacological activities, thus showing that it is a potential source of novel secondary metabolites. We reviewed the number of *Eupatorium* species that have been described in the State of Michoacan, and that can be available for harvesting, to make a phytochemical screening. It was found that 69 *Eupatorium* spp are growing in 69% of the Michoacan territory. Within this group there are ten species used in Mexican traditional medicine. It was evident the difficulty in taxonomic classifying these species since several *Eupatorium* spp have synonyms. It appeared that the number of native members should be reduced in the genus. A preliminary chemical analysis was conducted on the flowers of nine native *Eupatorium* spp by nuclear magnetic resonance of hexane extracts. It was determined that the major secondary metabolites belonged to the chemical families of terpenes and chromenes.

Keywords: Biodiversity; *Eupatorium*; Phytochemistry; Michoacan; Terpenes.

Resumen. En la búsqueda de nuevas moléculas con actividad farmacológica se efectuó un estudio de revisión importante en especies del género *Eupatorium* que habitan otras latitudes del mundo. Se obtuvo con ello que este género es una fuente potencial de metabolitos secundarios novedosos. Este hecho nos condujo a conocer el número de especies de *Eupatorium* que fueron descritas en el Estado de Michoacan, y cuáles estaban accesibles para su recolección e iniciar un escrutinio fitoquímico. Se detectaron 69 *Eupatorium* spp creciendo en el 69% del territorio, incluyendo diez especies utilizadas en la medicina tradicional mexicana. Debido a que especies de este género presentan sinonimia, se evidenció la dificultad para clasificar taxonómicamente a las especies de éste género; todo indicó que se reduciría el número de integrantes nativos. Un análisis químico preliminar de las flores de nueve *Eupatorium* spp nativas por resonancia magnética nuclear de los extractos hexánicos permitió determinar que las familias químicas principales fueron: terpenos y cromenos.

Palabras clave: Diversidad biológica; *Eupatorium*; Fitoquímica; Michoacán; Terpenos.

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INTRODUCTION

In the Michoacan State, there are sharp contrasts among different environments. This results in an extraordinary genetic wealth, with variations within and between species in the various ecosystems. It occupies the fifth place in national biodiversity (Estrategia para la Conservación y Uso Sustentable de la Diversidad Biológica de Michoacán, 2007). In this web of life, man is another member that depends of it for its welfare and survival. He makes use of plant natural resources such as species of the *Eupatorium* genus, which has approximately 1200 species and is one of the largest in the Family Asteraceae. It is widely distributed in five continents; however, the highest number of species is in the tropical, subtropical and temperate Americas (Katinas et al., 2007).

Several species of this genus are used in traditional medicine to relieve various illnesses of humans, animals and plants (Zardini, 1984; Editorial Committee of the Administration Bureau of Traditional Chinese Medicine 1998; Rondina et al., 2003; Sasikumar et al., 2005). It is estimated that in the different native Mexican pharmaceutical products and traditional uses, there is a core group of approximately 3000 medicinal plants used for treatment of common diseases. Only 1000 medicinal plants have been used for over 400 years, where several species of the genus *Eupatorium* have been used for the same purpose (Lozoya, 1994; Deciga et al., 2007; Biblioteca Digital de la Medicina Tradicional Mexicana. Acronym BDMTM-UNAM, 2009).

The ethnopharmacological information about medicinal plants has been an important basis for research and development of new drugs that currently contribute to human welfare by alleviating pain and improving their economy. Recurring research themes in the plant kingdom, as well as their economic impact for man, include the unabated search for new molecules (1) with specific non-toxic pharmacological effects, and (2) effective in controlling human diseases. In this respect, considerable phytochemical studies have been done in *Eupatorium* spp, as demonstrated by Zhang et al. (2008). They compiled 149 purified compounds during the past twenty years, some of which are immunomodulatory, cytotoxic and antimicrobial agents.

Obtaining phytochemical knowledge of the genus has various purposes such as (1) chemotaxonomy in fundamental plant biology, and (2) an important plant source in the search for new bioactive molecules against resistant organisms to conventional pesticides (e.g., weeds, insects, fungi, oomycetes, bacteria) (Assie et al., 2007; Sosa & Tonn, 2008). *Eupatorium* spp. has provided various biotechnological uses: (1) essential oils to deter insects, (2) had a lethal effect at the larval stage in the vector mosquito *A. aegypti*, and (3) has been used as feedstock for the production of petroleum substitutes (Belter, 2009). Eight oz (227 g) of *Eupatorium* essential oil have been offered at US\$ 10 for use in aromatherapy in alternative and

complementary medicine. These last uses lead to place *Eupatorium* diversity at an ecological risk in this region. Most species of the genus *Eupatorium*, however, have not been used in traditional medicine, and they have been considered as weeds. These species have been phytochemically unstudied and they might yield products of great importance to humans. Here is where the ethnobotanical knowledge becomes particularly important for a rational use of natural resources. These are arguments for knowing and conserving plant biodiversity. However, the link between undiscovered biodiversity and potential usefulness is largely conjectural. It is necessary to re-evaluate the biodiversity, and its associated potential phytochemistry, in any area of the planet (e.g., the Michoacan territory).

The objectives of this study were (1) to be aware of the *Eupatorium* species that have been described in the State of Michoacan, and which of them are accessible for harvesting, and (2) to conduct a qualitative, phytochemical screening in the collected plants.

MATERIALS AND METHODS

Study area. Three areas were studied: (1) the area where the genus *Eupatorium* has been historically present (20° 24', 17° 55' N; 100° 04', 103° 44' W; State of Michoacan, Mexico) (Fig. 1); (2) the harvesting area of *Eupatorium* spp., a circumference with a radius of 100 km, with central point at 19° 42' 10" N, 101° 11' 32" W. This point is located on Madero avenue, in front to the Cathedral of Morelia, State Capital; interval altitude 1400 – 3090 m.a.s.l., and (3) the extraction site of plants, adjacent to highways and main roads that converge to the State capital. All these study sites include disturbed ecosystems. Climate is temperate (6 to 28 °C) and rainfall varies between 700 to 1000 mm per year.

Fig. 1. State of Michoacan de Ocampo, Mexico (17° 55' - 20° 24' N; 100° 04' - 103° 44' W). The shaded area corresponds to the Michoacan territory where *Eupatorium* spp have been collected for over 100 years. Modified from <http://www.michoacan.gob-mx>
Fig. 1. Estado de Michoacán de Ocampo, México. Coordenadas geográficas, (20° 24' - 17° 55' N; 100° 04' - 103° 44' O). El área sombreada corresponde al territorio michoacano donde *Eupatorium* ha sido colectado por más de 100 años. Modificado de <http://www.michoacan.gob-mx>



Collection of information. It was compiled from various historical databases to determine the distribution and presence of *Eupatorium* spp in the state of Michoacan, Information was obtained from the Herbarium of the Faculty of Biology at the University of Michoacan; the first plant collection date was Nov 01, 1978 (Voucher 3067): it corresponded to *Eupatorium petiolare* Moc ex DC to this date with a total of 216 records. We also relied on the written record of phytochemistry research, obtained during 15 years, compiled in the Chemistry of Natural Products Laboratory of the Instituto de Investigaciones Químico Biológicas of the Universidad Michoacana de San Nicolas de Hidalgo (UMSNH). Any report of *Eupatorium* spp presence on any study area is considered within the geographical distribution of this species.

Collection and identification of the study plant species. Plant harvesting was guided by REN del Rio, and carried out by the authors of this work. Harvesting of specimens and plants of *Eupatorium* spp used for qualitative phytochemical analysis in this work was made at the flowering developmental morphology stage between October to April 2004 to 2010 (Table 1). Plants were prepared for botanical determination by botanists and further photographic records were taken. Plant species were herborized, authenticated and deposited in the Herbarium of the Facultad de Biología, UMSNH.

Obtaining extracts from the flowers of *Eupatorium* spp. *Eupatorium* spp flowers used in the qualitative chemical study were successively macerated with hexane. Maceration time was 3 days at room temperature, and it was protected from light. Each extraction stage within this process was done 3 times until exhaustion of all components. The flower extracts obtained from each stage were first mixed and then concentrated on a rotary evaporator.

Nuclear magnetic resonance spectroscopy (NMR). NMR Spectra of ^1H and ^{13}C were obtained on a Varian Mercury plus 400 spectrometer. The spectra of Hydrogen (^1H) were obtained at 400 MHz using eight repetitions. The spectral window was 6000 Hz.

RESULTS AND DISCUSSION

Distribution of *Eupatorium*. Michoacan has an important physiographic complexity, a variety of climates and soils, and about five thousand plant species that makes it an invaluable natural resource (Estrategia para la Conservacion y Uso Sustentable de la Diversidad Biológica de Michoacan, 2007). Little is known about the chemical and biological wealth of this genus, which in some cases has been used improperly. Within the geographical distribution of

Eupatorium spp, physiographic conditions, climate and soils are important factors for the species establishment in the territory of Michoacan. Generally, *Eupatorium* spp have been collected near water bodies or on the banks of streams and rivers in temperate, subtropical and tropical climates, with high relative humidity. They have also been collected at the margin of forests and at different forest types (Espinosa & Rodriguez, 1995; Fernández et al., 1998; Medina et al., 2000; Herbario de la Fac. de Biología-UMSNH; Laboratorio de Química de Productos Naturales-IIQB; Estrategia para la Conservación y Uso Sustentable de la Diversidad Biológica de Michoacán, 2007).

Sixty nine percent of the Michoacan territory was inhabited by some species initially reported as *Eupatorium* (Fig. 1). Of 113 municipalities of Michoacán, 42 of them did not have any reports of the presence of *Eupatorium*: these are marked as white areas in Fig. 1. It appears that there have not been any explorations in these areas for harvesting *Eupatorium* spp. However, the presence of *Eupatorium* spp has been reported around these areas, and it is likely that this genus inhabited them. We must keep in mind that the geographical distribution of *Eupatorium* species within the territory of Michoacan is specifically associated with the delimitation of natural regions.

***Eupatorium* spp.** The study of the genus *Eupatorium* in Michoacan begins in the last decade of the XIX century. This is evidenced by the earliest historical record made by the botanist C. Pringle, who collected a plant specimen in 1891 in Patzcuaro, Michoacan. This is the first occasion that the presence of *E. areolare* was reported (Espinosa & Rodriguez, 1995). Since then, 69 plant species of *Eupatorium* have been described, which still survive in Michoacan state. The number of species presented in this work was obtained from approximate floristic inventories and plant species initially classified as *Eupatorium* (Table 1). We found the remarkable presence of members of this genus given by the number of species. However, today is not yet feasible to make a direct and accurate calculation of the number of *Eupatorium* spp at the taxonomic level, and the possibility of relocating and finding new *Eupatorium* spp is not ruled out. The approximate floristic inventories that describe the plants in this area of Mexico allowed us to study the *Eupatorium* spp diversity in the region in detail. However, sources of false estimates and imprecise analysis of the geographical distribution and partitioning of the number of species present in this territory include: relocation of species; absence of information from remote areas; little or no dissemination of existing information, and unpublished plant research. The distribution and habitats of *Eupatorium* spp are clearly segregated from each other, and seem to depend on environmental factors.

Table 1. *Eupatorium* species recorded in Michoacan. Synonyms are indicated in brackets; ◀ = Medicinal; ‡ = Single Registration; ■ = Species with qualitative phytochemistry study in this work.

Tabla 1. Especies de *Eupatorium* registradas en Michoacán. Sinónimos están indicados entre corchetes; ◀ = Medicinal; ‡ = Registro único; ■ = Especies en estudio fitoquímico cualitativo en este trabajo.

<i>Eupatorium</i> spp		
<i>E. adenophorum</i> ■ Spreng [<i>E. glandulosum</i> HBK; <i>Ageratina adenophora</i> (Spreng) King & Rob]	<i>E. dolichobasis</i> ■ Mc Vaugh [<i>Ageratina dolichobasis</i> (Mc Vaugh) King & Rob]	<i>E. odoratum</i> ◀ L [<i>Chromolaena odorata</i> (L) King & Rob]
<i>E. adenospermum</i> Sch Bip [<i>Dyscritogyne adenosperma</i> (Sch Bip) King & Rob]	<i>E. aff dolichobasis</i> Mc Vaugh	<i>E. oligocephalum</i> DC [<i>E. erythrocomun</i> Rob; <i>Ageratina oligocephala</i> (DC) King & Rob]
<i>E. ageratifolium</i> ‡ DC	<i>E. epilobiodes</i> ‡ HBK	<i>E. oresbium</i> BL Rob
<i>E. albicaule</i> ◀ Sch Bip ex Klatt [<i>E. leucoderme</i> BL Rob]	<i>E. etlense</i> ‡ BL Rob	<i>E. palmeri</i> ‡ A Gray
<i>E. ageratoides</i> ‡ L f	<i>E. geminatum</i> Mc Vaugh	<i>E. pazcuarensis</i> HBK [<i>E. grandidentatum</i> DC; <i>E. skutchii</i> BL Rob; <i>Ageratina pazcuarensis</i> (HBK) King & Rob]
<i>E. areolare</i> ◀ ■ DC [<i>Piptothrix areolare</i> (DC) King & Rob; <i>Ageratina areolaris</i> (DC) Gage]	<i>E. glabratum</i> ◀ ■ HBK [<i>Ageratina glabrata</i> (HBK) King & Rob]	<i>E. peltrophum</i> ‡ BL Rob
<i>E. aff. areolare</i> ‡ DC	<i>E. glomeratum</i> ‡ DC	<i>E. petiolare</i> ◀ Moc ex DC [<i>Ageratina petiolaris</i> Moc ex DC King & Rob]
<i>E. arsenei</i> ■ BL Rob [<i>Ageratina arsenei</i> (Rob) King & Rob]	<i>E. gracilicaule</i> ‡ Sch Bip ex BL Rob	<i>E. polybotryum</i> DC [<i>Ophryosporus petraeus</i> BL Rob; <i>Dechachaeta ovatifolia</i> (DC) King]
<i>E. cf arsenei</i> BL Rob	<i>E. halbertianum</i> Mc Vaugh [<i>Ageratina halbertiana</i> (Mc Vaugh) King & Rob]	<i>E. prunellifolium</i> HBK [<i>E. saltivarii</i> Sch Bip; <i>Ageratina prunellifolia</i> (HBK) King & Rob]
<i>E. aschenbornianum</i> ◀ ■ S Schauer [<i>E. pichinchense</i> HBK; <i>E. aschenbornianum</i> Schauer; <i>E. bustamenta</i> DC; <i>Ageratina aschenborniana</i> (Schauer) King & Rob; <i>A. bustamenta</i> (DC) King & Rob; <i>A. pichinchensis</i> (HBK) King & Rob]	<i>E. hebebotryum</i> (DC) Hemsl [<i>Critonia hebebotrya</i> DC]	<i>E. pulchellum</i> HBK [<i>Chromolaena pulchella</i> (HBK) King & Rob]
	<i>E. hymenolepis</i> ‡ BL Rob	<i>E. pulchellum</i> var <i>angustifolium</i> ‡ Watson
	<i>E. incarnatum</i> ‡ Walter	<i>E. pycnocephalum</i> ◀ ■ Less [<i>Fleischmannia pycnocephala</i> (Less) King & Rob]
<i>E. bellidifolium</i> ‡ Benth	<i>E. incomptum</i> DC	<i>E. quadrangulare</i> DC [<i>E. thyrsoides</i> DC]
<i>E. bertholdii</i> var <i>stenophyllum</i> ‡ BL Rob	<i>E. isolepis</i> ‡ BL Rob [<i>Ageratina isolepis</i> (BL Rob) King & Rob]	<i>E. ramireziorum</i> Esp [<i>Ageratina ramireziorum</i> (Esp) Turner]
<i>E. blepharilepis</i> Sch Bip [<i>Ageratina blepharilepis</i> (Sch Bip) King & Rob]	<i>E. lasioneuron</i> ■ Hook & Arn [<i>Ageratina lasioneura</i> (Hook & Arn) King & Rob]	<i>E. rhomboideum</i> HBK [<i>Ageratina rhomboidea</i> (HBK) King & Rob]
<i>E. brevipes</i> DC [<i>Ageratina brevipes</i> (DC) King & Rob]	<i>E. lasium</i> ‡ BL Rob	<i>E. rivale</i> Greenm
<i>E. calophyllum</i> (Greene) BL Rob [<i>Ageratina calophylla</i> (Greene) King & Rob]	<i>E. ligustrinum</i> DC [<i>E. erythropappum</i> BL Rob; <i>Ageratina ligustrina</i> (DC) King & Rob]	<i>E. rubricaulis</i> HBK [<i>E. cylindricum</i> Mc Vaugh; <i>E. amplifolium</i> A Gray; <i>Ageratina rubricaulis</i> (HBK) King & Rob; <i>A. cylindrica</i> (Mc Vaugh) King & Rob]
<i>E. cardiophyllum</i> BL Rob [<i>Ageratina mcvaughii</i> (King & Rob)]	<i>E. longipes</i> A Gray [<i>Brickellia pedunculosa</i> (DC) Harc & Beaman; <i>Phanerostylis pedunculosa</i> (DC)]	<i>E. scabrellum</i> ‡ BL Rob
<i>E. ceriferum</i> Mc Vaugh [<i>Ageratina cerifera</i> (Mc Vaugh) King & Rob]	<i>E. macrophyllum</i> ‡ L	<i>E. schaffneri</i> Sch Bip ex BL Rob [<i>Ageratina schaffneri</i> (Sch Bip) King & Rob]
<i>E. choricephalum</i> BL Rob [<i>E. cilliatum</i> Less; <i>Ageratina coricephala</i> (BL Rob)]	<i>E. mairetianum</i> ◀ DC [<i>Ageratina mairetiana</i> (DC) King & Rob]	<i>E. aff schaffneri</i> ‡ Sch Bip ex BL Rob

<i>E. collinum</i> ◀ DC [<i>Chromolaena collina</i> (DC) King & Rob]	<i>E. aff mairetianum</i> ‡ DC	<i>E. solidaginifolium</i> ‡ A Gray
<i>E. collinum</i> var <i>mendezii</i> Mc Vaugh	<i>E. malacolepis</i> BL Rob	<i>E. sordidum</i> Less
<i>E. conspicuum</i> Kauth & Bouche [<i>Ageratina conspicua</i> (Kunth & Bouché) King & Rob]	<i>E. mariarum</i> ‡ BL Rob	<i>E. squarrosus</i> ◀ Cav [<i>Brickellia squarrosa</i> (Cav) BL Rob]
<i>E. crassirameum</i> BL Rob [<i>Ageratina crassiramea</i> (BL Rob) King & Rob]	<i>E. monanthum</i> Sch Bip	<i>E. triangulare</i> ‡ (DC) BL Rob
<i>E. cremastum</i> BL Rob	<i>E. muelleri</i> Sch Bip ex Klatt [<i>Ageratina muelleri</i> (Sch Bip ex Klatt) King & Rob]	<i>E. vernicosum</i> ‡ Sch Bip ex BL Rob
<i>E. deltoideum</i> Jacq [<i>Ageratina deltoides</i> (Jacq) King & Rob]	<i>E. nelsonii</i> BL Rob	<i>E. cf vernicosum</i> ‡ Sch Bip Ex Green

Eupatorium taxonomic considerations. The genus *Eupatorium* belongs to the tribe Eupatorieae, one of the thirteen tribes of the Asteraceae family. It brings together nearly 1200 species distributed mainly in tropical regions of America, Europe, Africa and Asia (Zhang et al., 2008). This genus has been under intense and critical taxonomic revision. The morphological features of the plant are often indistinguishable, and give rise to synonymous species (e.g. see Table 1). For example, the case of the medicinal plant *E. albicaule* Sch. Bip. ex Klatt which is synonymous with *E. leucoderme* B.L. Rob., or *E. quadrangulare* D.C. which is synonymous with *E. thyrsoideum* D.C. To date, a taxonomic level of species does not allow to make a direct and accurate calculation of the number of *Eupatorium* spp that inhabit the Michoacan territory. This is because, at least in part, species already identified as *Eupatorium* had been recently relocated to other taxa: for example, *E. adenophorum* Spreng has been relocated to the genus *Ageratina* as *A. adenophora* (Spreng) King & Rob., or *E. areolare* DC. has been relocated to the genus *Piptothrix* as *P. areolare* (DC.) King & Rob.

We demonstrated that 31 species referred to as *Eupatorium* have been recorded only once in the last 120 years (‡) (see Table 1). How do we explain it? It might be that (1) they have been transferred to other genera, (2) known as synonyms, or (3) have been true native, extinct plants. The evidence based on morphological characters indicates that the large plant group historically referred to as *Eupatorium* sp tends to decrease in Michoacán. Thirty eight species initially recorded as *Eupatorium* have been relocated into the genera *Ageratina* (28); *Brickellia* (2); *Chromolaena* (3); *Critonia* (1); *Dyscritogyne* (1); *Fleischmannia* (1); *Ophryosporus* (1), and *Piptothrix* (1), and the taxonomical position is doubtful for six of them (aff.; cf) (see Table 1).

This review of the genus *Eupatorium* has led to the generation of new genera, new species and new names. This was an activity that is currently enhanced by the advent of molecular taxonomy, complemented by cytologic examination to determine the ploidy [minimum level of ploidy = 10 chromosomes], hybrid derivatives, as well as the strategies of reproduction (apomixes and agamospermy) (Sullivan, 1972).

Therefore, we do not rule out the possibility of finding more species of this genus inhabiting in Michoacan. Currently, we are working on the biology of *Eupatorium* supported by molecular approaches using markers such as ITS, RFLP and other strategies of molecular biology and genetics (Ito et al., 2000; Peters et al., 2008).

Eupatorium spp in traditional medicine. Most *Eupatorium* species include plants with a large colonizing ability. However, some of them are considered weeds, and in some instances, a pest (*E. odoratum*) (Moni & George, 1959; Lvens, 1974; FAO). Other species have been incorporated into the human lifestyle with different applications, one of which is medicinal. In Mexico, at least 12 *Eupatorium* spp have been described with uses in traditional medicine (Table 1). *Eupatorium daliodes* DC Hemsley and *E. morifolium* Mill were not included in this study as they have not been detected in Michoacan (Magaña et al., 2010). All of them are used for the treatment of various human ailments with signs of microbial infection, parasites, stomach ache, body aches (head, muscle), central nervous system and liver disorders, as laxatives and antihistamines, cardiac stimulant, anticoagulant, astringent, antirheumatic, antiviral and immunostimulants (Zardini, 1984; Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1998; Rondina et al., 2003; Sasikumar et al., 2005). For example, *E. squarrosus* (common name, prodigiosa) and *E. petiolare* (common names, amargocilla, angel herb) are used with the above mentioned purposes in Michoacan.

These two species are often misidentified, and confused with *E. aschenbornianum* and *E. pazcuarensis*. Thus, plants of both species can be considered as medicinal plants. However, it appears that many species have no historical records of their medicinal uses. This observation is extended to many traditional medicinal plants; that is, there are no chemical studies and pharmacological effects that give occidental medical support to its therapeutic use.

In this study, at least 10 *Eupatorium* spp with medicinal use grew and prospered in this territory; 8 of them are used with the same medicinal purpose in other states, and they

are part of the local Mexican indigenous pharmacology. The lack of medicinal use given to some *Eupatorium* species in other Mexican latitudes might be the inhabitant ignorance or idiosyncrasy in that region because they are attached to tradition.

Michoacan has enough habitats conducive to the spread of *Eupatorium* spp used in traditional medicine without a negative impact on the native ecosystem. This can be considered an opportunity to (1) improve income through a systematic production in backyards at the rural environment, and (2) contribute to social rooting. Such activity can potentially prevent frequently observed rural conflicts derived from the harvest of this natural resource, disturbance of local growing sites and natural spreading, possible predation and extinction in the region. The major conflict with farm owners where they spread these species is theft (Damian et al., 2008).

Phytochemistry of the genus *Eupatorium*. The biological diversity in *Eupatorium* spp is the result of billions of years of evolution under the increasing pressures of human activities. The chemical constituents reported in the genus *Eupatorium* is good enough. However, Zhang et al. (2008) reported purified components of two species that grow in Michoacan, *E. adenophorum* and *E. odoratum*. Some *Eupatorium* sp are having little information on their phytochemistry: eg. *E. petiolare* (2- α -isovaleroyl-oxy-epiuric and kaurenoic acids, taraxasterol, benzyl 6-methoxy-salicylate, 11,13-dihydro-eupatoriopicrin and 2-hydroxy-6-methoxy-benzoic acid); *E. squarrosus* (atanasin, eupatolin, 5-hydroxy-4',6,7,8-trimethoxyflavone, gardenin B, glucoferido and santin, three derivatives from cativic acid and angeloyl-oxy-epoxy-brickelliol); *E. aschenbornianum* (enceclescine), *Ageratina pichinchensis* var. *bustamenta* (synonyms: *E. pichinchense*, *E. bustamenta*) (5-acetyl-3- β -angeloyl oxy-2- β -(1-hydroxy isopropyl)-2,3-dihydro benzofurane, 5-acetyl-3- β -angeloyloxy-2- β -(1-hydroxy isopropyl)-6-methoxy-2,3-dihydrobenzofurane, espeleton, encocalinol, O-methylencecalinol, encocaline, sonorol, taraxerol, (+)- β -eudesmol and a mixture of β -sitosterol/estigmasterol (Aguilar et al., 2009; Sanchez et al., 2010).

As suggested by botanists, phytochemical studies are needed for wild *Eupatorium* specimens harvested from sites located on the outskirts of Morelia, Michoacan. The plant species under study were marked with (■) in Table 1. Six of them are used in Mexican traditional medicine. Also, all of them are (1) members of non-timber forest, (2) weeds in agricultural and farm lands, and (3) predominant inhabitants of disturbed ecosystems. Preliminary chemical studies of *Eupatorium* species growing in Michoacan were done with the flowers. This was made for two reasons: flowers are an important organ for species identification, and the fact that they are rich in chemical compounds is of interest to establish chemical markers between species. A variety of chemical families were present in the hexanic extracts from

Table 2. ^1H NMR signals of the hexanic extracts from flowers of *Eupatorium* spp.

Tabla 2. Señales ^1H RMN de los extractos hexánicos obtenidos de flores de *Eupatorium* spp.

Species	^1H NMR signal (ppm)	Chemical family
<i>E. adenophorum</i>	7.2 - 7.5 ; 6.3 - 6.4 ; 5.5 - 5.6; 3.7; 0.8-2.6	Chromenes
<i>E. areolare</i>	7-8; 4-6.5; 0.9-3.7	Terpenes
<i>E. arsenei</i>	6.3-7.8; 4.8-5.8; 3-3.8; 1-2.9	Chromenes
<i>E. dolichobasis</i>	5-6; 3.5-4.6; 0.8-2.9	Terpenes
<i>E. glabratum</i>	7-8.2; 4-6.4; 0.9-3.2	Terpenes
<i>E. lasioneuron</i>	5-5.7; 0.8-2.8	Diterpenes
<i>E. pichinchense</i>	6-8.5; 3-5.5; 0.8-3	Terpenes
<i>E. pycnocephalum</i>	7-7.3; 4.4-5.5; 3.4-3.6; 0.7-2.8	Terpenes

Eupatorium spp flowers. In a qualitative chemical analysis using hydrogen (^1H) nuclear magnetic resonance techniques on flower extracts showed strong characteristic signals of terpenes and chromenes (Table 2). The presence of several species of the genus *Eupatorium* is a hint that they are successful colonizers and have few predators. However, an exhaustive study is required to know the exact number of species, and the possible existence of endemisms in the territory of Michoacan. With this in mind, it is necessary to take precautions in the harvesting processes, and to define the ecological environment for the botanical and molecular identification of species. Although most of this extraordinary plant richness is apparently not in danger of disappearing, it is possible that endemic *Eupatorium* species are already extinct.

In Mexico, the federal government has established that 2583 species are endangered in some categories. Also, it recognizes that most Michoacan ecosystems are severely degraded, and some of them, such as mangroves and reefs, are even in danger of disappearing (Estrategia para la Conservación y Uso Sustentable de la Diversidad Biológica de Michoacan, 2007).

This indicates that it is necessary to pay attention to the standard norms of use, production, handling, processing, distribution and marketing of each of *Eupatorium* species. This attitude will help to use this natural resource rationally (Mexican Official Standards related to this topic).

Of the species of *Eupatorium* without synonymy detected, it is necessary to know their natural abundance and accessibility, and make a taxonomic revision. These species, and all the native species of this genus, are ideal candidates for further biodirected phytochemical studies in the search for novel molecules with pharmacological action.

The study results of the phytochemical study on a species of successful propagation, easy access and harvesting as *Eupatorium* spp suggest that it is potentially possible to find

new active ingredients of secondary metabolites belonging to major chemical families like terpenes and chromenes. Our study provided complementary information on *Eupatorium* spp, an important member of the native Mexican flora.

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