Progress in the Chemistry of Organic Natural Products

A. Douglas Kinghorn · Heinz Falk
Simon Gibbons · Jun'ichi Kobayashi
Yoshinori Asakawa · Ji-Kai Liu *Editors*

108

# Progress in the Chemistry of Organic Natural Products

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Volume 108

With contributions by

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ISSN 2191-7043 ISSN 2192-4309 (electronic)
Progress in the Chemistry of Organic Natural Products
ISBN 978-3-030-01098-0 ISBN 978-3-030-01099-7 (eBook)
https://doi.org/10.1007/978-3-030-01099-7

Library of Congress Control Number: 2018965903



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The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

# Contents

# Chemistry and Biology of Selected Mexican Medicinal Plants

**Rachel Mata, Mario Figueroa, Andrés Navarrete, and Isabel Rivero-Cruz**

## Contents



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A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (eds.),
*Progress in the Chemistry of Organic Natural Products, Vol. 108*,
https://doi.org/10.1007/978-3-030-01099-7_1

## 1 Introduction

Mexico is a multifaceted and heterogeneous country with high cultural richness and 10–12% of the world's biodiversity. This country ranks 4th in the variety of vascular plants with about 31,000 different species; of this stock more than 3350 form part of the medicinal flora. When the Spanish conquerors arrived to ancient Mexico, they found existing civilizations with a holistic view of illnesses and healing. These early Mesoamericans inhabitants used religious, magic rituals and a variety of plant-based remedies to improve health. The abundance and variety of Mexican medicinal flora can be traced from published work written from the sixteenth century to modern times. Crucial and most important sources of information about traditional Mexican medicine were recently reviewed [1].

The use of herbal medicines survives to this day in modern Mexico; the original Aztec beliefs and practices are interlaced with strands of the European medicine introduced by the Spaniards in the sixteenth century. They are an integral element of alternative medical care and the best testimony of their efficacy and cultural value is the persistence of medicinal plants in present-day Mexican markets, where the highest percentage of medicinal and aromatic plants is sold.

For more than 100 years, researchers have explored Mexican medicinal flora from the ethnobotanical, anthropological, chemical, pharmacological, and biotechnological points of view; in a few cases some clinical investigations have been pursued. The most important investigations have been carried out at the Instituto Nacional de Antropología, Instituto Mexicano del Seguro Social, Universidad Autónoma de Nuevo León, Universidad Autónoma del Estado de Morelos, Instituto Tecnológico y de Estudios Superiores de Monterrey, Universidad Autónoma Metropolitana, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, and Universidad Nacional Autónoma de México. The no-longer existing Instituto Médico Nacional and Instituto Mexicano de Plantas Medicinales deserve special mention since they were devoted to the study of Mexican medicinal plants in different periods of the twentieth century. Both are good examples of important institutions dedicated to the comprehensive analysis of the national Materia Medica, and were pioneering institutions in bioprospecting matters.

In the twenty-first century, the commerce of medicinal plants in Mexico has grown due to a global resurgence of herbal-based remedies. Furthermore, according to a recent survey, 54% of health professionals and 49% of physicians have used medicinal plants as an alternative therapy for several diseases. Twenty-eight percent of health professionals and 26% of physicians, have recommended or prescribed medicinal plants to their patients, in particular for digestive and respiratory ailments; finally, 73%

of health professionals would agree to receiving academic information regarding the use and prescribing of medicinal plants [2].

Concomitantly, a loss of biodiversity, over-exploitation, biopiracy, and weak regulations on the use of medicinal plants are the major impediments to the growth of herbal medicine as an important national industry [3]. Therefore, current research on medicinal plants should also involve conservation issues and the sustainable search for bioactive natural products based on traditional knowledge, regulation, and quality control of the most important species; these are essential issues for the growth of a rational herbal medicine usage.

In the following sections, some work from the authors' laboratories will be highlighted. The most relevant phytochemical and pharmacological profiles of a selected group of plants widely used for treating major national health problems will be discussed.

## 2 Mexican Medicinal Plants Employed for Treating Major National Health Problems

### *2.1 Diabetes*

The global prevalence of diabetes in adults has been increasing over recent decades, making this disease a major public health threat in countries all over the world. The International Diabetes Federation estimated the global prevalence to be 425 million in 2017, which implied a health expenditure of 673 billion USD [4]. The prevalence of diabetes in adults aged 20–79 years is predicted to rise to 10.4% in 2040. Of the total diabetics, about 95% have type 2 diabetes mellitus (T2DM). Mexico is one of the countries most affected by this metabolic disease, in particular indigenous people owing to changes in their traditional lifestyle and the effects of industrialization on both environmental and sociocultural norms. In 2017, there were more than 12 million people affected by diabetes, representing a prime cause of mortality. In Mexico as in other regions of the world, people use plants to treat the symptoms of diabetes. More than 300 different plants have been described as reputedly beneficial for the diabetic patient [5–7], but most claims are subjective and few have received any suitable scientific evaluation. So far, about 200 plants have been investigated scientifically in Mexico in order to establish their antidiabetic potential. Most studies have been limited to the preclinical evaluation of extracts prepared with selected solvents using different pharmacological models [6]; the depth of their analysis is variable since some authors have reported in detail the mode of action of the extracts while others just measured their hypoglycemic activity. Other studies have determined both the active principles and the preclinical efficacy of the traditional preparations. Finally, only a very few studies have pursued in-depth clinical observations. Most of the work of the present author group falls into the second category, involving detailed phytochemical work coupled with substantial preclinical biological observations.

Some examples of our work on antidiabetic plants are described in the following sections. In addition, other investigations, from other authors and ourselves, carried out

after a survey on diabetic plants was published in 2005 [6], are summarized in the Appendix Table.

### 2.1.1 *Swietenia humilis*

*Swietenia humilis* Zuccarini (Meliaceae), locally known as "zopilote", "cobano", "flor de venadillo" and "caoba", is a medium-sized deciduous tree (Fig. 1). The species is regarded as one of the three true American mahogany species. It grows in a very wide ecological range within its native Pacific watershed of Central America and Mexico. The seeds are wind dispersed and highly valued for medicinal purposes. The plant is also a much appreciated hardwood species in the neotropics and is seriously threatened owing to overexploitation and habitat destruction. Therefore, a multilateral treaty called the Convention on International Trade in Endangered Species of Wild Fauna and Flora lists *S. humilis* in Appendix II (all parts and derivatives except the seeds) [8]. Also, it is categorized in the International Union for Conservation of Nature Red List of Threatened Species as "vulnerable" [9].

The medicinal use of the seeds of *S. humilis* can be traced to the sixteenth century; the Spanish royal physician Francisco Hernández, in his magnificent manuscript "Four Books on the Nature and Virtues of Plants and Animals for Medicinal Purposes in New Spain", described the antiulcer, astringent, antitussive, and emollient properties of these seeds. In the middle of the twentieth century, their astringent effects were also described [10]. In the present day, decoctions of the seeds of *S. humilis* (SHD), alone or in combination with other plants, are valued for treating indigestion, stomachache, amebic dysentery, and diarrhea. The ground raw seeds or their decoctions are also ingested as a blood depurative and antidiabetic agent [5, 6].

In general, for conducting our studies focused on the determination of any pharmacological properties of traditional extracts, first acute preclinical toxicity using the Lorke procedure is assessed [11]. This method measures acute toxicity for 14 days in mice using a range of doses between 10 and 5000 mg/kg, in two phases. The dried seeds and SHD (10–5000 mg/kg) showed no acute toxic effects when assessed by the Lorke procedure. The calculated $LD_{50}$ values of the preparation and crude drug were higher than 5000 mg/kg.

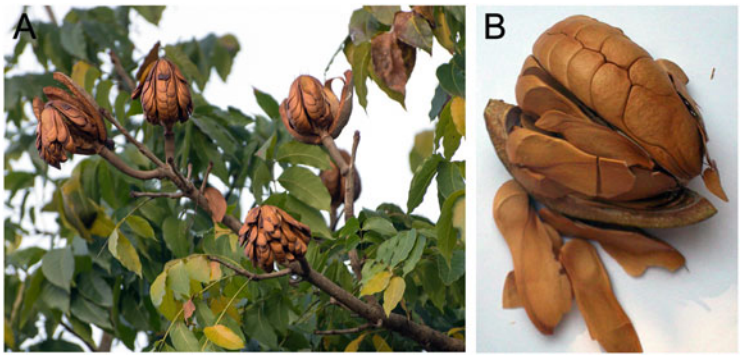



**Fig. 1** Leaves, stems (**A**), and seeds (**A** and **B**) of *Swietenia humilis*

Since the plant preparation lacked acute toxic effects, it was next tested for antidiabetic action in vivo by means of animal models using a standard protocol. By means of this protocol, initially the acute hypoglycemic activity in normoglycemic and hyperglycemic animals (ICR mice or Wistar rats) is assessed. If feasible, subchronic (14 days) or chronic (30 days) experiments are also performed. Then, the antihyperglycemic action of the extracts or purified compounds after a glucose (1 g/kg; oral glucose tolerance test, OGTT), sucrose (2 g/kg; oral sucrose tolerance test, OSTT) or starch (2 g/kg; oral starch tolerance test, OStTT) challenge is assessed using normal and hyperglycemic animals. These tests provide relevant information regarding peripheral utilization or absorption of glucose. In all tests, the animals are made hyperglycemic with streptozotocin (STZ, 130 mg/kg for mice; and 50 mg/kg for rats), after previous protection with nicotinamide (NAA, 40 mg/kg for mice; and 65 mg/kg for rats). After 7 days of NAA-STZ administration, the animals are generally hyperglycemic and can be included in the studies conducted subsequently. The NAA-STZ model affords a similar biochemical blood profile and pathogenesis to T2DM in humans. Glibenclamide (15 mg/kg), metformin (200 mg/kg) or acarbose (5 mg/kg) are used as positive controls, depending of the type of experiment. The percentage variation of glycemia for each group of animals is calculated with respect to the initial values at different periods of time. The results are plotted indicating blood glucose values or percentage of variation versus time at several doses [12].

In a series of experiments conducted in NAA-STZ hyperglycemic mice, SHD (100–316 mg/kg) caused a significant reduction in blood glucose levels and inhibited the postprandial peak provoked by a glucose load during an OGTT. On the other hand, SHD (100–316 mg/kg) did not inhibit the postprandial peak at any of the doses tested during an OSTT in normoglycemic mice, ruling out an inhibition of $\alpha$-glucosidases at the intestinal level [13].

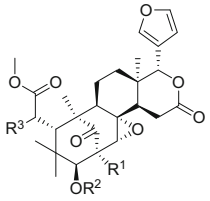
The antihyperglycemic, hypoglycemic, and hypolipidemic effects of *S. humilis* seeds were corroborated in rats with fructose-fed metabolic syndrome. SHD (100 and 316 mg/kg) caused a significant inhibition of the postprandial peak during an OGTT when compared with a vehicle-treated group. Moreover, daily administration of SHD (100 mg/kg) for a week provoked a significant hypoglycemic effect, and reductions in both serum triglycerides and uric acid, without any significant changes in fasting insulin levels or body weight. In addition, a reduction in the abdominal fat of the test animals, and an increment in hepatic glycogen, were observed. Altogether, the results suggested that the traditional preparation of *S. humilis* induced modifications in peripheral glucose uptake, rather than by inhibition of the intestinal $\alpha$-glucosidases. The reduction of the postprandial peak observed during the OGTT, and the increment of hepatic glycogen in rats with fructose-fed metabolic syndrome indicated that the hypoglycemic effect of SHD involves an insulin-sensitizing mechanism. The reduction in blood triglycerides is compatible with an increment in glucose uptake in adipose tissue, where energy is stored as triglycerides. These effects are also consistent with the use of this species as blood depurative (purifying) agent [13].

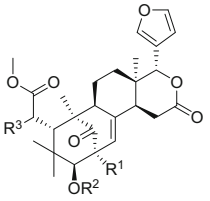
In order to identify the compounds responsible for these pharmacological effects, both the active aqueous and an organic extracts of *S. humulis* seeds were fractionated extensively by chromatographic procedures. These processes led to the isolation of

eight new limonoids of the mexicanolide type, namely, humilinolides A–H (**1**–**8**) along with humulin B (**9**), methyl-2-hydroxy-3$\beta$-isobutyroxy-1-oxomeliac-8(30)-enate (**10**), methyl-2-hydroxy-3$\beta$-tigloyloxy-1-oxomeliac-8(30)-enate (**11**), swietenin C (**12**), swietemahonin C (**13**) and 2-hydroxy-destigloyl-6-deoxyswietenine acetate (**14**) (Fig. 2) [13]. These mexicanolides can be categorized into two structural subclasses by considering the degree of oxidation at C-8/C-30 of the basic methyl-1-oxomeliacate nucleus. The first one comprises limonoids with an 8,30 double bond, while the second includes those with an 8,30 epoxide function. The compounds in each group differ in the number and position of oxygenated substituents. The acid residues esterifying the hydroxy group at C-3 could be either isobutyric, tiglic or acetic acid. All structures were elucidated using one- and two-dimensional NMR spectroscopic techniques, and with that of humulinolide G (**5**) confirmed by X-ray diffraction analysis [13].

Chromatographic analysis of SHD revealed that compounds **9**, **11**, and **14** are its major components, although the remaining limonoids isolated were also identified. These limonoids were isolated in adequate amounts to perform in vivo assays. As expected, the three major compounds (3.16–31.6 mg/kg) showed hypoglycemic and antihyperglycemic actions when tested in the NAA-STZ mice model using the acute hypoglycemic assay and the OGTT, respectively (Fig. 3). Although limonoids **9**, **11**, and **14** were found as the major hypoglycemic and antihyperglycemic limonoids of the decoction, the remaining compounds could also contribute to the pharmacological action displayed by SHD. Furthermore, they could be acting synergistically on different molecular targets to produce antidiabetic and hypolipidemic effects. Likewise, the mixture of components in SHD might enhance the bioavailability of one or several compounds of the extract, thus improving their pharmacological actions. It is worth mentioning that none of the isolates inhibited $\alpha$-glucosidases.

The antihyperalgesic effects of SHD and compound **14** were assessed in NAA-STZ hyperglycemic mice using the formalin method. The formalin test in mice is a valid and reliable model of nociception and is sensitive to various classes of analgesic drugs. The noxious stimulus is an injection of dilute formalin (1% in





**1** (humilinolide A) $R^1$ = OH, $R^2$ = A, $R^3$ = OH
**2** (humilinolide B) $R^1$ = OH, $R^2$ = A, $R^3$ = B
**6** (humilinolide F) $R^1$ = B, $R^2$ = C, $R^3$ = B
**8** (humilinolide H) $R^1$ = B, $R^2$ = A, $R^3$ = H
**9** (humulin B) $R^1$ = OH, $R^2$ = A, $R^3$ = H
**13** (swietemahonin C) $R^1$ = H, $R^2$ = A, $R^3$ = B

**3** (humilinolide C) $R^1$ = B, $R^2$ = C, $R^3$ = H
**4** (humilinolide D) $R^1$ = OH, $R^2$ = D, $R^3$ = B
**5** (humilinolide E) $R^1$ = OH, $R^2$ = C, $R^3$ = B
**7** (humilinolide G) $R^1$ = B, $R^2$ = A, $R^3$ = H
**10** $R^1$ = OH, $R^2$ = A, $R^3$ = H
**11** $R^1$ = OH, $R^2$ = C, $R^3$ = H
**12** (swietenin C) $R^1$ = H, $R^2$ = A, $R^3$ = OH
**14** $R^1$ = OH, $R^2$ = D, $R^3$ = H

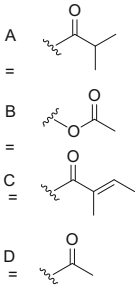


**Fig. 2** Limonoids isolated from *Swietenia humilis*

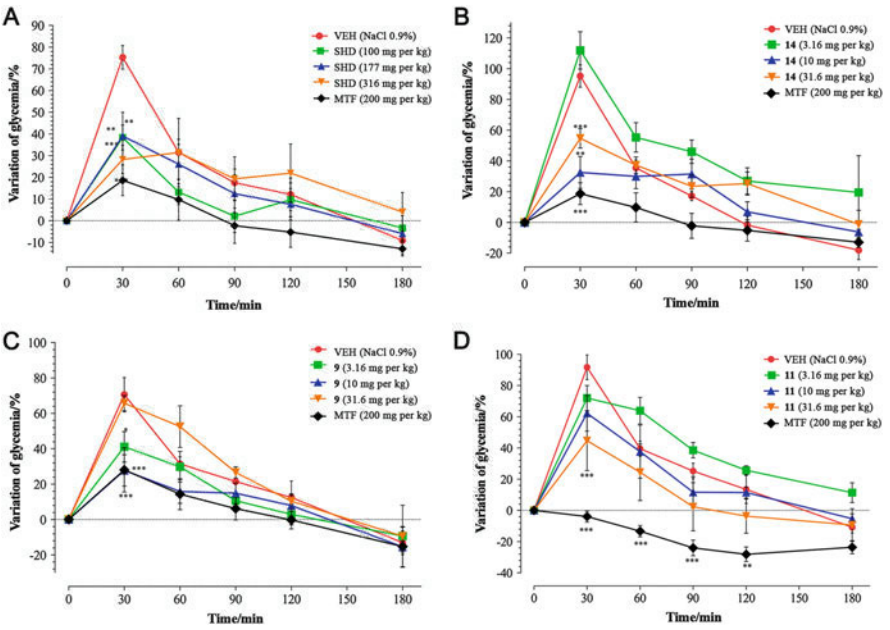



**Fig. 3** Effect of SHD (**A**), mexicanolide **14** (**B**), humulin B (**9**) (**C**), and methyl-2-hydroxy-3$\beta$-tigloyloxy-1-oxomeliac-8(30)-enate (**11**) (**D**) on blood glucose levels in NAA-STZ-hyperglycemic mice during an OGTT. VEH: vehicle; MTF: metformin. Values are expressed as the means from six data points ±SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Adapted from [13]

saline), placed under the skin of the dorsal surface of the right hind paw. The response observed is the amount of time the animals spend licking the injected paw. Two distinct periods of high licking activity can be identified, an early phase lasting for the first 5 min and a late phase lasting from 20 to 30 min after the injection of formalin. The two phases in the formalin test may have different nociceptive mechanisms. The early phase seems to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord; this pain can be inhibited by anti-inflammatory drugs [14]. Thus, local injection of SHD (10–177 μg) and mexicanolide **14** (0.5–3.5 μg) provoked a concentration-dependent antihyperalgesic action in NAA-STZ hyperglycemic mice (Fig. 4). Ketanserin (6 μg), a 5-$HT_{2A/C}$ receptor antagonist, and flumazenil (6 μg), a $GABA_A$ receptor antagonist, abolished the antihyperalgesic effect of mexicanolide **14** (3 μg) (Fig. 5). On the other hand, naloxone (3 μg), L-arginine (50 μg), and $N_\omega$-nitro-L-arginine methyl ester hydrochloride (L-NAME; 150 μg) diminished the antihyperalgesic effect of mexicanolide **14** (Fig. 6). The aqueous extract of the seeds possesses significant antihyperalgesic action [15]. Thus, *S. humilis* seeds have shown also promising results for managing secondary complications (neuropathic pain) of diabetes.

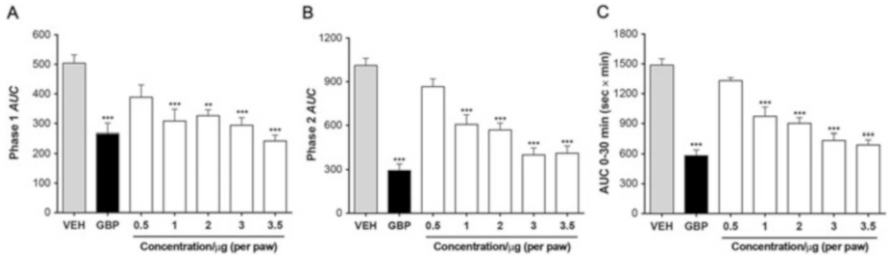



**Fig. 4** Antihyperalgesic effect of mexicanolide **14** in NAA-STZ hyperglycemic mice during phases 1 (**A**), 2 (**B**), and the total area under the curve (**C**) in the formalin test. VEH: vehicle; GBP: gabapentin (30 μg per paw) was used as positive control. Each bar represents the mean area under the curve (AUC, time of licking against time, sec × min) from six data points ±SEM. $*p < 0.01$ and $**p < 0.001$. Adapted from [15]

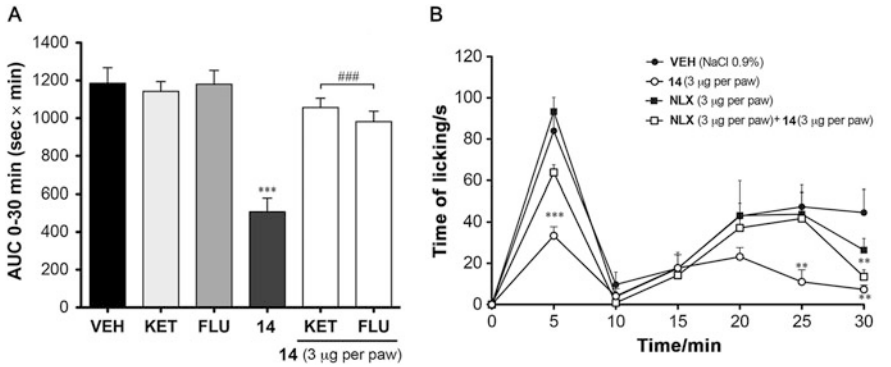



**Fig. 5** Possible antihyperalgesic mechanism of mexicanolide **14** (3 μg per paw) in NAA-STZ hyperglycemic mice during the formalin test: serotoninergic, GABAergic (**A**), and opioid modulation (**B**). VEH: vehicle, ketanserin (KET, 6 μg per paw), flumazenil (FLU, 6 μg per paw), and naloxone (NLX, 3 μg per paw). (**A**) Each bar represents the mean area under the curve (AUC, time of licking against time, sec × min) from six data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. (**B**) Each point represents the mean of the time of licking (sec) from six data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Adapted from [15]

*Swietenia humilis* and/or its limonoids represent promising alternatives for development as safer and cheaper phytotherapeutic agents. The overall results described in the paragraphs above support the use of seeds of this tropical species for treating diabetes in contemporary Mexico. Finally, it is worth mentioning that the potential antiamebiasis effects of the limonoids and extracts from this plant were tested, with negative results being obtained. Moreover, most of the limonoid constituents and an organic extract from *S. humilis* were active against the European corn borer, *Ostrinia nubilalis*, affecting important life cycle parameters such as reduction of the % pupation and the % of adult emergence, in a similar way to the positive control toosendanin [16, 17]. They also inhibited radical growth of a several weed species

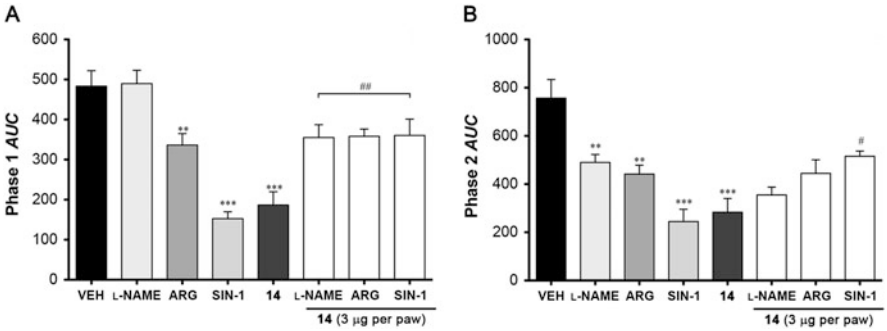



**Fig. 6** Possible antihyperalgesic mechanism of mexicanolide **14** (3 µg per paw) in NAA-STZ hyperglycemic mice during phases 1 (**A**) and 2 (**B**) on the formalin test: nitrergic modulation. VEH: vehicle, L-NAME (150 µg per paw), L-arginine (ARG, 50 µg per paw), and 3-morpholinosydnonimine hydrochloride (SIN-1, 200 µg per paw). Each bar represents the mean area under the curve (AUC, time of licking against time, sec × min) from six data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Adapted from [15]

when tested in vitro [18]. In consequence, this species seems valuable not only as a medicinal agent but also as a pesticide.

### 2.1.2 Mexican "Copalchis": *Hintonia latiflora*, *Hintonia standleyana*, and *Exostema caribaeum*

*Hintonia latiflora* (Sessé & Moc. ex DC.) Bullock (Rubiaceae) is a species endemic to Mexico, while *H. standleyana* Bullock has a wider distribution area up to Northern Central America. *Hintonia standleyana* was considered to be synonym of *H. latiflora*, which is still widely accepted by some authors, however, recent molecular evidence has revealed that these two species are significantly different [19–21]. Both species are known commonly as "copalquin" and "copalchi", among other colloquial names. The plants are shrubs or trees up to 8 m tall, with gray stems; the leaves are bright green and covered with hairs on the back (Fig. 7). The main area


**Fig. 7** Mexican "Copalchis": *Hintonia latiflora* (**A**), *Hintonia standleyana* (**B**) and *Exostema caribaeum* (**C**)

supplying the commercial "copalchi" is the northern state of Guerrero, Mexico. Teas from the bark of these species are used in modern Mexico for a variety of health problems, including malaria, stomach ulcers, diabetes, obesity, infections and fevers. In addition, the Tarahumaras have used *H. latiflora* on body sores [22].

*Exostema caribaeum* (Jacq.) Schult. (Rubiaceae), the Caribbean prince wood, is an evergreen slender shrub or small tree up to 12 m height (Fig. 7). The plant occurs on all islands within the Bahamian Archipelago, as well as the rest of the Caribbean region, Florida, Mexico, and Central America. In Mexico, the plant is gathered from the wild for local use as a medicine to treat fevers, especially those related to malaria, and also a source of lighting and timber. This species is also regarded as "copalchi", and in some local markets its stem bark is mixed with those of *H. latiflora* or *H. standleyana* [23].

The hypoglycemic and diuretic properties of *H. latiflora* were discovered clinically by researchers at the Instituto Médico Nacional in Mexico City at the beginning of twentieth century (Fig. 8). They also discovered some chemical compounds present that were later on rediscovered by German, French, and Mexican researchers. It is notable that in 1913, when the Instituto Médico Nacional closed, "copalchi" was reintroduced in Europe for the treatment of diabetes. Later on, researchers in Germany and France corroborated the earlier work of the Mexican scientists. Recently, the most relevant historical aspects about this species as well as the research carried out by other scientists were reviewed [12]. Perhaps the most relevant aspect of these historical events was that, after the Royal Botanical Expedition to New Spain (1787–1803), led by Martín Sessé and José Mociño, *H. latiflora*,

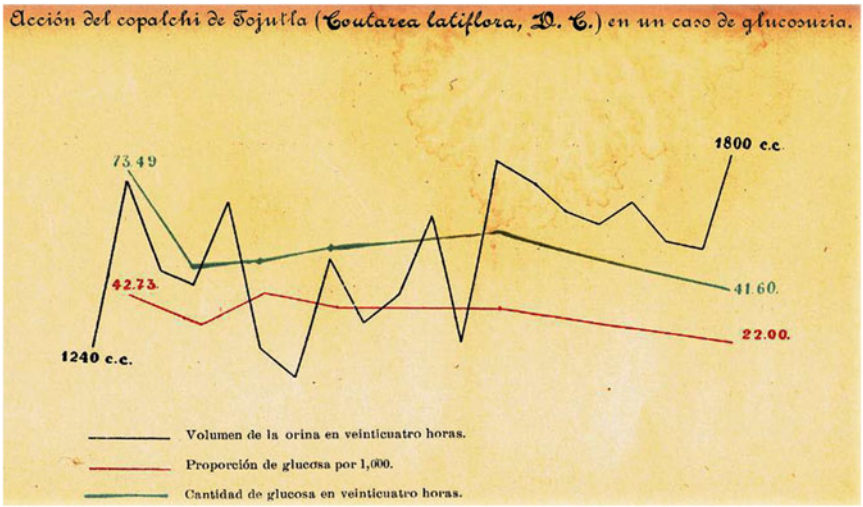
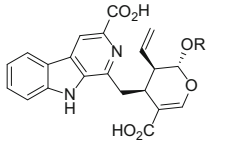


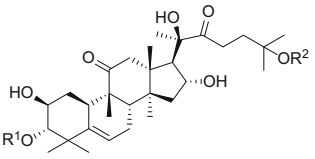

**Fig. 8** Hypoglycemic and diuretic effects exerted by a *Hintonia latiflora* hydroalcoholic extract in a clinical trial conducted at IMN, Mexico City. Urine volume during a 24-h period (black line); amount of glucose in urine during a 24-h period (green line); proportion of glucose per liter (red line). Adapted from [12]

under its synonym *Coutarea latiflora* Sessé & Moc. ex DC., and *E. caribaeum* appeared in the list of the most important "Medicinal Plants of New Spain". They were also included in the well-known "Torner Collection" of Sessé and Mociño biological illustrations. Thus, in the following paragraphs, we will review mostly the work carried out by our group.

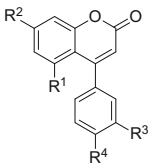
Phytochemical analysis of the stem bark of these three plants allowed the discovery of cucurbitacins in the Rubiaceae family, as well as the characterization of several 4-phenylcoumarins, with most being new chemical entities, and the indole alkaloid desoxycordifolinic acid (**15**) [23–31]. The basic core of the cucurbitacins **16**–**19** is dihydrocucurbitacin F (**16**) (Fig. 9). The 4-phenylcoumarins **20**–**36** of the three species are 5,7,3′,4′- or 5,7,4′-substituted with oxygenated functionalities, with the former having the most common pattern; the sugar unit is usually a monosaccharide (*β*-D-galactose, *β*-D-glucose, 6″-acetyl-*β*-D-glucose or 6″-acetyl-*β*-D-galactose), although some disaccharides have been found (*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranose or *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranose) (Fig. 9). In all cases, the saccharide unit is attached to the hydroxy group at C-5. During the course of our investigations it was demonstrated that 4-phenylcoumarins undergo oxidative cyclization under aerobic alkaline conditions to give oxido-4-phenylcoumarins.

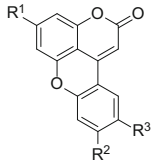



**15** (desoxycordifolinic acid) R = *β*-D-glucopyranosyl




**16** (dihydrocucurbitacin F) $R^1$ = H, $R^2$ = H
**17** $R^1$ = H, $R^2$ = Ac
**18** $R^1$ = *β*-D-glucopyranosyl, $R^2$ = H
**19** $R^1$ = *β*-D-glucopyranosyl, $R^2$ = Ac




**20** $R^1$ = $R^2$ = $R^4$ = $OCH_3$, $R^3$ = H
**21** $R^1$ = $R^2$ = $R^4$ = $OCH_3$, $R^3$ = OH
**22** $R^1$ = *O*-*β*-D-galactopyranosyl, $R^2$ = $OCH_3$, $R^3$ = $R^4$ = OH
**23** $R^1$ = $R^3$ = $R^4$ = OH, $R^2$ = $OCH_3$
**24** $R^1$ = *O*-*β*-D-glucopyranosyl, $R^2$ = $OCH_3$, $R^3$ = $R^4$ = OH
**25** $R^1$ = *O*-*β*-D-glucopyranosyl, $R^2$ = $R^3$ = $R^4$ = OH
**26** $R^1$ = $R^2$ = $R^3$ = $R^4$ = OH
**27** $R^1$ = 6″-*O*-acetyl-*O*-*β*-D-glucopyranosyl, $R^2$ = $R^3$ = $R^4$ = OH
**28** $R^1$ = *O*-*β*-D-galactopyranosyl, $R^2$ = $OCH_3$, $R^3$ = H, $R^4$ = OH
**29** $R^1$ = *O*-*β*-D-glucopyranosyl, $R^2$ = $R^4$ = $OCH_3$, $R^3$ = OH
**30** $R^1$ = 6″-*O*-acetyl-*O*-*β*-D-galactopyranosyl, $R^2$ = $R^4$ = OH, $R^3$ = H
**31** $R^1$ = 6″-*O*-acetyl-*O*-*β*-D-galactopyranosyl, $R^2$ = $R^3$ = $R^4$ = OH
**32** $R^1$ = *O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyl, $R^2$ = $OCH_3$, $R^3$ = $R^4$ = OH
**33** $R^1$ = *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl, $R^2$ = $OCH_3$, $R^3$ = $R^4$ = OH
**34** $R^1$ = *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl, $R^2$ = $R^4$ = $OCH_3$, $R^3$ = H

**35** $R^1$ = $OCH_3$, $R^2$ = $R^3$ = OH
**36** $R^1$ = $R^2$ = $OCH_3$, $R^3$ = OH

**Fig. 9** Compounds isolated from Mexican "Copalchis"

Thus, 7-methoxy-5,3′,4′-trihydroxy-4-phenylcoumarin (**23**) was converted to 7-methoxy-4′,5′-dihydroxy-4-phenyl-5,2′-oxido-coumarin (**35**) by treatment with potassium hydroxide in methanol. Since the reaction took place only in basic conditions and in the presence of air, it might proceed via an oxidative phenol coupling process.

Preclinical toxicity studies have revealed that none of the aqueous (traditional preparations) extracts from the stem bark of the two above-mentioned *Hintonia* species and *E. caribaeum* were toxic to mice ($LD_{50} > 5$ g/kg). These results thus suggest the preclinical safety of the traditional preparations of these three plants [32, 33]. The organic extracts of *H. latiflora* and *E. caribaeum*, however, showed $LD_{50}$ values of 2900 and 700 mg/kg, respectively; the extract of *E. caribaeum* generated tremors, respiratory distress as well as decreases in motor activity and in body weight, by 27.1% with respect to the vehicle-treated animals. The organic extract of *H. standleyana* had an $LD_{50}$ of > 5 g/kg. Moreover, none of the extracts induced mutagenic effects when assayed by the Ames test [33]. Rivera et al. [34] reported that a methanol extract from *H. latiflora* induced genotoxic effects, piloerection, excitability, dyspnea, anoxia, mydriasis, tachycardia, overcrowding, decreased muscle tone, burying behavior, and ambulatory movements in a dose-dependent manner in mice. These effects were only observed during the first 24 h of the experiment. At the end of the study (15 days after treatment), all surviving mice showed a normal behavior [34]. Our group has worked extensively with *Hintonia* species and never observed such effects, even in long-term experiments. Unfortunately, the authors did not provide chromatographic profiles of their extract nor a voucher number to compare the plant material they analyzed [34].

The long-term hypoglycemic effect of the organic extracts ($CH_2Cl_2$:MeOH = 1:1) of the three species (*H. latiflora, H. standleyana*, and *E. caribaeum*) and a commercial mixture of "copalchi" (composed by *H. standleyana* and *E. caribaeum*), and compounds **18**, **22**, **24**, **25**, and **32** (15 mg/kg each time) was established (Fig. 10) [31]. The extract of *H. latiflora* and compound **25** restored blood glucose levels to normal values, with the effect being comparable to that of glibenclamide. Compounds **22** and **24** also restored blood glucose levels to near normal values by the end of the experiment. During this study, it was also demonstrated that the extract of *H. latiflora* regulated both hepatic glycogen and plasma insulin levels ($p < 0.05$) (Fig. 11). These data suggested that its hypoglycemic effect is due in part to stimulation of insulin secretion and regulation of hepatic glycogen metabolism. Comparison of the hypoglycemic activity of the 4-phenylcoumarins tested established that the most active compounds possess a free hydroxy group at C-7 in the 4-phenylcoumarin core. On the other hand, comparison of the activity of all glycosides tested indicated that the nature of the sugar moiety (glucose, galactose, *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranose or *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranose) had little or no influence on the resultant biological activity [31]. The hypoglycemic activity of such cucurbitacin-type compounds was demonstrated for the first time in these studies.

Infusions of the stem bark of both *Hintonia* species and *E. caribaeum* also showed hypoglycemic and antihyperglycemic effects. The later was demonstrated

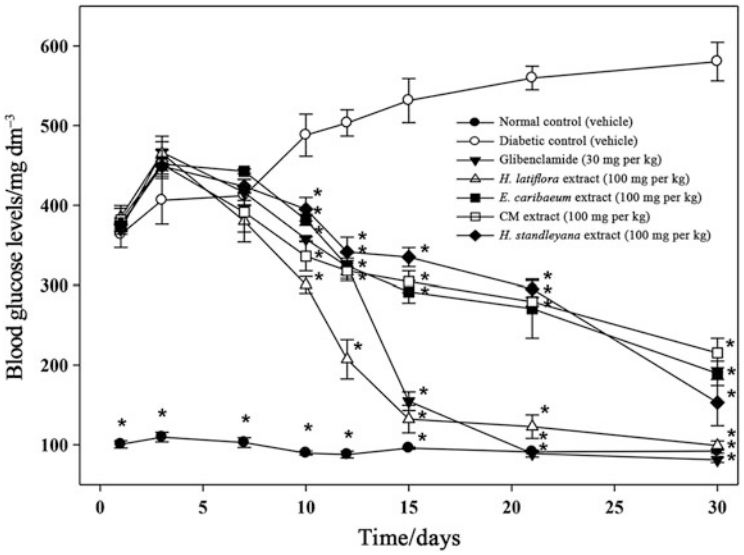



**Fig. 10** Long-term effect of extracts of *Hintonia latiflora*, *Hintonia standleyana*, *Exostema caribaeum*, and CM (commercial mixture of "copalchi") on blood glucose levels in NAA-STZ-diabetic rats. Each value is the mean from six data points ±SEM. *$p < 0.05$. Adapted from [31]

during an OSTT in mice suggesting that the plants contained inhibitors of $\alpha$-glucosidases [12]. In addition, the major components of the infusions were 4-phenylcoumarin glycosides: in the case of *H. latiflora*, the most abundant compound was **33**, for *H. standleyana*, the most relevant was **32**, while for *E. caribaeum* this was **22**. The $\alpha$-glucosidase inhibitory activities of several glycosides present in the infusions and the aglycones **23** and **26** were demonstrated in vitro using different enzymes. This assay was carried out using a well-known spectrophotocolorimetric procedure that measures the ability of any $\alpha$-glucosidases (baker's yeast, *Ruminococcus obeum* or mammalian) to hydrolyze a suitable substrate (*p*-nitrophenyl-$\alpha$-D-glucopyranoside) in the presence of the potential inhibitor; acarbose was also used as positive control. Docking studies, using AutoDock software, predicted that the aglycone **23** ($IC_{50} = 3.0$ μ*M* vs. 0.41 m*M* for acarbose), which turned out to be the most active inhibitor, binds to the yeast $\alpha$-glucosidases in the same pocket as acarbose. Coumarin **23** and glycoside **32** were also very active in an OStTT, thus indicating that both compounds possess also $\alpha$-amylase inhibitory activity [12].

For the crude drug (stem bark) of the three species, reliable, reproducible, and accurate high-performance liquid chromatography (HPLC)-UV methods were developed for the quantitative determination of the active compounds (Fig. 12). These methods were included in the second edition of the "Mexican Herbal Pharmacopoeia" [23, 35]. The development of the composition and identity tests for the three "copalchi" species have been very useful to detect the adulteration of

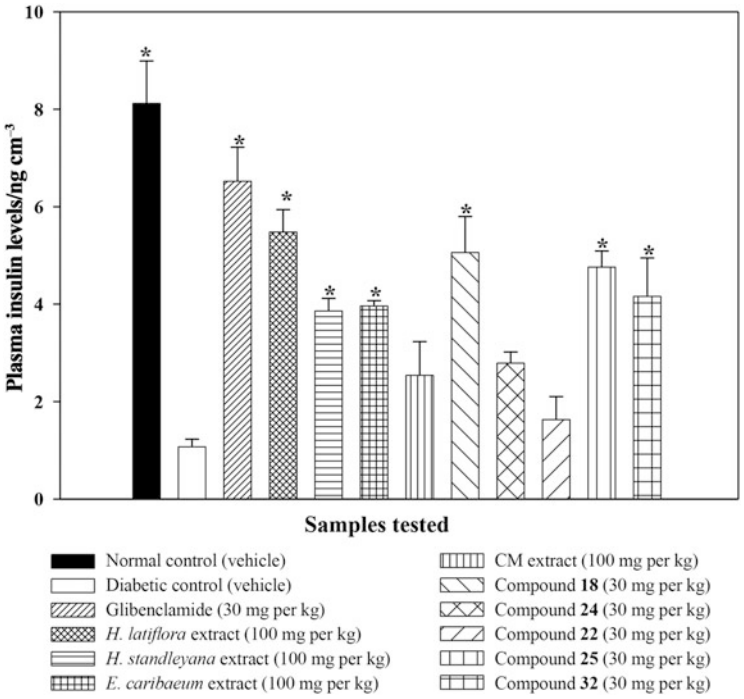



**Fig. 11** Effect of extracts of *Hintonia latiflora*, *Hintonia standleyana*, *Exostema caribaeum*, and CM (commercial mixture of "copalchi"), cucurbitacin **18**, and 4-phenylcoumarins **22**, **24**, **25**, and **32** on plasma insulin levels in STZ-diabetic rats. Each value is the mean from six data points ±SEM. $^*p < 0.05$. Adapted from [31]

*H. latiflora* with *E. caribaeum*. Indeed, the analysis of the commercial crude drugs or preparations made up with "copalchi" samples, Mexican or from abroad, have revealed clearly that most preparations contain a mixture of the two "copalchi" components, with *E. caribeum* almost always the more abundant in the mixture.

The organic extracts [$CH_2Cl_2$-MeOH (1:1)] and infusions from the leaves of *H. standleyana* and *H. latiflora* were also hypoglycemic and antihyperglycemic, in both normal and hyperglycemic rats. These extracts did not provoke death or damage, behavioral alterations, lesions or bleeding of the internal tissues to the animals, throughout the experiments conducted [32]. Therefore, the leaves of *Hintonia* species represent a therapeutic alternative to the stem bark in terms of conservation, since these species have been extensively exploited and commercialized locally and outside of Mexico from harvesting wild plants. In consequence, the populations of the plants are now scarce and in danger of extinction. From the active leaf extracts, three additional 4-phenylcoumarins (**37**–**39**) were obtained (Fig. 13) [32]. In addition, HPLC profiles of the leaf extracts of both plants revealed the presence of several hypoglycemic 4-phenylcoumarins isolated from the stem bark as well as ursolic (**40**) and chlorogenic (**41**) acids (Fig. 13). The overall results indicated

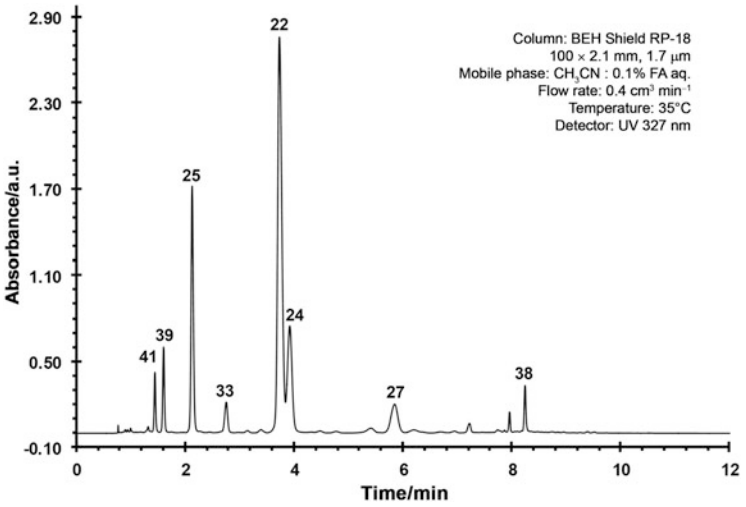
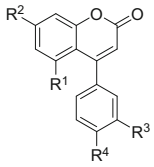
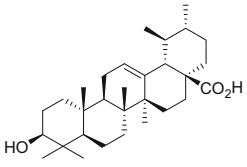


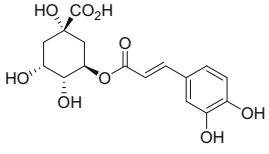

**Fig. 12** UHPLC-PDA chromatogram of *Exostema caribaeum* stem bark aqueous extract under optimized conditions; detection wavelength 327 nm. Adapted from [23]




**37** $R^1$ = 6"-*O*-acetyl-5-*O*-*β*-D-galactopyranosyl, $R^2$ = $R^4$ = OH, $R^3$ =H
**38** $R^1$ = 6"-*O*-acetyl-5-*O*-*β*-D-galactopyranosyl, $R^2$ = $R^3$ = $R^4$ = OH
**39** $R^1$ = *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl, $R^2$ = $R^4$ = $OCH_3$, $R^3$ = H




**40** (ursolic acid)




**41** (chlorogenic acid)

**Fig. 13** Structures of 4-phenylcoumarins **37**–**39**, and ursolic (**40**) and chlorogenic (**41**) acids

that the leaves of both species possess similar antidiabetic actions to their stem bark. Phenological and geographical analysis of the leaves from two different regions of Mexico (Chihuahua and Michoacán), using a validated UPLC method, revealed that the best harvesting season for the leaves of *H. latiflora* is between the leaf renovation and senescence stages, avoiding the flowering period [32]. In addition, no significant differences were found among the two different geographical populations analyzed.

Recently, a dry concentrated extract from the stem bark of *H. latiflora* in capsule form was tested in an open prospective clinical study in 41 dietetically stabilized subjects with T2DM for 6 months [36]. The results revealed that fasting and postprandial glucose levels and $HbA_{1c}$ values all declined significantly. Moreover, the tolerance was excellent, and liver and lipid values tended to be positively affected. In particular, no side effects and no hypoglycemic episodes or worsening of diabetic symptoms occurred. These results were in agreement with earlier studies [36]. Thus, the use of *Hintonia* dry extract for treating mild to moderate T2DM can be regarded as safe and useful in cases where dietary measures alone cannot provide adequate control of the disease.

Vierling and collaborators showed also that an extract of *H. latiflora* exerted a vasodilatory effect in vitro in aortic rings of the guinea pig and in vivo in rabbits [37]. Aortic rings pre-contracted with noradrenaline (NA) could be relaxed completely by the extract ($EC_{50}$ = 51.98 mg/dm$^3$). The aglycone also inhibited NA-induced contractions of aortic rings ($EC_{50}$ = 32.55 mg/dm$^3$) and in aortic cells suppressed calcium transients evoked by vasopressin at a concentration of 60 mg/dm$^3$, suggesting a possible inhibition of G-protein-induced intracellular calcium release. Ultrasound measurements in conscious rabbits showed that the extract induced vasodilation and lowering of blood flow velocity in the abdominal aorta and the carotid artery. The combination of a blood glucose-lowering with a vasodilating effect may be helpful for reducing vascular long-term complications in patients with T2DM.

During our earlier work on *H. latiflora*, the in vitro anti-*Plasmodium falciparum* activity of the extracts or isolates was not demonstrated. The same type of results were obtained by Noster and Kraus in Germany [38], who found only a moderate effect in vitro with *E. caribaeum* extracts. However, in a paper by Argotte-Ramos et al. [39], it was reported that an ethyl acetate extract of the stem bark of *H. latiflora* provoked suppression of induced parasitemia with *P. berghei* in mice. Bioassay-directed fractionation of the active extract using in vitro and in vivo assays indicated that **23** is the active principle. Also, Rivera and coworkers [34] found that a methanolic extract of *H. latiflora* had an excellent chemosuppression of *P. yoelii* total parasitemia and schizonts number in CD1 male mice in a 4-day test protocol. As indicated above, in this work no voucher nor chemical composition of the plant was provided. Hence, there is an uncertainty about the precise identity of the plant material that was actually analyzed.

The organic extract of *H. standleyana* showed a potent antinociceptive effect when tested in hot-plate and writhing tests [29]. The hot-plate test is a suitable method to evaluate central antinociceptive activity. In addition, this model measures animal behavior and has good sensitivity and specificity. It is based on the principle that when rodents are placed onto a hot surface they will initially demonstrate aversive behaviors to the thermal stimulus by licking their paws and jumping. Compounds that alter the nociceptive threshold increase the latency period to the observed licking/jumping. On the other hand, the acetic acid-induced writhing test, is a classical visceral pain model useful to detect painful symptoms associated with inflammatory disorders of the internal organs such as the stomach or intestines.

The metabolite responsible for this antinociceptive activity of the extract was found to be compound **18**, which significantly reduced the acetic acid-induced abdominal constrictions in mice. In addition, this compound produced a significant increase in thermal latency in the hot-plate test (Fig. 14) [29]. The effect of **18** was

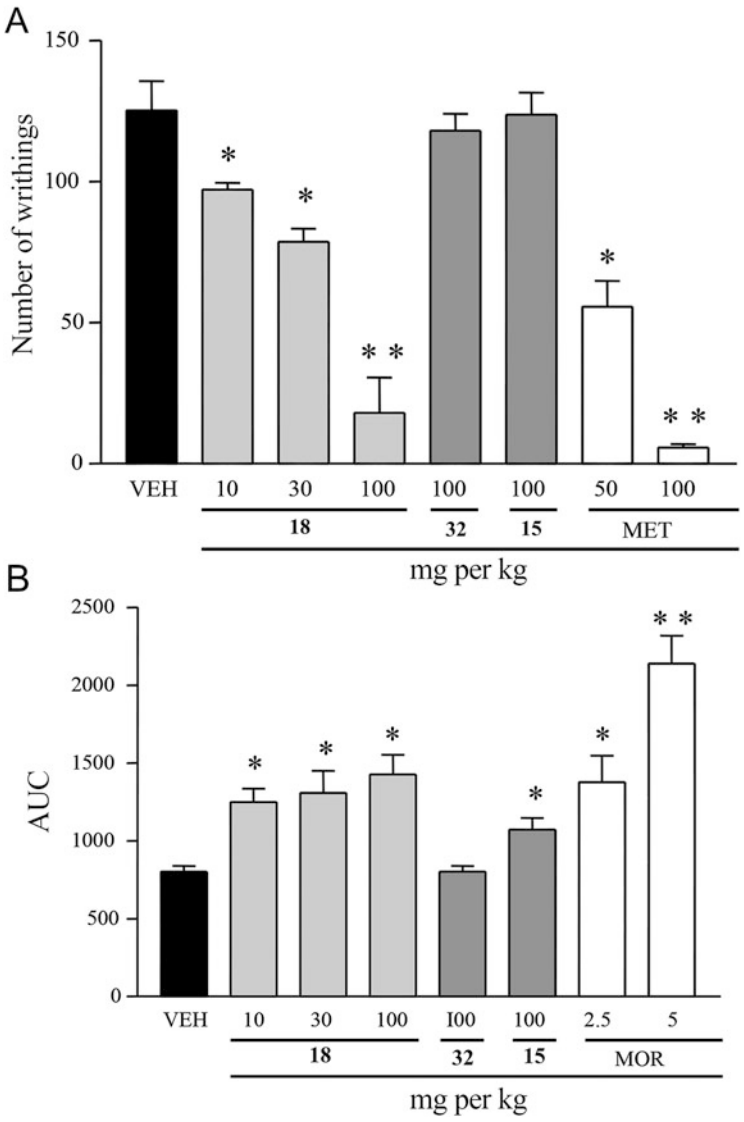



**Fig. 14** Antinociceptive effects of desoxycordifolinic acid (**15**), cucurbitacin **18** and 4-phenylcoumarin **32** in mice submitted to the writhing (**A**) and hot-plate (**B**) tests. Metamizol (MET) and morphine (MOR) were used as positive controls in the writhing and hot-plate tests. In both cases, bars are the means from six data points ±SEM. *$p < 0.05$ and **$p < 0.01$. Adapted from [29]

mediated by the nitric oxide-cyclic GMP pathway at the peripheral and/or central levels, opening of ATP-sensitive $K^+$ channels, and activation of the opioid receptors or increasing the levels of endogenous opioids. Altogether, these results suggested that the extract of *H. standleyana* investigated is able to reduce inflammatory and central pain in mice [29].

The aqueous extracts from the stem bark and leaves of *H. latiflora* and *H. standleyana* (80.5%, 80.2%, 75.1%, and 76.9% of gastroprotection, respectively), as well as compounds **32** ($ED_{50} = 15$ mg/kg) and **38** ($ED_{50} = 26$ mg/kg), were able to inhibit ethanol-induced gastric lesions in rats [40]. Intragastric application of absolute ethanol to produce gastric lesions in experimental animals is a well-known and reproducible method to investigate cytoprotective agents. The mode of action of **32** and **38** involved non-protein sulfhydryl endogenous compounds, because when the rats were pretreated with *N*-ethylmaleimide (NEM), a sulfhydryl alkylator, their gastroprotective action was inhibited [40].

Both *Hintonia* species contain more than one active constituents that act on different targets. Accordingly, these plants exhibit a wide range of biological properties, which collectively could be useful for treating a multifactorial disease such as diabetes.

### 2.1.3 *Salvia circinata*

*Salvia circinata* Cav. (syn. *S. amarissima* Ortega) (Lamiaceae) is a perennial shrub native to Mexico (Fig. 15). It grows up to 1.5 m tall. The whitish green oval leaves are rough or wrinkled and usually downy. The flowers, usually pale blue, feature tubular two-lipped corollas and produce nutlet fruits [41]. *Salvia circinata* was also listed as a medicinal in the catalog of plants from the Royal Botanical Expeditions to New Spain. A tea brewed from the aerial parts of the plant is used in Mexican folk medicine for treating ulcers, helminthiasis, and diabetes. According to the Lorke criterion, this tea did not provoke behavior alterations, macroscopic tissue injury, or weight loss, when tested during 14-day in mice; the estimated $LD_{50}$ was higher than 5 g/kg [41].

Single oral administration of the traditional preparation (100–570 mg/kg) to normal and NAA-STZ hyperglycemic mice induced a perceptible decrease of blood glucose level. During an OSTT, the infusion (31.6, 100, and 316 mg/kg) also significantly reduced the postprandial peak when compared with a vehicle-treated group; the effect observed was comparable to that of the positive control, acarbose. However, the preparation (100–570 mg/kg) did not induce a significant drop in the postprandial peak after a glucose challenge in normal and hyperglycemic mice. These results strongly suggested that the antihyperglycemic effect of *S. circinata* infusion could be due to the presence of $\alpha$-glucosidase inhibitors, which may be able to prevent postprandial hypersecretion of insulin and reactive hypoglycemia [41]. The active compounds included a few new glucosides of

**Fig. 15** *Salvia circinata*

tricyclic neo-clerodane type diterpenoids with the six-membered rings *trans*-fused, and the side chain fragment forming ethylbutenolides, ethylfuran or linear substructures. These terpenoids were given the trivial names amarisolides A–E (**42**–**46**) (Fig. 16). Several flavonoids (**47**–**50**) were also among the active principles (Fig. 16). All compounds were characterized on the basis of their spectroscopic properties. The absolute configurations at the stereocenters of diterpenoids **42**–**46** were established by comparison of the experimentally obtained and calculated electronic circular dichroism spectra [41].

Compounds **42**–**50** were tested in vitro against rat small intestine $\alpha$-glucosidase. The more active compounds were the flavonoids, in particular, compound **47**, which showed an $IC_{50}$ value of 39 $\mu M$ and was 2.5 times more active than acarbose ($IC_{50} = 100\ \mu M$). Flavonoids **48**–**50** exhibited $IC_{50}$ values of 810, 200, and 1800 $\mu M$, respectively. Regarding the diterpenoids, the most active compound was **42**, with an $IC_{50}$ value of 500 $\mu M$. Compounds **42** and **48** were also tested against a recombinant $\alpha$-glucosidase with maltase-glucoamylase activity from *Ruminococcus obeum*, a bacterium found in the human intestine that is involved in carbohydrate metabolism. This enzyme is phylogenetically closer to human *N*-maltase-glucoamylase than those of rats. The results of the assays showed that **42** and **48** inhibited the activity of the pure enzyme with $IC_{50}$ values of 400 and 60 $\mu M$,

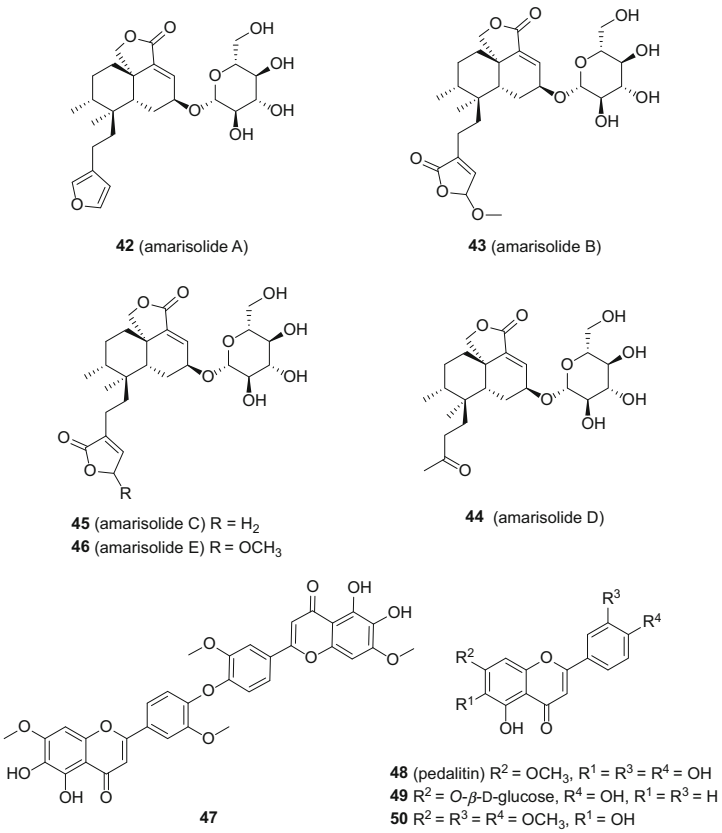



**Fig. 16** Compounds isolated from *Salvia circinata*

respectively ($IC_{50} = 1030\ \mu M$ for acarbose). In vivo evaluation of these compounds reduced significantly the postprandial peak in a dose-dependent manner during an OSTT in mice (Fig. 17). In both cases, the effect was comparable to that of the positive control, thus revealing their antihyperglycemic potential. Finally, docking and molecular dynamics analyses of the *R. obeum* $\alpha$-glucosidase complexes with **42**, **48**, and acarbose, predicted that the three products bind to the catalytic site of the enzyme (Fig. 18). The molecular dynamics studies indicated that the binding of the compounds was stable during the period of analysis (20 ns) [41].

*Salvia circinata* biosynthesizes also several *seco*-clerodane diterpenoids, some of which are moderately cytotoxic against human cancer cell lines and exhibited modulatory activity in a breast cancer cell line resistant to vinblastine [42, 43].

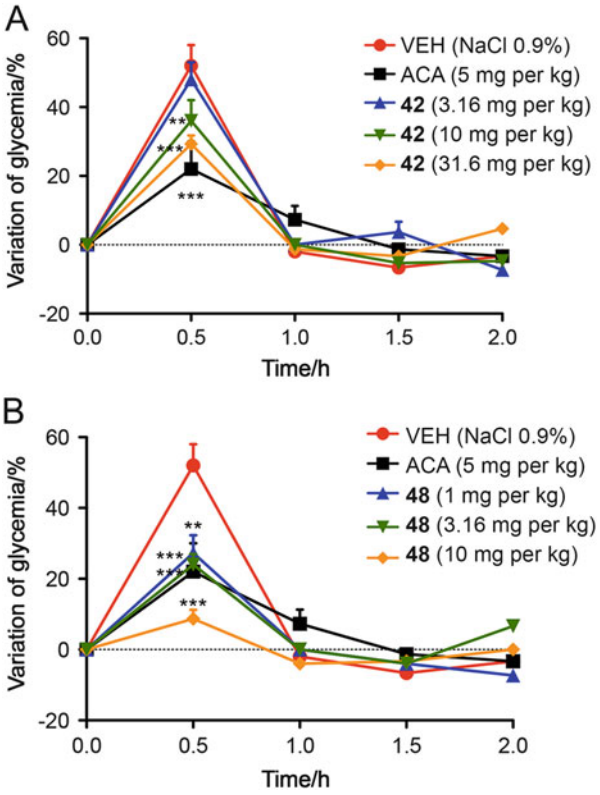



**Fig. 17** Antihyperglycemic action of amarisolide A (**42**) (**A**) and pedalitin (**48**) (**B**) in normoglycemic mice during an OSTT. VEH: vehicle; ACA: acarbose. Each point represents the mean from six data points ±SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$. Adapted from [41]

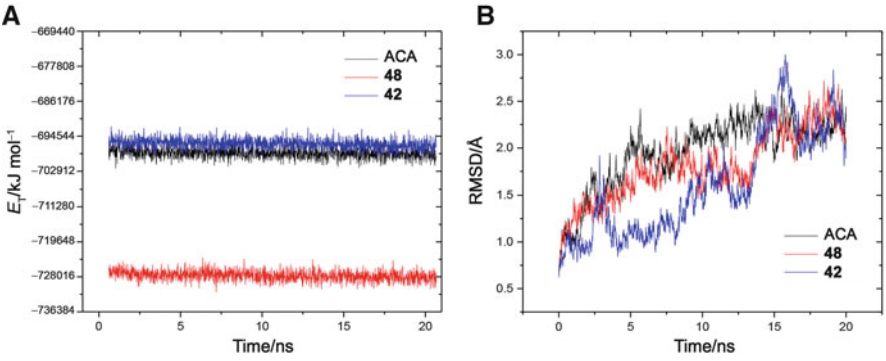



**Fig. 18** Molecular dynamics trajectory analysis: Total energy of the system as a function of the time of acarbose (ACA), amarisolide A (**42**) and pedalitin (**48**) (**A**), and RMSD as a function of time (**B**). Adapted from [41]

## 2.2 *Smooth Muscle-Relaxant Agents for Gastrointestinal and Cardiovascular Illnesses*

Ethnobotanical reports refer to the use of several herbs for the treatment of stomachache (with or without diarrhea), pains of hepatic and splenic origin, or related functional disorders (irritable bowel syndrome, abdominal bloating, and dyspepsia). More than 400 plant extracts have been examined for their spasmolytic action [44–49], but only a few studies have resulted in the isolation and identification of the active principles concerned.

Hypertension affects around 48 million of adults in Mexico and is expected to increase with rising rates of obesity. Hypertension greatly increases the risk of cardiovascular diseases such as ischemic heart disease (the second leading cause of death in Mexico) and stroke (the third leading cause of death in Mexico). It is also a co-morbidity very common in diabetic patients [50]. Different plants with antihypertensive properties are used in Mexican folk medicine [51]. A preliminary pharmacological survey of plants used in Mexico for the treatment of hypertension indicated that 186 plant species belonging to 163 genera and 76 families are used for treating hypertension. The authors pointed out that 47% of the total had been studied at least once from the phytochemical point of view, with 74% subjected to investigations using mostly in vitro pharmacological assays.

On the basis of the above considerations, we investigated three orchid species and the results are summarized in the next few paragraphs. It is worth mentioning that two papers summarizing some of the work carried out in Mexico regarding spasmolytic plants and their active principles were published recently [52, 53]. In addition, the Appendix Table summarizes selected medicinal plants with important smooth muscle-relaxant properties.

### 2.2.1 *Scaphyglottis livida*, *Maxillaria densa*, and *Nidema boothii*

Mexico produces about 1260 species of orchids with many have been used medicinally since prehispanic times, but, nevertheless, only 9 have been subjected to pharmacological and chemical studies [54]. *Scaphyglottis livida* (Lindley) Schltr. (Orchidaceae) (Fig. 19) is an epiphytic orchid found from Mexico to tropical South America. It is widely distributed along the tropical forests of the state of Veracruz, where the plant colonizes coffee farms and contributes to the shade conditions required for coffee growth. Important characteristics of the plant are the presence of cylindrical pseudobulbs, 5–6 cm long, 0.3 cm wide, unifoliated, arising from each rhizome; the leaves up to 14 cm long and 0.1 cm wide and are linear and grass-like. The inflorescence is fasciculate, with a single flower opening in time. The flower is small with bracts 5 mm long. In Los Tuxtlas in the state of Veracruz, the ground herb is applied topically to the body of humans to eliminate ectoparasites, and for treating wounds; the decoction is employed for treating stomachache and to prevent miscarriage [55, 56].

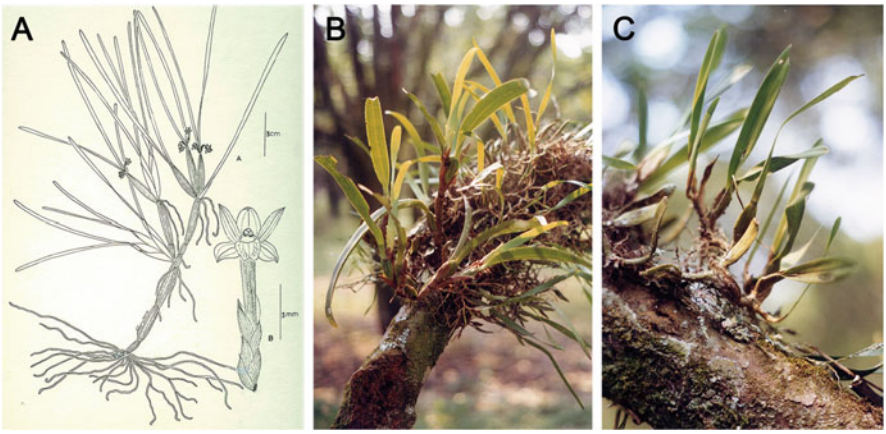



**Fig. 19** *Scaphyglottis livida* (**A**), *Maxillaria densa* (**B**), and *Nidema boothii* (**C**)

For assessing the potential smooth muscle relaxant-effect, the plant extracts and compounds were analyzed by means of classical isolated tissue bath assays [14, 55, 57]. These tests are useful for recording responses elicited by increasing concentrations of the test materials on the isometric contractions of guinea-pig or rat ilea and rat thoracic aortic rings (with and without the endothelium), among other contractile tissues. The power of these techniques is in their simplicity and versatility; by recording responses elicited by increasing concentrations of the test material, in the presence or absence of antagonists, a myriad of information can be derived about the pharmacological characteristics of each compound from the extracts and the receptor to which they might bind.

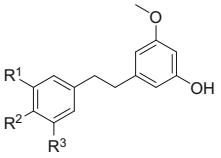
The application of the classical assays mentioned above showed that an organic extract of the whole plant of *S. livida* inhibited the spontaneous contractions of the rat ileum in a concentration-dependent manner ($IC_{50} = 6.0$ μg/cm$^3$) (Table 1) [58]. Bioassay-guided fractionation of the active extract led to the isolation of a series of stilbenoids including the bibenzyls gigantol (**51**) and batatasin III (**52**) as well as the phenanthrene derivatives coelonin (**53**), 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**54**), and denthyrsinin (**55**) (Fig. 20). These compounds showed also noted spasmolytic action, better than or comparable with papaverine. Different pharmacological experiments demonstrated that the smooth muscle-relaxant effect of these stilbenoids was mediated by neuronal release of nitric oxide.

Gigantol (**51**) produced the generation of NO/cGMP in the rat whole ileum as detected by a radioimmunoassay [55]. Compound **51** also inhibited the complex $Ca^{2+}$-calmodulin (CaM)-CaM-sensitive phosphodiesterase 1 (PDE1) and potentiated the antispasmodic action of chlorpromazine, a classical CaM inhibitor [57]. In addition, it quenched the extrinsic fluorescence of the human CaM biosensor, *h*CaMeM124C-mBBr (Fig. 21) [59]. Altogether, these results suggested that gigantol (**51**) is a CaM inhibitor and that its smooth muscle-relaxant activity is

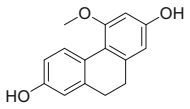
**Table 1** Inhibition of spontaneous contractions of the isolated rat ileum induced by an extract and compounds from *Scaphyglottis livida*

| Compound | $E_{max}/\%^{a,b}$ | $IC_{50}/\mu M^{c}$ | Potency[c] |
|---|---|---|---|
| Papaverine | 96.70 ± 5.02 | 1.55 ± 0.12 | 1.00 |
| Extract | 82.10 ± 6.02 | 6.06 ± 1.02[d] | – |
| Gigantol (**51**) | 93.50 ± 3.02 | 5.83 ± 0.55 | 0.27 |
| Batatasin III (**52**) | 85.20 ± 2.08 | 0.74 ± 0.07 | 2.10 |
| Lusianthridin (**53**) | 80.00 ± 1.98 | 0.95 ± 0.03 | 1.63 |
| 3,7-Dihydroxy-2,4-dimethoxyphenanthrene (**54**) | 83.59 ± 1.30 | 0.66 ± 0.01 | 2.33 |
| Denthyrsinin (**55**) | 88.05 ± 1.80 | 7.13 ± 0.42 | 0.22 |

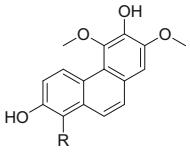
[a]Indicates the percentage of maximum inhibition
[b]Means ±SEM; $n = 6$; $p < 0.05$
[c]Potency was obtained by the formula: $IC_{50}/\mu M_{papaverine}/IC_{50}/\mu M_{compound}$, assuming a value of 1.00 for papaverine
[d]The $IC_{50}$ of the extract is expressed in μg/cm$^3$




**51** (gigantol) $R^1 = OCH_3$, $R^2 = OH$, $R^3 = H$
**52** (batatasin III) $R^1 = R^2 = H$, $R^3 = OH$




**53** (coelonin)




**54** R = H
**55** (denthyrsinin) R = $OCH_3$

**Fig. 20** Stilbenoids and phenanthrenes of *Scaphyglottis livida*

also mediated by CaM. More recently, the presence of gigantol (**51**) was reported as the spasmolytic agent of the medicinal Mexican orchid *Encyclia michuacana* [60].

Subsequently, we have screened several Mexican orchid extracts for their ability to relax the spontaneous rat ileum contraction and selected *Maxillaria densa* Lindley and *Nidema boothii* (Lindl.) Schltr. (Fig. 19) for fractionation. Following a similar strategy as described for *S. livida*, *M. densa* yielded six phenanthrene derivatives,