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STUDY OF THE ETHNOPHARMACOLOGICAL POTENTIAL OF *Catasetum integerrimum* Hook. IN THE HUASTECA POTOSINA

TESIS QUE PARA OBTENER EL GRADO DE

DOCTORADO EN CIENCIAS AMBIENTALES

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In memory of my grandma Victoria Hernández Esteban

and all the people who heal with medicinal plants

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“There is a time for everything, and a season for every activity under the heavens (Ecclesiastes 3:1)”

ÍNDICE

ABSTRACT

Catasetum integerrimum Hook. is an epiphytic orchid distributed in tropical areas such as the Huasteca Potosina. In this region, the population uses this orchid for treating kidney and gastrointestinal disease and diabetes mellitus. This research aimed to evaluate and validate the medicinal potential of *C. integerrimum* with a comparative study between wild plants and plants propagated by *in vitro* culture. Moreover, this research contributes to updating scientific knowledge about Mexican orchids with medicinal potential and their biotechnological uses. The achievement of these objectives was possible through the development of six stages of the project including i) a systematic review of the endemic orchids of Mexico, their distribution in the country, and their biotechnological uses, ii) a review concerning the biotechnological uses of the medicinal plants worldwide, iii) the contribution of the first review on Mexican orchids with medicinal potential, iv) a contribution on the medicinal properties, phytochemicals and biotechnological use of orchids of the *Catasetum* genus, v) the establishment of an *in vitro* method for biotechnological propagation of unlimited plant material and vi) pharmacological tests to evaluate the medicinal potential conferred on this orchid. To comply with the experimental stages, interviews were carried out in four municipalities of the Huasteca Potosina, Cd. Valles, Axtla de Terrazas, Matlapa, and Xilitla to record the medicinal uses that the population confers on this orchid. Subsequently, *C. integerrimum* capsules were collected before dehiscence and transported to the laboratory for performing an aseptic process. Seeds were cultivated in basal MS medium. After six months of culture, vitroplants were used as explants to establish a micropropagation protocol by direct organogenesis. The results showed that the best explant was the pseudobulbs. Pseudobulbs developed 1 shoot per explant with 5.50 ± 0.18 leaves per shoot and 4.37 ± 0.37 roots per explant with a root length of 4.88 ± 0.20 cm. With enough plant material, a microwave-assisted ethanolic extraction of the vitroplants (EEV) and the pseudobulbs obtained from wild orchids (EEP) was carried out. Furthermore, a phytochemical profile and DPPH and ABTS assays were performed to evaluate the antioxidant potential in both extracts. The phytochemical profile registered the presence of phytosterol alkaloids, terpenoids, sesquiterpene lactones, flavones, coumarins, phenolic hydroxyls, and glycosides. These same phytochemicals were present in the EEP. Regarding the antioxidant potential, EEP had the best antioxidant activity. The ABTS assay showed a 78% inhibition of free radicals in the presence of the sample at 6 minutes. The DPPH assay showed a 52% inhibition of free radicals in the presence of the sample at 20 minutes. Finally, to validate the medicinal uses reported by the Nahuatl population, pharmacological tests were carried out to evaluate the diuretic and antidiarrheal activity. The results showed that 100 mg/kg EEP did not show a diuretic effect. EEP showed effectiveness by inhibiting the diarrheal process by 76%. Likewise, it was observed that EEP retarded the onset of diarrhea by 50% compared to the effect caused by the reference drug loperamide. EEP also significantly ($p<0.05$) reduced gastrointestinal transit. EEP at 10 mg/kg showed the best decrease in intestinal fluid accumulation, compared to the activity shown by 2.5 mg/kg loperamide. The mean effective dose (ED_{50}) of EEP, obtained by linear regression, was 2 mg/Kg in the castor oil-induced diarrhea. These results provide the first micropropagation protocol by direct organogenesis with an unlimited production of vitroplants, which can contribute to the conservation and study of this species. Moreover, this research validated that the EEP of *C. integerrimum* has a high antidiarrheal potential. Finally, it is important to mention that this study is the first to demonstrate the antidiarrheal medicinal potential of this orchid.

Keywords: Mexican orchids, antidiarrheal potential, medicinal orchids, direct organogenesis, *Catasetum*

RESUMEN

Catasetum integerrimum Hook. es una orquídea epífita distribuida en zonas tropicales como la Huasteca Potosina. En esta región, la población utiliza esta orquídea para el tratamiento de enfermedades renales, gastrointestinales y diabetes mellitus. Esta investigación tuvo como objetivo evaluar y validar el potencial medicinal de *C. integerrimum* con un estudio comparativo entre plantas silvestres y plantas propagadas por cultivo *in vitro*. Además, esta investigación contribuye a actualizar el conocimiento científico sobre las orquídeas mexicanas con potencial medicinal y sus usos biotecnológicos. El logro de estos objetivos fue posible a través del desarrollo de seis etapas del proyecto que incluyen i) una revisión sistemática de las orquídeas endémicas de México, su distribución en el país y sus usos biotecnológicos, ii) una revisión sobre los usos biotecnológicos de las plantas medicinales a nivel mundial, iii) el aporte de la primera revisión sobre orquídeas mexicanas con potencial medicinal, iv) un aporte sobre las propiedades medicinales, fitoquímicas y uso biotecnológico de las orquídeas del género *Catasetum*, v) el establecimiento de un método *in vitro* para la propagación de material vegetal ilimitado y vi) pruebas farmacológicas para evaluar el potencial medicinal conferido a esta orquídea. Para cumplir con las etapas experimentales se realizaron entrevistas en cuatro municipios de la Huasteca Potosina, Cd. Valles, Axtla de Terrazas, Matlapa y Xilitla para registrar los usos medicinales que la población le confiere a esta orquídea. Posteriormente, se recolectaron las cápsulas de *C. integerrimum* antes de la dehiscencia y fueron transportadas al laboratorio para realizar un proceso aséptico. Las semillas se cultivaron en medio MS basal. Después de seis meses de cultivo, las vitroplantas se utilizaron como explantes para establecer un protocolo de micropropagación por organogénesis directa. Los resultados mostraron que el mejor explante fueron los pseudobulbos. Los pseudobulbos desarrollaron 1 brote por explante con 5,50 ± 0,18 hojas por brote y 4,37 ± 0,37 raíces por explante con una longitud de raíz de 4,88 ± 0,20 cm. Con suficiente material vegetal se realizó una extracción etanólica asistida por microondas de las vitroplantas (EEV) y los pseudobulbos obtenidos de orquídeas silvestres (EEP). Además, se realizó un perfil fitoquímico y ensayos de DPPH y

ABTS para evaluar el potencial antioxidante de ambos extractos. El perfil fitoquímico registró la presencia de alcaloides de fitoesteroles, terpenoides, lactonas sesquiterpénicas, flavonas, cumarinas, hidroxilos fenólicos y glucósidos. Estos mismos fitoquímicos estaban presentes en el EEP. En cuanto al potencial antioxidante, EEP tuvo la mejor actividad antioxidante. El ensayo ABTS mostró un 78% de inhibición de radicales libres en presencia de la muestra a los 6 minutos. El ensayo DPPH mostró un 52% de inhibición de radicales libres en presencia de la muestra a los 20 minutos. Finalmente, para validar los usos medicinales reportados por la población náhuatl, se realizaron pruebas farmacológicas para evaluar la actividad diurética y antidiarreica. Los resultados mostraron que 100 mg/kg de EEP no mostraron un efecto diurético. EEP mostró efectividad al inhibir el proceso diarreico en un 76%. Asimismo, se observó que EEP retardó la aparición de diarrea en un 50% en comparación con el efecto provocado por el fármaco de referencia loperamida. EEP también redujo significativamente ($p<0,05$) el tránsito gastrointestinal. EEP a 10 mg/kg mostró la mejor disminución en la acumulación de líquido intestinal, en comparación con la actividad mostrada por 2,5 mg/kg de loperamida. La dosis efectiva media (DE_{50}) de EEP, obtenida por regresión lineal, fue de 2 mg/Kg en la diarrea inducida por aceite de ricino. Estos resultados proporcionan el primer protocolo de micropropagación por organogénesis directa con una producción ilimitada de vitroplantas, que pueden contribuir a la conservación y estudio de esta especie. Además, esta investigación validó que el EEP de *C. integerrimum* tiene un alto potencial antidiarreico. Finalmente, es importante mencionar que este estudio es el primero en demostrar el potencial medicinal antidiarreico de esta orquídea.

Palabras clave: orquídeas mexicanas, potencial antidiarreico, orquídeas medicinales, organogénesis directa, *Catasetum*

INTRODUCTION

Mexico has about 1,400 species of orchids distributed in its ecosystems. Of this amount, 40% are considered endemic to the Mexican territory (Castillo-Pérez et al., 2019). However, despite this vast diversity of orchids, 181 species are listed in some risk category according to NOM-059-SEMARNAT-2010. Internationally, orchids have been used mostly as ornamental plants due to the beauty and eccentricity of their flowers. However, there are some species that are used in the food field, such as *Vanilla planifolia*. Moreover, some orchids have been used for medicinal purposes (Gantait et al., 2021).

According to the Royal Botanical Garden at Kew, approximately 2.3% of the total species of the Orchidaceae family have been reported to have some medicinal property. At the national level, there is no precise data on the number of medicinal orchid species, but it is known that since pre-Hispanic times some species were already beginning to be used for the treatment of some conditions and diseases (Castillo-Pérez et al., 2019).

In the state of San Luis Potosí, 152 species of orchids divided into 70 genera have been documented, distributed mainly in the humid mountain forest ecosystem (Fortanelli-Martínez et al., 2022). This ecosystem is found mainly in the Huasteca Potosina region, where 67 species have been recorded just for the municipality of Xilitla (Ramírez-Palomenque et al., 2019).

Among the several species of orchids with distribution in the Huasteca Potosina, it found *Catasetum integerrimum*. This species is distributed in the tropical forest, low deciduous and sub-deciduous forest ecosystems and its present from the Gulf of Mexico to countries such as Guatemala, Honduras, Belize, El Salvador and Nicaragua (Salazar et al., 1990).

Currently, there are records of the use of *C. integerrimum* in traditional medicine in several parts of Mexico for the treatment of tumors, kidney and gastrointestinal diseases, skin problems, snake bites, and even diabetes mellitus (Alayón-Gamboa 2011; Alonso-Castro et al. 2011; Cox-Tamay 2013; Cruz-García et al. 2014; Castillo-Pérez et al., 2022). However, until the moment there are no scientific reports that validate these empirical medicinal properties.

The ornamental use of *C. integerrimum* and the fame it has acquired as a medicinal plant could be causing the decline of wild populations in regions such as the Huasteca Potosina, which implies urgently proposing sustainable strategies that reduce or solve this problem. Therefore, the objective of the present research was to evaluate the ethnopharmacological potential conferred to the orchid *C. integerrimum* with a comparative study between wild plants and vitroplants.

Justification

The Orchidaceae family is one of the largest families in the plant kingdom. Despite being a family of cosmopolitan species, a large number of orchids are threatened or endangered throughout the world. For this, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) places the entire Orchidaceae family under protection in its Appendix II.

Due to the eccentricity of their flowers, a large number of orchids in Mexico are collected for ornamental use, others for use in religious festivities, food use and also for their medicinal properties. These activities, together with the extraction of wild specimens and illegal trade, have placed 181 species in some category of risk (SEMARNAT, 2010).

Among the orchids collected for their medicinal properties is *C. integerrimum*, an orchid that has been reported in the state of Tabasco with properties against tumors (Alayón-Gamboa, 2011) and in the Huasteca region of the state of San Luis Potosí, it is used by the Tének and Nahuatl populations to treat gastrointestinal and renal problems and against diabetes mellitus (Castillo-Pérez et al., 2022).

The empirical medicinal properties conferred to *C. integerrimum* in the Huasteca Potosina, have caused the uncontrolled extraction of specimens in several municipalities such as Cd. Valles, Axtla de Terrazas, Matlapa and Tamazunchale. Therefore, it is necessary to establish a prevention and control strategy to study the medicinal properties of this orchid, but without breaching its wild populations. Moreover, the information that is available on the medicinal properties of this orchid is null.

In this context, the implementation of techniques based on plant biotechnology, such as *in vitro* micropropagation, could be an excellent strategy, since, with the massive propagation of *C. integerrimum*, sufficient plant material can be obtained to make extracts and test pharmacological tests necessary to validate the medicinal properties that have been conferred on this orchid.

General Objective

To contribute for the conservation and knowledge of the medicinal properties of the *C. integerrimum* orchid, the aim of this study was to evaluate and validate its pharmacological potential with a comparative study between wild plants and vitroplants.

Specific Objectives

1. To register the ethnopharmacological use of *C. integerrimum* in the Huasteca Potosina region.
2. To establish the *in vitro* culture by direct organogenesis way of the *C. integerrimum* orchid.
3. To determine the phytochemical profile and antioxidant capacity of ethanolic extracts from wild and propagated plant material of *C. integerrimum*.
4. To evaluate the pharmacological properties (diuretic effect and antidiarrheal potential) of *C. integerrimum* with laboratory tests.

Thesis Structure

The knowledge of the medicinal properties of *C. integerrimum* and the establishment of an *in vitro* protocol for its propagation are described in the following seven Chapters. Six Chapters correspond to six scientific articles that comprise the core of this thesis. The final Chapter includes a general discussion for the importance and contributions made with this research. Furthermore, conclusions, recommendations and future prospects are included.

Chapter I focuses on a systematic review concerning to the endemic orchids of Mexico. In this review, works on biotechnological micropropagation, ecology and distribution of endemic orchids in natural protected areas of Mexico are gathered and discussed. This work shows the huge diversity of Mexican endemic orchids and the threats they suffer. Chapter II corresponds to the biotechnological approaches that have been established for the conservation of medicinal plants around the world. In Chapter III, the diversity of medicinal orchids that exist in Mexico is analysed for the first time. This is followed by a critical review of the ethnomedicinal uses, phytochemistry, medicinal potential and biotechnological strategies for the conservation of orchids of the genus *Catasetum* (Chapter IV), where the gap in knowledge that exists about the study of this species is revealed. Chapter V presents the establishment of a protocol for the micropropagation of *C. integerrimum* (Orchidaceae) through seed germination and direct shoot regeneration from pseudobulbs and roots is reported. Finally, in Chapter VI the results of the pharmacological studies carried out to validate the medicinal potential of *C. integerrimum* are presented.

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CHAPTER I

The endemic orchids of México: a review.

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Abstract

Orchids are the most extensive family of flowering plants in the kingdom Plantae. There are between 30,000 - 35,000 species, belonging to 850 genera, which are distributed in almost all the ecosystems of the planet, except in polar zones and extreme deserts. In Mexico there are more than 1,200 species of the Orchidaceae family, divided into approximately 170 taxonomic genera, of which approximately 40% are endemic to the Mexican territory. In this review are discussed aspects related to the distribution, ecology, conservation, biotechnological applications and uses for the orchid species that grow and develop specifically in Mexico. Under this idea, the geographical zones where their ecological niches are located are described, highlighting if these zones belong to priority conservation areas for Mexico. In addition, all works about the Mexican orchids related with micropropagation, medicinal uses, beneficial applications for humans, and associated microorganisms to endemic orchids of Mexico are discussed in depth. In summary, this work presented a general overview of all aspects related to endemic orchids of Mexico, allowing to expand the possibilities and perspectives to propose new studies and research focused on the use, propagation and conservation of these beautiful and interesting plants.

Keywords:

mexican orchids, orchid conservation, ecology of orchids, endemic species.

Introduction

Orchidaceae family includes 850 genera with more than 30,000 species and is considered the largest and most specialized family of Angiosperms (Chase et al. 2003, 2015; Dressler 2005; Hágsater et al. 2015). Members of this plant family are distributed throughout the world, and more than 50% of these species are concentrated in tropical areas of the world (Chase et al. 2003, 2015; Givnish et al. 2015; Hágsater et al. 2015). Orchids have particular characteristics, including the structure of its flowers and roots, its pollination processes, and its the ecological interactions established with other living organisms, such as plants, insects, fungi, and bacteria (Bronstein et al. 2014; Phillips et al. 2014; Selosse 2014; Nurfadilah et al. 2016).

In Mexico, there are approximately 1,200 species of orchids, of which approximately 40% are endemic and distributed throughout the country (Espejo-Serna 2012; Hágsater et al. 2015). Some species of Mexican orchids are very attractive to horticulturists, collectors, and general public, so they are extracted from its natural habitats and sold illegally, which lead to their threat of extinction (Halbinger and Soto 1997; Merritt et al. 2014; Rewicz et al. 2015). Currently, orchids from Mexico are considered the second most threatened plant family in terms of illegal trafficking of species, something that occurs even in natural protected areas of Mexico (Solano-Gómez et al. 2010; Alvarado-Martínez 2012; Cruz-Garcia et al. 2015).

The micropropagation of threatened or in danger of extinction orchids by the tissue culture system, and their subsequent reintegration into their ecological niches, have been used as biotechnological strategies to help their conservation (Moreno-Martínez and Menchaca-García 2007; Santos-Díaz and Carranza-Álvarez 2009). The application of mycorrhizas to promote the growth and development of orchids under natural conditions is another strategy that had been used to propagate and conserve some Mexican species (Oja et al. 2014; Jacquemyn et al. 2015; Rasmussen et al. 2015; Reiter et al. 2016). Considering the peculiarity and the ecological importance of the Mexican orchids, in this paper we review and discuss the distribution, uses, as well as the biotechnological and ecological studies that have been carried out about these plants.

This review also focuses on the conservation of these plants, many of which are categorized as threatened or in danger of extinction.

General aspects of orchids

Orchids are considered the most evolved family of flowering plants and the family with the second largest number of species (Chase et al. 2003, 2015; Hágsater et al. 2015). Orchids have developed evolutive adaptations in their physiology and morphology (e.g., floral morphology, which is the ability to develop and grow in different habitats), as well as their pollination and their specificity with pollinators. These characteristics have allowed its growth and development in almost all ecosystems from the world (Williams and Whitten 1983; Nilsson 1992; Wasserthal 1997; Ng and Hew 2000; Ehlers et al. 2002; Rudall and Bateman 2002; Johnson et al. 2003; Schiestl et al. 2003; Cozzolino and Widmer 2005; Schiestl 2005; Jersáková et al. 2006; Ramírez et al. 2007; Leitch et al. 2009; Phillips et al. 2014; Whitehead and Peakall 2014; Hágsater et al. 2015; Phillips et al. 2017).

Orchids can be classified as epiphytes, terrestrial or lithophytic. Epiphytic orchids grow in tropical areas on different tree species without being parasitic and have developed specialized roots for obtaining nutrients from the environment (Ceja-Romero et al. 2008; Hurtado-Alza et al. 2017). The flowers of many orchids are considered exotic and of high commercial value in horticulture (Mondragon 2009; Mohanty et al. 2012; Liu et al. 2015). Terrestrial orchids have developed tubers that grow underground, which enables them to easily obtain water and essential nutrients for its development (Rasmussen and Whigham 2002; McCormick et al. 2004; Juárez et al. 2016). Lithophytic or rupicolous orchids grow on rocks and have developed rhizomes for obtaining nutrients from humus deposits formed in the holes, cracks or cavities (Weston et al. 2005; Putz 2007; Hágsater et al. 2015).

Most of the genera in the Orchidaceae produce bisexual flowers (Chase and Hills 1992; Fritz and Nilsson 1996; Menchaca-García 2011). The orchids flowers have three sepals and three petals, one petal is modified and is called lip, and it is where pollination agents arrive to obtain the pollen to pollinate other flowers (Moreira-Muñoz 2009; Hágsater et al. 2015; Madrigal-Bedoya and Pabón-Mora 2017). The orchid pollen is amalgamated in specialized structures called pollinia, and each orchid species have different number of pollinia (Martija-Ochoa 2003; Quiroga et al. 2010; Menchaca-García 2011). Interestingly, the seeds in orchids lack endosperm, and need a symbiosis with fungi, which provide nutrients, for its germination (Johansen and Rasmussen 1992; Ordóñez et al. 2012; Chávez et al. 2015).

Most of the Mexican orchids are classified as tropical and epiphytes (Soto-Arenas et al. 2007; Hágsater et al. 2015). Although there are terrestrial orchids in Mexico, these are found in a lesser proportion compared to the epiphytic orchids (Sharp et al. 1950; Hágsater et al. 2015). In addition, orchids belonging to the Laelias, Stanhopeas, and Rhynchosteles genera develop flowers with characteristic odors and attractive combinations of colours (e.g., *Laelia anceps*, *Rhynchostele cervantesii*, and *Stanhopea tigrina*). Many Mexican endemic species are considered rare and attractive to collectors. *Prosthechea mariae* (Fig. 1), cataloged as one of the most beautiful and most coveted Mexican orchids in the world (Dressler and Pollard 1974; Soto 2002), is classified as an endangered species (NOM-059-SEMARNAT 2010).

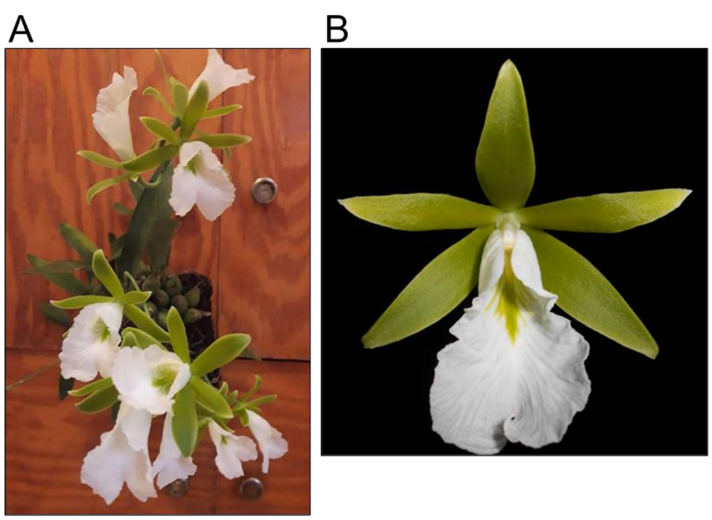

Fig. 1 *Prosthechea mariae*, endemic species considered one of the most beautiful in Mexico: a, Complete plant. b, Flower detail

The Mexican endemic orchids

Mexico is one of the most biodiverse countries in the world, hosting 12% (ca. 30,000) of all vascular plants in the world (Mittermeier and Goettsch 1992; Espejo-Serna 2012; Jiménez-Sierra et al. 2014). Mexico has also significant number of endemic vascular plant species (Rzedowski 1991a; Mittermeier and Goettsch 1992; Rzedowski 1996; Espejo-Serna 2012), which are remarkable components of its biodiversity, and contribute to determinate its importance and richness, and serve as indicators of healthy environmental factors such as soil and climate (Rzedowski 1991b; Espejo-Serna 2012; Martínez-Meyer et al. 2014).

In 2012, Espejo-Serna reported 596 species of the Orchidaceae endemic to Mexico, including 6 varieties and 8 subspecies. These species constitute 46.3% of the 1200 species of orchids growing in Mexico (Soto-Arenas et al. 2007; Hágsater et al. 2015). Interestingly, of all Mexican endemic species, 43.6% have a restricted distribution to only one state, whereas 56.4% of the species can be found in two or more states of country (Supplementary Table 1).

Sates with the largest number of endemic orchid species are Jalisco (western Mexico), Oaxaca, and Chiapas (southern Mexico), with 59, 76, and 29, respectively. Twenty two of the thirty-two states in Mexico have at least one endemic orchid. For example, Baja California, Puebla, Chihuahua, Coahuila, and Tamaulipas (northern Mexico), Puebla and Zacatecas (central Mexico) have only one endemic species restricted to its territory (Fig. 2).

Of the total Mexican endemic orchids, 73 species (40.3%) are classified under some category of protection according to the Norma Oficial Mexicana (NOM-059-SEMARNAT 2010). Of these, 37 species are cataloged under special protection, 28 as threatened, seven as endangered, and one (*Laelia gouldiana*) is considered as extinct (Table 1).

Mexico has the highest percentage of endemic orchids in Latin America. The percentage of endemic orchids in the Mexican territory is higher than the percentages of endemic orchid species reported in Colombia (41.7%) and Ecuador (42.6%), two of the countries with the largest number of endemic orchids in Latin America (Cribb et al. 2003; Meisel and Woodward 2005; Sarmiento-Téllez and

Betancur 2006; Flores-Escobar et al. 2008). Unfortunately, the Orchidaceae is the second most threatened family of vascular plants, surpassed only by the Cactaceae (McMahan and Walter 1989; Alvarado-Martínez 2012). The main causes affecting the conservation of orchids are: i) excessive looting and illegal national and international trade, ii) deforestation; and iii) wildfire, activity that its even being practiced in protected areas.

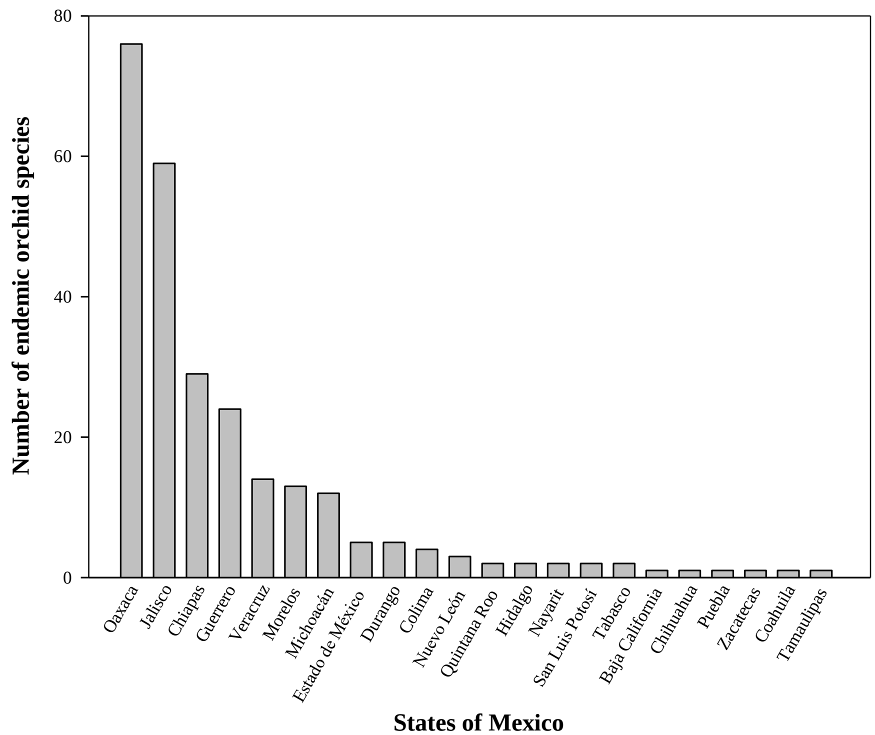

Fig. 2 States of Mexico that have endemic orchids

Table 1 Species of endemic orchids of Mexico propagated by plant tissue culture

Endemic Species	Explant used	Reference
Bletia urbana Dressler[3]	Seeds	Rangel-Villafranco 2004
Laelia albida Bateman ex Lindl.	Seeds	Santos-Hernández et al. 2005
Encyclia adenocaula (Lex.) Schltr. ssp. *adenocaula*[3]	Seeds	Ávila-Díaz and Salgado-Garciglia 2006
Prosthechea citrina (Lex.) W.E. Higgins[4]		
Laelia albida Bateman ex Lindl.		
Laelia autumnalis (Lex.) Lindl.		

Laelia speciosa (Kunth) Schltr.[4]		
Stanhopea tigrina Lindl.[3]	Seeds	Moreno-Martínez and Menchaca-García 2007
Prosthechea mariae (Ames) W.E. Higgins[3]	Seeds	Suárez-Quijada et al. 2007
Stanhopea tigrina Lindl.[3]	Seeds	Tinoco-Juárez and Mata-Rosas 2007
Laelia anceps Lindl.[2]		
Laelia eyermaniana Rchb.f.	Seeds	Francisco-Nava 2008
Encyclia adenocaula (Lex.) Schltr. ssp. *adenocaula*[3]	Seeds	Ruiz et al. 2008
Laelia speciosa (Kunth) Schltr.[4]	Seeds	Ávila-Diaz et al. 2009
Cuitlauzina pendula Lex.[3]	Seeds	Mata-Rosas and Salazar-Rojas 2009
Mormodes tuxtlensis Salazar		
Prosthechea mariae (Ames) W.E. Higgins[3]	Leaves and PLB´s*	Santos-Díaz and Carranza-Álvarez 2009
Laelia speciosa (Kunth) Schltr.[4]	Leaves	Sarabia-Ochoa et al. 2010
Laelia speciosa (Kunth) Schltr.[4]	Seeds	Aguilar-Morales and López-Escamilla 2013
Barkeria uniflora (Lex.) Dressler et Halb.	Seeds	Duarte-Salinas 2014
Laelia speciosa (Kunth) Schltr.[4]	Seeds	González-Castellanos 2014
Laelia anceps Lindl.[2]	Seeds	Castillo-Pérez 2016
Stanhopea tigrina Lindl.[3]	Seeds	Carranza-Álvarez et al. 2016
Prosthechea mariae (Ames) W.E. Higgins[3]		
Myrmecophila grandiflora (Lindl.) Carnevali, J.L. Tapia et I. Ramírez		
Laelia anceps Lindl.[2]		
Encyclia parviflora (Regel) Whitner		
Prosthechea citrina (Lex.) W.E. Higgins[4]	Seeds	Cazarez-Favela et al. 2016
Cuitlauzina pendula Lex.[3]	Seeds	Altamirano-López, Cabezas-García and García-Hernández 2017

*PLB´s = Protocorm Like Bodies
Different numbers denote different categories according to NOM-059-SEMARNAT-2010: [1]Probably extinct; [2]In danger of extinction; [3]Threatened; [4]In special protection

Distribution of the endemic orchids in the natural protected areas of Mexico

The natural protected areas of Mexico (Áreas Naturales Protegidas ANP, its acronym in Spanish) are organized into 6 main groups: i) biosphere reserves, ii) national parks, iii) natural monuments, iv) natural resources protected areas, v) protected areas of flora and fauna, and vi) Mexican sanctuaries. So far 2013, the Comisión Nacioal de Áreas Protegidas (CONANP, for its acronym in Spanish) decreed 176 ANPs throughout Mexico, equivalent to 25,394,779 ha., which represent 12.9% of the Mexican territory (Figueroa and Sánchez-Cordero 2008; CONANP 2018).

Floristic lists including orchids have been reported for only 60 (34.0%) of the natural protected areas of Mexico and the remaining 116 (66.0%) have not have reports on floristic listings or have not orchids within. The national parks (18 sites) and the flora and fauna protective areas (14 sites) have the largest number of sites where endemic orchids can be found (Supplementary Table 2).

The ANPs with the largest number of endemic orchids are El Tepozteco National Park, located in Mexico City (CDMX) and the state of Morelos (both states located in central Mexico), where 67 species of endemic orchids are distribuited (Espejo-Serna et al. 2002) and the flora and fauna protection area called Corredor Biológico Chichinautzin, located in the states of CDMX, as well as the states of Mexico and Morelos, where 66 orchid species are found (Pulido-Esparza et al. 2009). These data indicate that most of the Mexican endemic orchids are distributed in the center of the country.

Plant tissue culture for the conservation of Mexican endemic orchids

Plant tissue culture (PTC), a biotechnological tool carried out under controlled and aseptic conditions (Abdelnour-Esquivel and Escalant 1994; Calva-Calva and Pérez-Vargas 2005; Shahzad et al. 2017), is based on the totipotentiality of a plant cell, which in under appropriate concentration of nutrients and phytohormones has the ability to create a new complete plant (Thorpe 1994; Krikorian 1995; Thorpe 2007; Morales-Rubio et al. 2016). The main advantages of *in vitro* micropropagation include: i) propagation of a greater number of plants in a shorter time, ii) production of plants regardless of season, iii) propagation and conservation of a large number of plants in a small space, and iv) easy transportation of propagated plants (Razdan 1993; Abdelnour-Esquivel and Escalant 1994; Carranza-Álvarez et al. 2016).

Considering that approximately 40.3% of the Mexican endemic orchids are cataloged in some risk category (NOM-059-SEMARNAT 2010) in the last decade several investigations have been carried out to establish propagation protocols through biotechnological techniques such as PTC (some examples can be found in Ávila-Diaz and Salgado-Garciglia 2006; Flores-Escobar et al. 2008; Mayo-Mosqueda et al. 2010; Carranza-Álvarez et al. 2016, among others). Of the 596 Mexican endemic orchids, only 16 species (2.68%) have been propagated using PTC. Furthermore, nine of these orchids are listed under some risk category (NOM-059-SEMARNAT 2010) (Table 1). One of these species is *Laelia speciosa*, one of the most beautiful species of the genus *Laelia*, and one of the most exploited species in Mexico. This orchid currently is classified in special protection category (Halbinger and Soto 1997; Ávila-Diaz and Salgado-Garciglia 2006; González-Castellanos 2014).

Other species of Mexican orchids that have been propagated by PTC are *Encyclia adenocaula*, *Prosthechea citrina*, *Laelia albida*, *Laelia autumnalis*, and *Laelia speciosa* (Table 1; Fig. 3a-e). These orchids carry out important functions in their ecosystems, such as interactions with mycorrhizal fungi or with other microorganisms, and interactions with other plants (Luyando-Moreno et al. 2011; Morales-Hernández et al. 2016). In addition, *Encyclia adenocaula*, *Prosthechea citrina* and *Laelia speciosa*, cataloged as in extreme danger of extinction, have been propagated by PTC (Ávila-Diaz and Salgado-Garciglia 2006). Recently, Carranza-Álvarez et al. (2016) established propagation protocols for *Stanhopea tigrina*, *Prosthechea mariae*, *Myrmecophila grandiflora*, *Laelia anceps*, and *Encyclia parviflora*, which are endemic to Mexico and can be found in the wetland region Ciénega de Cabezas, located in Tamasopo, San Luis Potosí, a priority area for the conservation of both plant and animal species (Fig. 3g-k).

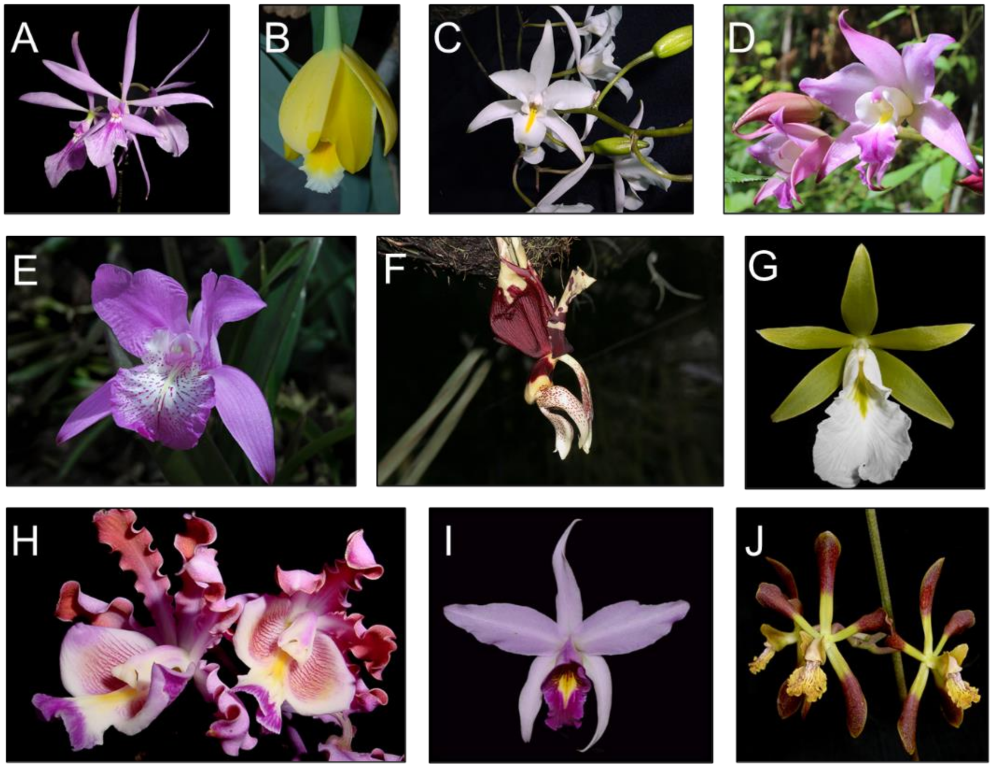

Fig. 3 Species that have been propagated by plant tissue culture: a, *Encyclia adenocaula*; b, *Prosthechea citrina*; c, *Laelia albida*; d, *Laelia autumnalis*; e, *Laelia speciose*; f, *Stanhopea tigrina*; g, *Prosthechea mariae*; h, *Myrmecophila grandiflora*; i, *Laelia anceps*; j, *Encyclia parviflora*

Table 1 shows that seeds are the explant most commonly used to establish tissue culture protocols because of their capability to conserve genetic diversity (Hamrick and Godt 1996; Gaudencio-Sedano et al. 2015; Aguilar-Morales et al. 2016). Despite the presence of some laboratories dedicated to the micropropagation of orchids in Mexico, many institutions do not have formal programs for the *ex situ* conservation or acclimatization of orchids (Téllez-Velasco 2011), limiting the propagation of Mexican orchids.

Organisms associated with endemic orchids of Mexico

Orchids, distributed in almost all terrestrial ecosystems, have important roles in ecology due to the large number of interactions established with plants, animals, and microorganisms (Granados-Sánchez et al. 2003; Ramos-Zambrano et al. 2007; Hágsater et al. 2015; Martínez-Salamanca and Romero-Escobar 2016).

Plant-plant and plant-animal interactions have been widely studied around the world for several orchid species (Nilsson et al. 1987; Bohman et al. 2017), for example, i) the relationships established between phorophytes (plants that support epiphytic organisms) and orchids; ii) the pollination processes between orchids and different pollination agents such as insects or birds; and iii) the mutualistic interactions established between orchids (housing for insects) and ants (protection against herbivores) (Hernández-Rosas 2000; Almeida and Figueiredo 2003; Mújica et al. 2010; Breitkopf et al. 2015; Morales-Hernández et al. 2016; Rasmussen and Rasmussen 2016; Bohman et al. 2017). Unfortunately, the interactions established between Mexican orchids and other organisms have been poorly studied. The most studied relationship of the Mexican orchids with other plants is likely the link established with phorophytes, trees where epiphytic orchids develop and complete their life cycle (Benzing 1987; Ceja-Romero et al. 2008). Additionally, it has been described that certain orchids develop specificity for a species of host tree (Benzing 1987; Migenis and Ackerman 1993; Mújica et al. 2010; Valverde and Bernal 2010; Wagner et al. 2015). For example, the Mexican orchids *Encyclia adenocaula*, *Erycina hyalinobulbon*, *Encyclia parviflora*, *Hintonella mexicana*, *Isochilus unilateralis*, *Laelia autumnalis*, *Oncidium hastatum* and *Stanhopea tigrina* have been found in association with *Quercus* trees (Table 2).

During the pollination process, pollination agents transfer pollen from the anther to the stigma allowing fertilization and future formation of the fruit (Hágsater et al. 2015; Juárez et al. 2016). The pollination agents have developed structural and physiological adaptations to carry out the pollination process with greater efficiency. Some of these adaptations are brushlike tongues, long and thin beaks, and small villi on their bodies that collect pollinia and allow its transportation (Van Der Niet et al. 2014; Gross et al. 2016; Bohman et al. 2017). Only six species (1%) of Mexican endemic orchids have been studied their interactions with insects. The association between Laelia anceps, Artorima erubescens, and *Myrmecophila grandiflora* and its pollination insects, *Bombus medius* and *Xylocopa violacea* (Apidae) have been investigated. The interactions between *Cuitlauzina pendula*, *Stanhopea martiana*, and *Oncidium tigrinum* and the phytophagous insects

Table 2 List of organisms associated with Mexican endemic orchids

Orchid	Type of association	Organism	Taxonomy family	Role of the associated organisms	Reference
Laelia autumnalis (Lex.) Lindl.	Plant – Plant	*Arbutus xalapensis*	Ericaceae	Phorophyte	Luyando-Moreno et al. 2011
Isochilus unilateralis B.L. Rob.	Plant – Plant	*Quercus germana*	Fagales	Phorophyte	García-Balcázar 2012
Stanhopea tigrina Bateman ex Lindl.		*Quercus germana*	Fagaceae		
Encyclia parviflora (Regel) Whitner		*Quercus sartorii*	Fagaceae		
Encyclia adenocaula (Lex.) Schltr. ssp. *adenocaula*	Plant – Plant	*Quercus* spp.	Fagaceae	Phorophyte	Morales-Hernández, González-Razo and Pérez-Chávez 2016
Erycina hyalinobulbon (Lex.) N.H. Williams et M.W. Chase					
Hintonella mexicana Ames					
Oncidium hastatum (Bateman) Lindl.					
Laelia anceps Lindl.	Plant – Animal	*Bombus medius*	Apidae	Pollinator agent	Rodríguez-Flores, Barney-Guillermo and Vazquez-Torres 1996
Artorima erubescens (Lindl.) Dressler et G.E. Pollard	Plant – Animal	ONS*	FNS**	Pollinator agent	Leopardi 2011
Cuitlauzina pendula Lex.	Plant – Animal	*Macrosiphum lutem*	Apidae	Phytophagous insect	González-Díaz et al. 2012

Stanhopea martiana Lindl. ex Bateman		*Toxoptera aurantii*	Aphidoidea		
Oncidium tigrinum Lex.					
Myrmecophila grandiflora (Lindl.) Carnevali, J.L. Tapia et I. Ramírez	Plant – Animal	*Xylocopa violacea*	Apidae	Pollinator agent	Ortiz-Santos 2015
	Plant – Animal	*Crematogaster* spp.	Formicidae	Protective insect	
		Camponotus spp.			
		Pseudomyrmex spp.			

*ONS = Organism not specified; **FNS = Family not specified

Macrosiphum lutem and *Toxoptera aurantia* have also been studied. The association of *M. grandiflora* and the ants that inhabit its hollow pseudobulbs indicated the protective role of ants on this orchid (Ortíz-Santos 2015; Table 2). The interactive role of mycorrhizal fungi in orchid species has been reported by Oja et al. 2014; Dearnaley and Cameron 2017; Phillips et al. 2014; Rodríguez-Echeverría et al. 2016; Jacquemyn et al. 2017. This association is fundamental for the development and survival of most species in the Orchidaceae family, especially during the seed germination (Knudson 1922; Arditti 1967; Hágsater et al. 2015).

The interaction of orchids and microorganisms living in their roots has been reported in two Mexican orchids (0.33% of the endemic orchids) (Table 3).

For example, 16 mycorrhizal fungi were isolated from *Laelia speciosa*. Similarly, five different genera of bacteria were isolated from *Laelia furfuracea*, all belonging to the Proteobacteria, but their biological functions are not yet known. However, their ability to survive inside plants apparently without any competition, suggests its potential functions as agents of biological control (Table 4). Therefore, it is necessary to carry out more studies in Mexican orchids to understand the symbiotic role of microorganisms living in these plant species.

Uses of the Mexican endemic orchids

In some regions of the world, orchids represent an important non-timber source of economic resources (Hinsley 2011; Menchaca-García et al. 2012; Cruz-Garcia et al. 2015; Emeterio-Lara et al. 2016), mainly due to their ornamental and medicinal uses (Hinsley et al. 2015; Akhter et al. 2017; Chowlu et al. 2017; Khajuria et al. 2017; Tsering et al. 2017). In southern Mexico, many endemic orchids are commercialized for its medicinal or ceremonial use (Vergara-Galicia et al. 2008, 2010, 2013; Solano-Gómez et al. 2010; Cox-Tamay 2013; Cano-Asseleih et al. 2015; Rojas-Olivos et al. 2017). *Laelia autumnalis* has been widely used for ornamental, ceremonial, and medicinal purposes. Other orchids that are extracted from their natural habitat for ceremonial purposes are *Artorima erubescens*, *Laelia albida*, *L. anceps*, *L. furfuracea*, *L. gouldiana*, and *Prosthechea karwinskii* (Table 4). The looting and commercialization of orchids for the purposes above described

Table 3 List of isolated microorganisms from endemic orchids of Mexico

Orchid	Used tissue	Microorganism	Type of isolated microorganism	Taxonomic division	Reference
Laelia furfurácea Lindl.	Roots, leafs and pseudobulbs	*Azospirillum* sp.	Bacteria	Proteobacteria	Ramos-Zambrano, Jiménez-Salgado and Tapia-Hernández 2007
		Enterobacter sp.			
		Pseudomonas sp.			
		Acetobactereas sp.			
		Herbaspirillum sp.			
Laelia speciosa (Kunth) Schltr.	Seeds and plantlets	*Alternaria* sp.	Fungus	Ascomycota	Garibay-Orijel, Oyama and Ávila-Díaz 2011
		Cylindrocarpon sp.			
		Curvularia sp.			
		Fusarium sp.			
		Myrmecridium sp.			
		Neonectria sp.			
		Penicillium sp.			
		Tetracladium sp.			
	Capsule and seeds	*Alternaria longissima*	Fungus	Ascomycota	Ávila-Díaz et al. 2013
	Capsule	*Hypocreales* sp.			
		Lasiosphaeriaceae sp.			
	Roots	*Curvularia affinis*			
		Cylindrocarpon pauciseptatum			
		Fusarium solani			

	Roots, capsule and seeds	*Helotiales* sp.			
	Capsule	*Myrmecridium schulzeri*			
		Sordariomycete sp.			
	Roots	*Nectriaceae*			
		Tricholomataceae		Basidiomycota	
		Atractiellales sp.			
		Basidiomycete			

Table 4 List of uses and applications for endemic orchids of Mexico

Specie	Use or application	Part used	Reference
Laelia autumnalis (Lex.) Lindl.	Antihypertensive effect	Full plant	Vergara-Galicia et al. 2008
Artorima erubescens (Lindl.) Dressler et G.E. Pollard	Ceremonial use	Flowers	Solano-Gómez et al. 2010
Laelia albida Bateman ex Lindl.			
Laelia anceps Lindl.			
Laelia autumnalis (Lex.) Lindl.			
Laelia furfuracea Lindl.			
Laelia gouldiana Rchb.f.			
Prosthechea karwinskii (Mart.) Soto Arenas et Salazar			
Rhynchostele áptera (Lex.) Soto Arenas et Salazar			
Laelia anceps Lindl.	Antihypertensive effect	Roots	Vergara-Galicia et al. 2010
Laelia autumnalis (Lex.) Lindl.	Ornamental use	Full plant	Beltrán-Rodriguez, Martínez-Rivera and Paulo-Maya 2012
	Ceremonial use		

Myrmecophila christinae Carnevali et GómezJuárez var. ibarrae Carnevali et J.L. Tapia	Treatment against wounds	Pseudobulbs	Cox-Tamay 2013
Arpophyllum spicatum Lex.	Treatment against dysentery	Not specified	
Epidendrum anisatum Lex.			
Laelia autumnalis (Lex.) Lindl.	Treatment against cough		
Laelia speciosa (Kunth) Schltr.	Vasorelaxant effect	Roots	Vergara-Galicia et al. 2013
Laelia anceps Lindl.			
Prosthechea karwinskii (Mart.) Soto Arenas et Salazar	Medicinal, ceremonial, edible, and cosmetic uses	Pseudobulbs , leaves and flowers	Cruz-García, Solano-Gómez and Lagunez-Rivera 2014
Prosthechea citrina (Lex.) W.E. Higgins	Restoration material	Pseudobulbs	Núñez-Vázquez 2014
Mormodes maculata (Klotzsch) L.O. Williams var. *unicolor* (Hook.) L.O. Williams	Treatment against inflammation		Cano-Asseleih, Menchaca-García and Ruíz-Cruz 2015
Oestlundia luteorosea (A. Rich. et Galeotti) W.E. Higgins	Treatment to reduce or remove pain in head bumps		
Artorima erubescens (Lindl.) Dressler et G.E. Pollard	Obtaining economic resources	Full plant	Cruz-García et al. 2015
Barkeria scandens (Lex.) Dressler et Halb.		Flowers	
Barkeria vanneriana Rchb.f.			
Cuitlauzina pendula Lex.			
Encyclia kienastii (Rchb.f.) Dressler et G.E. Pollard		Full plant	
Encyclia microbulbon (Hook.) Schltr.			
Encyclia rzedowskiana Soto Arenas			

Epidendrum camposii Hágsater			
Epidendrum juergensenii Rchb.f.			
Epidendrum lignosum Lex.		Flowers	
Laelia albida Bateman ex Lindl.		Full plant	
Laelia furfurácea Lindl.			
Laelia speciosa (Kunth) Schltr.			
Oncidium brachyandrum Lindl.			
Oncidium hastatum (Bateman) Lindl.			
Oncidium reflexum Lindl.			
Oncidium unguiculatum Lindl.		Full plant	
Prosthechea concolor (Lex.) W.E. Higgins		Pseudobulbs	
Prosthechea ghiesbreghtiana (A. Rich. et Galeotti) W.E. Higgins		Pseudobulbs	
Prosthechea karwinskii (Mart.) Soto Arenas et Salazar		Flowers	
Prosthechea semiaperta (Hágsater) W.E. Higgins		Full plant	
Rhynchostele cervantesii (Lex.) Soto Arenas et Salazar		Full plant	
Laelia autumnalis (Lex.) Lindl.	Ceremonial use	Flowers	Cox-Tamay 2016
Laelia autumnalis (Lex.) Lindl.	Ornamental use	Full plant	Emeterio-Lara et al. 2016
Prosthechea karwinskii (Mart.) Soto Arenas et Salazar			
Oncidium unguiculatum Lindl.			
Stanhopea hernandezii (Kunth) Schltr.			
Prosthechea karwinskii (Mart.) Soto Arenas et Salazar	Diabetes treatment	Pseudobulbs, leaves and flowers	Rojas-Olivos et al. 2017
	Anti-inflammatory effect		

have contributed to the slow but sure extinction of the Mexican orchids. In 2015, Cruz-Garcia et al. reported the use of 22 Mexican endemic orchids as a source for obtaining economic earning through its illegal sale in different markets in southern Mexico. This pattern has not been officially reported in other states in central and northern Mexico, however, is a common practice in many markets throughout Mexico. For example, in the popular markets of the Huasteca Potosina in San Luis Potosí state, species such as Laelia anceps, Prosthechea mariae, and Stanhopea tigrina, which are threatened or in danger of extinction, are illegally sold (Fig. 4). Although several Mexican endemic orchids have been used empirically for the treat of several diseases, including diabetes, inflammatory processes, dysentery, and cough (Table 4), only three orchid species of the Laelia genus have been scientifically studied for its pharmacological effects (Vergara- Galicia et al. 2008, 2010, 2013; Table 4).

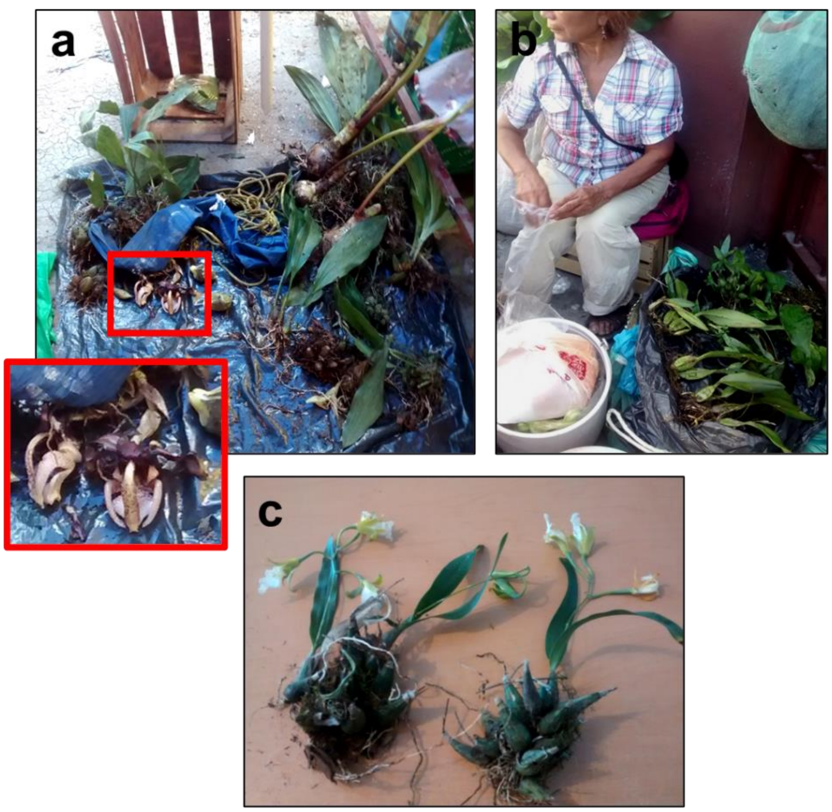

Fig. 4 Representative image of Mexican endemic orchids sold in popular markets of the Huasteca Potosina (in the San Luis Potosí state). a, *Stanhopea tigrina*. Notice highlighted in a red square, flowers of this species. b, Pseudobulbs from *Laelia anceps*. c, *Prosthechea mariae* with flowers.

Conclusions and perspectives

Endemic orchids, besides being part of the ecological identity of the country, develop essential functions in its ecological niches. The extinction of any of these species contributes undoubtedly to the extinction of other species of plants, animals, and microorganisms in its environment.

Orchids are the most commercialized plants in the world due to its flowers beauty and exotic characteristics. The Orchidaceae has been mainly studied from a botanical and taxonomic approach. In the last decade, a genomic approach for orchids has been used to analyze its ecology and physiology. In Mexico there are many endemic orchids, however most of these plant species have not been evaluated to understand its diverse pharmacological and/or ecological aspects, and therefore represents a wide field to be exploited. Currently, our work group is assessing different pharmacological effects of some Mexican endemic orchids in different in vitro and in vivo models.

Mexico is one of the countries with the highest number of endemic orchids in the world, but it is also one of the countries with high indices of illegal traffic of these species, and high rates of deforestation. These actions are even being carried out in natural areas, protected by the government, where species of orchids were declared at risk or in danger of extinction. Therefore, the conservation of Mexican orchids should be aided by national and international governments to protect its local biodiversity.

It is important to contribute to the study of the Mexican endemic orchids, considering the new advances of next generation sequencing (NGS) technologies, as well as the use of genomics for assessing the holobiome and hologenoma of the orchids, which allow understanding a comprehensive approach of the physiology of these plants. The functions played by the microorganisms associated with orchids, during the germination and growth of these plants, also need to be studied.

The use of metagenomics, transcriptomics, proteomics, and metabolomics should be considered to understand how orchids, being non-parasitic epiphytic plants, can uptake nutrients and adapt to different climatic conditions. Studies, at the genomic and molecular levels, of interactions that these plants establish with other plants and animals should not be neglected. Undoubtedly, this techniques and tissue in vitro culture would contribute to the propagation of orchids, increasing the number of species in nature and promoting the study of medicinal and biotechnological applications of Mexican endemic orchids.

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Supplementary material

Supplementary Table 1 Distribution and state of conservation of all endemic orchids of Mexico

Name	**Distribution**	**Conservation status***
Acianthera chrysantha (Lindl.) Pridgeon et M.W. Chase	Several states	5
Acianthera eximia (L.O. Williams) Solano	Oaxaca	3
Acianthera greenwoodii Soto Arenas	Oaxaca	5
Acianthera hartwegiifolia (H. Wendl. et Kraenzl.) Solano et Soto Arenas	Several states	5
Acianthera majakoluckae Soto Arenas et Solano	Several states	5
Acianthera martinezii (Luer) Luer	Chiapas	5
Acianthera obscura (A. Rich. et Galeotti) Pridgeon et M.W. Chase	Several states	5
Acianthera sotoana Solano	Several states	5
Acianthera unguicallosa (Ames et C. Schweinf.) Solano	Colima	4
Acineta hagsateri Salazar et Soto Arenas	Guerrero	5
Alamania punicea Lex. ssp. *greenwoodiana* Soto Arenas et R. Jiménez	Several states	5
Alamania punicea Lex. ssp. *punicea*	Several states	5
Anathallis greenwoodii Soto Arenas et Salazar	Oaxaca	5
Anathallis involuta (L.O. Williams) Solano et Soto Arenas	Several states	5
Anathallis oblanceolata (L.O. Williams) Solano et Soto Arenas	Several states	3
Anathallis scariosa (Lex.) Pridgeon et M.W. Chase	Several states	5
Arpophyllum laxiflorum Pfitzer	Several states	5
Arpophyllum spicatum Lex.	Several states	5
Artorima erubescens (Lindl.) Dressler et G.E. Pollard	Several states	5
Aulosepalum oestlundii (Burns-Bal.) Catling	Guerrero	5
Aulosepalum ramentaceum (Lindl.) Garay	Several states	5
Aulosepalum riodelayense (Burns-Bal.) Salazar	Oaxaca	5

Aulosepalum tenuiflorum (Greenm.) Garay	Several states	5
Barkeria barkeriola Rchb.f.	Several states	5
Barkeria dorotheae Halb.	Several states	3
Barkeria fritz-halbingeriana Soto Arenas	Oaxaca	5
Barkeria melanocaulon A. Rich. et Galeotti	Oaxaca	3
Barkeria naevosa (Lindl.) Schltr.	Several states	5
Barkeria palmeri (Rolfe) Schltr.	Several states	5
Barkeria scandens (Lex.) Dressler et Halb.	Several states	4
Barkeria shoemakeri Halb.	Several states	4
Barkeria strophinx (Rchb.f.) Halb.	Michoacán	3
Barkeria uniflora (Lex.) Dressler et Halb.	Several states	5
Barkeria vanneriana Rchb.f.	Several states	5
Barkeria whartoniana (C. Schweinf.) Soto Arenas	Oaxaca	4
Bletia adenocarpa Rchb.f.	Several states	5
Bletia amabilis C. Schweinf.	Several states	5
Bletia coccinea Lex.	Several states	5
Bletia concolor Dressler	Several states	5
Bletia ensifolia L.O. Williams	Several states	5
Bletia greenmaniana L.O. Williams	Several states	5
Bletia greenwoodiana Sosa	Durango	5
Bletia lilacina A. Rich. et Galeotti	Several states	5
Bletia macristhmochila Greenm.	Several states	5
Bletia neglecta Sosa	Several states	5
Bletia nelsonii Ames	Several states	5
Bletia parkinsonii Hook.	Several states	5
Bletia punctata Lex.	Several states	5
Bletia riparia Sosa et Palestina	Veracruz	5

Bletia similis Dressler	Michoacán	5
Bletia urbana Dressler	Several states	3
Bletia villae Soto Arenas	Durango	5
Bletia warfordiana Sosa	Jalisco	5
Brachystele luzmariana Szlach. et R. González	Jalisco	5
Brachystele tamayoana Szlach., Rutk. et Mytnik	Baja California	5
Brassia signata Rchb.f.	Several states	5
Bulbophyllum cirrhosum L.O. Williams	Several states	5
Bulbophyllum nagelii L.O. Williams	Several states	5
Bulbophyllum solteroi R. González	Several states	5
Camaridium atratum (Lex.) M.A. Blanco	Several states	5
Camaridium oestlundianum (L.O. Williams) M.A. Blanco	Guerrero	5
Camaridium rhombeum (Lindl.) M.A. Blanco	Several states	5
Camaridium soconuscanum (Breedlove et Mally) M.A. Blanco	Chiapas	5
Catasetum laminatum Lindl.	Several states	5
Catasetum pendulum Dodson	Several states	5
Chysis addita Dressler	Chiapas	5
Chysis limminghei Linden et Rchb.f.	Tabasco	3
Clowesia dodsoniana E. Aguirre	Several satates	5
Clowesia glaucoglossa (Rchb.f.) Dodson	Michoacán	4
Clowesia rosea Lindl.	Several states	3
Clowesia thylaciochila (Lem.) Dodson	Several states	5
Corallorhiza ehrenbergii Rchb.f.	Several states	5
Corallorhiza fimbriata Schltr.	Several states	5
Corallorhiza macrantha Schltr.	Several states	4
Corallorhiza pringlei Greenm.	Several states	5
Corallorhiza williamsii Correll	Morelos	5

Cranichis ciliilabia C. Schweinf.	Several states	5
Cranichis gracilis L.O. Williams	Several states	5
Cranichis subumbellata A. Rich. et Galeotti	Several states	5
Cuitlauzina dubia (S. Rosillo) E. Yáñez et Soto Arenas ex Solano	Several states	5
Cuitlauzina pendula Lex.	Several states	3
Cuitlauzina pygmaea (Lindl.) M.W. Chase et N.H. Williams	Several states	5
Cyclopogon luteo-albus (A. Rich. et Galeotti) Schltr.	Several states	5
Cyclopogon pringlei (S. Watson) Soto Arenas	Several states	5
Cyclopogon saccatus A. Rich. et Galeotti	Several states	5
Cypripedium molle Lindl.	Several states	5
Deiregyne albovaginata (C. Schweinf.) Garay	Several states	5
Deiregyne chartacea (L.O. Williams) Garay	Jalisco	5
Deiregyne densiflora (C. Schweinf.) Salazar et Soto Arenas	Several states	5
Deiregyne diaphana (Lindl.) Garay	Oaxaca	5
Deiregyne falcata (L.O. Williams) Garay	Several states	5
Deiregyne nonantzin (R. González ex McVaugh) Catling	Jalisco	5
Deiregyne pandurata Garay	Durango	5
Deiregyne pseudopyramidalis (L.O. Williams) Garay	Several states	5
Deiregyne ramirezii R. González	Jalisco	5
Deiregyne rhombilabia Garay	Several states	5
Deiregyne sheviakiana (Szlach.) Espejo et López Ferr.	Chiapas	5
Deiregyne tenorioi Soto Areanas et Salazar	Several states	5
Deiregyne velata (B.L. Rob. et Fernald) Garay	Chihuahua	5
Dichromanthus yucundaa Salazar et GarcíaMend.	Oaxaca	5
Domingoa gemma (Rchb.f.) van der Berg et Soto Arenas	Puebla	5
Domingoa kienastii (Rchb.f.) Dressler	Several states	5
Elleanthus teotepecensis Soto Arenas	Guerrero	5

Encyclia adenocarpa (Lex.) Schltr.	Several states	5
Encyclia adenocaula (Lex.) Schltr. ssp. *adenocaula*	Several states	3
Encyclia adenocaula (Lex.) Schltr. ssp. *kennedyi* (Fowlie et Withner) Soto Arenas	Several states	3
Encyclia aenicta Dressler et G.E. Pollard	Several states	5
Encyclia atrorubens (Rolfe) Schltr.	Several states	4
Encyclia calderoniae Soto Arenas	Oaxaca	5
Encyclia candollei (Lindl.) Schltr.	Several states	5
Encyclia chiapasensis Withner et D.G. Hunt	Chiapas	5
Encyclia contrerasii R. González	Colima	5
Encyclia flabellata (Lindl.) B. Thurst. et W. Thurst.	Veracruz	5
Encyclia halbingeriana Hágsater et Soto Arenas	Michoacán	5
Encyclia huertae Soto Arenas et R. Jiménez	Michoacán	5
Encyclia kienastii (Rchb.f.) Dressler et G.E. Pollard	Oaxaca	2
Encyclia lorata Dressler et G.E. Pollard	Guerrero	4
Encyclia meliosma (Rchb.f.) Schltr.	Several states	5
Encyclia microbulbon (Hook.) Schltr.	Several states	5
Encyclia nizandensis Pérez-García et Hágsater	Oaxaca	5
Encyclia oestlundii (Ames, F.T. Hubb. et C. Schweinf.) Hágsater et Stermitz	Guerrero	5
Encyclia ovulum (Lindl.) Schltr.	Sinaloa	5
Encyclia parviflora (Regel) Whitner	Several states	5
Encyclia pollardiana (Withner) Dressler et G.E. Pollard	Several states	4
Encyclia rzedowskiana Soto Arenas	Oaxaca	5
Encyclia spatella (Rchb.f.) Schltr.	Jalisco	5
Encyclia suaveolens Dressler	Several states	5
Encyclia trachycarpa (Lindl.) Schltr.	Several states	5
Encyclia uxpanapensis Salazar	Veracruz	5
Epidendrum alabastrialatum G.E. Pollard ex Hágsater	Several states	4

Epidendrum anisatum Lex.	Several states	5
Epidendrum camposii Hágsater	Oaxaca	5
Epidendrum chimalapense Hágsater et Salazar	Several states	5
Epidendrum chlorops Rchb.f.	Several states	5
Epidendrum cilioccidentale Hágsater et L. Sánchez	Several states	5
Epidendrum citrosmum Hágsater	Several states	5
Epidendrum costatum A. Rich. et Galeotti	Oaxaca	5
Epidendrum cusii Hágsater	Several states	5
Epidendrum dorsocarinatum Hágsater	Estado de México	4
Epidendrum dressleri Hágsater	Several states	4
Epidendrum erectifolium Hágaster et L. Sánchez	Chiapas	5
Epidendrum examinis S. Rosillo	Jalisco	5
Epidendrum falcatum Lindl.	Several states	5
Epidendrum gasteriferum Scheeren	Oaxaca	5
Epidendrum gomezii Schltr.	Several states	5
Epidendrum gonzalez-tamayoi Hágsater	Several states	5
Epidendrum greenwoodii Hágsater	Oaxaca	5
Epidendrum guerrerense Hágsater et GarcíaCruz	Guerrero	5
Epidendrum hagsateri Christenson	Several states	5
Epidendrum hueycatenangense Hágsater et García-Cruz	Guerrero	5
Epidendrum ibarrae R. González	Jalisco	5
Epidendrum incomptoides Ames, F.T. Hubb. et C. Schweinf.	Chiapas	4
Epidendrum juergensenii Rchb.f.	Oaxaca	5
Epidendrum lignosum Lex.	Several states	5
Epidendrum longicaule (L.O. Williams) L.O. Williams	Several states	5
Epidendrum longipetalum A. Rich. et Galeotti	Several states	5
Epidendrum lowilliamsii García-Cruz	Several states	5

Epidendrum macdougallii (Hágsater) Hágsater	Several states	5
Epidendrum macroclinium Hágsater	Several states	5
Epidendrum magnificum Schltr.	Guerrero	5
Epidendrum marmoratum A. Rich. et Galeotti	Several states	5
Epidendrum matudae L.O. Williams	Several states	5
Epidendrum melistagoides Hágstaer et L. Sánchez	Several states	5
Epidendrum miserum Lindl.	Several states	5
Epidendrum mixtecanum Hágsater et García-Cruz	Oaxaca	5
Epidendrum mocinoi Hágsater	Several states	5
Epidendrum motozintlensis Hágstaer et L. Sánchez	Chiapas	5
Epidendrum nagelii L.O. Williams	Guerrero	5
Epidendrum neogaliciense Hágsater et R. González	Jalisco	5
Epidendrum oaxacanum Rolfe	Oaxaca	5
Epidendrum pastranae Hágsater	Several states	5
Epidendrum pollardii Hágsater	Several states	5
Epidendrum rosilloi Hágsater	Several states	5
Epidendrum rowleyi Withner et G.E. Pollard	Several states	5
Epidendrum stallforthianum Kraenzl.	Veracruz	5
Epidendrum succulentum Hágsater	Several states	5
Epidendrum tortipetalum Scheeren	Several states	5
Epidendrum tuxtlense Hágsater, García-Cruz et L. Sánchez	Several states	5
Epidendrum tziscaoense Hágsater	Chiapas	5
Epidendrum vandifolium Lindl.	Several states	5
Erycina echinata (Kunth) Lindl.	Several states	5
Erycina hyalinobulbon (Lex.) N.H. Williams et M.W. Chase	Several states	5
Funkiella laxispica (Catling) Salazar et Soto Arenas	Oaxaca	5
Funkiella markowskiana (Szlach.) Salazar et Soto Arenas	Oaxaca	5

Funkiella porphyricola (Schltr.) Salazar et Soto Arenas	Several states	5
Funkiella rubrocallosa (B.L. Rob. et Greenm.) Salazar et Soto Arenas	Several states	5
Galeandra greenwoodiana Warford	Several states	5
Galeoglossum cactorum Salazar et ChávezRendón	Oaxaca	5
Galeoglossum prescottioides A. Rich. et Galeotti	Oaxaca	5
Galeoglossum thysanochilum (B.L. Rob. et Greenm.) Salazar	Oaxaca	5
Galeoglossum tubulosum (Lindl.) Salazar et Soto Arenas	Several states	5
Galeottia grandiflora A. Rich.	Several states	2
Galeottiella sarcoglossa (A. Rich. et Galeotti) Schltr.	Several states	4
Gongora galeottiana A. Rich.	Several states	5
Goodyera brachyceras (A. Rich. et Galeotti) Garay et G.A. Romero	Oaxaca	5
Goodyera dolabripetala (Ames) Schltr.	Several states	5
Goodyera fimbrilabia Ormerod	Chiapas	5
Goodyera purpusii Ormerod	Veracruz	5
Goodyera zacuapanensis Ormerod	Veracruz	5
Govenia alba A. Rich. et Galeotti	Several states	5
Govenia bella E.W. Greenw.	Oaxaca	5
Govenia capitata Lindl.	Several States	5
Govenia elliptica S. Watson	Nuevo León	5
Govenia jouyana R. González	Jalisco	5
Govenia praecox Salazar et E.W. Greenw.	Veracruz	5
Govenia purpusii Schltr.	Several states	5
Govenia rubellilabia García-Cruz	Chiapas	5
Govenia tequilana Dressler et Hágsater	Several states	4
Habenaria acalcarata Espejo et López Ferr.	Chiapas	5
Habenaria agapitae R. González et Reynoso	Jalisco	5
Habenaria agrestis R. González et Cuev.-Fig.	Several states	5

Habenaria atrata R. González et Cuev.-Fig.	Jalisco	5
Habenaria brevilabiata A. Rich. et Galeotti	Several states	5
Habenaria calicis R. González	Morelos	5
Habenaria carvajaliana R. González et Cuev.-Fig.	Jalisco	5
Habenaria castroi R. González et Cuev.-Fig.	Zacatecas	5
Habenaria cortesii R. González et Cuev.-Fig.	Jalisco	5
Habenaria cuevasiana R. González et Cuev.-Fig.	Jalisco	5
Habenaria diffusa A. Rich et Galeotti	Estado de México	5
Habenaria entomantha (Lex.) Lindl.	Several states	5
Habenaria felipensis Ames	Oaxaca	5
Habenaria filifera S. Watson	Several states	5
Habenaria flexuosa Lindl.	Oaxaca	5
Habenaria gonzaleztamayoi García-Cruz, R. Jiménez et L. Sánchez	Several states	5
Habenaria greenwoodiana R. González	Jalisco	5
Habenaria horaliae R. González	Michoacán	5
Habenaria ibarrae R. González	Jalisco	5
Habenaria ixtlanensis E.W. Greenw.	Oaxaca	5
Habenaria jardeliana R. González et Cuev.-Fig.	Jalisco	5
Habenaria kariniae R. González et Cuev.-Fig.	Several states	5
Habenaria leon-ibarrae R. Jiménez et Carnevali	Quintana Roo	5
Habenaria lizbethae R. González et Cuev.-Fig.	Jalisco	5
Habenaria luzmariana R. González	Jalisco	5
Habenaria mariae R. González et Cuev.-Fig.	Jalisco	5
Habenaria matudae Salazar	Several states	5
Habenaria mcvaughiana R. González	Several states	5
Habenaria micheliana R. González et Cuev.-Fig.	Jalisco	5
Habenaria mitodes Garay et W. Kittr.	Several states	5

Habenaria nogeirana R. González et Cuev.-Fig.	Jalisco	5
Habenaria oreophila Greenm.	Several states	5
Habenaria ortiziana R. González	Estado de México	5
Habenaria pinzonii R. González et Cuev.-Fig.	Several states	5
Habenaria pseudofilifera R. González et Cuev.-Fig.	Jalisco	5
Habenaria rosilloana R. González	Michoacán	5
Habenaria rosulifolia Espejo et López-Ferr.	Morelos	5
Habenaria rotundifolia Conz.	Oaxaca	5
Habenaria ruizii R. González	Jalisco	5
Habenaria rzedowskiana R. González	Several states	5
Habenaria rzedowskii R. González	Michoacán	5
Habenaria schaffneri S. Watson	Several states	5
Habenaria socorroae R. González et Cuev.-Fig.	Jalisco	5
Habenaria subauriculata B.L. Rob. et Greenm.	Several states	5
Habenaria szlachetkoana R. González et Cuev.-Fig.	Jalisco	5
Habenaria talaensis R. González et Cuev.-Fig.	Jalisco	5
Habenaria tequilana R. González et Cuev.-Fig.	Jalisco	5
Habenaria uncata R. Jiménez, L. Sánchez et García-Cruz	Morelos	5
Habenaria virens A. Rich. et Galeotti	Several states	5
Habenaria xochitliae R. González	Jalisco	5
Habenaria zamudioana R. González	Michoacán	5
Hagsatera brachycolumna (L.O. Williams) R. González	Several states	4
Hagsatera rosilloi R. González	Jalisco	5
Helleriella guerrerensis Dressler et Hágsater	Guerrero	5
Hexalectris brevicaulis L.O. Williams	Several states	5
Hexalectris fallax M.I. Rodríguez et R. González	Several states	5
Hexalectris grandiflora (A. Rich. et Galeotti) L.O. Williams	Several states	5

Hexalectris nitida L.O. Williams	Several states	5
Hexalectris parviflora L.O. Williams	Several states	5
Hexalectris revoluta Correll	Several states	5
Hexalectris spicata (Walter) Barnhart var. *arizonica* (S. Watson) Catling et V.S. Engel	Several states	5
Hexalectris warnockii Ames et Correll	Several states	5
Hintonella mexicana Ames	Several states	5
Homalopetalum pachyphyllum (L.O. Williams) Dressler	Several states	5
Homalopetalum pumilum (Ames) Dressler	Several states	5
Isochilus bracteatus (Lex.) López-Ferr. et Espejo	Several states	5
Isochilus langlassei Schltr.	Several states	5
Isochilus oaxacanus Salazar et Soto Arenas	Oaxaca	5
Isochilus unilateralis B.L. Rob.	Several states	5
Jacquiniella cernua (Lindl.) Dressler	Several states	5
Kionophyon pollardiana Szlach., Rutk., et Mytnik	Oaxaca	5
Kionophyton sawyeri (Standl. et L.O. Williams) Garay	Several states	5
Kraenzlinella hintonii (L.O. Williams) Solano	Guerrero	4
Kreodanthus casillasii R. González	Jalisco	5
Laelia albida Bateman ex Lindl.	Several states	5
Laelia anceps Lindl.	Several states	2
Laelia aurea Navarro	Several states	5
Laelia autumnalis (Lex.) Lindl.	Several states	5
Laelia crawshayana Rchb.	Jalisco	5
Laelia eyermaniana Rchb.f.	Several states	5
Laelia furfuracea Lindl.	Oaxaca	5
Laelia gouldiana Rchb.f.	Hidalgo	1
Laelia speciosa (Kunth) Schltr.	Several states	4
Leochilus carinatus (Knowles et Westc.) Lindl.	Several states	5

Leochilus crocodiliceps (Rchb.f.) Kraenzl.	Several states	5
Lepanthes acuminata Schltr. ssp. *ernestii* Salazar et Soto Arenas	Chiapas	5
Lepanthes ancylopetala Dressler	Chiapas	4
Lepanthes aprica Catling et V.R. Catling	Oaxaca	5
Lepanthes attenuata Salazar, Soto Arenas et O. Suárez	Several states	5
Lepanthes avis Rchb.f.	Several states	5
Lepanthes brachystele Salazar et Soto Arenas	Several states	5
Lepanthes breedlovei Salazar et Soto Arenas	Several states	5
Lepanthes calopetala Salazar et Soto Arenas	Several states	5
Lepanthes camposii Salazar et Soto Arenas	Several states	5
Lepanthes catlingii Salazar, Soto Arenas et O. Suárez	Oaxaca	5
Lepanthes chiangii Salazar, Soto Arenas et O. Suárez	Oaxaca	5
Lepanthes cryptostele Salazar et Soto Arenas	Guerrero	5
Lepanthes erythroxantha Salazar et Soto Arenas	Oaxaca	5
Lepanthes gabriellae Salazar et Soto Arenas	Oaxaca	5
Lepanthes galeottiana Salazar et Soto Arenas	Oaxaca	5
Lepanthes greenwoodii Salazar et Soto Arenas	Oaxaca	5
Lepanthes guerrerensis Salazar et Soto Arenas	Several states	5
Lepanthes hagsateri Salazar et Soto Arenas	Guerrero	5
Lepanthes machorroi Salazar et Soto Arenas	Oaxaca	5
Lepanthes maldonadoae Soto Arenas	Chiapas	5
Lepanthes mariae Salazar et Soto Arenas	Oaxaca	5
Lepanthes martinezii Salazar et Soto Arenas	Several states	5
Lepanthes matudana Salazar et Soto Arenas	Chiapas	5
Lepanthes maxima Salazar et Soto Arenas	Chiapas	5
Lepanthes mazatlanensis Solano et Reynaud	Oaxaca	5
Lepanthes minima Salazar, Soto Arenas et O. Suárez	Oaxaca	5

Lepanthes mixe Salazar et Soto Arenas	Oaxaca	5
Lepanthes moorei C. Schweinf.	Several states	5
Lepanthes motozintlensis Salazar et Soto Arenas	Chiapas	5
Lepanthes nagelii Salazar et Soto Arenas	Several states	5
Lepanthes nigriscapa R.E. Schult. et G.W. Dillon	Oaxaca	5
Lepanthes oaxacana Salazar, Soto Arenas et O. Suárez	Oaxaca	5
Lepanthes oreophila Catling et V.R. Catling	Oaxaca	5
Lepanthes papilionacea Salazar, Soto Arenas et O. Suárez	Oaxaca	5
Lepanthes pollardii Hespenh.	Oaxaca	5
Lepanthes rekoi R.E. Schult.	Several states	5
Lepanthes schiedei Rchb.f.	Several states	5
Lepanthes schultesii Salazar et Soto Arenas	Oaxaca	5
Lepanthes sousae Salazar et Soto Arenas	Oaxaca	5
Lepanthes suarezii Salazar et Soto Arenas	Oaxaca	5
Lepanthes thurstoniorum Salazar, Soto Arenas et O. Suárez	Several states	5
Lepanthes totontepecensis Salazar et Soto Arenas	Oaxaca	5
Lepanthes vivipara Salazar et Soto Arenas	Chiapas	5
Lepanthes wendtii Salazar et Soto Arenas	Oaxaca	5
Lepanthes yuvilensis Catling	Several states	5
Liparis cordiformis C. Schweinf.	Several states	5
Liparis draculoides E.W. Greenw.	Several states	5
Liparis greenwoodiana Espejo	Several states	5
Liparis lindeniana (A. Rich. et Galeotti) Hemsl.	Veracruz	5
Liparis madrensis Soto Arenas, Salazar et R. Jiménez	Durango	5
Liparis volcanica R. González et Zamudio	Several states	5
Lockhartia galeottiana A. Rich. ex Soto Arenas	Several states	5
Lycaste crinita Lindl.	Several states	5

Macroclinium lexarzanum (Hágsater et R. González) Dodson	Several states	5
Macroclinium pachybulbon (Hágsater et R. González) Dodson	Several states	5
Malaxis abieticola Salazar et Soto Arenas	Several states	5
Malaxis adenotropa R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis alvaroi García-Cruz, R. Jiménez et L. Sánchez	Several states	5
Malaxis amplexicolumna E. Greenw. et R. González	Chiapas	5
Malaxis andersoniana R. González, Lisb. Hern. et E. Ramírez	Estado de México	5
Malaxis brachystachya (Rchb.f.) Kuntze	Several states	5
Malaxis casillasii R. González	Jalisco	5
Malaxis chiarae R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis chica Todzia	Nuevo León	5
Malaxis contrerasii R. González	Jalisco	5
Malaxis elliptica A. Rich. et Galeotti	Morelos	5
Malaxis elviae R. González	Jalisco	5
Malaxis espejoi R. González, Lizb. Hern. & E. Ramírez	Jalisco	5
Malaxis greenwoodiana Salazar et Soto Arenas	Chiapas	4
Malaxis hagsateri Salazar	Guerrero	4
Malaxis hintonii Todzia	Several states	5
Malaxis javesiae (Rchb.f.) Ames	Several states	5
Malaxis lizbethiae R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis luceroana R. González	Several states	5
Malaxis lyonnetii Salazar	Morelos	5
Malaxis macrostachya (Lex.) Kuntze	Several states	5
Malaxis macvaughiana R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis marthaleidae R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis martinezii R. González	Jalisco	5
Malaxis micheliana R. González, Lisb. Hern. et E. Ramírez	Jalisco	5

Malaxis molotensis Salazar et de Santiago	Guerrero	5
Malaxis myurus (Lindl.) Kuntze	Several states	5
Malaxis nelsonii Ames	Durango	5
Malaxis novogaliciana McVaugh	Several states	5
Malaxis ocreata (S. Watson) Ames	Several states	5
Malaxis perezii R. González	Jalisco	5
Malaxis pringlei (S. Watson) Ames	Several states	5
Malaxis ramirezii R. González	Jalisco	5
Malaxis reichei (Schltr.) Ames et C. Schweinf.	Several states	5
Malaxis ribana Espejo et López-Ferr.	Morelos	5
Malaxis roblesgilana R. González	Nayarit	5
Malaxis rodrigueziana R. González	Several states	5
Malaxis rosei Ames	Several states	5
Malaxis rosilloi R. González et E.W. Greenw.	Several states	5
Malaxis rositae R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis ruizii R. González	Jalisco	5
Malaxis rzedowskiana R. González	Estado de México	5
Malaxis salazarii Catling	Several states	5
Malaxis streptopetala (B.L. Rob. et Greenm.) Ames	Several states	5
Malaxis stricta L.O. Williams	Morelos	5
Malaxis tamayoana Garay et W. Kittr.	Jalisco	5
Malaxis tepicana Ames	Several states	5
Malaxis tequilensis R. González, Lisb. Hern. et E. Ramírez	Jalisco	5
Malaxis urbana E.W. Greenw.	Oaxaca	5
Malaxis wendtii Salazar	Several states	5
Malaxis xerophila Salazar et L.I. Cabrera	Several states	5
Malaxis zempoalensis López-Ferr. et Espejo	Morelos	5

Maxillariella mexicana (J.T. Atwood) M.A. Blanco et Carnevali	Several states	5
Meiracyllium gemma Rchb.f.	Several states	5
Mesadenus chiangii (M. Johnst.) Garay	Coahuila	5
Mesadenus tenuissimus (L.O. Williams) Garay	Morelos	5
Mexipedium xerophyticum (Soto Arenas, Salazar et Hágsater) V.A. Albert et M.W. Chase	Oaxaca	2
Microchilus mexicanus (Ames) Ormerod	Chiapas	5
Microepidendrum subulatifolium (A. Rich. et Galeotti) W.E. Higgins	Several states	5
Mormodes badia Rolfe ex W. Watson	Several states	5
Mormodes cozticxochitl Salazar	Several states	5
Mormodes luxata Lindl.	Several states	5
Mormodes maculata (Klotzsch) L.O. Williams var. *maculata*	Oaxaca	3
Mormodes maculata (Klotzsch) L.O. Williams var. *unicolor* (Hook.) L.O. Williams	Several states	3
Mormodes oestlundiana Salazar et Hágsater	Guerrero	5
Mormodes pardalinata S. Rosillo	Several states	5
Mormodes porphyrophlebia Salazar	Several states	3
Mormodes ramirezii S. Rosillo	Jalisco	5
Mormodes sanguineoclaustra Fowlie	Guerrero	2
Mormodes tezontle S. Rosillo	Several states	5
Mormodes tuxtlensis Salazar	Veracruz	5
Mormodes uncia Rchb.f.	Oaxaca	2
Mormodes williamsii hort. ex G. Nicholson	Jalisco	5
Mormolyca sotoana (Carnevali et Gómez-Juárez) M.A. Blanco	Chiapas	5
Myrmecophila christinae Carnevali et GómezJuárez var. *ibarrae* Carnevali et J.L. Tapia	Quintana Roo	5
Myrmecophila galeottiana (A. Rich.) Rolfe	Several states	5
Myrmecophila grandiflora (Lindl.) Carnevali, J.L. Tapia et I. Ramírez	Several states	5
Myrmecophila laguna-herrerae Carnevali, L. Ibarra et J.L. Tapia	Several states	5
Nemaconia dresslerana (Soto Arenas) van den Berg, Salazar et Soto Arenas	Morelos	4

Nemaconia longipetala (Correll) van den Berg, Salzar et Soto Arenas	Several states	5
Notylia leucantha Salazar	Oaxaca	5
Notylia orbicularis A. Rich. et Galeotti ssp. *warfordiae* Salazar	Several states	5
Notylia tamaulipensis Rchb.f.	Tamaulipas	5
Oestlundia cyanocolumna (Ames, F.T. Hubb. et C. Schweinf.) W.E. Higgins	Several states	5
Oestlundia distantiflora (A. Rich. et Galeotti) W.E. Higgins	Several states	4
Oestlundia luteorosea (A. Rich. et Galeotti) W.E. Higgins	Several states	5
Oestlundia tenuissima (Ames, F.T. Hubb. et C. Schweinf.) W.E. Higgins	Several states	5
Oncidium brachyandrum Lindl.	Several states	5
Oncidium durangense Hágsater	Several states	5
Oncidium geertianum C. Morren	Several states	5
Oncidium ghiesbreghtianum A. Rich. et Galeotti	Several states	5
Oncidium graminifolium (Lindl.) Lindl.	Several states	5
Oncidium hagsaterianum R. Jiménez et Soto Arenas	Veracruz	5
Oncidium hastatum (Bateman) Lindl.	Several states	5
Oncidium hintonii L.O. Williams	Several states	5
Oncidium incurvum F. Barker ex Lindl.	Several states	3
Oncidium karwinskii (Lindl.) Lindl.	Several states	5
Oncidium lindleyi (Galeotti ex Lindl.) R. Jiménez et Soto Arenas	Several states	5
Oncidium microstigma Rchb.f.	Morelos	5
Oncidium oblongatum Lindl.	Several states	5
Oncidium oviedomotae Hágsater	Michoacán	5
Oncidium pollardii Dodson et Hágsater	Oaxaca	3
Oncidium reflexum Lindl.	Several states	5
Oncidium reichenheimii (Linden et Rchb.f.) Garay et Stacy	Several states	5
Oncidium sotoanum R. Jiménez et Hágsater ssp. *papalosmum* R. Jiménez	Several states	5
Oncidium stelligerum Rchb.f.	Several states	4

Oncidium suave Lindl.	Several states	5
Oncidium tigrinum Lex.	Several states	3
Oncidium unguiculatum Lindl.	Several states	3
Ornithidium tonsoniae (Soto Arenas) Senghas	Several states	4
Ornithocephalus biloborostratus Salazar et R. González	Michoacán	5
Ornithocephalus obergiae Soto Arenas	Veracruz	5
Ornithocephalus torresii Salazar et Soto Arenas	Chiapas	5
Ornithocephalus tripterus Schltr.	Several states	5
Pachyphyllum mexicanum Dressler et Hágsater	Oaxaca	4
Papperitzia leiboldii (Rchb.f.) Rchb.f.	Several states	4
Physogyne garayana R. González et Szlach.	Colima	5
Physogyne gonzalezii (L.O. Williams) Garay	Oaxaca	4
Physogyne sparsiflora (C. Schweinf.) Garay	Several states	5
Platanthera brevifolia (Greene) Kraenzl.	Several states	5
Platanthera calderoniae López-Ferr. et Espejo	Michoacán	5
Platanthera nubigena A. Rich. et Galeotti	Veracruz	5
Platanthera volcanica Lindl.	Several states	5
Polystachya mcvaughiana Soto Arenas	Several states	5
Ponera exilis Dressler	Several states	5
Ponthieva hildae R. González et Soltero	Jalisco	5
Ponthieva rinconii Salazar	Veracruz	5
Ponthieva schaffneri (Rchb.f.) E.W. Greenw.	Several states	5
Potosia kusibabiana Szlach., Mytnik et Rutk.	Nuevo León	5
Potosia praetermissa Szlach., Mytnik et Rutk.	San Luis Potosí	5
Potosia tamayoana Szlach., Mytnik et Rutk.	Several states	5
Prescottia lindeniana A. Rich. et Galeotti	Chiapas	5
Prescottia pachyrrhiza A. Rich. et Galeotti	Oaxaca	5

Prosthechea bicamerata (Rchb.f.) W.E. Higgins	Several states	5
Prosthechea brachiata (A. Rich. et Galeotti) W.E. Higgins	Several states	5
Prosthechea citrina (Lex.) W.E. Higgins	Several states	4
Prosthechea concolor (Lex.) W.E. Higgins	Several states	5
Prosthechea cretacea (Dressler et G.E. Pollard) W.E. Higgins	Several states	5
Prosthechea favoris (Rchb.f.) W.E. Higgins	Several states	5
Prosthechea fragrans (Sw.) W.E. Higgins	Several states	5
Prosthechea ghiesbreghtiana (A. Rich. et Galeotti) W.E. Higgins	Several states	5
Prosthechea greenwoodiana (Aguirre-Olav.) W.E. Higgins	Oaxaca	5
Prosthechea hastata (Lindl.) W.E. Higgins	Several states	5
Prosthechea karwinskii (Mart.) Soto Arenas et Salazar	Oaxaca	5
Prosthechea linkiana (Klotzsch) W.E. Higgins	Several states	5
Prosthechea magnispatha (Ames, F.T. Hubb. et C. Schweinf.) W.E. Higgins	Several states	5
Prosthechea mariae (Ames) W.E. Higgins	Several states	3
Prosthechea mulasii Soto Arenas et L. Cerv.	Guerrero	5
Prosthechea obpiribulbon (Hágsater) W.E. Higgins	Several states	5
Prosthechea pastoris (Lex.) Espejo et López-Ferr.	Several states	5
Prosthechea pringlei (Rolfe) W.E. Higgins	Several states	5
Prosthechea pterocarpa (Lindl.) W.E. Higgins	Several states	5
Prosthechea punctulata (Rchb.f.) Soto Arenas et Salazar	Several states	5
Prosthechea semiaperta (Hágsater) W.E. Higgins	Several states	5
Prosthechea tripunctata (Lindl.) W.E. Higgins	Several states	5
Prosthechea trulla (Rchb.f.) W.E. Higgins	Several states	5
Prosthechea varicosa (Bateman ex Lindl.) W.E. Higgins ssp. *leiobulbon* (Hook.) Dressler et G.E. Pollard	Several states	5
Pseudogoodyera pseudogoodyerioides (L.O. Williams) R. González et Szlach.	Several states	4
Rhynchostele aptera (Lex.) Soto Arenas et Salazar	Several states	5

Rhynchostele candidula (Rchb.f.) Soto Arenas et Salazar	Several states	5
Rhynchostele cervantesii (Lex.) Soto Arenas et Salazar	Several states	3
Rhynchostele cordata (Lindl.) Soto Arenas et Salazar	Several states	3
Rhynchostele ehrenbergii (Link, Klotzsch et Otto) Soto Arenas et Salazar	Several states	3
Rhynchostele galeottiana (A. Rich. et Galeotti) Soto Arenas et Salazar	Several states	4
Rhynchostele londesboroughiana (Rchb.f.) Soto Arenas et Salazar	Guerrero	3
Rhynchostele maculata (Lex.) Soto Arenas et Salazar	Several states	5
Rhynchostele madrensis (Rchb.f.) Soto Arenas et Salazar	Several states	3
Rodriguezia dressleriana R. González	Several states	4
Rossioglossum hagsaterianum Soto Arenas	Nayarit	5
Rossioglossum insleayi (Barker ex Lindl.) Garay et H.A. Kenn.	Several states	3
Rossioglossum splendens (Rchb.f.) Garay et H.A. Kenn.	Several states	3
Sarcoglottis lobata (Lindl.) P.N. Don	Hidalgo	5
Sarcoglottis scintillans (E.W. Greenw.) Salazar et Soto Arenas	Several states	5
Schiedeella affinis (C. Schweinf.)	Several states	5
Schiedeella crenulata (L.O. Williams) Espejo et López-Ferr.	Several states	5
Schiedeella durangensis (Ames et C. Schwienf.) Buns-Bal.	Several states	5
Schiedeella garayana R. González	Jalisco	5
Schiedeella llaveana (Lindl.) Schltr. var. *alinae* Szlach.	Several states	5
Schiedeella llaveana (Lindl.) Schltr. var. *guerrerensis* Szlach.	Guerrero	5
Schiedeella nagelii (L.O. Williams) Garay	San Luis Potosí	4
Schiedeella romeroana Szlach.	Oaxaca	5
Schiedeella tenella (L.O. Williams) Burns.-Bal.	Several states	5
Schiedeella williamsiana Szlach., Rutk. et Mytnik	Jalisco	5
Sigmatostalix mexicana L.O. Williams	Guerrero	4
Sobralia galeottiana A. Rich.	Several states	5
Sotoa confusa (Garay) Salazar	Several states	5
Specklinia digitale (Luer) Pridgeon et M.W. Chase	Several states	3

Spiranthes delitescens Sheviak	Several states	5
Spiranthes graminea Lindl.	Several states	5
Stanhopea dodsoniana Salazar et Soto Arenas	Several states	5
Stanhopea hernandezii (Kunth) Schltr.	Several states	5
Stanhopea intermedia Klinge	Several states	5
Stanhopea maculosa Knowles et Westc.	Several states	5
Stanhopea martiana Lindl. ex Bateman	Several states	5
Stanhopea pseudoradiosa Jenny et R. González	Oaxaca	5
Stanhopea tigrina Bateman ex. Lindl.	Several states	3
Stelis aeolica Solano et Soto Arenas	Chiapas	5
Stelis annedamoniae Solano	Several states	5
Stelis aristocratica (L.O. Williams) Solano et Soto Arenas	Guerrero	5
Stelis chiapensis Solano	Several states	5
Stelis desantiagoi Solano et Salazar	Guerrero	5
Stelis fulva Schltr.	Several states	5
Stelis hagsateri Solano	Chiapas	5
Stelis martinezii Solano	Several states	5
Stelis nagelii Solano	Several states	5
Stelis nigriflora (L.O. Williams) Pridgeon et M.W. Chase	Morelos	4
Stelis nonresupinata Solano et Soto Arenas	Several states	5
Stelis oaxacana Solano	Oaxaca	5
Stelis oestlundiana (L.O. Williams) Pridgeon et M.W. Chase	Several states	5
Stelis resupinata (Ames) Pridgeon et M.W. Chase	Several states	5
Stelis retusa (Lex.) Pridgeon et M.W. Chase	Several states	5
Stelis rufobrunnea (Lindl.) L.O. Williams	Several states	5
Stelis salazarii Solano	Oaxaca	5
Stelis sanguinolenta (Garay et W. Kittr.) Solano	Jalisco	5
Stelis soconuscana Solano	Chiapas	5

Stelis sotoana Solano	Oaxaca	5
Stelis sotoarenasii Solano	Oaxaca	5
Stelis veracrucensis Solano	Oaxaca	5
Stelis wendtii Solano	Oaxaca	5
Stelis xerophila (Schltr.) Soto Arenas	Several states	5
Svenkoeltzia congestiflora (L.O. Williams) Burns.-Bal.	Several states	5
Svenkoeltzia luzmariana R. González	Jalisco	5
Svenkoeltzia pamelae Szlach., Rutk. et Mytnik	Oaxaca	5
Svenkoeltzia patriciae R. González	Jalisco	5
Tamayorkis hintonii (Todzia) R. González et Szlach.	Several states	5
Tamayorkis porphyrea (Ridl.) Salazar et Soto Arenas	Several states	5
Tamayorkis wendtii (Salazar) R. González et Szlach.	Several states	5
Trichocentrum andreanum (Cogn.) R. Jiménez et Carnevali	Several states	5
Trichocentrum andrewsiae (R. Jiménez et Carnevali) R. Jiménez et Carnevali	Several states	5
Trichocentrum biorbiculare (Balam et Cetzal) R. Jiménez et Solano	Several states	5
Trichocentrum cosymbephorum (Morren) R. Jiménez et Carnevali	Several states	5
Trichocentrum flavovirens (L.O. Williams) M.W. Chase et N.H. Williams	Several states	4
Trichocentrum hoegei Rchb.f.	Several states	4
Trichocentrum leptotifolium (Cetzal et Carnevali) R. Jiménez et Solano	Several states	5
Trichocentrum longifolium (Lindl.) R. Jiménez	Several states	5
Trichocentrum margalefii (Hágsater) M.W. Chase et N.H. Williams	Several states	5
Trichocentrum nataliae Balam et Carnevali	Jalisco	5
Trichocentrum oestlundianum (L.O. Williams) M.W. Chase et N.H. Williams	Several states	5
Trichocentrum pendulum (Carnevali et Cetzal) R. Jiménez et Solano	Several states	5
Trichocentrum sierracaracolense (Cetzal et Balam) R. Jiménez et Solano	Chiapas	5
Trichocentrum stramineum (Lindl.) M.W. Chase et N.H. Williams	Veracruz	5
Trichocentrum teboana (R. Jiménez, Carnevali et J.L. Tapia) R. Jiménez et Carnevali	Tabasco	5
Trichocentrum yucatanense (Cetzal et Carnevali) R. Jiménez et Solano	Several states	5

Trichopilia galeottiana A. Rich.	Several states	2
Trichosalpinx nageliana Soto Arenas	Several states	5
Trichosalpinx tamayoana Soto Arenas	Colima	5
Triphora mexicana (S. Watson) Schltr.	Several states	5
Triphora yucatanensis Ames	Several states	5

*The conservation status was taken of Espejo-Serna (2012), with some modifications and updates. The categories and abbreviations used to describe the conservation status of each orchid correspond to that described in the Official Mexican Norms: 1, probably extinct; 2, in danger of extinction; 3, threatened; 4, in special protection and 5, uncategorized species (NOM-059-SEMARNAT-2010).

Supplementary Table 2 Distribution of endemic orchids in the Federal Natural Protected Areas of Mexico

Type of Federal Natural Procted Area*	Official name	State (s)	Endemic orchids	Reference
PN01	Desierto de Los Leones	Estado de México	*Corallorhiza macrantha* Schltr.	CONANP 2006a
PN02	Iztaccíhuatl-Popocatépetl	Ciudad de México, Morelos y Puebla	*Bletia macristhmochila* Greenm.	Luna-Rosales et al. 2007; CONANP 2013a
			Bletia neglecta Sosa	
			Corallorhiza macrantha Schltr.	
			Erycina hyalinobulbon (Lex.) N.H. Williams et M.W. Chase	
			Laelia autumnalis (Lex.) Lindl.	
			Malaxis salazarii Catling	
			Mesadenus tenuissimus (L.O. Williams) Garay	
			Platanthera brevifolia (Greene) Kraenzl.	
			Prosthechea linkiana (Klotzsch) W.E. Higgins	
			Stelis retusa (Lex.) Pridgeon et M.W. Chase	
PN03	Grutas de	Guerrero	*Liparis greenwoodiana* Espejo	CONANP 2006b

	Cacahuamilpa			
PN04	Lagunas de Zempoala	Estado de México, Morelos	11 species, Without access to the list	Bonilla-Barbosa and Viana-Lases 1997
PN05	El Tepozteco	Ciudad de México, Morelos	*Bletia adenocarpa* Rchb.f.	CONANP 2008a; Block and Meave 2015
			Bletia coccinea Lex.	
			Bletia greenmaniana L.O. Williams	
			Bletia lilacina A. Rich. et Galeotti	
			Bletia macristhmochila Greenm.	
			Bletia neglecta Sosa	
			Bletia parkinsonii Hook.	
			Bletia punctata Lex.	
			Bulbophyllum nagelii L.O. Williams	
			Clowesia thylaciochila (Lem.) Dodson	
			Corallorhiza ehrenbergii Rchb.f.	
			Corallorhiza williamsii Correll	
			Cranichis subumbellata A. Rich. et Galeotti	
			Cyclopogon saccatus A. Rich. et Galeotti	
			Deiregyne rhombilabia Garay	
			Encyclia aenicta Dressler et G.E. Pollard	
			Encyclia microbulbon (Hook.) Schltr.	
			Encyclia spatella (Rchb.f.) Schltr.	
			Epidendrum anisatum Lex.	
			Epidendrum matudae L.O. Williams	
			Epidendrum miserum Lindl.	
			Galeottiella sarcoglossa (A. Rich. et Galeotti) Schltr.	
			Habenaria calicis R. González	

			Habenaria entomantha (Lex.) Lindl.	
			Habenaria filifera S. Watson	
			Habenaria flexuosa Lindl.	
			Habenaria oreophila Greenm.	
			Habenaria rosulifolia Espejo et López-Ferr.	
			Habenaria rzedowskiana R. González	
			Habenaria uncata R. Jiménez, L. Sánchez et García-Cruz	
			Habenaria virens A. Rich. et Galeotti	
			Hexalectris grandiflora (A. Rich. et Galeotti) L.O. Williams	
			Hintonella mexicana Ames	
			Isochilus bracteatus (Lex.) López-Ferr. et Espejo	
			Laelia autumnalis (Lex.) Lindl.	
			Leochilus carinatus (Knowles et Westc.) Lindl.	
			Lepanthes nagelii Salazar et Soto Arenas	
			Liparis cordiformis C. Schweinf.	
			Liparis greenwoodiana Espejo	
			Malaxis abieticola Salazar et Soto Arenas	
			Malaxis alvaroi García-Cruz, R. Jiménez et L. Sánchez	
			Malaxis myurus (Lindl.) Kuntze	
			Malaxis ocreata (S. Watson) Ames	
			Malaxis ribana Espejo et López-Ferr.	
			Malaxis rosei Ames	
			Malaxis rosilloi R. González et E.W. Greenw.	
			Malaxis salazarii Catling	

			Malaxis streptopetala (B.L. Rob. et Greenm.) Ames	
			Malaxis stricta L.O. Williams	
			Oncidium brachyandrum Lindl.	
			Oncidium geertianum C. Morren	
			Oncidium graminifolium (Lindl.) Lindl.	
			Oncidium microstigma Rchb.f.	
			Oncidium reflexum Lindl.	
			Oncidium reichenheimii (Linden et Rchb.f.) Garay et Stacy	
			Oncidium suave Lindl.	
			Oncidium unguiculatum Lindl.	
			Platanthera brevifolia (Greene) Kraenzl.	
			Platanthera volcanica Lindl.	
			Ponthieva hildae R. González et Soltero	
			Ponthieva schaffneri (Rchb.f.) E.W. Greenw.	
			Prosthechea linkiana (Klotzsch) W.E. Higgins	
			Prosthechea pringlei (Rolfe) W.E. Higgins	
			Rhynchostele cervantesii (Lex.) Soto Arenas et Salazar	
			Schiedeella crenulata (L.O. Williams) Espejo et López-Ferr.	
			Spiranthes graminea Lindl.	
			Stanhopea hernandezii (Kunth) Schltr.	
PN06	Cofre de Perote	Veracruz	Without endemic species	Vázquez-Rámirez 2014
PN07	Benito Juárez	Oaxaca	*Acianthera chrysantha* (Lindl.) Pridgeon et M.W. Chase	CONANP 2014a

			Artorima erubescens (Lindl.) Dressler et G.E. Pollard	
			Bletia punctata Lex.	
			Encyclia microbulbon (Hook.) Schltr.	
			Epidendrum lignosum Lex.	
			Epidendrum costatum A. Rich. et Galeotti	
			Epidendrum hagsateri Christenson	
			Epidendrum oaxacanum Rolfe	
			Govenia capitata Lindl.	
			Laelia albida Bateman ex Lindl.	
			Laelia furfuracea Lindl.	
			Malaxis myurus (Lindl.) Kuntze	
			Malaxis streptopetala (B.L. Rob. et Greenm.) Ames	
			Oncidium graminifolium (Lindl.) Lindl.	
			Prosthechea semiaperta (Hágsater) W.E. Higgins	
			Rhynchostele cervantesii (Lex.) Soto Arenas et Salazar	
			Rhynchostele maculata (Lex.) Soto Arenas et Salazar	
PN08	La Malinche	Puebla, Tlaxcala	Without endemic species	López-Domínguez, Acosta-Pérez and Sánchez-Hernández 2004; Martínez-y-Pérez et al. 2011
PN09	Barranca del Cupatitzio	Michoacán	*Laelia autumnalis* (Lex.) Lindl.	CONANP 2006c
PN10	Lagunas de Montebello	Chiapas	*Arpophyllum laxiflorum* Pfitzer	Beutelspacher-Baigts and Moreno-Molina
			Cyclopogon luteo-albus (A. Rich. et Galeotti)	

			Schltr.	2011; CONANP 2007a
			Epidendrum chlorops Rchb.f.	
			Epidendrum hagsateri Christenson	
			Epidendrum tziscaoense Hágsater	
			Habenaria virens A. Rich. et Galeotti	
			Homalopetalum pumilum (Ames) Dressler	
			Lepanthes breedlovei Salazar et Soto Arenas	
			Lepanthes maxima Salazar et Soto Arenas	
			Lepanthes minima Salazar, Soto Arenas et O. Suárez	
			Oestlundia luteorosea (A. Rich. et Galeotti) W.E. Higgins	
			Oncidium lindleyi (Galeotti ex Lindl.) R. Jiménez et Soto Arenas	
			Oncidium sotoanum R. Jiménez et Hágsater ssp. *papalosmum* R. Jiménez	
			Rhynchostele cordata (Lindl.) Soto Arenas et Salazar	
PN11	General Juan N. Álvarez	Guerrero	*Bletia neglecta* Sosa	Bustamante-García 2012
			Epidendrum anisatum Lex.	
			Oncidium graminifolium (Lindl.) Lindl.	
			Schiedeella llaveana (Lindl.) Schltr. var. *guerrerensis* Szlach.	
			Sotoa confusa (Garay) Salazar	
PN12	Cañón del Sumidero	Chiapas	*Epidendrum erectifolium* Hágaster et L. Sánchez	Espinosa-Jiménez, Pérez-Farrera and Martínez-Camilo 2011
			Govenia alba A. Rich. et Galeotti	
			Habenaria matudae Salazar	
			Ornithocephalus tripterus Schltr.	

			Pseudogoodyera pseudogoodyerioides (L.O. Williams) R. González et Szlach.	
			Trichocentrum andreanum (Cogn.) R. Jiménez et Carnevali	
PN13	Cascada de Bassaseachic	Chihuahua	*Malaxis salazarii* Catling	CONANP 2016a
PN14	Palenque	Chiapas	Without endemic species	Gómez-Domínguez et al. 2015
PN15	El Chico	Hidalgo	*Malaxis rosei* Ames	CONANP 2005a
			Malaxis streptopetala (B.L. Rob. et Greenm.) Ames	
PN16	Huatulco	Oaxaca	*Barkeria shoemakeri* Halb.	Salas-Morales et al. 2007
			Clowesia dodsoniana E. Aguirre	
			Encyclia rzedowskiana Soto Arenas	
			Erycina echinata (Kunth) Lindl.	
PN17	Dzibilchantún	Yucatán	Without endemic species	CONANP 2016b
APFF01	Nevado de Toluca	Estado de México	*Corallorhiza macrantha* Schltr.	CONANP 2013b
			Epidendrum anisatum Lex.	
			Laelia gouldiana Rchb.f.	
			Malaxis rosei Ames	
			Rhynchostele cervantesii (Lex.) Soto Arenas et Salazar	
APFF02	Pico de Tancítaro	Michoacán	*Epidendrum anisatum* Lex.	García-Ruíz 2001
			Epidendrum longipetalum A. Rich. et Galeotti	
			Govenia purpusii Schltr.	
			Liparis draculoides E.W. Greenw.	
			Oncidium reichenheimii (Linden et Rchb.f.) Garay et Stacy	
APFF03	La Primavera	Jalisco	*Bletia adenocarpa* Rchb.f.	CONANP 2000

			Bletia amabilis C. Schweinf.	
			Bletia coccinea Lex.	
			Bletia ensifolia L.O. Williams	
			Bletia greenmaniana L.O. Williams	
			Bletia macristhmochila Greenm.	
			Clowesia rosea Lindl.	
			Cyclopogon pringlei (S. Watson) Soto Arenas	
			Deiregyne albovaginata (C. Schweinf.) Garay	
			Deiregyne chartacea (L.O. Williams) Garay	
			Epidendrum rosilloi Hágsater	
			Habenaria diffusa A. Rich et Galeotti	
			Habenaria entomantha (Lex.) Lindl.	
			Habenaria filifera S. Watson	
			Habenaria schaffneri S. Watson	
			Hexalectris brevicaulis L.O. Williams	
			Hexalectris grandiflora (A. Rich. et Galeotti) L.O. Williams	
			Laelia albida Bateman ex Lindl.	
			Malaxis ocreata (S. Watson) Ames	
			Oncidium graminifolium (Lindl.) Lindl.	
APFF04	Maderas del Carmen	Cohahuila	*Hexalectris grandiflora* (A. Rich. et Galeotti) L.O. Williams	CONANP 2013
			Malaxis wendtii Salazar	
APFF05	Cañón del Usumacinta	Tabasco	Without endemic species	CONANP 2015a
APFF06	Cañón de Santa Elena	Chihuahua	*Hexalectris warnockii* Ames et Correll	SEMARNAP 1997; CONANP 2008b
APFF07	Uaymil	Quinatana Roo	*Without endemic species*	CONANP 2014b

APFF08	Sierra de Quila	Jalisco	*Bletia ensifolia* L.O. Williams	Guerrero-Nuño 1994
			Encyclia meliosma (Rchb.f.) Schltr.	
			Laelia autumnalis (Lex.) Lindl.	
			Laelia speciosa (Kunth) Schltr.	
			Malaxis tepicana Ames	
APFF09	Corredor biológico Chichinautzin	Ciudad de México, Estado de México, Morelos	*Bletia adenocarpa* Rchb.f.	Pulido-Esparza, Espejo-Serna and López-Ferrari 2009
			Bletia coccinea Lex.	
			Bletia lilacina A. Rich. et Galeotti	
			Bletia macristhmochila Greenm.	
			Bletia neglecta Sosa	
			Bletia parkinsonii Hook.	
			Bletia punctata Lex.	
			Bulbophyllum nagelii L.O. Williams	
			Clowesia thylaciochila (Lem.) Dodson	
			Corallorhiza ehrenbergii Rchb.f.	
			Corallorhiza williamsii Correll	
			Cranichis subumbellata A. Rich. et Galeotti	
			Cyclopogon saccatus A. Rich. et Galeotti	
			Deiregyne rhombilabia Garay	
			Encyclia microbulbon (Hook.) Schltr.	
			Encyclia spatella (Rchb.f.) Schltr.	
			Epidendrum anisatum Lex.	
			Epidendrum matudae L.O. Williams	
			Epidendrum miserum Lindl.	
			Galeottiella sarcoglossa (A. Rich. et Galeotti) Schltr.	
			Habenaria calicis R. González	

			Habenaria entomantha (Lex.) Lindl.	
			Habenaria filifera S. Watson	
			Habenaria flexuosa Lindl.	
			Habenaria oreophila Greenm.	
			Habenaria rosulifolia Espejo et López-Ferr.	
			Habenaria uncata R. Jiménez, L. Sánchez et García-Cruz	
			Habenaria virens A. Rich. et Galeotti	
			Hexalectris grandiflora (A. Rich. et Galeotti) L.O. Williams	
			Hintonella mexicana Ames	
			Isochilus bracteatus (Lex.) López-Ferr. et Espejo	
			Laelia autumnalis (Lex.) Lindl.	
			Leochilus carinatus (Knowles et Westc.) Lindl.	
			Lepanthes nagelii Salazar et Soto Arenas	
			Liparis cordiformis C. Schweinf.	
			Liparis greenwoodiana Espejo	
			Malaxis abieticola Salazar et Soto Arenas	
			Malaxis alvaroi García-Cruz, R. Jiménez et L. Sánchez	
			Malaxis brachystachya (Rchb.f.) Kuntze	
			Malaxis myurus (Lindl.) Kuntze	
			Malaxis ribana Espejo et López-Ferr.	
			Malaxis rosei Ames	
			Malaxis rosilloi R. González et E.W. Greenw.	
			Malaxis salazarii Catling	
			Malaxis streptopetala (B.L. Rob. et Greenm.)	

			Ames	
			Malaxis stricta L.O. Williams	
			Oncidium brachyandrum Lindl.	
			Oncidium geertianum C. Morren	
			Oncidium graminifolium (Lindl.) Lindl.	
			Oncidium microstigma Rchb.f.	
			Oncidium reichenheimii (Linden et Rchb.f.) Garay et Stacy	
			Oncidium unguiculatum Lindl.	
			Platanthera brevifolia (Greene) Kraenzl.	
			Platanthera volcanica Lindl.	
			Ponthieva hildae R. González et Soltero	
			Ponthieva schaffneri (Rchb.f.) E.W. Greenw.	
			Prosthechea linkiana (Klotzsch) W.E. Higgins	
			Prosthechea pringlei (Rolfe) W.E. Higgins	
			Prosthechea varicosa (Bateman ex Lindl.) W.E. Higgins ssp. *leiobulbon* (Hook.) Dressler et G.E. Pollard	
			Rhynchostele aptera (Lex.) Soto Arenas et Salazar	
			Rhynchostele cervantesii (Lex.) Soto Arenas et Salazar	
			Rhynchostele maculata (Lex.) Soto Arenas et Salazar	
			Schiedeella crenulata (L.O. Williams) Espejo et López-Ferr.	
			Schiedeella garayana R. González	
			Spiranthes graminea Lindl.	
			Stanhopea hernandezii (Kunth) Schltr.	

APFF10	Laguna de Terminos	Campeche	Without endemic species	Endañú-Huerta et al. 2017
APFF11	Bala'an K'aax	Quintana Roo	Without endemic species	CONANP 2007b
APFF12	Nahá	Chiapas	Without endemic species	Durán-Fernández et al. 2016
APFF13	Metzabok	Chiapas	Without endemic species	CONANP 2006d
APFF14	Sierra de Álamos-Río Cuchujaqui	Sonora	*Encyclia adenocarpa* (Lex.) Schltr.	CONANP 2015b
			Laelia autumnalis (Lex.) Lindl.	
			Laelia eyermaniana Rchb.f.	
			Laelia speciosa (Kunth) Schltr.	
RB01	Reserva de la biosfera de la Mariposa Monarca	Michoacán	*Govenia capitata* Lindl.	Cornejo-Tenorio and Ibarra Manríquez 2008
RB02	Montes Azules	Chiapas	Without endemic species	SEMARNAP 2000a
RB03	La Michilia	Durango	*Habenaria entomantha* (Lex.) Lindl.	González-Elizondo, González-Elizondo and Cortés-Ortiz 1993
RB04	Selva El Ocote	Chiapas	*Oncidium hagsaterianum* R. Jiménez et Soto Arenas	SEMARNAT 2001
RB05	El Triunfo	Chiapas	*Triphora mexicana* (S. Watson) Schltr.	Martínez-Meléndez, Pérez-Farrera and Farrera-Sarmiento 2008; Martínez-Camilo, Pérez-Farrera and Martínez-Meléndez 2012; Pérez-Farrera et al. 2012
			Epidendrum erectifolium Hágaster et L. Sánchez	
			Lepanthes matudana Salazar et Soto Arenas	
			Mormodes tuxtlensis Salazar	
			Rhynchostele cordata (Lindl.) Soto Arenas et Salazar	
			Epidendrum erectifolium Hágaster et L. Sánchez	
			Govenia alba A. Rich. et Galeotti	
			Prosthechea varicosa (Bateman ex Lindl.)	

			W.E. Higgins ssp. *leiobulbon* (Hook.) Dressler et G.E. Pollard	
			Stelis soconuscana Solano	
RB06	La Sepultura	Chiapas	*Cyclopogon luteo-albus* (A. Rich. et Galeotti) Schltr.	Reyes-García, Souza-Sánchez and León-Velasco 2006
			Goodyera dolabripetala (Ames) Schltr.	
			Habenaria virens A. Rich. et Galeotti	
			Malaxis greenwoodiana Salazar et Soto Arenas	
			Oncidium incurvum F. Barker ex Lindl.	
			Prosthechea varicosa (Bateman ex Lindl.) W.E. Higgins ssp. *leiobulbon* (Hook.) Dressler et G.E. Pollard	
			Rhynchostele cordata (Lindl.) Soto Arenas et Salazar	
			Trichopilia galeottiana A. Rich.	
RB07	Sierra de Manantlán	Colima, Jalisco	*Clowesia thylaciochila* (Lem.) Dodson	SEMARNAP 2000b
			Cuitlauzina pendula Lex.	
			Malaxis rosilloi R. González et E.W. Greenw.	
			Malaxis tamayoana Garay et W. Kittr.	
			Malaxis tepicana Ames	
			Oncidium tigrinum Lex.	
			Rossioglossum splendens (Rchb.f.) Garay et H.A. Kenn.	
			Stanhopea maculosa Knowles et Westc.	
RB08	Calakmul	Campeche	Without endemic species	Martínez, Sousa and Ramos-Álvarez 2001
RB09	Pantanos de Centla	Tabasco	*Laelia anceps* Lindl.	SEMARNAP 2000c
RB10	Sierra del Abra	San Luis Potosí	*Epidendrum falcatum* Lindl.	CONANP 2014c

	Tanchipa		*Prosthechea mariae* (Ames) W.E. Higgins	
			Isochilus unilateralis B.L. Rob.	
			Laelia gouldiana Rchb.f.	
			Laelia speciosa (Kunth) Schltr.	
			Stanhopea tigrina Lindl.	
RB11	Zicuirán Infiernillo	Michoacán	*Barkeria shoemakeri* Halb.	CONANP 2014d
			Bletia coccinea Lex.	
			Erycina echinata (Kunth) Lindl.	
			Habenaria filifera S. Watson	
			Habenaria flexuosa Lindl.	
RB12	Sierra de La Laguna	Baja California Sur	*Corallorhiza fimbriata* Schltr.	CONANP 2003a
			Habenaria entomantha (Lex.) Lindl.	
			Hexalectris warnockii Ames et Correll	
RB13	Volcán Tacaná	Chiapas	Without endemic species	CONANP 2013d
RB14	Barranca de Metztitlán	Hidalgo	*Laelia autumnalis* (Lex.) Lindl.	CONANP 2003b
			Laelia gouldiana Rchb.f.	
			Laelia speciosa (Kunth) Schltr.	
RB15	Sierra de Huautla	Morelos	*Encyclia adenocarpa* (Lex.) Schltr.	CONANP 2005b
			Oncidium microstigma Rchb.f.	
RB16	Los Petenes	Campeche	Without endemic species	CONANP 2006e
RB17	Ría Lagartos	Yucatán	Without endemic species	CONANP 2007c
RB18	Los Tuxtlas	Veracruz	*Arpophyllum spicatum* Lex.	CONANP 2006f
			Encyclia parviflora (Regel) Whitner	
			Epidendrum macroclinium Hágsater	
			Mormodes maculata (Klotzsch) L.O. Williams var. *maculata*	
			Mormodes tuxtlensis Salazar	

			Oncidium hagsaterianum R. Jiménez et Soto Arenas	
			Rhynchostele cordata (Lindl.) Soto Arenas et Salazar	
			Stanhopea dodsoniana Salazar et Soto Arenas	
RB19	Tehuacán-Cuicatlán	Oaxaca, Puebla	*Barkeria melanocaulon* A. Rich. et Galeotti	CONANP 2013e
			Barkeria scandens (Lex.) Dressler et Halb.	
			Deiregyne falcata (L.O. Williams) Garay	
			Deiregyne rhombilabia Garay	
			Deiregyne tenorioi Soto Areanas et Salazar	
			Domingoa kienastii (Rchb.f.) Dressler	
			Epidendrum lignosum Lex.	
			Prosthechea citrina (Lex.) W.E. Higgins	
			Govenia capitata Lindl.	
			Govenia tequilana Dressler et Hágsater	
			Habenaria subauriculata B.L. Rob. et Greenm.	
			Laelia albida Bateman ex Lindl.	
			Laelia anceps Lindl.	
			Laelia furfuracea Lindl.	
			Mesadenus tenuissimus (L.O. Williams) Garay	
			Oncidium brachyandrum Lindl.	
			Oncidium suave Lindl.	
			Prosthechea concolor (Lex.) W.E. Higgins	
			Prosthechea pterocarpa (Lindl.) W.E. Higgins	
			Prosthechea semiaperta (Hágsater) W.E. Higgins	
			Prosthechea tripunctata (Lindl.) W.E. Higgins	

			Rhynchostele aptera (Lex.) Soto Arenas et Salazar	
			Rhynchostele maculata (Lex.) Soto Arenas et Salazar	
			Schiedeella romeroana Szlach.	
RB20	Sierra Gorda	Queretaro	*Arpophyllum spicatum* Lex.	SEMARNAP 1999
			Bletia neglecta Sosa	
			Corallorhiza ehrenbergii Rchb.f.	
			Cranichis subumbellata A. Rich. et Galeotti	
			Cyclopogon luteo-albus (A. Rich. et Galeotti) Schltr.	
			Epidendrum longipetalum A. Rich. et Galeotti	
			Laelia anceps Lindl.	
RB21	Sian Ka'an	Quinatana Roo	Without endemic species	CONANP 2014b
RB22	Arrecifes de Sian Ka'an	Quinatana Roo	Without endemic species	CONANP 2014b
APRN01	Cuenca hidrográfica del río Necaxa	Puebla	*Encyclia candollei* (Lindl.) Schltr.	CONANP 2013f
			Isochilus unilateralis B.L. Rob.	
			Malaxis salazarii Catling	
			Oncidium incurvum F. Barker ex Lindl.	
			Trichocentrum stramineum (Lindl.) M.W. Chase et N.H. Williams	
			Ornithocephalus tripterus Schltr.	
			Stanhopea tigrina Lindl.	
APRN02	Zona de Protección Forestal "La Frailescana"	Chiapas	Without endemic species	Bachem and Rojas 1994; Pérez-Farrera et al. 2006
MN01	Yaxchilán	Chiapas	Without endemic species	Meave et al. 2008
MN02	Río Bravo del Norte	Chihuahua,	*Hexalectris nitida* L.O. Williams	CONANP 2013g

		Cohahuila	*Hexalectris revoluta* Correll	
			Hexalectris warnockii Ames et Correll	
MN03	Yagul	Oaxaca	*Barkeria vanneriana* Rchb.f.	CONANP 2013h
			Deiregyne diaphana (Lindl.) Garay	

*(PN, National Park; APFF, Flora and Fauna Protection Area; RB, Biosphere Reserve; APRN, Natural Resources Protection Area; and MN, National Monument)

CHAPTER II

Biotechnological approaches for conservation of medicinal plants

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Introduction

Medicinal plants are an important global resource. Health systems in developed countries use folk medicine for primary health care. In some countries or regions, traditional medicine is called alternative or complementary medicine (Rosenbloom, Chaudhary, & Castro-Eschenbach, 2011; WHO, 2019). The World Health Organization (WHO) estimates that more than 80% of the world population use traditional medicine, mainly based on medicinal plants. In some countries, health professionals recommend the use of medicinal plants as an alternative treatment (Alonso-Castro et al., 2017). The International Union for Conservation of Nature and the World Wildlife Fund have recorded approximately 60,000 angiosperms used for medicinal purposes worldwide (Chen et al., 2016; Jamshidi-Kia, Lorigooini, & Amini-Khoei, 2018). A medicinal plant is defined as any plant species that contains secondary metabolites that can be used for therapeutic purposes or can be used as precursors for the synthesis of new drugs (Penso, 1980; Seca & Pinto, 2019; Yuan, Ma, Ye, & Piao, 2016).

However, it is estimated that approximately 15,000 wild populations of medicinal plants are threatened due to their extensive commercialization (Brower, 2008; Wang, Usmanovich, Luo, Xu, & Wu, 2019). Therefore, it is necessary to establish useful strategies for the conservation of medicinal herbs.

This chapter contains evidence on the diversity and overexploitation of medicinal plants around the world. In addition, a special emphasis on the conservation of medicinal plants is given, addressing some strategies with biotechnological approaches such as the plant tissue culture (PTC), a technique that has managed to improve the yield of medicinal plants. The information will be useful to provide some strategies that promote a sustainable use of medicinal plants.

Knowledge about diversity of medicinal plants in the last decade

Plant and cultural diversity in the world offer new alternatives for the implementation of crops and products based on medicinal plants. However, a large number of plant species, distributed in many regions of the Earth, remain to be scientifically studied. A bibliographic search of scientific reports from the last decade was conducted on academic databases to analyse the diversity of medicinal plants in several regions of the planet and was carried out. The search of information was based on three pairs of keywords: medicinal plants, global diversity, and bioactive compounds.

The results show that the Asian continent, leading by India, presents the highest number of scientific reports (36%) (Fig. 1) (Table 1). The information contained in Table 1 does not duplicate medicinal plants. India has approximately 17,000 species of higher plants, due to its wide range of ecosystems, of which 45% are used for medicinal purposes and are enlisted in traditional medicinal systems such as Ayurveda (Singh, 2015).

Some reports cited in Table 1 indicate that ethnobotanical studies were performed for the first time in different countries, regions, and natural protected areas (i.e., Ullah et al., 2013). In addition, the ethnomedicinal uses of different herb species were documented for the first time (i.e., Eisenman, Zaurov, & Struwe, 2013; Shrestha, Shrestha, Koju, Shrestha, & Wang, 2016). This indicates that the biodiversity of many areas in the world remains to be studied. Many studies were carried out with indigenous populations, which preserve their ancient information. However, many studies pointed out the need to preserve folk knowledge (Eddouks, Ajebli, & Hebi, 2017; Ribeiro, Bieski, Balogun, & de Oliveira Martins, 2017).

Ethnobotanical studies are necessary to preserve local ethnomedicinal information of medicinal plants and update their medicinal uses. Urbanization and loss of biodiversity are among the causes of the loss of traditional knowledge. In addition, many studies remark the need to carry out conservation programs in the studied areas (Li & Xing, 2016). Some plant species reported in Table 1 are classified as endangered (Rahmawati, Mustofa, & Haryanti, 2020). The information clearly indicates that medicinal plants continue to be used for primary health care in many regions of the world (Sarri, Mouyet, Benziane, & Cheriet, 2014).

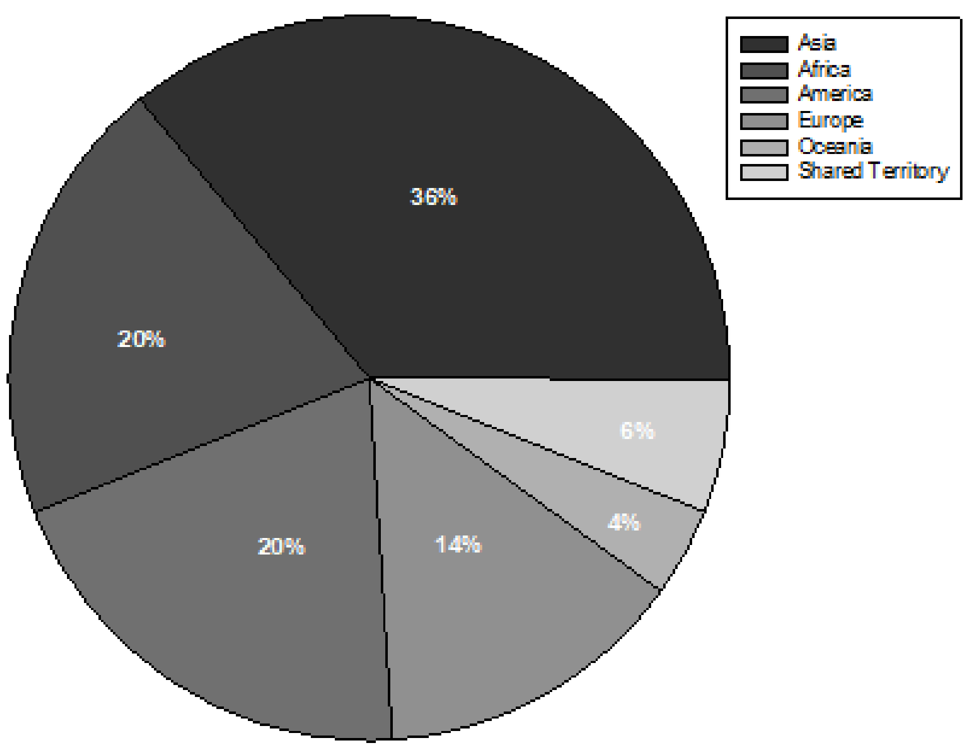

Fig. 1. Percentage of scientific research that studies the diversity of medicinal plants in the world (n = 50). (Credit: Own elaboration.)

Furthermore, medicinal plants are now used to treat current emergent diseases such as obesity, AIDS, diabetes, and malaria (Alonso-Castro, Domínguez, Zapata-Morales, & Carranza-Álvarez, 2015; Ngarivhume, van'tKlooster, de Jong, & Van der Westhuizen, 2015).

There is also a need to validate the ethnomedicinal uses by carrying out pharmacological and toxicological studies with plants mentioned in Table 1. The use of medicinal plants dates from centuries in countries like India, Japan, China, and Mexico (Jeyaprakash, Lego, Payum, Rathinavel, & Jayakumar, 2017; Leonti, Sticher, & Heinrich, 2003).

The use of medicinal plants is deeply rooted in the Asian continent. In contrast, Europe and Oceania showed less than 10 studies, which represent 18% of the diversity of medicinal plants in the last decade. Spain and Italy (in Europe) and three islands of Vanuatu and Northern New South Wales (in Oceania) are the countries where some research has been carried out on this topic (Table 1).

In addition, developed countries such as the United States, the United Kingdom, Switzerland, Germany, and France do not have diverse studies on their medicinal plants during the last decade. In these countries, many pharmaceutical laboratories are situated as following: Pfizer, Merck, Johnson & Johnson, Abbott Laboratories, and Lilly in the United States of America; GlaxoSmithKline (GSK) and AstraZeneca, located in the United Kingdom; Novartis and Roche in Switzerland; and Sanofi-Aventis, in France (Hasegawa & Hambrecht, 2003; McCay, Lemer, & Wu, 2009).

These countries are also considered as the world's largest producers and exporters of drugs. Germany, Switzerland, and the United States are the main exporters of medicines. In contrast the African continent, possessing a great diversity of plants, only exports 0.2% of all medicines in the world (Stevens & Huys, 2017). Countries like Russia do not make the information available for the international scientific community (Shikov et al., 2014).

Table 1. Studies that record the diversity of medicinal plants in several parts of the world in the last decade.

Region	Country	Continent	Diversity (Number of species registered)	Relevant notes	Reference
Khulna City	Bangladesh	Asia	67	The use of medicinal plants has a great impact among allopathic physicians	Akber et al., 2011
Chittagong Hill Tracts region	Bangladesh	Asia	73	Chakma population has an extensive use of medicinal plants	Rahmatullah et al., 2012
Hainan Island	China	Asia	264	The loss of folk knowledge might occur due to degradation of the local environment	Li and Xing, 2016
Medog County, Tibet	China	Asia	37	The documentation of medicinal plants in this region had not been previously carried out	Yang, Chen, et al. (2020) and Yang, Yang, et al. (2020)
Almora District, Central Himalaya Region	India	Asia	188	Some medicinal plants require urgent actions for their protection and conservation	Kumari, Joshi, and Tewari (2011)
Odisha, East Coast of The Bay of Bengal	India	Asia	68	This research provides a list of medicinal plants, some of them without pharmacological studies	Panda et al., 2014
Indian Himalayan Region	India	Asia	90	Invasive medicinal plants can cause loss of local biodiversity	Sekar, 2012
Ladakh, Kashmir	India	Asia	111	Ethnobotanical studies are necessary to update the information in India	Dar and Qazi (2017)

Daying Ering Memorial Wildlife Sanctuary, Arunachal Pradesh	India	Asia	73	Most of the plants lack pharmacological studies that validate their ethnomedicinal uses	Jeyaprakash et al., 2017
Manipur	India	Asia	145	Medicinal plants with magico-religious practices are mainly used by male members of the Zeliangrong community. Some side effects of excessive consumption of medicinal herbs are reported	Panmei, Gajurel, and Singh (2019)
Karo community, Sumatra Septentrional	Indonesia	Asia	344	There is a high demand to obtain medicinal herbs in the traditional Kabanjahe market. Women possess more knowledge of medicinal plants than men	Silalahi, Walujo, Supriatna, and Mangunwardoyo (2015)
Central Sulawesi Province	Indonesia	Asia	89	Some of the medicinal plants are classified as endangered species. Local conservation should be encouraged.	Rahmawati et al., 2020
Whole country	Kyrgyzstan	Asia	200	This is the first work reporting the ethnomedicinal use of plant species in this country	Eisenman et al., 2013
Sankhuwasabha district	Nepal	Asia	48	Medicinal plants are mainly used to treat gastro-intestinal disorders. The work reported the undocumented use of 10 medicinal plants	Shrestha et al., 2016
Wana district south Waziristan agency	Pakistan	Asia	50	This is the first report documenting the ethnomedicinal use of plant species in this region	Ullah et al., 2013

Whole country	Thailand	Asia	2187	Most of plant species are used for the treatment of gastrointestinal disorders. The conservation of local herbs should be carried out	Phumthum et al., 2018
Whole country	Uzbekistan	Asia	400	This is the first report documenting the ethnomedicinal use of plant species in this country	Eisenman et al., 2013
Bac Huong Hoa nature reserve	Vietnam	Asia	111	This is the first report documenting the ethnomedicinal use of plant species in this nature reserve. The medicinal properties of 8 plants are reported for the first time	Lee et al., 2019
M'Sila city	Algeria	Africa	58	Medicinal plants could be incorporated in the national health systems to reduce costs	Boudjelal et al., 2013
M'Sila city	Algeria	Africa	36	Medicinal plants are used for primary health care problems, including gastrointestinal and respiratory acute diseases	Sarri et al., 2014
Administrative departments of Tiaret, Saida, Naama, Djelfa and M'sila	Algeria	Africa	97	This is the first report documenting the ethnomedicinal use of plant species in this region. Many uses of herbs were documented for the first time	Miara, Bendif, Hammou, and Teixidor-Toneu (2018)
Ankober District, North Shewa Zone, Amhara Region	Ethiopia	Africa	158	Plants are used for primary health care needs in human and livestock. The local knowledge is deep-rooted	Lulekal, 2014
Whole country	Gabon	Africa	217	27 tons of medicinal plant-based products are sold in Gabon's main markets, equivalent to $ 1.5 million per	Towns, Quiroz, Guinee, de Boer, and van

				year	Andel (2014)
Whole country	Guinea-Bissau	Africa	218	The transmission of local knowledge about medicinal plants relies on original dialects among communities	Catarino, Havik, and Romeiras (2016)
Daraa-Tafilalet region (Province of Errachidia)	Morocco	Africa	194	Preservation of traditional knowledge is encouraged. Local population have a great expertise on the use of medicinal plants	Eddouks et al., 2017
Whole country	Nigeria	Africa	115	Many medicinal plants used for the empirical treatment of diabetes lacked preclinical studies	Ezuruike and Prieto, 2014
Arua, Dokolo, Mbale, Bushenyi, Iganga, Rakai, Luwero and Kaabong districts	Uganda	Africa	236	Opportunistic infections such AIDS, malaria, among others are treated with medicinal plants. The concomitant use of retroviral agents and medicinal plants is a common practice	Anywar et al., 2020
Chipinge district in the Manicaland Province	Zimbabwe	Africa	28	The medicinal plants used for the treatment of malaria is documented	Ngarivhume et al., 2015
Whole continent	Whole continent	Africa	More than 5400	This research mentions the largest amount of medicinal plants from Africa exported for their sale in markets around the world	Van Wyk, 2015
Misiones province, in the subtropics of Argentina	Argentina	America	509	The updated information about medicinal uses of herbs is documented. Many plants need to be included in conservation programs	Kujawska, Hilgert, Keller, and Gil (2017)

Salta province (South American Gran Chaco region)	Argentina	America	115	This is the first report documenting the ethnomedicinal use of native plant species in this region. The adaptation on knowledge about the use of medicinal plants from other indigenous people is documented.	Suárez, 2019
Caatinga (semi-arid region)	Brazil	America	108	The knowledge transmission about exotic medicinal plants from the region has occurred during centuries	Rangel, Ramos, de Amorim, and de Albuquerque (2010)
North Araguaia microregion	Brazil	America	309	This is the first report documenting the ethnomedicinal use of native plant species in this region. The study preserves local folk knowledge	Ribeiro et al., 2017
Ecuadorian Amazon	Ecuador	America	101	This study proposes a new methodology for ethnobotanical studies based on phylogenetic biases	Arias, Cevallos, Gaoue, Fadiman, and Hindle (2020)
Huasteca Potosina	Mexico	America	73	The information here reported updated local ethnomedicinal uses of medicinal plants	Alonso-Castro et al., 2012
Xalpatlahuac, Guerrero	Mexico	America	67	The use of medicinal plants is an essential practice among inhabitants from one of the poorest municipalities in the country	Juárez-Vázquez et al., 2013
Mexico, Central America and the Caribbean	Several countries	America	139	This report indicates that medicinal plants with diuretic and anti-diabetic effects are now used for weight-loss	Alonso-Castro et al., 2015
Several states	Mexico	America	77	Health professionals prescribe medicinal plants to treat diseases	Alonso-Castro et al., 2017

Several states	Mexico	America	343	The information of medicinal plants used for gastrointestinal illnesses is discussed	Sharma, del Carmen Flores-Vallejo, Cardoso-Taketa, and Villarreal (2017)
South-Eastern area of the Partenio Regional Park	Italy	Europe	87	Medicinal plants are reported for human and veterinary use. Preservation of local knowledge and biodiversity is encouraged.	Menale and Muoio, 2014
Mainarde Mountains (central-southern Apennine)	Italy	Europe	106	This is the first work reporting the ethnomedicinal use of plant species in a natural protected area	Fortini, Di Marzio, Guarrera, and Iorizzi (2016)
Arribes del Duero	Spain	Europe	70	Magical and medicinal purposes of plant species are informed	González, García-Barriuso, and Amich (2010)
Balearic Islands, Mediterranean Sea	Spain	Europe	121	Provides the first report about ethnobotanical information of the Mallorca island	Carrió and Vallés, 2012
Navarra region	Spain	Europe	19	Medicinal plants used for the treatment of most common eye problems are indicated	Calvo and Cavero, 2016
South of Alava	Spain	Europe	36	Edible plants with medicinal uses are reported	Alarcón, Pardo-de-Santayana, Priestley, Morales, and Heinrich (2015)

Whole country	Switzerland	Europe	768	Neighbouring countries have influenced the folk medicinal knowledge in Switzerland	Dal Cero, Saller, and Weckerle (2014)
Whole continent	Whole continent	Europe	400	Some threatened medicinal plants are reported in this study	Allen et al., 2014
Northern New South Wales	Australia	Oceania	32	The indigenous community of Yaegl rely on the use of medicinal plants for primary health care	Packer et al., 2012
Three islands of Vanuatu	Vanuatu	Oceanía	133	This is the first work reporting the folk medicinal use of plant species in this region	Bradacs, Heilmann, and Weckerle (2011)
Central region of Abyan governorate	Yemen	Africa/Asia	195	This is the first work reporting the folk medicinal use of plant species in this region	Al-Fatimi, 2019
The Russian Federation	Russia	Europa/Asia	32	Information about the use of medicinal plants should be accessible to everyone.	Shikov et al., 2014
Ağrı Province	Turkey	Europa/Asia	118	Medicinal plants in this region have a potential to develop new pharmacological products	Dalar, Mukemre, Unal, and Ozgokce (2018)

The problems of subtracting medicinal herbs

Urban sprawl around the world has contributed to the destruction of several ecosystems, including forests, jungles, mangrove, and marine ecosystems. The loss of many plant species, as well as the imminent removal of some others, has been the consequence of this intrusion and other anthropogenic actions such as forest fires, loss of habitats, and illegal extraction of species for commercialization (Scanes, 2018). In this context, medicinal plants are a main target of many traffickers. In many cases medicinal plants are illegally extracted from their ecosystems and can be used locally or transported over long distances to big, urbanized cities, which cause the loss of biodiversity (Rajeswara-Rao, 2016). The main threats from medicinal plants are habitat destruction, bioprospecting, and their overexploitation for illegal trafficking (Chi et al., 2017).

The methods for the conservation of medicinal plants are increasingly necessary, due the accelerated loss of species and natural habitats in the world. It is estimated that approximately 15,000 species of medicinal plants may be endangered worldwide. Therefore, the discovery of new drugs could be missed (Chen et al., 2016; Van-Wyk & Wink, 2018).

The Global Convention on Biological Diversity, the Convention on International Trade in Endangered Species (CITES), and The National Center for the Preservation of Medicinal Herbs (NCPMH) have worked on the conservation of medicinal species. Nevertheless, more scientific and governmental efforts about the loss of medicinal plants should be carried out. On the other hand, some works have been published studying the commercialization of medicinal species products worldwide (Gänger, 2015; Street, Stirk, & Van Staden, 2008; Vasisht, Sharma, & Karan, 2016). For instance, many countries in Latin America illegally export medicinal plants. The trade of medicinal plants has been increased during the last decade because people are afraid of allopathic medicine because of its undesirable side effects (Gänger, 2015; Robbins, 2000).

Pharmaceutical industry that produces plant-based medicines is often accused to commit acts of biopiracy, which is defined as the practice of patenting and overexploiting traditional remedies, obtaining great profits, from which the indigenous communities will not obtain any economic benefit (Efferth et al., 2016; Zakrzewski, 2002). In 2001 a group of researchers found more than 5000 patent references from 90 medicinal plants (80% from India) (Robertson, 2008).

Finally, it is important to highlight those countries with high biodiversity on Earth face problems with the illegal trade of medicinal plants. Aboriginal population from Mexico has an ancient knowledge of traditional medicine. However, this population struggle to obtain economical resources, and many times, they need to commercialize medicinal plants to obtain an economic income (Barreda, 2001; Martínez-Moreno, Alvarado-Flores, Mendoza-Cruz, & Basurto-Peña, 2006).

Conservation strategies for medicinal plants

In the last two decades, the demand for wild populations of medicinal plants has been increased between 8% and 15% annually in Europe, North America, and Asia, causing the reproductive capacity of some species to be irreversibly reduced (Gupta, 2017; Majeed, 2017). Therefore, some strategies for the conservation of medicinal plants, such as in situ to *ex situ* conservation methods, have been developed. The declaration of protected natural areas around the world and the establishment of wildlife nurseries are essential for the retention of plants in their natural habitats (*in situ* conservation). Worldwide, approximately 13,000 protected areas have been established, representing 13.2 million km^2 or 8.81% of the Earth's land surface (Huang et al., 2002). Furthermore, the establishment of wildlife nurseries has contributed to species-oriented cultivation and the domestication of endangered medicinal plants in protected areas or natural habitats (Chen et al., 2016; Prins, 1996).

In situ conservation is important because in several regions of the planet, a large percentage of medicinal plants are endemic, and their ecological interactions with other plants and animals, as well as the environmental conditions of each region, ease the production of their bioactive compounds. The production of bioactive compounds by medicinal plants can be diminished under cultivation conditions (Kusari, Hertweck, & Spiteller, 2012; Núñez-Pons & Avila, 2015).

Ex situ conservation processes are important methods for the conservation of medicinal plants threatened by illegal trafficking. *Ex situ* conservation is often applied in plants (for instance, Orchidaceae family) with slow growth rates or high environmental specifications to thrive (Merritt, Hay, Swarts, Sommerville, & Dixon, 2014; Yadav, 2016).

Ethnobiological gardens and germplasm and seeds banks are also important processes for *ex situ* conservation (Chen et al., 2016; Chen & Sun, 2018; Que et al., 2016; Singh, Ansari, Singh, Singh, & Pal, 2017). *Ex situ* conservation methods based on plant tissue culture have the ability to function as a large raw material factory by producing large quantities of plant material that can be used for the study and the production of bioactive compounds (Kasagana & Karumuri, 2011; Niazian, 2019).

Plant tissue culture: A strategy for conservation of medicinal plants

Biotechnological processes based on PTC offer an integrated approach for the rapid multiplication and production of secondary metabolites with applications in the pharmaceutical, agricultural, cosmetic, and food industries (Bhatia, Sharma, Dahiya, & Bera, 2015; Vanisree et al., 2004). PTC technologies have a great impact on *ex situ* conservation of plant genetic resources due the international exchange of germplasm (Streczynski et al., 2019).

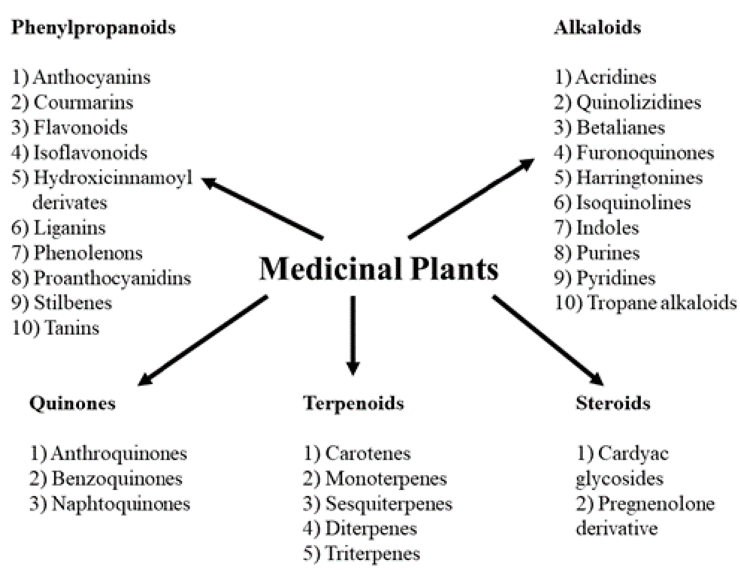

Fig. 2. Classification of plant-derived compounds. (Credit: Own elaboration).

The establishment of *in vitro* cell cultures in the last decade has been an effective technique to produce specific medicinal compounds and finding new bioactive compounds. Fig. 2 shows different groups of bioactive compounds found in medicinal plants. With the use of plant biotechnology, many bioactive compounds are now synthesized by callus production.

Some advantages offered by the PTC are the following: (1) a higher production of plants in smaller facilities, (2) efficient intercontinental transfer of germplasm and vitroplants, (3) production of plants free from pathogenic microorganisms, (4) the production of the plants that is independent on the climate conditions, and (5) the production of the bioactive compounds that can be regulated (Cardoso, Oliveira, & Cardoso, 2019).

The cultivation of root sections of medicinal plants is a novel technique in which the genetic and biosynthetic stability of the plant is maintained, which provides accelerated growth and easy maintenance. From this methodology, many bioactive compounds have been synthesized (Kumar, Kumar, Nehra, Maan, & Kumar, 2017; Yang, Chen, et al., 2020; Yang, Yang, et al., 2020).

PTC has contributed to the production of many bioactive compounds (i.e., phenylpropanoids, alkaloids, quinones, terpenoids, steroids, essential oils, and amino acids) with pharmacological properties (Dias, Sousa, Alves, & Ferreira, 2016; Ochoa-Villarreal et al., 2016). In addition, research in several fields of experimental biology indicates that PTC will be one of the most recurrent techniques to produce bioactive compounds (Isah et al., 2018). The advantage of this methodology relies on providing a continuous and reliable source of natural products.

The Research Laboratory of Environmental Sciences from the Universidad Autonoma de San Luis Potosi (UASLP) in San Luis Potosi, Mexico, has implemented a line of research focused on the study of bioactive compounds in species of the Orchidaceae family, especially with taxa that are distributed in the Huasteca Potosina region. This laboratory has also developed propagation protocols for more than 15 orchids (including vanilla) (Fig. 3). Research is carried out with the aim of conserving these orchid species and proposing sustainable and alternative methods for the study and production of bioactive compounds.

Fig. 3. (A) Cultivation room of the Environmental Sciences Research Laboratory, UASLP Mexico and (B) Vitroplant of *Vanilla planifolia* (Orchidaceae). (Photocredits: Castillo-Pérez, L.J.).

Table 2 shows a list of 20 representative studies about the obtention of bioactive compounds with medicinal or industrial potential using PTC during the last decade. This technique has been widely used since the beginning of the 20th century and has shown a significant demand. *In vitro* culture is an efficient strategy to produce plant species and their bioactive compounds (Table 2). *In vitro* culture also allows the alteration of biochemical pathways to ease the overproduction of specific bioactive compounds.

Table 2. Bioactive compounds isolated using plant tissue cultures in the last decade.

Plant name	Bioactive compound	Culture type	Reference
Aloe arborescens Mill	Iridoids, phenolics, flavonoids and condensed tannins	Shoot	Amoo, Aremu, and Van-Staden (2012)

Arnebia hispidissima (Lehm). DC.	Alkannin	Callus and cell suspension	Shekhawat and Shekhawat, 2011
Baliospermum montanum (Wïlld.) Muell. Arg.	Steroids, triterpenoids, glycosides, saponins, alkaloids, flavanoids, phenolic compounds, and tannins	Callus	Johnson, Wesely, Hussain, and Selvan (2010)
Dendrobium fimbriatum Hook.	β-Sitosterol	Shoots	Paul, Joshi, Gurjar, Shailajan, and Kumaria (2017)
Dendrobium moniliforme (L.) Sw.	Polysaccharides, polyphenolics, and flavonoids	Suspension	Cui, Murthy, Moh, Cui, and Paek (2015)
Hypericum perforatum L.	Phenols and flavonoids	Roots	Cui, Chakrabarty, Lee, and Paek (2010)
Merwilla plumbea (Lindl.) Speta	Total phenolic, flavonoid, gallotannin and condensed tannin	Shoot	Baskaran, Ncube, and Van Staden (2012)
Moringa oleifera Lam	Kaempferol and quercetin	Suspension	Muhammad, Pauzi, Arulselvan, Abas, and Fakurazi (2013)
Opuntia robusta Wendl.	Phenolic acids and flavonoids	Callus	Astello-García, Robles-Martínez,Barba-de la Rosa, and del Socorro Santos-Díaz (2013)
Panax quinquefolius L.	Phenolic compounds	Suspension	Uchendu, Paliyath, Brown, and Saxena (2011)
Platanthera edgeworthii (Hook.f. ex Collett) R.K. Gupta	Phenols	Callus	Giri et al., 2012

Passiflora spp.	Flavonoid C-glycosides	Callus and suspension	Ozarowski and Thiem, 2013
Prunella vulgaris L.	Alkaloid, saponins, phenolics and tannins	Suspension	Rasool, Ganai, Akbar, Kamili, and Masood (2010)
Randia echinocarpa Moc. and Sessé ex DC.	Melanins	Callus	Valenzuela-Atondo et al., 2020
Ruta graveolens L.	Phenolic acids and furanocoumarins	Suspension	Szopa, Ekiert, Szewczyk, and Fugas (2012)
Saussurea involucrata (Kar. et Kir.)	Flavonoids (syringin and rutin)	Callus	Kuo et al., 2015
Schisandra chinensis (Turcz.) Baill.	Dibenzocyclooctadiene lignans	Suspension	Szopa, Ekiert, and Ekiert (2017)
Sedum roseum (L.) Scop.	Salidroside, polysaccharides, phenolics, and flavonoids	Callus	Li et al., 2016
Swertia chirayita H. Karst.	Lignans, alkaloids, flavonoids, terpenoids, iridoids, secoiridoids, and other compounds such as chiratin, ophelicacid, palmitic acid, oleic acid, and stearic acid.	Suspension	Kumar and Van Staden, 2016
Zaleya decandra (L.) Burm.f.	Betalains	Callus	Radfar, Sudarshana, and Niranjan (2012)

Conclusions

The diversity of medicinal plants around the world is huge. However, some regions on the planet have not reported research on this topic. Asia, Africa, and America are the continents with most of the research on medicinal plants. Europe and Oceania have very few studies on this topic. There are some laws and recommendations for the conservation and sustainable use of medicinal plants. However, only a small portion of medicinal plants has accomplished sufficient protection through conventional conservation in nature reserves or botanical gardens. PTC has been proved to be an excellent biotechnological technique for the propagation, conservation, and production of medicinal plants.

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CHAPTER III

Medicinal orchids of Mexico: a review

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Abstract

Some species of the Orchidaceae family are used in traditional Mexican medicine. However, until now there is no official report of all the species used. This review provides for a first time a comprehensive analysis of medical traditional uses, pharmacological reports and phytochemicals isolated from Mexican orchids. Bibliographic investigation was carried out by analysing recognized books and peer-reviewed papers, consulting worldwide accepted scientific databases. The information search was carried out in English and Spanish (due to Mexico belongs to a Spanish-speaking country) and was based on the following groups of keywords: Mexican medicinal orchids, Mexican orchid phytochemistry, and Mexican therapeutic orchids. The current taxonomy of the species was validated using the websites "The Plant List" and "Tropicos". Sixty-four Mexican orchids with medicinal potential have been recorded, of which only 15 have been experimentally analysed. The remaining 49 plant species are in use in empirical treatment of several diseases. These orchids are dispersed in 14 states of the Mexican Republic, mainly in the south of the country. The most common pharmacological activities reported are anti-inflammatory, vasorelaxant, antinociceptive, antioxidant, spasmolytic, antihypertensive and hallucinogenic. Phytochemicals of Mexican medicinal orchids have been evaluated *in vivo* or *in vitro* only in 13 species of orchids. Clearly, this review indicates that it is time to increase the number of experimental studies and to begin to conduct clinical trials with those medicinal Mexican orchids. Also, the mechanisms of action by which plant extracts and their active compounds exert medicinal effects remain to be studied.

Keywords: medicinal orchids, pharmacological orchids, ethnobotanical orchids

Introduction

Orchids are the most diverse group of flowering plants on the planet. Currently, there are more than 30,000 species spread in a wide range of biogeographic regions. The distribution of the Orchidaceae family in the world is considered cosmopolitan due they can be found in most of the planet's ecosystems, with the exception of the polar extremes and the most arid deserts (Hágsater et al., 2005).

Mexico has more than 1,400 orchid species, and the most common use that is given to these plants is ornamental, due to the beautiful flowers that develop in different shapes and colors. However, several orchids in the country have an apparently medicinal importance, and others such as *Vanilla planifolia*, are used in the food industry (Castillo-Pérez et al., 2019). Despite the great diversity of orchids that exist in the country, wild populations are decreasing due to anthropogenic activities, and currently 188 orchids are threatened and listed in the Mexican national red list of threatened plants called NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

Despite that some Mexican orchids are used for medicinal purposes, very few have scientific research for the validation of their bioactive compounds or their pharmacological activity (Castillo-Pérez et al., 2019). The validation of these elements is essential for the development of drugs that can contribute to the prevention or treatment of several diseases. Therefore, it is crucial to critically analyse the knowledge that is currently available on the ethnopharmacology of Mexican orchids and determine the gaps between traditional knowledge and evidence-based research.

Until the moment, there is no similar review published on the aspects we discuss in this article about medicinal orchids from Mexico. Therefore, the main objective of this review is to provide an updated and complete data on the distribution, ethnomedical uses, phytochemical and pharmacology activity of the Mexican orchid species with medicinal potential. For this, a bibliographic search of scientific reports in academic databases was carried out to analyse the diversity of medicinal orchids in Mexico. The information search was carried out in English and Spanish (due to Mexico belongs to a Spanish-speaking country) and was based on the following groups of keywords: Mexican medicinal orchids, Mexican orchid phytochemistry, and Mexican therapeutic orchids. We search the most relevant data in "PubMed", "ScienceDirect", "Scopus", "Web of Science" and "Google Scholar", in addition physical and digital books were consulted. The current taxonomy of the species was validated using the websites "The Plant List" and "Tropicos".

Distribution of medicinal orchids in Mexico

The distribution of the orchid species in Mexico is very unequal. The states located in the south of the country such as Veracruz, Oaxaca, Chiapas and Guerrero have the largest diversity of orchids in the country, while most of the states located in the north, have desert climates that difficult their germination and development (Soto-Arenas et al., 2007). This marked distribution of the Orchidaceae family in the southern states of Mexico, could be related with the fact that most of the states that use orchids as medicinal plants are found in this region (Fig. 1).

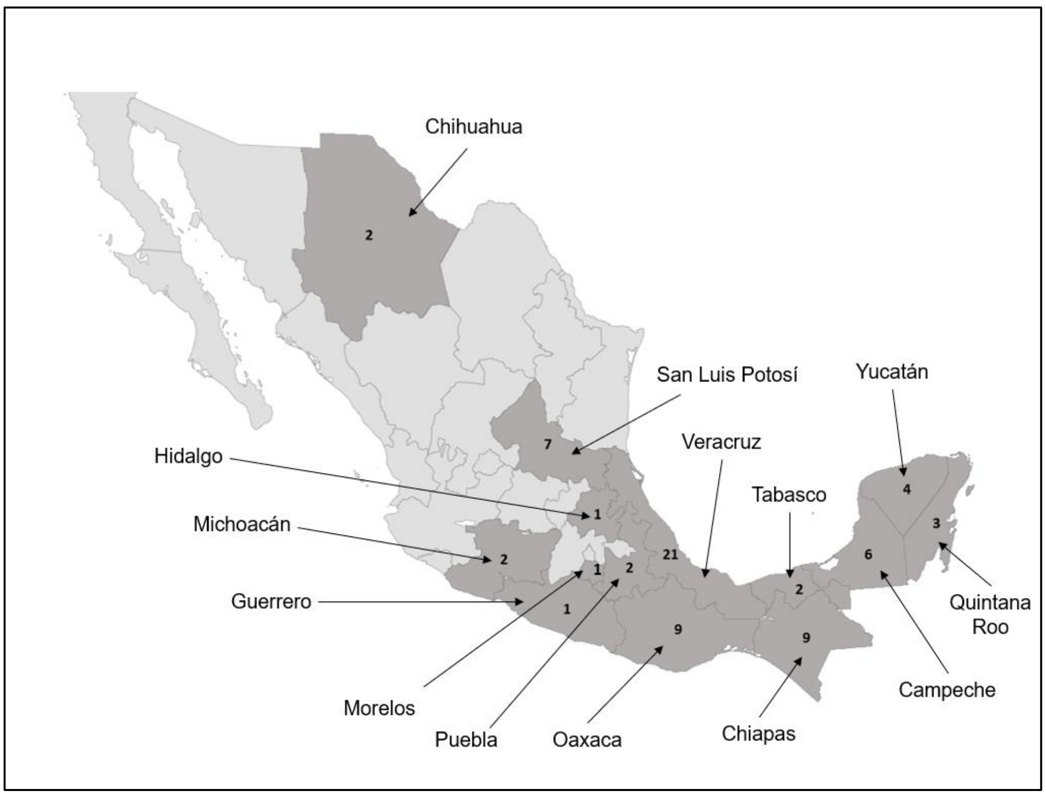

Fig. 1. Number of orchids with medicinal potential used in every state in the Mexican Republic.

In the Table 1, we present 64 orchid species with medicinal potential used in Mexico. These orchids were reported in 14 states of the Mexican Republic (Fig. 1). However, for some species a specific state is not reported, and only the ethnic group that uses the plant is recorded. However, this data is very inaccurate since the ethnic groups are usually spread in two or more states, for example, Mayans, who exist in several states in southern Mexico. In six species, neither the state nor the ethnic group that uses this plant were recorded. This means that this orchid has only been reported as medicinal without giving further details of its use, for example, the orchids of the genera *Trichocentrum*, *T. brachyphyllum* and *T. cavendishianum* (Table 1).

Veracruz was the state that present the highest number of medicinal orchids (21 species). It was followed by the states of Chiapas (9 spp.), Oaxaca (9 spp.), San Luis Potosí (7 spp.) and Campeche (6 spp.) (Fig. 1). Most of these territories have ecosystems such as cloud forest or rainforest, which are ideal for the growth and development of orchids (Hágsater et al., 2005; Soto-Arenas et al., 2007). Is important to mention that these states are located in the southern of Mexico, with the exception of San Luis Potosí, that is located in the central zone of the country, but has a tropical region covered by jungles and rain forest called Huasteca Potosina. The characteristics in this region make it a suitable site for the growth of orchids and their use in ethnomedicine (Fortanelli-Martínez et al., 2022).

Interestingly, the only state located in the northern of Mexico that have a report of medicinal orchids was Chihuahua. In this state two species were reported, Tepehuan ethnic group uses a species of the genus *Malaxis* for gastrointestinal diseases and the ethnic group of the Tarahumara used *Trichocentrum cebolleta* as a hallucinogenic plant during their religious rituals (Bye-Jr, 1979; Stermitz et al., 1983; Schultes et al., 2000). Other ethnic groups that usually use orchids as medicinal plants, are the Mayans for which are reported eight species and the Nahuatl that reported seven species which are used to treat several ailments, especially those related to gastrointestinal diseases (Table 1).

Table 1. Medicinal species of the Orchidaceae family in Mexico.

Species name	Common name	Plant part used	Preparation way	Ethnopharma-cological uses	State, region, or ethnicity	References
Arpophyllum spicatum Lex.	Tzauhxilotl	Pseudobulbs and stem	Decoction	Gastrointestinal disorders (dysentery)	Nahuatl	Urbina (1903); Hernández (1942); Lawler (1984); Teoh (2019)
Bletia campanulata Lex.	Tzacuxochitl	Corms	Infusion	Gastrointestinal disorders (dysentery)	Nahuatl	Cox-Tamay (2013); Teoh (2019)
Bletia coccinea Lex.	Tonalxochitl	Corms	Infusion	Gastrointestinal disorders (dysentery)	Nahuatl	Urbina (1903); Hernández (1942); Lawler (1984)
Bletia purpurea (Lam.) A.DC.	NM	Corms	Infusion; Crushed	Gastrointestinal disorders (dysentery)	Nahuatl; Tzetzal (Chiapas)	Correll (1950); García-Peña and Peña (1981)
Bletia reflexa Lindl.	Tzautli	Corms	Infusion	Gastrointestinal disorders (dysentery)	Nahuatl	Urbina (1903); Hernández (1942); Lawler (1984)
Calanthe calanthoides (A. Rich. & Galeotti) Hamer & Garay	NM	Corms and flowers	Crushed or powdered	Cicatrize wounds or burns; Nosebleed (epistaxis)	NM	Uphof (1968); Lawler (1984); Teoh (2019)
Camaridium densum	NM	Pseudobulbs	Infusion	Gastrointestinal	Catemaco,	Estrada et al.

(Lindl.) M.A.Blanco		and leaves		disorders (dysentery). Treatment of painful complaints. Relaxant agent	Veracruz	(1999); Estrada et al. (2004); Hágsater et al. (2005); Déciga-Campos et al. (2007); Valencia-Islas et al. (2002); De et al. (2014)
Catasetum integerrimum Hook.	Chinela; Cola de pato; Chi'i tku'uk; Trompa de puerco; Xøj cuxp tso'nabie; Nabie	Pseudobulbs	Crushed; Infusion	Diabetes, diarrhoea, renal disorders, cicatrize wounds or burns, inflamed blows, against "Nacidos" (small inflamed tumor or grain on the skin), Viper bite and dermatological problems	Zapoteca (Oaxaca); Mayans (Quintana Roo and Tabasco); Nahuatl (San Luis Potosí)	Lawler (1984); Aguilar et al. (1994); Argueta et al. (1994); Hágsater et al. (2005); Zolla and Argueta, (2009); Alayón-Gamboa (2011); Alonso-Castro et al. (2011); Cox-Tamay (2013); Cruz-García et al. (2014)
Catasetum maculatum	Ch'it cuc	Flowers	Cooked and	Sores and	Mayans	Teoh (2019)

Kunth			grounded	tumours	(Yucatan)	
Chysis laevis Lindl.	NM	Pseudobulbs and roots	Crushed and boiled in alcohol or water	Decrease fever	NM	Teoh (2019)
Cyrtopodium macrobulbon (Lex.) G.A.Romero & Carnevali	Caña or cañaveral	Pseudobulbs	Crushed, roasted and infusion	Cicatrize wounds or burns, inflamed blows, back affectation and pain urinary ailments	Acapulco, Guerrero; Tlaxiaco, Oaxaca; Tamasopo, San Luis Potosí; Chiapas	Berlin et al. (1974); García-Peña and Peña (1981); Morales-Sánchez et al. (2014)
Deiregyne eriophora (B.L.Rob. & Greenm.) Garay	Cecetzi; Margaretilla	NM	NM	Asthma	Tlaquilpa, Veracruz	Sánchez et al. (2003)
Dichaea muricata (Sw.) Lindl.	NM	NM	Wash prepared	Conjunctivitis	NA	Kong et al. (2003)
Dichaea neglecta Schltr.	Chhitë xkudzu belhë; Espinazo de culebra	Branches	NM	Scare or bite caused by snake and swing scare	Santiago Camotlán and Totontepec Villa de Morelos, Oaxaca	Pérez-Nicolás et al. (2017)
Dichromanthus cinnabarinus (Lex.) Garay	Cutzis (for all species of Dichromanthus genera)	Leaves	Crushed and mixed with monnina (*Monina* sp.) leaf	Cold	San Juan Chamula, Chiapas	Aguilar et al. (1994)
Epidendrum anisatum Lex.	NM	Stems	Infusion	Gastrointestinal	Purepechan	Hágsater et al.

				disorders (as dysentery)		(2005)
Epidendrum rigidum Jacq.	NM	NM	NM	NM	Quintana Roo	Alayón-Gamboa (2011)
Epidendrum cnemidophorum Lindl.	Ech'wamal	Leaves	Infusion	Digestive disorders that produce flatulence	Oxchuc, Chiapas	Cabrera (2006)
Epidendrum radicans Pav. ex Lindl.	Semperavil ak'; Kilon vet; Tz'emani'; Krus tz'emani; Muk'tikil	Flowers and leaves	Mixed with Brugmansia leaves; mixed with *Polygonum punctatum*; Infusion; Crushed	Dandruff and grains, cough, burns and whooping	San Juan Chamula, San Andrés Larraínzar and San Pablo Chalchihuitá, Chiapas	Aguilar et al. (1994)
Epidendrum umbelliferum J.F.Gmel.	Siempre viva	Leaves	Infusion	Cholesterol lowering, ear pain, grains and stimulate sleep	Atzalan, Veracruz	Cano-Asseleih et al. (2005)
Epidendrum verrucosum Sw.	Jutz'ep	NM	Crushed	Against "nacidos"	Oxchuc, Chiapas	Aguilar et al. (1994)
Habenaria floribunda Lindl.	Clavo cochinillo	Leaves	Infusion	Vaginal haemorrhage	Soteapan, Veracruz	Cano-Asseleih et al. (2005)
Isochilus latibracteatus A. Rich. & Galeotti	NM	NM	NM	Abdominal colic and gastrointestinal disorders	Soteapan, Veracruz	Cano-Asseleih et al. (2005); Teoh (2019)
Isochilus major Cham. & Schltdl.	NM	Leaves	Crushed	Inflamed blows	Ixtaczoqui, Veracruz	Cano-Asseleih et al. (2005);

						Teoh (2019)
Isochilus sp.	NM	Stems	Infusion	Gastrointestinal disorders	Ancient Mexican	Hágsater et al. (2005)
Laelia anceps Lindl.	Flor de San Miguel; Flor de Todos Santos; Vara de San Diego	Pseudobulbs	NM	Against pain and inflammation	Coatepec, Veracruz; San Luis Potosí	Jimarez-Montiel (2009); Vergara-Galicia et al. (2010a); Vergara-Galicia et al. (2013)
Laelia autumnalis (Lex.) Lindl.	Camote de San Diego; Flor de las Animas; Flor de Encino; Flor de la Calavera; Flor de Todos Santos; Flor de Catarina; Lirio de San Francisco	Pseudobulbs, leaves and flowers	Infusion; Crushed and diluted in alcohol	Circulatory disorders, cough, hypertension, respiratory disorders, heal the waist in postpartum and gastrointestinal disorders (as dysentery)	Zirahuen, Michoacán; Puente de Ixtla, Tepoztlan and Tetela del Volcán, Morelos	Martínez (1969); De la Cruz and Badiano, (1991); Aguilar et al. (1994); Zenteno et al. (1995) Hágsater et al. (2005); Aguirre-Crespo et al. (2008); Vergara-Galicia et al. (2010b); Pant (2013); Esquivel-Gutiérrez et al. (2012); Teoh

						(2019)
Laelia furfuracea Lindl.	Gihtsl; Ita ndeka morada; Lirio morado; Lirio de San Francisco; Monja morada	Flowers	Infusion	Cough	San Pedro y San Pablo Teposcolula , Oaxaca	López-Pérez (2016)
Laelia speciosa (Kunth) Schltr.	Chichiltictep etzaxochitl (ancient Nahuatl, red and sticky flower of the hill) Deanta; Flor de Corpus; Flor de mayo; Itzamahua	Pseudobulbs and flowers	Infusion	Cough and inflamed blows	Valle del Mezquital, Hidalgo	Aguilar et al. (1994); Vergara-Galicia et al. (2013)
Malaxis sp.	Jengibre	Corms	Infusion	Gastrointestinal disorders (as dysentery)	Tepehuanes (Chihuahua)	Hágsater et al. (2005)
Maxillariella tenuifolia (Lind l.) M.A.Blanco & Carnevali	Kowa nokcha	Roots	Crushed	Gastrointestinal disorders (as dysentery)	Hueyapan de Ocampo and Soteapan, Veracruz	Leonti (2002); Leonti et al. (2003)
Mormodes maculata var.	Flor de	Pseudobulbs	Crushed	Inflammation	Zongolica,	Cano-Asseleih

unicolor (Hook.) L.O.Williams	mayo			caused by sprains dislocations	Veracruz	et al. (2005)
Myrmecophila christinae Carnevali & Gómez-Juárez	Flor de confesionario; cuerno; Homikim; Xonikni; X-yonixin	Pseudobulbs	Infusion	Pregnancy pain and wounds	Mayans in Calakmul, Campeche	Alayón-Gamboa (2011); Teoh (2019)
Myrmecophila tibicinis (Bateman ex Lindl.) Rolfe	Hom-ikim	Pseudobulbs	Infusion or juice	Pregnancy pain to facilitate childbirth	Mayans (Yucatan)	Hartmann (1972); García-Peña and Peña (1981); Aguilar et al. (1994); Teoh (2019)
Nidema boothii (Lindl.) Schltr.	NM	Whole plant	NM	Is not used as a traditional or alternative remedy	Catemaco, Veracruz	Hernández-Romero et al. (2004)
Oestlundia luteorosea (A.Rich. & Galeotti) W.E.Higgins	Topixcamohtli	Pseudobulbs	Crushed in alcohol	Inflamed blows and headache	Zongolica, Veracruz	Cano-Asseleih et al. (2005)
Oncidium graminifolium (Lindl.) Lindl.	Näjx pïj or tsäj pïj	Pseudobulbs	Infusion	Renal disorders	Santa Maria Tlahuitoltepec, Oaxaca	Martínez (2015)
Oncidium sphacelatum Lindl.	Flor de mayo; Chorizo con huevo; Anis nikte´	Leaves	Warmed	Air pain	Misantla, Veracruz	Aguilar et al. (1994)

Ornithocephalus inflexus Lindl.	Puuts´muku y	Sap	Spread	The sap is used against insect bites	Mayans in Calakmul, Campeche	Alayón-Gamboa (2011)
Pleurothallis cardiothallis Rchb.f.	Oo quia' dsea ñu	Whole plant	Infusion Chewed	Gastrointestinal disorders, female sterility and to conceive male children	Zapotec (Oaxaca); Populaca (Veracruz)	Hágsater et al. (2005); Zolla and Argueta, (2009)
Ponera striata Lindl.	NM	NM	NM	NM	Mayans in Calakmul, Campeche	Alayón-Gamboa (2011)
Prosthechea citrina (Lex.) Withner	Cozticcoatz ontecoxochi tl	Pseudobulbs	Pseudobulbs cut in half are used as poultice	Cicatrize wounds	Jesús del Monte and Ichaqueo, Michoacán	García-Peña and Peña (1981); Pant (2013); Teoh (2019)
Prosthechea karwinskii (Mart.) Christenson	Ita ndeca amarilla; Lirio Amarillo; Monja amarilla	Pseudobulbs, leaves and flowers	Infusion Poultice	Diabetes, bleeding, cicatrize wounds or burns, cough, inflamed blows and prevent risk of abortion	Tlaxiaco and Villa Sola de Vega, Oaxaca	Urbina (1903); Hernández (1942); Mijangos-Ricardez (2010); Cruz-García et al. (2014); López-Pérez (2016); Rojas-Olivos et al. (2017); Barragán-Zarate et al. (2020)
Prosthechea michuacana (	Aguanoso;	Pseudobulbs	Crushed	Diabetes,	Ejutla de	Tovar-Gijón et

Lex.) W.E.Higgins	Camote de agua; Näjx pïj o tsä jpïj		Chewed Infusion	cicatrize wounds or burns, circulatory disorders, hangover, inflamed blows and renal disorders	Crespo, Santa Catarina Ixtepeji, Santa Maria Tlahuitoltepec and Tlaxiaco, Oaxaca	al. (2006); Cervantes-Reyes (2008); Pérez-Gutiérrez and Vargas-Solis (2009); Neira-González (2009); Pérez-Gutiérrez (2010); Pérez-Gutiérrez et al., (2010a,b); Pérez-Gutiérrez et al. (2011);
Prosthechea panthera (Rchb.f.) W.E.Higgins	Jazmin	Roots	Boiled two bundles of roots	Scabies	Amatenango del Valle, Chiapas	Aguilar et al. (1994)
Prosthechea pastoris (Lex.) Espejo & López-Ferr.	Tzacutli	Pseudobulbs	Infusion	Gastrointestinal disorders (as dysentery)	Nahuatl	García-Peña and Peña (1981); Teoh (2019)
Prosthechea sp.	Tzauhtli	Pseudobulbs	Infusion Chewed	Adhesive for poultices in bone fractures, bleeding, Gastrointestinal disorders (as diarrhea and dysentery)	Purepechan	Urbina (1903); Hernández (1942); Navarro (1992)

Prosthechea vitellina (Lindl.) W.E.Higgins	Tzauxochitl	Pseudobulbs	NM	Gastrointestinal disorders (as dysentery)	Nahuatl	Hernández (1942)
Rhyncholaelia digbyana (Lindl.) Schltr.	Ch´it ku´uk, Piita, Xk´ubeenbaj nunup´le	Leaves	Crushed and placed in the wound	Cicatrize wounds or burns	Mayans in Calakmul, Campeche	Alayón-Gamboa (2011); Cox-Tamay (2013); Teoh (2019)
Rhynchostele bictoniensis (Bateman) Soto Arenas & Salazar	Uch'al vo'; Camote de agua	Pseudobulbs and roots	Squeezed and mixed with salt: Boiled	Baby cramps and headache	San Juan Chamula, Chiapas	Aguilar et al. (1994)
Scaphyglottis fasciculata Hook.	Parasita menuda	NM	Infusion	Anti-inflammatory, antinociceptive, and relaxing activities	Catemaco, Veracruz	Garrido (1997); Cano-Asseleih et al. (2005)
Scaphyglottis livida (Lindl.) Schltr.	Parasita	NM	Topically	To eliminate parasites, relieve stomachache, relax muscles, colic treatment, insect repellent and prevent miscarriage	Los Tuxtlas (Veracruz)	Estrada et al. (1999b); Estrada-Soto et al. (2006); Déciga-Campos et al. (2007)
Sobralia macrantha Lindl.	Atempanxochichocane;Cabolín; Lirio de San	Leaves	Crushed; Liquated in water and add lemon	Cough, fever and inflamed blows	Xocoyolo and Cuetzalan, Puebla;	Hartmann (1972); Gutiérrez-Báez (1994)

	Antonio		and honey		Chiconquiaco, Veracruz	
Specklinia grobyi (Bateman ex Lindl.) F.Barros	NM	NM	NA	NM	Mayans in Calakmul, Campeche	Alayón-Gamboa (2011)
Stanhopea hernandezii (Kunth) Schltr.	Coatzontecomaxochitl: Cabeza de víbora	Flowers	Mixed with red corn and other herbs to make tortillas	Sunstroke and weakness	Ancient Mexican	Urbina (1903); Hernández (1942); Enciso-Díaz (2013); Pant (2013); Teoh (2019)
Stanhopea oculata (Lodd.) Lindl.	Tehuanxochitl; Flor de la bestia	Pseudobulbs	Crushed and boiled	Reduce pain in labor women	Zongolica, Veracruz	Cano-Asseleih et al. (2005)
Stanhopea tigrina Bateman ex Lindl.	Cabeza de vibora; Toritos	Pseudobulbs and roots	Powdered	To treat sunstroke, weakness and renal disorders	Huasteca veracruzana (Veracruz); Huasteca Potosina (San Luis Potosí)	Teoh (2019); Castillo-Pérez et al. (2021)
Trichocentrum ascendens (Lindl.) M.W.Chase & N.H.Williams	Cola de rata; Putsche, putsubehe, puts'ubche, puts'sche, puts'subche	Whole plant	Crushed	Inflammation caused by splinter. “Limpia” (a ritual to prevent, diagnose or cure a disease set)	Soteapan, Veracruz; Yucatán; Quintana Roo	Argueta et al. (1994); Cano-Asseleih et al. (2005); Rodríguez-Castro (2009); Zolla and Argueta, (2009)

Trichocentrum brachyphyllum (Lindl.) R.Jiménez	Orejas de burro	NM	Dried and chewed	NM	NM	Willaman and Li (1970)
Trichocentrum cavendishianum (Bateman) M.W.Chase & N.H.Williams	Orejas de burro	NM	NM	To treat the artery hypertension	NM	Alemán-Pantitlán (2008, 2011)
Trichocentrum cebolleta (Jacq.) M.W.Chase & N.H.Williams	Cola de iguana; Cola de rata	Leaves	Crushed; boiled	Hallucinogen or to treat abdominal colic	Tarahumara (Chihuahua)	Willaman and Li (1970); Bye-Jr (1979); Stermitz et al. (1983); Schultes and Hofmann (2000); Teoh (2019)
Trichocentrum luridum (Lindl.) M.W.Chase & N.H.Williams	Orejas de burro	Whole plant	NM	Treatment of heart conditions and asthma	Campeche, Chiapas, Oaxaca, San Luís Potosí, Tabasco, Veracruz, Yucatán	Alonso-González et al. (1997); Vera-Ku et al. (2017); Teoh (2019)
Vanilla mexicana Mill.	Vainilla sin perfume	NM	NM	Hypochondria, hysteria and induces menstruation	NM	Teoh (2019)
Vanilla planifolia Jacks. ex Andrews	Tlilxochitl; Xanat; Vainilla	Fruits and flowers	Flowers squeezed in water1	Gastrointestinal disorders, against nervous	Misantla, Veracruz; Huasteca	De Sahagún (1829); Hernández

			Flowers powered and placed into a Magnolia flower.1 Flavoring chocolate or drink water2. Mixed with mecaxochitl provoke the "regla" (menstruation). Aromatherapy Infusion Decoction	problems, lowering fever, accelerate the birth child and attract dead fetuses, provoke menstruation, facilitates digestion, skin tumours, dermatological problems, for the treatment of hysteria, impotence, rheumatism and to increase the energy of muscular systems	Potosina (San Luis Potosí); Puebla	(1942); Martínez (1969); De la Cruz and Badiano, (1991); Aguilar et al. (1994); Rain (2004); Mourtzinos et al. (2009); Zolla and Argueta, (2009)

On the other hand, the medicinal properties of the orchid *Trichocentrum luridum* have been registered in seven states of the Mexican Republic. This is the orchid with the greatest use in Mexico (Table 1). Regarding to the diversity of medicinal orchids, 33 genera were recorded. The genus *Prosthechea*, with seven species presents the largest amount of useful medicinal orchids in Mexico (Fig. 2; Table 1). It is followed by the genera *Epidendrum* (6 spp.), *Trichocentrum* (5 spp.), *Bletia* (4 spp.) and *Laelia* (4 spp.). In 18.2% of the genera, only two species of orchids are used medicinally, and in 60.6% of the genera this use is restricted to one species (Fig. 2).

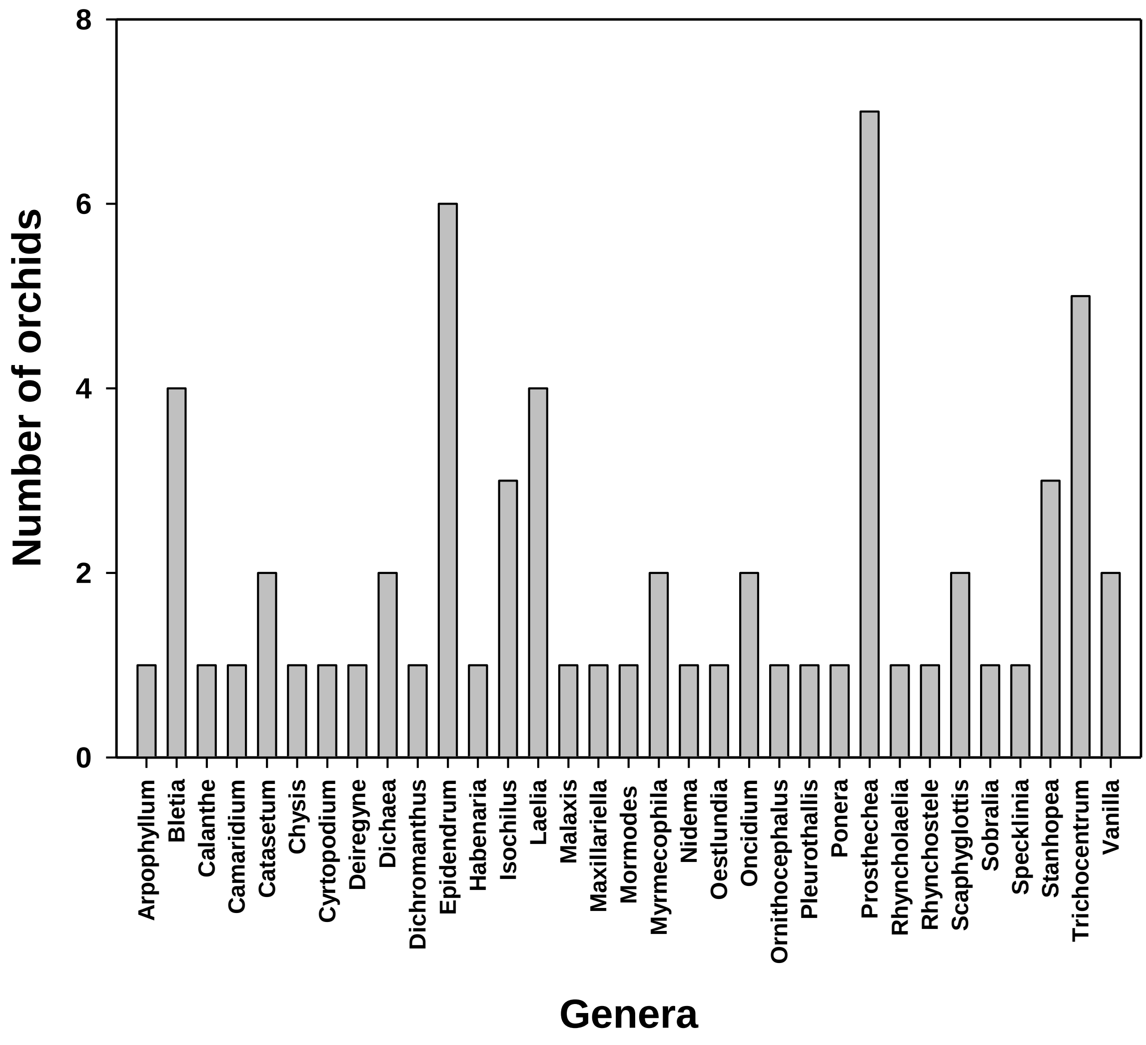

Fig. 2. Genera of Mexican orchids with medicinal potential.

Ethnomedical uses of Mexican orchids

The wide plant and cultural diversity that Mexico possess has resulted in a vast amount of ethnobotanical knowledge. The people of indigenous areas far from large cities in most Mexican states, preserve a large number of empirical medicinal recipes that have been passed down from generation to generation until the present day (Monroy and Ayala, 2003; Medellín-Morales et al., 2017). These recipes have influenced in the development of current methodologies that seek to verify, with the application of the scientific method, the validity of the therapeutic properties assigned to these plants. It is estimated that 90% of Mexicans use medicinal plants for the empirical treatment of different diseases. From the 30,000 species of vascular plants that the country possesses, between 3,500 and 4,000 are used for therapeutic purposes. However, the chemical, pharmacological and biomedical validation of its bioactive compounds has been carried out only in 5% of these species (Juárez-Rosete et al., 2013).

Whit respect to the Orchidaceae family, currently, there is no scientific report that provides information about the number of species that are considered medicinal in the country. In this research, we recorded 64 orchid species with medicinal use and distribution in Mexico. Of these medicinal orchids, 76.6% are used empirically and their biological activity has not been evaluated. Nevertheless, in 15 species (23.4%) the biological activity obtained by the scientific method is reported.

In the Mexican traditional medicine, 18 orchids (28.1%) (*Arpophyllum spicatum*, *Bletia reflexa*, *B. coccinea*, *B. campanulata*, *B. purpurea*, *Camaridium densum*, *Epidendrum anisatum*, *E. cnemidophorum*, *Isochilus lactibracteus*, *Isochilus* sp., *Laelia autumnalis*, *Malaxis* sp., *Maxillariella tenuifolia*, *Pleurothallis cardiothallis*, *P. pastoris*, *Prosthechea* sp. (probably *P. concolor*), *P. vitellina* and *Vanilla planifolia*) are used to treat diseases related to the digestive system, such as dysentery and diarrhea. These gastrointestinal disorders represent the major condition or disease for which orchids are used in Mexico (Table 1).

Interestingly, three Mexican orchids, *C. integerrimum*, *Prosthechea karwinskii* and *Prosthechea michuacana*, have been reported with the potential to treat diabetes mellitus (Table 1); a disease that until 2012 affected to more than 6.4 million of Mexican people, and ranked to Mexico on the list of the 10 countries with the highest number of people with diabetes in the world (Rojas-Martínez et al., 2018).

On the other hand, the orchids *Dichaea muricata*, *Ponera striata*, *Specklinia grobyi*, *Trichocentrum brachyphyllum* and *Vanilla mexicana* were registered as medicinal, but the data for these species are scarce, some of them only mentioned they use, and other just the place or ethnicity that uses them. In the case of *Trichocentrum cavendishianum*, it is only mentioned as a medicinal species, but the part used, the ethnopharmacological use, or the place or ethnic group that uses it are not recorded (Table 1).

The most common way to take advantage of the medicinal properties of these species, is to realize an infusion which is consumed orally in the form of tea (Table 1). Some species of orchids are mixed with other plant species or some additive ingredients. For example, the leaves of *Dichromanthus cinnabarinus* are used to treat the common cold by mixing them with leaves of *Monina* sp. to form a kind of poultice (Aguilar et al., 1994). The leaves and flowers of *Epidendrum radicans* are mixed with the leaves of *Brugmansia* sp. or with *Polygonum punctatum* and are used to eliminate dandruff, pimples, coughs and burns (Aguilar et al., 1994). *Sobralia macrantha* is liquefied with water, lemon and honey to decrease cough, fever and inflammatory processes (Hartmann, 1972; Gutiérrez-Báez, 1994). Finally, the orchid *Stanhopea hernandezii* is mixed with red corn and other herbs to make tortillas, which are consumed for decrease weakness and a condition called "sunburn" (Urbina, 1903; Hernández, 1942) (Table 1).

Pharmacology activity

The pharmacological activity of 15 Mexican orchids species has been reported in *in vivo* and *in vitro* models, this number is equivalent to 23.4% of the 64 species that are used in traditional Mexican medicine (Table 2). The most common pharmacological activities reported are anti-inflammatory (5 spp.), vasorelaxant (4 spp.), antinociceptive (3 spp.), antioxidant (3 spp.), spasmolytic (3 spp.), antihypertensive (2 spp.) and hallucinogenic (2 spp.) (Fig. 3).

Table 2. Pharmacology activities of plant extracts from Orchidaceae in Mexico.

Plant species	Plant part analyzed	Activity studies	*In vivo* (1) or *in vitro* (2)	Tested extracts	References
Camaridium densum	Whole plant	Antinociceptive	(1)	Dichloromethane and methanol extract	Déciga-Campos et al., 2007
		Spasmolytic	(2)	Trichloromethane and methanol extract	Estrada et al., 1999a; Estrada et al., 2004
Cyrtopodium macrobulbon	Pseudobulbs	Antinociceptive	(1)	Aqueous and dichloromethane extract	Morales-Sánchez et al., 2014; Yáñez-Barrientos et al., 2022
Laelia anceps	Roots	Vasorelaxant and antihypertensive	(1) and (2)	Methanolic extract	Vergara-Galicia et al., 2010a
	Pseudobulbs, roots and leaves	Vasorelaxant	(2)	Hexane and Dichloromethane extracts	Vergara-Galicia et al., 2013
Laelia autumnalis	Pseudobulbs	Haemagglutinating	(2)	NM	Zenteno et al., 1995
	Aerial parts	Vasorelaxant	(2)	Methanolic extract	Aguirre-Crespo et al., 2005
	Roots, pseudobulbs, leaves and plantlets generated *in vitro*	Vasorelaxant	(2)	Methanolic extract	Vergara-Galicia et al., 2010b
	NM	Vasorelaxant Antihypertensive	(2) (1)	Methanolic extract	Vergara-Galicia et al., 2008

Laelia speciosa	Roots and pseudobulbs	Vasorelaxant	(2)	Hexane, dichloromethane and methanolic extracts	Vergara-Galicia et al., 2013
Nidema boothii	Whole plant	Spasmolytic	(2)	Trichloromethane and methanol extract	Estrada et al., 2002
				Dichloromethane and methanol extract	Hernández-Romero et al., 2004
Prosthechea karwinskii	Pseudobulbs, leaves and flowers	Anticoagulant	(2)	Hydroalcoholic extracts	Barragán-Zarate et al., 2016
		Antioxidant	(1)	Hydroalcoholic extracts	Rojas-Olivos et al., 2017
	Leaves	Anti-inflammatory and gastroprotective effects	(1)	Hydroalcoholic extracts	Barragán-Zarate et al., 2020
Prosthechea michuacana	Bulbs	Antidiabetic and antihyperlipidemic	(1)	Hexane, chloroform, and methanol extracts	Pérez-Gutierrez and Hoyo-Vadillo, 2011
	Bulbs	Antiglycation	(1) and (2)	Methanol extract	Pérez-Gutierrez, 2013
	Leaves, bulbs and roots	Anti-inflammatory, hypoglycemic, and wound healing potential	(1)	Hexane, chloroform, and methanol extracts	Cervantes-Reyes, 2008; Pérez-Gutiérrez and Vargas-Solís, 2009
	Bulbs	Antioxidant	(2)	Chloroform extract	Pérez-Gutiérrez et al., 2010b
	Bulbs	Hepatoprotective	(1)	Methanol extract	Pérez-Gutiérrez and Vargas-Solís, 2009; Pérez-

	Bulbs	Nephroprotec-tive	(1)	Methanolic extract	Gutiérrez et al., 2011 Pérez-Gutiérrez et al., 2010a
Scaphyglottis livida	Whole plant	Antinociceptive	(1)	Methanol and dichloromethane extract	Déciga-Campos et al., 2005
	Whole plant	Antinociceptive and anti-inflammatory	(1)	Dichlorome-thane and methanol extract	Déciga-Campos et al., 2007
	Whole plant	Spasmolytic	(2)	Trichlorome-thane and methanol extract	Estrada et al., 1999; Estrada et al., 2002; Mathison et al., 2010
	NM	Vasorelaxant	(2)	Trichlorome-thane and methanol extract	Estrada-Soto et al., 2006
Stanhopea hernandezii	NM	Antifatigue	NM	Hexanic and Dichlorome-thane/methanol extract	Enciso et al., 2015
Trichocentrum cavendishianum	NM	Anti-hypertensive and vasorelaxant	NM	NM	Aleman-Pantitlán, 2008, 2011
Trichocentrum cebolleta	Leaves	Hallucinogenic	NM	Chloroform, methanol, and hexane extracts	Stermitz et al., 1983
Trichocentrum luridum	Leaves	Bronchodilator	(1)	Hydroalco-holic extract	Alonso-González et al., 1997
Vanilla planifolia	NM	Anticarcinogen	(1)	NM	Ho et al., 2009, 2012; Liang et al., 2009; Lirdprapamongkol et al., 2009, 2010

Leaves	Anticarcinogen	(2)	Ethanolic extract	Vijaybabu and Punnagai, 2019
Stems	Anticarcinogen	(2)	Ethanolic extract	Amalnath et al., 2020
NM	Anti-depressant	(1)	NM	Shoeb et al., 2013; Xu et al., 2015
NM	Anti-inflammatory	(1)	NM	Niazi et al., 2014
NM	Antimicrobial	(2)	NM	Choo et al., 2006
NM	Antimutagenic	(2)	NM	King et al., 2007
Fruits	Antioxidant	(2)	Hydroalco-holic extracts	Shyamala et al., 2007
NM	Antioxidant and antiapoptotic	(1)	NM	Dhanalakshmi et al., 2015
NM	Anti-sickling effect	(1)	NM	Hannemann et al., 2014
NM	Aphrodisiac	(1)	NM	Maskeri et al., 2012
NM	Anti-stress and anti-depressant	(1)	NM	Abo-youssef et al., 2016
NM	Neuroprotective	(1)	NM	Anand et al., 2019
NM	Modifying effect in Alzheimer's disease	(2)	NM	Song et al., 2016

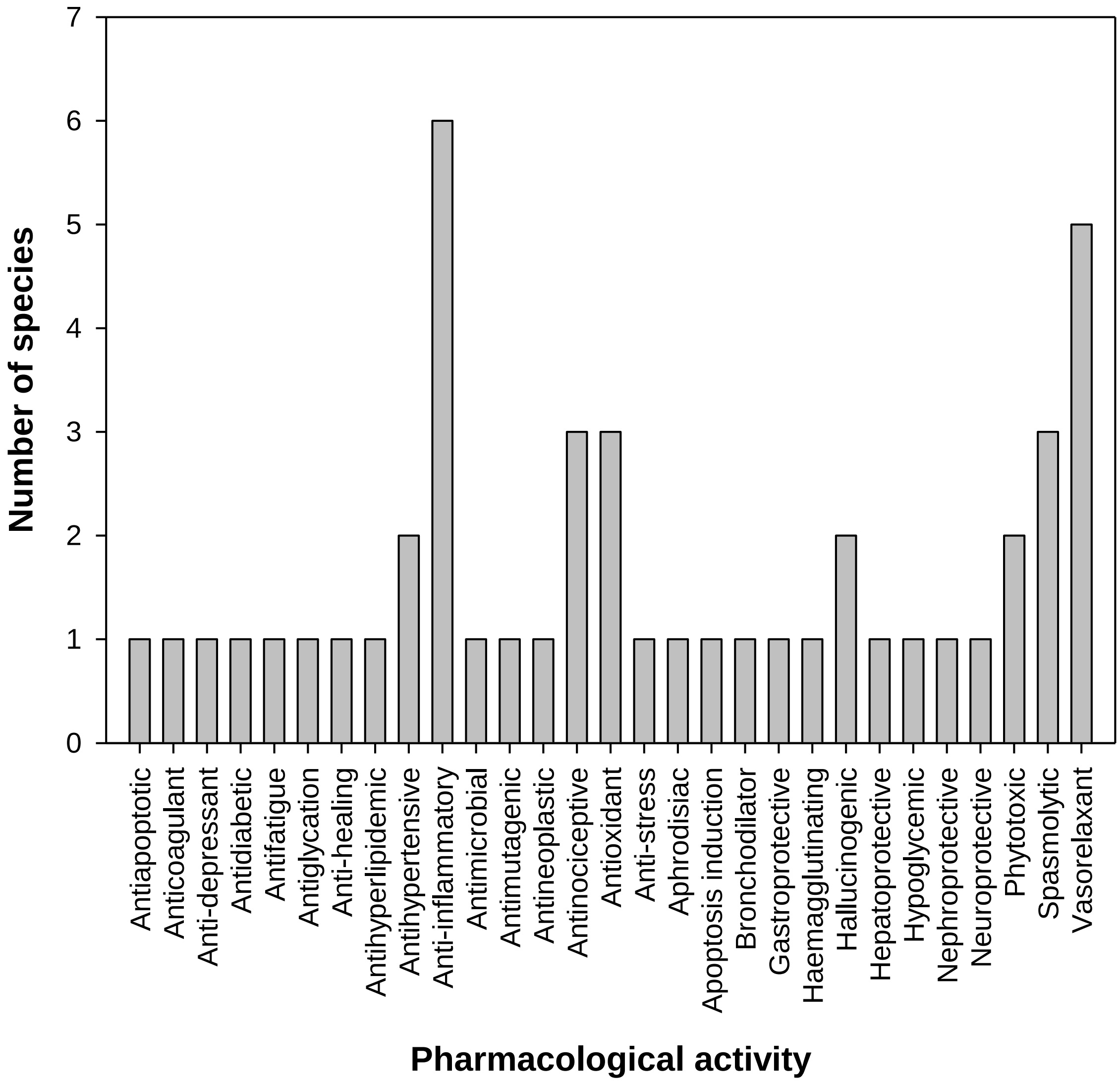

Fig. 3. Diversity of pharmacological activities recorded in Mexican orchids with medicinal potential.

It is important to mention that, for the species *Trichocentrum cavendishianum* only the pharmacological activity is mentioned, but no further details are indicated, which results in a gap in knowledge about this orchid which must be aborded in future research (Table 2). The most studied species in terms of its pharmacological properties is *Vanilla planifolia* (Table 2).

However, most research focuses on the study and effects of the vanillin (4-hydroxy-3-methoxybenzaldehyde), molecule which is not exclusive to the species *V. planifolia* and is found in other species of the genus, such as *V. tahitensis* and

V. pompona (Dhanalakshmi et al., 2015) and can even be synthesized in the laboratory. Therefore, it is important to start developing research that studies other plant sections of this species, such as the stem, leaves, roots or flowers. For the other species of Mexican orchids, pharmacological research is described and analysed in detail in the following sections.

Anti-inflammatory activity

The anti-inflammatory activity was verified in six species of Mexican orchids (Table 2; Fig. 3). In *Laelia furfuracea*, an endemic orchid from the state of Oaxaca, this activity was studied with the model of sub plantar edema with carrageenan in Wistar rats, and an inhibition of 43.36% of inflammation was observed using a dose of 1000 µg/paw of the leaf extract. These data suggest that this species could be used as a potent anti-inflammatory in traditional medicine (López-Pérez, 2016). In the epiphytic orchid *Prosthechea karwinskii*, the hydroalcoholic extract of the leaves showed anti-inflammatory activity due to the presence of compounds such as quinic acid, malic acid, neochlorogenic acid, chlorogenic acid, rutin, embeline, pinelic acid and azelaic acid and also induced the protection of the gastric mucosa. These results suggest that this orchid could be used to combat inflammation related to oxidative stress (Barragán-Zarate et al., 2020).

In the case of the terrestrial orchid *Prosthechea michuacana*, Cervantes-Reyes (2008) determined the anti-inflammatory activity using the model of edema induced with TPA and observed the inhibition of inflammation of 34.88% with the extracts of leaves and roots, while, for pseudobulbs an inhibition percentage of 25.58% was recorded. On the other hand, Pérez-Gutiérrez and Vargas-Solís (2009) found that the extract from the aerial parts and pseudobulb of *P. michuacana* has significant anti-inflammatory activity, since it was proven to block the release of prostaglandins and cyclooxygenase in the later phase of the acute inflammatory process.

Interestingly, the combined methanol-dichloromethane whole plant extract of *Scaphyglottis livida* reduced the inflammatory process induced by carrageenan. These data suggest that this orchid poses a significant dose-dependent anti-inflammatory effect in rats and mice (Déciga-Campos et al., 2007). Finally, Table 2 reports three investigations that adjudge an anti-inflammatory potential to the vanillin molecule, one of the natural and major components of *Vanilla planifolia*. Eun-Ju et al. (2008), realized vascular permeability tests and observed that vanillin showed an inhibition of the inflammatory process of 14.2%, 40.6% and 49.8% at concentrations of 25, 50 and 100 mg/kg, respectively. For their part, Kwon et al. (2013) developed a new inflammation-responsive polymeric vanillin antioxidant prodrug called poly vanillin oxalate (PVO), which achieved potent anti-inflammatory activity by reducing the expression of proinflammatory cytokines in activated macrophages *in vitro* and in vivo. Finally, Niazi et al. (2014) determined that the administration of doses of 50 and 100 mg/kg of vanillin, a significant decrease in the inflammation observed in the volume of the mice paw was achieved, which they related to its antihistamine activity.

Vasorelaxant activity

The vasorelaxant activity has been verified in five orchid species, three belonging to the genus *Laelia*. The first is *Laelia anceps*, two different works have been reported for this orchid; in the first Vergara-Galicia et al., (2010a) made a methanolic extract with the plant roots and when administering it they observed that an induced relaxation process was presented in aortic rings pre-contracted with norepinephrine (NE), 5-hydroxytryptamine creatinine sulphate (5-HT) and potassium chloride (KCl). Interestingly, the vasorelaxant effect of the extract was not reduced by ODQ, 1-alprenolol, TEA, glibenclamide, and 2-AP. This effect was associated with the presence of the compound 2,7-dihydroxy-3,4,9-trimethoxyphenanthrene, which was characterized by the HPLC technique. In the second, Vergara-Galicia et al., (2013) made two extracts, one with hexane and the other with dichloromethane, and although the pseudobulbs and leaves were used, the root extracts were the most active and where most vasorelaxant compounds were found. A concentration-dependent relaxation process was observed in precontracted aortic rings with and without endothelium. These results suggest that the secondary metabolites responsible for the vasorelaxant activity in *L. anceps*, belong to a group of compounds of medium and low polarity.

In the case of *Laelia autumnalis*, three research have been registered. Aguirre-Crespo et al., (2005) made a methanolic extract with the aerial parts of the orchid and observed the induction of a relaxation process dependent on concentration and independent of the endothelium in the aorta of rats. Furthermore, they determined that the use of L-NAME blocks relaxation, indicating that the vasodilatory properties of the extract are mediated by the endothelium due to the release of nitric oxide. Moreover, Vergara-Galicia et al., (2010b) made methanolic extracts using roots, pseudobulbs, leaves and seedlings generated *in vitro*. This is one of the few works in which orchid vitroplants have been used to investigate the vasorelaxant action. However, the roots and pseudobulbs of *L. autumnalis* were the extracts that produced the best vasorelaxant effect in a concentration-dependent and endothelium-independent way on contractions induced by norepinephrine (NE) and potassium chloride (KCl) in rings rat thoracic aorta. Leaf and *in vitro* seedling extracts did not show relevant vasorelaxant activity. Finally, Vergara-Galicia et al., (2008) observed that 0.15 - 50 µg/mL induced relaxation in aortic rings precontracted with KCl and determined that the extract of this orchid caused an inhibition of the sustained contraction of serotonin. Finally, it was determined that the vasorelaxant effect of rat aortic rings is carried out through an endothelium-independent pathway, which implies the blockade of Ca^{2+} channels and a possible enhanced concentration of cGMP.

Laelia speciosa it's a third species of the *Laelia* genus where vasorelaxant activity was investigated, specifically with hexane, dichloromethane and methanol extracts from roots and pseudobulbs. The results showed that all extracts tested induced a concentration-dependent relaxation process in precontracted aortic rings with and without endothelium. However, the dichloromethane and hexane root extracts were less potent than the positive controls used (carbachol and sodium nitroprusside). These results suggest that the secondary metabolites responsible for the vasorelaxant activity belong to a group of compounds with medium and low polarity, and the roots were the main tissues of the plant where the vasorelaxant compounds are stored (Vergara-Galicia et al., 2013).

In the case of the orchid *Scaphyglottis livida*, the stilbenoid compounds gigantol and 3,7-dihydroxy-2,4-dimethoxyphenantrene were found to induce a significant concentration-dependent relaxation of norepinephrine-evoked contractions in endothelial-bearing rat aortic rings intact and naked (Estrada-Soto et al., 2006). These works indicate that orchids have a huge vasorelaxant effect and that they should continue to be investigated from this perspective, due they are a potential source of drugs that could counteract blood pressure, a condition that currently afflicts millions of people around the world.

Antinociceptive activity

Research on antinociceptive activity is important to find biomolecules in plant species that can be used for pain treatment. This biological activity was verified in three Mexican medicinal orchids. Firstly, combined dichloromethane-methanol extract of *Camaridium densum*, orchid formerly known as *Maxillaria densa*, reduced acetic acid-induced abdominal writhing but failed to produce antinociception in the hot-plate test. In addition, the researchers were able to isolate two chemical compounds from this orchid, fimbriol A and erianthridin, which partially reduced the contortions induced by acetic acid (Déciga-Campos et al., 2007).

A second orchid species with antinociceptive potential is *Cyrtopodium macrobulbon*. Two research teams in Mexico have studied this orchid, specifically the pseudobulbs. In the case of Morales-Sánchez et al. (2014) they evaluated an aqueous extract and an organic extract. They used the methods of contortions induced by acetic acid and the hot plate test and observed that only the organic extract of pseudobulbs showed an antinociceptive effect when administered at doses of 100 and 316 mg/kg.

On the other hand, Yáñez-Barrientos et al. (2022) also analysed the pseudobulbs of this orchid. They make an ethanolic extract with pseudobulbs and using three methodologies to determine the antinociceptive effect. They were observed that 17.8 mg/kg of ethanolic extract promotes an antinociceptive effect by the acetic acid test. However, in a second phase, using the formalin test, 29 mg/kg were needed to induce said activity. Interestingly, it was also observed that a dose of just 10.6 mg/kg of *C. macrobulbon* extract combined with naproxen induced an excellent synergistic response in pain inhibition.

Finally, for the orchid *Scaphyglottis livida*, Deciga-Campos et al. (2005) used the contortion test and analysed the antinociceptive effect of a methanolic extract and another made with dichloromethane using the whole plant. The results showed that doses of 150, 300 and 600 mg/kg of the orchid methanolic extract produced a significant reduction in the number of stretches in the acetic acid-induced contortion test. Subsequently, the antinociceptive effect of four compounds isolated from *S. livida* was analyzed using the hot plate test. It was observed that the compounds 5α-lanosta-24,24-dimethyl-9(11),25-dien-3β-ol and gigantol significantly increased hot plate latency compared to vehicle-treated mice (Déciga-Campos et al., 2007).

Antioxidant activity

Only in three Mexican medicinal orchids has antioxidant activity been scientifically registered, two of them belonging to the genus *Prosthechea*. In the case of the orchid *P. karwinskii*, the antioxidant capacity was determined by the DPPH method and the concentration required to inhibit 50% of free radicals (IC50), with these data the antioxidant activity index (AAI) was generated. The highest antioxidant activity index was found in the leaf extract (AAI = 5.7), followed by the flower extract (AAI = 1.276 and finally the pseudobulb extract (AAI = 0.925) (Rojas-Olivos et al., 2017).

With respect to the orchid *P. michuacana*, Pérez-Gutiérrez et al., (2010) used the pseudobulbs, and determined the antioxidant activity through the total content of phenols, the DPPH radical scavenging effect and the scavenging ABTS radicals test. Interestingly, this study reported the extraction and isolation of six compounds: two triterpenoids, 3α-acetoxy, 24-hydroxy-24-methyl-5α-lanosta-9(11),25-diene and 3α-acetoxy, 24- hydroxy -24-methyl-5α-lanosta-9(11)ene, the stilbene α-α´-dihydro,3´,5´,2-trimethoxy-3-hydroxy-4-acetyl-4´-isopentenylstilbene, the phenanthrene 4,6,7-trihydroxy-2-methoxy-8-(methylbut-2-enyl)phenanthrene-1,1´-4´,6´,7´-trihydroxy-2´-methoxy-8´-(methylbut-2´-enyl) phenanthrene, the diterpene 12-hydroxy-3β,7β,18α-triacetoxy-8,11,13-abietatriene and the gigantol. Of these compounds isolated from *P. michuacana*, stilbene, phenanthrene, diterpene and gigantol showed the highest values of antioxidant capacity. In the case of triterpenes, none exhibited this property. These results provide medical advances pointing out chemical compounds that can be used for the treatment of oxidative tissue damage, caused by the generation of reactive oxygen species.

The third orchid with studies on its antioxidant properties is *Vanilla planifolia*. Interestingly, for determinate this properties Shyamala et al. (2007) used vanilla fruits cured in 60% aqueous ethyl alcohol. They identified and purified the compounds vanillic acid, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 4-hydroxybenzaldehyde and vanillin. The antioxidant activity of the extract was measured by the DPPH methods and using the compound β-carotene-linoleate, where they determined 43 and 26% of antioxidant activity, respectively. Authors mention that these results are higher than those reported for the pure vanillin molecule, which is widely used to investigate biological properties of *Vanilla* species.

Spasmolytic activity

Three orchids used to reduce pain caused by contractures, spasms or injuries were recorded. In the orchid *Camaridium densum*, two compounds derived from phenanthrenes obtained from a whole plant extract with dichloromethane-methanol showed significant smooth muscle relaxant properties verified by the methodology that evaluates the nitric oxide/cGMP system (Estrada et al., 1999a). Subsequently, this same biological activity was also reported for five *C. densum* compounds, but of the stilbene type. The compounds 2,5-Dihydroxy-3,4-dimethoxyphenanthrene, fimbriol-A, nodol, gymnopusin and erianthridin, caused a concentration-dependent inhibition of spontaneous contractions of the rat ileum with potencies comparable to papaverine (Estrada et al., 2004).

In the case of the epiphytic orchid *Nidema boothii*, Estrada et al. (2002) made an extract of the whole plant with trichloromethane-methanol and mention that a compound called nidemin showed spasmolytic activity, but no further details are given on the methodology or dose that they used to achieve this effect. However, Hernández-Romero et al. (2004) investigated the same orchid in a dichloromethane extract with whole plant methanol and reported that 6.26 ± 2.5 µg/mL of the extract inhibited guinea-pig ileum contractions. Furthermore, they suggest that for maximum spasmolytic activity, the bibenzyl compounds found in the *N. boothii* extract must have oxygenated substituents on the molecular aromatic rings.

The third orchid for which spasmolytic activity was investigated is *Scaphyglottis livida*. Estrada et al., (1999b) reported five aromatic compounds that induced a concentration-dependent inhibition of contraction in the rat ileum. The compounds are: 3,4′-dihydroxy-5,5′-dimethoxybibenzyl, batatasin III, coelonin, 3,7-dihydroxy-2,4-dimethoxyphenanthrene, and 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene. Subsequently, the discovery of the triterpenoid 9,19-cyclolanosta-24,24-dimethyl-25-en-3β-y-trans-p-hydroxycinnamate expanded the number of compounds with said biological activity in this orchid (Estrada et al., 2002). Finally, Mathison et al. (2010), determined that the molecular mechanism of the relaxing action induced by aromatic compounds of *S. livida* in the rat ileum was the nitric oxide/cGMP system.

Antihypertensive activity

Arterial hypertension is one of the diseases that has had the greatest impact on the world. This condition represents a predisposition for the development of diseases such as metabolic syndrome, diabetes, kidney dysfunction, heart failure and stroke. Therefore, it is urgent to develop research for the creation of drugs that contribute to the treatment of these diseases.

In Mexico, there are two orchids of the genus *Laelia* in which an effective antihypertensive activity has been proven. The first of these is *Laelia autumnalis*, in which a methanolic extract was studied and it was observed that 100 mg/kg significantly decreased systolic and diastolic blood pressure, as well as heart rate in spontaneously hypertensive rats. Furthermore, the authors suggest that the antihypertensive effect is caused by the blockade of Ca^{2+} channels and a possible enhanced concentration of cGMP (Vergara-Galicia et al., 2008).

The second species that has been analysed is *Laelia anceps*, from which a methanolic extract of roots was studied and it was found that oral administration of 100 mg/kg produces a significant decrease in systolic and diastolic blood pressure in hypertensive rats. For this species, it was determined that the reduction of the transient contraction induced by norepinephrine in solution free of Ca^{2+} ions and the inhibition induced by the increase in external calcium suggest that the antihypertensive effect caused by the extract is conducted by blocking the channels of Ca^{2+} (Vergara-Galicia et al., 2010a).

The results that the species of the *Laelia* genus have provided in terms of the discovery of metabolites with hypertensive activity, suggest that this genus of orchids could be an excellent source of these compounds. Interestingly, in Mexico there are 11 species belonging to this genus of which, as shown in this research, only two have been studied.

Other pharmacological activities

Other pharmacological activities have been conferred to Mexican medicinal orchids. For example, Zenteno et al., (1995) reported that in *Laelia autumnalis* the pseudobulbs have haemagglutinating property, a biological response against microorganisms such as viruses. In humans it is used to determine the viral load. On the other hand, Barragán-Zarate et al. (2016) verified the anticoagulant activity in the orchid *Prosthechea karwinskii*. They used hydroalcoholic extracts of pseudobulbs, leaves and flowers, and observed that the three extracts prolonged the activated Partial Thromboplastin Time (aPTT), inhibiting coagulation by a process similar to anticoagulant drugs. Moreover, in this orchid gastroprotective effects derived from a hydroalcoholic extract of the leaves were observed (Barragán-Zarate et al., 2020).

Interestingly, in the *Prosthechea michuacana* orchid, several biological activities related to diabetes mellitus have been found, for example, bulb extracts based on hexane, chloroform and methanol showed antidiabetic and antihyperlipidemic activity (Pérez-Gutiérrez and Hoyo-Vadillo, 2011), as well as antiglycan activity (Pérez-Gutiérrez, 2013). In this sense, capability of the compounds found in this orchid to inhibit *in vitro* glycation, makes them potent anti-glycation agents, which can be of great value in preventing the pathogenesis of diabetes associated with glycation. Other biological activities that have been verified in the bulbs of this Mexican medicinal orchid are hepatoprotective and nephroprotective (Table 2). These data place *P. michuacana* as an important orchid for the manufacture of possible drugs for the treatment of diabetes mellitus, one of the diseases that currently most afflicts Mexico and the world.

In *Stanhopea hernandezii*, antifatigue properties were documented in hexanic extracts of pseudobulbs as well as in extracts with dichloromethane/methanol of pseudobulbs and roots. This property was confirmed by the significant increase in latency time during forced swimming tests using a murine model. For the orchid *Trichocentrum luridum*, the leaves were the ones that showed bronchodilator activity, which is related to its use in the treatment of asthma (Alonso-González et al., 1997). Finally, in the case of *Vanilla planifolia*, it is worth discussing the biological activities of this orchid separately, because what has been studied mainly is the vanilla molecule and very rarely the extract of some section of the plant. Therefore, we could consider the vanillin molecule as an excellent medicinal phytochemical compound, but not the *Vanilla planifolia* plant, due in many cases, the molecule is synthesized in laboratory, and it’s not extracted naturally. However, in the Table 2 we show some proven medicinal properties of vanillin. It is also observed that only in three works some section of the plant was worked on.

Phytochemicals

The use of orchids for the empirical treatment of different diseases has caused to several studies that provide scientific evidence to justify the medicinal use of these species. The bioactive compounds of the Orchidaceae family have begun to be studied approximately 25 years ago, and despite the great variety of species that this family possess, they have been little explored (Pérez-Gutiérrez, 2010).

Phytochemical studies have been carried out in several places of the world from extracts of roots, leaves, pseudobulbs, stems and flowers of different species and hybrids orchids (Sut et al., 2017). Due to these techniques, it has been possible to isolate and identify a wide range of bioactive compounds. In this review we group the bioactive compounds of Mexican medicinal orchids into three general groups, terpenes, phenolic compounds and alkaloids, within which other subgroups that are produced during the biosynthesis of these general compounds (Fig. 4). However, it is important to mention that no alkaloid extracted from a Mexican medicinal orchid has been scientifically reported, although in the case of *Trichocentrum cebolleta*, which is reported to have hallucinogenic potential, these compounds could exist (Stermitz et al., 1983). In this research, of the 64 orchids reported with medicinal use in Mexico, only in 13 species (20.31%) the phytochemicals have been investigated and registered (Table 3).

Terpenes

Terpenes are considered the largest group of plant secondary metabolites, with more than 55,000 known chemical structures (Salha et al., 2019). Terpenes are generally insoluble in water and are formed by the union of five-carbon compounds called isoprene. Some terpenes produced by orchids and other plants perform to attract insects, promote pollination, as toxins and repellents to defend plants from attacks by insects, herbivores, or pathogens, or as a response to abiotic stress (Huang et al., 2010). Also, significant antibiotic, antifungal, antiviral, and anticancer activity has been proved in these molecules, and they have even been used in agro-industrial processes. Also, significant antibiotic, antifungal, antiviral, and anticancer activity has been proved in these molecules, and they have even been used in agro-industrial processes (Rajesh and Howard, 2003; Astani et al., 2010; Salha et al., 2019).

The production of terpenes with biological importance has been reported in seven species of Mexican orchids (Table 3). In the aerial parts of *Cyrtopodium macrobulbon*, the presence of three volatile terpenes named 6,10,14-trimethyl-2-pentadecanone, eucalyptol and isobornyl formate was reported. Among these terpenes, only eucalyptol has been associated with antinociceptive pharmacological properties (Morales-Sanchez et al., 2014). In the case of *Epidendrum rigidum*, two triterpenes were isolated, 24,24-dimethyl-9,19-cyclolanostane-25-en-3β-ol and 24-methyl-9,19-cyclolanost-25-en-3β-ol, both are considered as pharmaceutical intermediates and have been used for the treatment of asthma (Waizel and Waizel, 2009).

Another orchid in which phytochemicals have been studied is *Laeila furfuracea*. López-Pérez (2016) reported the presence of sesquiterpenlactones, saponins and cardiotonic glycosides in this epiphytic orchid. Among these molecules, sesquiterpenlactones and saponins have been identified as potent antioxidants. Cardiotonic glycosides produce effects on the heart muscle, specifically on Na^+/K^+ ATPases. In addition, in regulated doses they slow down and strengthen the heartbeat and are prescribed for the treatment of heart diseases (Taiz and Zeiger, 2002).

In the phytochemical analysis carried out on the hydroethanolic extracts of the orchids *Prosthechea karwinskii* and *Stanhopea hernandezii*, the presence of triterpenes was reported. However, these molecules were not characterized. Similarly, in *Scaphyglottis livida* the presence of four triterpenes was found, 5α-lanosta-24,24-dimethyl-9(11),25-dien-3β-ol (LDD), 5α-lanosta-24(S)- methyl-9(11),25-dien-3β-ol, 24,24-dimethyl-9,19-cyclolanosta-9(11),25-dien-3-one (cyclobalanone) and 9,19-cyclolanosta-24,24-dimethyl-25-en-3β-yl trans-p-hydroxycinnamate. Of these molecules, LDD has been reported that has antinociceptive and anti-inflammatory activity and it is suggested that its pharmacological effect may be due to the activation of opioid receptors or an increase in endogenous opiates (Déciga-Campos et al., 2007).

Two compounds have been reported for the congener specie *S. fasciculata*. Identity and composition tests performed with HPLC, and GC chromatographic profiles revealed that this orchid produced volatile compounds, one of which is linalool (3,7-dimethyl-1,6-octadien-3-ol), a terpene (Morales-Sanchez et al., 2014). Interestingly, it has been proven in laboratory animals that linalool has neurotoxic and neuropharmacological activity (Sabogal-Guáqueta et al., 2016). On the other hand, in traditional medicine this terpene is used specifically for induce sleep and anticonvulsant (Batista et al., 2010).

Two triterpenes were isolated from the chloroform extract of *P. michuacana* orchid bulbs, 3α-acetoxy,24-hydroxy-24-methyl-5α-lanosta-9(11),25-diene and 3α-acetoxy,24-hydroxy-24-methyl-5α-lanosta-9(11)ene, and a diterpene was identified as 12-hydroxy-3β,7β,18α-triacetoxy-8,11,13-abietatriene. According to the results obtained in the DPPH radical scavenging tests, this diterpene has significant antioxidant activity (Pérez-Gutiérrez et al., 2010).

Finally, for the epiphytic orchid *Vanilla planifolia*, the presence of terpenoids in hexane and methanol/ethanol extracts from leaves and stems has been reported in the literature (Andrade-Andrade et al., 2018; Díaz-Bautista et al., 2018). However, the chemical characterization of these extracts for the identification of the compounds contained has not been carried out, so this could be an important field of study that adds information on orchid terpenes.

Phenolic compounds

Phenolic compounds are secondary metabolites that have an aromatic ring with a hydroxyl substituent and the majority are of plant origin. Some of these compounds act as a defense against herbivores and pathogens, as a mechanical support, pollinator attractor and in plant allelopathy processes (Marchiosi et al., 2020). On the other hand, in phenolic compounds we also find some molecules that are derived from them, such as stilbenoids and phenanthrenes, the latter being widely found in the Orchidaceae family (Vasas, 2021). Orchid phenanthrenes have shown promising pharmacological activities, mainly antiproliferative effects against cancer cells, antimicrobial, anti-inflammatory, and antioxidant effects. About Mexican medicinal orchids, the isolation and identification of phenolic compounds is reported in all of them (Table 3). The findings in Mexican medicinal orchids related to phenolic compounds are detailed below.

In the case of the orchid *Camaridium densum*, several phenanthrene derivatives have been isolated and identified that represent the main bioactive molecules of this orchid, these compounds are: 9,10-dihydro-2,5-dihydroxy-3,4-dimethoxyphenanthrene, 2,5-dihydroxy-3,4-dimethoxyphenanthrene, nudol, erianthridin, fimbriol-A, gymnopusin. The latter five compounds have been confirmed to have spasmolytic activity on the rat ileum (Estrada et al., 2004). According to Déciga-Campos et al. (2007), fimbriol-A and erianthridin found in *C. densum* showed antinociceptive activity in the nociception model acetic acid-induced. In addition, it has been reported that gigantol and gymnopusin also isolated from *C. densum* are antagonists of the protein calmodulin (CaM), which leads to an explanation of the spasmolytic activity (Mata et al., 2014).

In *C. macrobulbon*, 10 different phenolic compounds were isolated from an organic extract of the pseudobulbs. Among these phenolic compounds are three compounds that are derivatives of p-coumaric acid, n-hexacosyl-trans-p-coumarate, n-octacosyl-trans-p-coumarate and n-triacontyl-trans-p-coumarate. Studies indicate that p-coumaric acid and its derivatives can protect mammalian cells in culture against oxidative stress and genotoxicity (Grajales-Hernández, 2015). Additionally, p-coumaric acids have been shown to act as antioxidants and anticancer agents (Mura-Mordones, 2014). The rest of the phenolic compounds isolated from *C. macrobulbon* were the stilbenoids 4-methoxy-benzylalcohol, 4-hydroxybenzaldehyde, 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol, confusarin, gigantol, batatasin III and ephemeranthol B (Morales-Sánchez, et al., 2014). Of these, gigantol and ephemeratrol B are known to possess antinociceptive, anti-inflammatory, and antioxidant activity.

In the epiphytic orchid *Laelia anceps*, has been reported the presence of a flavonol (5-hydroxy-3,7,4'-trimethoxyflavonol) and two phenanthrenes (9,10-dihydro-2,5-dimethoxyphenanthrene-1,7-diol and 2,7-dihydroxy-3,4,9-trimethoxyphenantrene). Phenanthrene-type compounds are credited with an anti-inflammatory effect. About flavonol, it has been reported in the literature that it has antimutagenic, antifungal, antibacterial and cytotoxic inhibition activity (Jimarez-Montiel, 2009).

In *L. furfuracea* actually the presence of 13 phenolic compounds have been recorded. Some of the most relevant, detected in the hydroethanolic extract of leaves, are syringic acid acetate, which is used in the prevention of cardiovascular diseases, reduces coagulation times, has antioxidant, anti-inflammatory, neuro and hepatoprotective activity (Srinivasulu et al., 2018). Protocatechuic acid, which possesses antithrombotic and antiplatelet activity, inhibits granular secretion of dense granules and α-granules, and attenuates the activation of glycoproteins IIb/IIIa (Kim et al., 2012). On the other hand, rosmarinic acid which has antioxidant activity, platelet inhibition and anticoagulant (Shi et al., 2012). The flavone luteolin-7,3´-di-O-glucoside has antiplatelet and vasorelaxant activity and the compounds Kaempferol-3-O-rutinoside and Kaempferol-7-O-glucoside for which anticoagulant activity has been reported. Another interesting molecule is the stilbenoid 4-hydroxybenzyl alcohol (BHA), which has been shown to have antiangiogenic, anti-inflammatory and antinociceptive activity. Finally, malic acid and caffeic acid, which have antioxidant, anti-inflammatory, and anticancer activity (López-Pérez, 2016).

The orchid *Nidema boothii* is not generally used as a traditional or alternative remedy. However, scientific evidence proves that this orchid has a variety of phenolic compounds, mostly stilbenoids and derivatives of phenanthrenes. According to Hernández-Romero et al. (2004), nine phenolic compounds have been found, among them, six are derivatives of phenanthrenes (1,5,7-trimethoxyphenanthrene-2,6-diol; 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol; 2,4-dimethoxyphenanthrene-3,7-diol; ephemeranthol B; ephemeranthoquinone and lusianthridin) and three stilbenoids (aloifol II, batatasin III and gigantol). Studies have shown that the compounds aloifol II, 1,5,7-trimethoxy-9,10-dihydrophenanthrene2,6-diol, 1,5,7-trimethoxyphenanthrene2,6-diol, ephemeranthoquinone, gigantol, 2,4-dimethoxyphenanthrene-3,7-diol, lusianthridin and batatasin III have spasmolytic activity concentration-dependent in mouse ileum (Estrada et al., 1999b). Also, has been shown that aloifol and gigantol exert spasmolytic activity through the inhibition process mediated by cadmodulin.

In *Prosthechea karwinskii*, was identified the presence of phenolic constituents such as 7-glucoside, caffeic acid, tyrosol, apigenin, vanillin, p-coumaric acid and ferulic acid. These compounds are known for their cardioprotective activity due to their ability to inhibit cholesterol oxidation (Rojas-Olivos et al., 2017). In addition, has been reported that some phenolic compounds can inhibit the growth of adipose tissue due to their antiangiogenic activity (González-Castejón and Rodríguez-Casado, 2011). In addition, Barragán-Zárate et al. (2020), reported the presence of compounds derived from caffeoyl-quinic acid (chlorogenic acid and neochlorogenic acid) and flavonoid glycosides (rutin and kaempferol-3-O-rutinoside), which have been used for their anti-inflammatory and diabetic activity.

The above mentioned is due to the neochlorogenic acid and rutin inhibit the signaling pathways of the mitogen-activated protein kinase (MAP kinases) and the kappa-light chain of the nuclear factor of activated β cells (NF-κβ) (Wang et al., 2012; Kim et al., 2015). In addition, these last two compounds and chlorogenic acid decrease the expression of cyclooxygenase-2 (COX-2). Also, chlorogenic acid and embelin have been reported to blunt the proinflammatory cytokines interleukin 1 beta (IL-1β), TNF-α, and interleukin 6 (IL-6) and pinellic acid to inhibit the production of prostaglandins, leukotrienes, and oxide nitric (NO) in cell cultures (Barragán-Zárate et al., 2020).

For the *P. michuacana* orchid, the presence of 10 phenolic compounds has been evidenced in the scientific literature. Particularly in the study carried out by Pérez-Gutiérrez et al. (2010b), isolated a phenanthrene from the chloroform extract (4,6,7-trihydroxy-2- methoxy-8-(methylbut-2-enyl) phenanthrene-1,1'-4',6',7'-trihydroxy-2'-methoxy-8'-(methylbut-2'-enyl) phenanthrene) and two stilbenzenes (α-α'-dihydro,3',5',2-trimethoxy-3-hydroxy- 4-acetyl- 4'-isopentenylstilbene and gigantol). These compounds were reported to have antioxidant and lipid antiperoxidation activity, which are important for the treatment of oxidative tissue damage caused by the generation of reactive oxygen species. An additional study by Pérez-Gutiérrez et al. (2011) managed to isolate four flavones (scutellarein 6-methyl ether, dihydroquercetin, apigenin-7-O-glucoside, apigenin-7-neohesperidoside) and one flavonoid (apigenin-6-O-β-d-glucopyranosyl-3-O- α-l-rhamnopyranoside). Of these, the compounds scutellarein 6-methyl ether, apigenin-7-neohesperidoside and apigenin-6-O-β-d-glucopyranosyl-3-O-α-l-rhamnopyranoside have been reported to have moderate hepatoprotective activity, according to the results obtained in the biological study carried out with carbon tetrachloride (CCl_4). In addition, it is known that dihydroquercetin has antioxidant and hepatoprotective activity and inhibits the process of spontaneous lipid peroxidation in the liver (Pérez-Gutierrez et al., 2011).

In *S. livida*, seven phenolic compounds have been identified, including four stilbenes: gigantol, 3,4'-dihydroxy-3',4,5-trimethoxybibenzyl (DTB), batatasin III and 3,4-dihydroxy-5 ,5-dimethoxybibenzyl and three are phenanthrene derivatives: coelonin, 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (Déciga-Campos et al., 2007). These compounds induce a concentration-dependent inhibition of spontaneous contractions in tests carried out with rat ileum. In particular, the compound 3,4-dihydroxy-5,5-dimethoxybibenzyl has its spasmolytic action possibly on the nitric oxide/cGMP system, since that compound increased the content of cyclic GMP (Estrada et al., 1999b). On the other hand, for coelonin, studies indicate that it has high anti-inflammatory activity, since significantly inhibits the expression of interleukin-1β, interleukin-6 and tumor necrosis factor-α (Jiang et al., 2019).

From the epiphytic orchid *Vanilla planifolia* have been isolated and identified a wide variety of phenolic compounds. However, the therapeutic or pharmacological activity of most of these compounds has not been demonstrated in the scientific literature. The pharmacological effects of some phenolic compounds are listed below. According to Shyamala et al. (2007) report in their study that the compounds 4-hydroxy-3-methoxybenzyl alcohol and 4-hydroxybenzyl alcohol exhibited antioxidant activity of 65% and 45% by the beta-carotene-linoleate method and 90% and 50% by the DPPH method. In contrast, 4-hydroxy-3-methoxybenzaldehyde (vanillin) had much lower antioxidant activity compared to the compounds previously mentioned (Shyamala et al., 2007). On the other hand, vanillin has been reported to have antitumor potential and is known to have antimicrobial properties, including inhibition of bacterial biofilms. It has been reported that at a concentration of 500 $\mu g \cdot mL^{-1}$ this compound significantly reduces the development of biofilms by *Candida albicans* (Raut et al., 2013). Finally, Kim et al. (2011) reported that 4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol) shown to have neuroprotective, antioxidant, and antiapoptotic effects in a study of 1-methyl-4-phenylpyridinium neurotoxin-induced cytotoxicity in MN9D dopaminergic cells.

Other reported compounds

In the epiphytic orchid *L. autumnalis*, the lectin, N-acetyl-D-galactosamine, which is considered a glycoprotein, was purified. Interestingly, this lectin possesses the biological activity of agglutinating desalted human erythrocytes of human blood groups A, O and B (Zenteno et al., 1995).

Interestingly, it was observed that there are no reports about the presence of alkaloids identified in the analysed orchids. There is a report carried out by Andrade-Andrade et al. (2018) who indicate the presence of alkaloids in *V. planifolia*. However, the isolation and identification of the compounds was not made.

Conclusions

Traditional medicine serves the health needs of about 80% of the world's population, and Mexican orchids contain a large number of phytochemicals can be used as a promising source of medicine. Although there are a few scientific studies on the pharmacological activity of the Mexican orchids, we can say there is potential of these plants as an anti-inflammatory, vasorelaxant, antinociceptive, antioxidant, spasmolytic, antihypertensive and other pharmacological activities. Although, orchidaceous preparations should typically be subjected to the precise scientific clarification and standardization to confirm traditional knowledge.

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CHAPTER IV

Ethnomedicinal uses, phytochemistry, medicinal potential and biotechnology strategies for the conservation of orchids from the *Catasetum* genus

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Abstract

Catasetum is a genus of the Orchidaceae family with approximately 170 species, some of which have been reported with medicinal properties in different countries of America. The species of this genus are mostly epiphytic plants and are one of the few genera in the Orchidaceae family that develops male and female flowers. *Catasetum* is distributed from Mexico, throughout tropical America and reach the North of Argentina. Medicinally, some species are used to treat gastrointestinal diseases, abscesses, asthma, kidney and urinary infections, diabetes mellitus, and others. Propagation methods for large-scale multiplication of some species of this genus are available, but a very few species have actually been propagated. Despite medicinal reports, the species of this genus have been little studied. Therefore, this chapter highlights several research areas where there are gaps and potential research areas to harness the potential of *Catasetum* genus.

Keywords

Medicinal orchids, *Catasetum*, Medicinal plant, Gastrointestinal disease, Mexican orchids

Introduction

The obtention of new drugs from plant secondary metabolites plays an important interest in the pharmaceutical industry [1]. Many medicinal plants from the Solanaceae, Asteraceae, and Fabaceae families have been studied, whereas other plant families like Apiaceae, Ranunculaceae, and Orchidaceae lack scientific information validating their medicinal properties [2].

The Orchidaceae family is the most diverse in the plant kingdom and represents an important part of the biodiversity in the Neotropics. This family has a wide distribution in this area. Some of the genera, including *Laelia*, *Stanhopea*, *Cyrtropodium*, and *Epidendrum* belonging to this family have reports of medicinal properties [3]. These orchid genera have shown biological activities as antihypertensive, antipyretic, anti-inflammatory, antinociceptive, antidysentery, among others [4, 5, 6, 7].

A particular and low-studied genus within the Orchidaceae family is the *Catasetum* genus, which possesses approximately 170 species and is widely distributed in the neotropical region of America [8]. However, some *Catasetum* hybrids have been successfully cultivated and adapted to other regions, being grown in Europe, Asia and America [9]. They have diverse growth habits, mostly species are epiphytic (Fig. 1a), but some species present terrestrial, lithophyte or saprophyte development [8]. Moreover, are sexually dimorphic and exhibiting male and female flowers (Fig. 1b and 1c) [10]. Another important data is these orchids present mycorrhizal and myrmecophile ecological interactions for their growth and defense, and the male bees of the *Euglossini* tribe is the main pollinating agent (Fig. 1d) [10, 11].

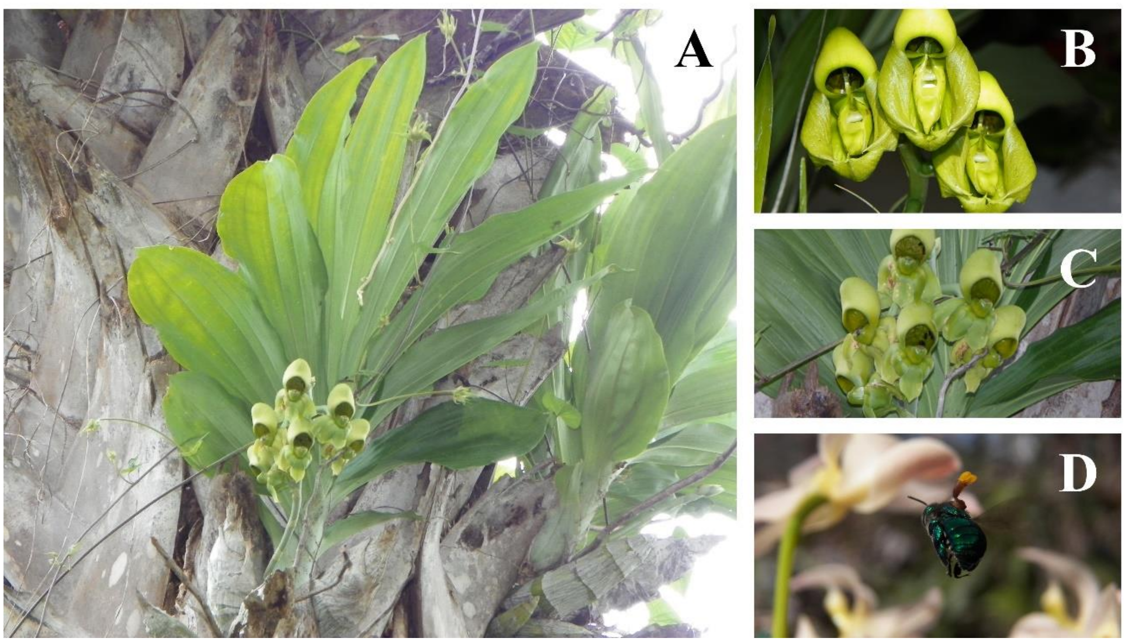

Fig. 1. *C. integerrimum* (Orchidaceae) a common example of the genus *Catasetum*. (a) Whole plant in situ with epiphytic growth; (b) Male flowers; (c) Female flowers; (d) Interaction with the bee of the Euglossini tribe, the main pollinator of the genus *Catasetum*

Currently, species of the *Catasetum* genus are used mainly as ornamental plants in several parts of the world. Some species of this genus have medicinal properties attributed to different population groups around the world. The objective of this chapter is to summarize all the research findings available on various aspects, such as botanical description and distribution, ethnopharmacology, phytochemistry and conservation of the *Catasetum* genus.

The information search was based on the following groups of keywords: *Catasetum* orchids, Medicinal *Catasetum*, Phytochemical *Catasetum*, Biotechnology *Catasetum* and Ecology of *Catasetum*. We search the most relevant data in "PubMed", "ScienceDirect", "Scopus", "Web of Science" and "Google Scholar", in addition physical and digital books were consulted. The current taxonomy of the species was validated using the website of The World Flora Online (http://www.worldfloraonline.org/). The article search was carried out from 15 March 2022 to 15 August 2022. Based on all the compiled information, research gap has also been discussed. This chapter provides the basis for further studies on the conservation and development of identifying better therapeutic agents and health products from the *Catasetum* orchids.

Botanical description of the species

This section describes the general characteristics shared by *Catasetum* orchids. We suggest consulting the taxonomic keys provided in the botanical bibliography for the specific description of any species from this genus. Most *Catasetum* orchids are epiphytic, perennial, medium-sized with a height among 30 to 70 cm, composed of pseudobulbs ovoid to fusiform and fibrous roots at the base. The plants have leaves that can be oblong-lanceolate to elliptic and deciduous. The flowers are terminal racemes, and some species develop non-resupinate, unisexual, dimorphic and fragrant flowers. The column in *Catasetum* species is short and truncate and have pollinial vestigial. After pollination, these species develop ellipsoid and glaucous capsules with seeds minute and powdery. Mostly of pseudobulbs are fleshy, smooth, shining, greenish, covered with membranous sheath, and slightly mucilaginous [12]. The flowering of these species varies throughout the year and some species have the ability to developing flowers more than once a year. Anatomical and histochemical studies revealed the presence of endophytic mycorrhizal fungus in the root and protocorm [13]. The anatomical similarity between rhizomes and pseudobulbs indicates that species can be propagated from its rhizomes as well as pseudobulbs.

Habitat, distribution and ecology

Catasetum orchids have different development forms, some species are epiphytic, others are terrestrial or lithophyte, and some species have even been described with saprophyte growth. The species of the *Catasetum* genus usually develop mycorrhizal and myrmecophile interactions for their growth, plant development, and defense. Another interesting ecological aspect is that the wide majority of *Catasetum* species share their main pollinating agent, the male bees of the *Euglossini* tribe, also known as orchid bees [8, 10, 11].

The *Catasetum* genus is present only in the neotropical region of the American continent and has approximately 170 species. Brazil encompasses the largest number of *Catasetum* orchids [14, 15, 16]. In the case of Mexico, two of the most important orchids of the *Catasetum* genus, *C. integerrimum* and *C. laminatum*, are distributed in the states of Tamaulipas, San Luis Potosí, Hidalgo, Veracruz, Puebla, Querétaro, Oaxaca, Chiapas, Tabasco, Campeche, Yucatan and Quintana Roo [12].

Table 1 shows the few available studies published about the habitat and ecology of the *Catasetum* orchids. Most of these species are distributed in tropical forests, which is not surprising since many of these orchids are epiphytes. Interestingly, *C. discolor* grows in more arid ecosystems in countries like Bolivia, Brazil, and Venezuela [8, 17].

Table 1. Some ecological aspects of *Catasetum* orchids.

Species	Habitat	Main growth type	Pollinator species	Flowering season	References
Catasetum arietinum F.E.L. Miranda and K.G. Lacerda	Neotropical cloud forests	Epiphyte	*Euglossa nanomelanotricha* *Euglossa securigera*	February – July	[18]

Catasetum integerrimum Hook	Tropical deciduous and semi-deciduous forests, warm oak and palm forests, forests, neotropical cloud forests, and montane forests	Epiphyte	*Eulaema cingulata* *Eulaema polichroma* *Eulaema cingulate* *Eulaema meriana* *Exaerate frontalis*	April – November	[12, 19]
Catasetum uncatum Rolfe	Short palm stems in dry forest	Epiphyte	*Euglossa nanomelanotricha* *Euglossa carolina*	March – May	[20]
Catasetum pusillum C. Schweinf	Semi-humid forests	Lithophyte and terrestrial	*Euglossa sp.*	February – May	[21]
Catasetum saccatum Lindl.	Primary forests	Epiphyte	*Eufriesea violacens* *Euglossa augaspis* *Euglossa chalybeata* *Euglossa cordata* *Euglossa ignita* *Euglossa imperialis* *Eulaema cingulata*	NM*	[8, 21]
Catasetum peruvianum Dodson and D.E. Benn	Primary and secondary forests	Epiphyte	NM	NM	[21]
Catasetum cernnum (Lindl.) Rchb.f.	Tropical and riparian forests	Epiphyte	*Eufriesea violacea*	NM	[22]
Catasetum ochraceum Lindl.	Tropical dry forests	Terrestrial	*Euglossa modestior* *Euglossa gaiani* *Euglossa deceptrix* *Euglossia liopoda*	NM	[23, 24]

Catasetum macrocarpum Rich. ex Kunth	Tropical forests bordering rivers	Epiphyte and terrestrial	*Eulaema bombiformis* *Eulaema nigrita*	Starts in January	[17, 25, 26]
Catasetum discolor (Lindl.) Lindl	Savannah, in sand	Terrestrial	*Eulaema bombiformis* *Eulaema bomboides* *Eulaema cingulata* *Eulaema nigrita* *Eulaema meriana* *Euglossa ignita*	May – November	[17, 8]
Catasetum longifolium Lindl.	NM	Epiphyte	*Eulaema bombiformis* *Eulaema meriana*	NM	[17]
Catasetum maculatum Kunth	NM	Epiphyte	*Eulaema polychroma* *Eulaema meriana* *Eulaema cingulata*	Twice a year, March and July	[27]
Catasetum viridiflavum Hook	Tropical cloud forest	Epiphyte	*Eulaema cingulata* *Eulaema nigrita* *Eulaema marcii* *Exaerete frontalis*	April – December	[28, 8]
Catasetum galeritum Rchb. f.	Tropical cloud forest	Epiphyte	*Eufriesea superba*	NM	[29]
Catasetum gardneri Schltr.	NM	Epiphyte	*Eufriesea auriceps* *Eufriesea violacens* *Eufriesea combinata* *Eulaema cingulata* *Euglossa sp.*	NM	[29, 30]
Catasetum barbatum (Lindl.) Lindl	NM	Epiphyte	*Euglossa augaspis* *Euglossa cognata* *Euglossa cordata* *Euglossa mixta* *Eulaema cingulata*	NM	[29]

Catasetum macroglossum Rchb. f.	NM	Epiphyte	*Eulaema cingulata* *Eulaema tropica* *Eulaema bomboides* *Eulaema speciosa* *Eulaema polychroma*	NM	[29, 31]

*NM: Not mentioned

Catasetum orchids have their pollinating species. Nevertheless, there are few records about the pollinating organisms of these orchids, including insects of the Hymenoptera order, Apidae family, and Euglossini tribe, specifically two genera, *Eufriesea*, *Euglossa* and *Eulaema* (Table 1). However, this work found records of 16 *Catasetum* species, which represents a gap in the ecological knowledge of these species.

Another ecological aspect with limited information is the time of flowering in these orchids. For example, of the 16 species presented in this work, this data is only known in eight of the sixteen species. Interestingly, some species such as *C. integerrimum* and *C. viridiflavum* flower for most of the year (Table 1) [8, 19].

Ethnomedicinal uses

The medicinal uses conferred on the *Catasetum* orchids have been documented in several reports (Table 2). Firstly, it was recorded in 1958 that the ashes of pseudobulbs from C. maculatum were used for the treatment of inflammations, abscesses, sores, and warts [32]. Afterward, Arenas and Moreno-Azorero (1977) [33] documented using *C. gardneri* pseudobulbs as a sterilant. The application of this orchid was recommended in conjunction with the rhizomes of another plant denominate *Typha latifolia*. To obtain the sterilizing effect, both parts of the plants should be boiled in water and consumed at the morning. The consumption of the pseudobulbs of *C. gardneri* as an infusion is registered as a contraceptive method by residents of indigenous regions from Paraguay and Brazil.

Table 2. Ethnopharmacological uses of the genus *Catasetum*.

Species	Plant section used	Prepara-tion way	Ethnopharmacolo-gical uses	Country where it is used	References
Catasetum maculatum Kunth	Pseudobulb	Plaster	Treatment of inflammations, abscesses, sores and warts	Mexico	[32, 34]
Catasetum gardneri Schltr.	Pseudobulb	Infusion with rhizome of *Typha latifolia*	Sterilization	Paraguay	[33]
		Infusion	Contraceptive	Paraguay and Brazil	[35]
Catasetum barbatum (Lindl.) Lindl.	Aerial parts	NM	Asthma and lumbago	Paraguay	[36]
Catasetum integerri-mum Hook	Leaf	NM	Treatment of pimples	Mexico	[37]
	All plant	NM	Dermatological diseases		[38]
	Pseudobulb	Liquefied with water	Supplement against kidney and urinary infections		[39]
	NM	Infusion with leaf of *Laelia autumnalis*	Cough treatment		[34]
		NM	Snake bite		[40]
			Cure of tumors and in the treatment of abscesses and wounds		[41]
			Burns and wounds		[42]

			Antidiarrheal		[35]
	Pseudobulb, leaf, root, capsule	Infusion or liquefied with water	Treatment of colitis, diabetes, high blood pressure, kidney conditions and cancer		[43, 44]
Catasetum expansum Rchb. f.	Stem	Plaster or poultice	Treatment of broken bones and bone fractures	Ecuador	[45]
Catasetum macroglo-ssum Rchb. f.	Pseudobulb	Plaster	Treatment of broken bones and bone fractures Anti-inflammatory and antirheumatic	Ecuador	[15, 46]

*NM: Not mentioned

C. barbatum is another species of the *Catasetum* genus documented as medicinal. This species is used in traditional medicine from Paraguay for the treatment of asthma and lumbago. However, there is no information on the preparation of this plant for the treatment of these diseases [36].

One of the *Catasetum* species with the most records of medicinal properties is *C. integerrimum* (Table 2). In the late 1980s, this species was reported to be useful for treating viper bites [40]. Subsequently, it was reported that the leaves of this species were used by Mayan communities in the state of Yucatan, Mexico for the treatment of "large grains", which possible may allude to tumors [37].

Another investigation carried out by Alonso-Castro et al., (2011) [38] mentioned the entire use of the orchid for the treatment of dermatological conditions, and Cox-Tamay (2013) [41], documented its application in the treatment of tumors, abscesses and wounds by communities of Yucatan, Mexico. Another use that has been conferred to *C. integerrimum* is in the treatment of burns and wounds [42], and recently in the state of Veracruz, Mexico, its application is used for treating diarrhea [35]. However, the information on which plant part should be used for the medicinal purpose, the way of administration, and the way of preparation are frequently omitted in the scientific literature.

In the Huasteca Potosina region, pseudobulbs of *C. integerrimum* are traded in local markets with other medicinal plants and fruits (Fig. 2). The inhabitants of this region comment that the pseudobulbs should be prepared as an infusion with water and orally consumed for treating kidney, gastrointestinal, urinary tract infections, and against diabetes mellitus [39].

Other *Catasetum* species documented with medicinal properties are *C. expansum* and *C. macroglossum*, used in communities in Provincias del Rio, Ecuador. According to Zambrano-Intriago et al., (2015) [45], *C. expansum* is used in the treatment of broken bones and bone fractures, through the preparation of a plaster or poultice, made from the scape floral, which is then applied to the affected area. On the other hand, Ramos-Corrales et al., (2011) [46] mention that *C. macroglossum* is used in the treatment of inflammation, pain and broken bones, also being applied by making a poultice from the pseudobulbs. Likewise, Ramos et al., (2012) [15], reported the topical use of the pseudobulbs of *C. macroglossum* as anti-inflammatory and anti-rheumatic in middle lands and forests of Ecuador.

Fig. 2. Pseudobulbs on sale of *C. integerrimum* in a local market in the municipality of Matlapa, Huasteca Potosina, Mexico.

Finally, some reports documented the consumption of the stem floral wand of some *Catasetum* species for the reduction of headaches in Shuar communities (Ecuador). However, the *Catasetum* species was not reported [47]. Likewise, Kunow (1958) and Teoh (2019) [32, 35] reported the use of *Catasetum maculatum* used in traditional Mayan medicine for treating external tumors and abscesses. The *Catasetum* genus has a wide variety of medicinal applications. However, the studies that support these properties are scarce.

Phytochemicals isolated and pharmacological activities

As shown in Table 3, the studies on the secondary metabolites isolated *Catasetum* species and their pharmacological actions are limited to three species. Four compounds, including the phenanthrene 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene were isolated from an ethanolic extract of the aerial parts of *C. barbatum* and tested on their anti-inflammatory and antinociceptive activity through the carrageenan-induced plantar edema test and the histamine-induced contortion tests in rats [36].

Table 3. Biological activities and phytochemicals isolated of *Catasetum*.

Species	Plant part analyzed	Biological activity studies	Tested extracts	Isolated chemical compounds	References
Catasetum barbatum (Lindl.) Lindl	Aerial parts	Anti-inflammatory activity by carrageenan-induced plantar edema	Ethanolic extract	1) 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene 2) 2,7-dihydroxy-3,4-dimethoxyphenanthrene; 3) 2,7-dihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene 4) 2,7-diacetoxy-3,4-dimethoxy-9,10-dihydrophenanthrene	[36]

Catasetum integerrimum Hook	Leaf, root and pseudobulb	Cytotoxic activity by breast cancer cell lines (MCF-7 and MDA-MB231)	Ethyl acetate extract	1) ferulic acid 2) gallic acid 3) p-coumaric acid 4) p-hydroxybenzoic acid 5) syringic acid 6) vanillin 7) phloretin 8) galangin 9) naringenin 10) quercetin 11) rutin	[42]
	Root	Antioxidant activity by DPPH and ABTS assays	Ethanolic extract	1) flavonoids 2) phenols 3) reducing sugars 4) alkaloids 5) tannins 6) saponins 7) sterols 8) terpenes	[48]
Catasetum macroglossum Rchb. f.	Pseudobulb	Anti-inflammatory activity by carrageenan-induced plantar edema	Aqueous extract	1) reducing sugars 2) flavonoids 3) glucomannan 4) phenanthrene 5) stilbene	[15]
		Antioxidant activity by DPPH assay	Ethanolic extract	1) phenols 2) flavonoids 3) 1,5-anhydro-D-sorbitol 4) xylitol 5) octanedioic acid 6) fructose 7) linoleic acid	[49, 50]

Currently, *C. integerrimum* is one of the orchids most studied under different approaches. There are two studies carried out to verify its pharmacological activities. First, 25 µg/mL ethyl acetate extract of leaves, roots, and pseudobulbs of *C. integerrimum* showed cytotoxic activity by 79.47% and 97.79% on breast cancer cell lines MCF-7 and MDA-MB231, respectively. The compounds identified included phenolic acids (ferulic, gallic, p-coumaric, p-hydroxybenzoic, syringic, and vanillin) and flavonoids (phloretin, galangin, naringenin, quercetin, and rutin) [42].

The antioxidant activity of a root extract from *C. integerrimum* and its metabolites showed antioxidant activity in the ABTS and DPPH assays. The phytochemical qualitative test revealed the presence of sterols, unsaturations, flavonoids, and coumarins in wild plants and vitroplants (Table 3). This is one of the first studies reporting the phytochemical profile of *Catasetum* vitroplants [48].

Aqueous extract prepared from *C. macroglossum* pseudobulbs showed anti-inflammatory activity on the carrageenan-induced plantar edema test in Wistar rats. These properties were attributed to the presence of flavonoids. An HPLC-DAD analysis determined the presence of phenanthrenic and stilbenic dihydroderivatives [15]. Recent current works on *C. macroglossum* suggested the presence of phenols, flavonoids, various sugars, and some fatty acids in this plant species (Table 3). Some of these compounds have antioxidant effects, which can confer add value to many of these edible orchids. The presence of biological activity in *Catasetum* species confirms the traditional use of these orchids, demonstrating the need for more ethnobotanical studies.

Propagation and cultivation effort

Current biotechnological efforts in plants are an integral part of the works associated with *in vitro* and ex vitro conservation and propagation, genetic transformation, acclimatization, and product development from several plant genera and species [51]. Several works were published on biotechnological studies about the *Catasetum* genus (Table 4), focusing on the propagation and *in vitro* conservation of these species from various types of explants, denoting a preference for the conservation of the genus, but with little research focused on the acclimatization and development of products from species with phytochemicals of pharmacological potential

Table 4. Propagation effort by plant tissue culture techniques in *Catasetum* species

Species	Explant type used	Composition of the culture media	Response obtained	References
Catasetum boyi Mansf.	Seed	30 mg L^{-1} sucrose 2 g L^{-1} fertilizer B and G 100 mg L^{-1} coconut water 2 g L^{-1} activated carbon 4 g L^{-1} agar	90% seed germination was obtained	[52]
Catasetum gardneri Schltr.	Protocorm	MS basal medium modified with ½ macronutrients	Vitroplants were obtained by direct organogenesis way with growth of 6 cm per explant, 3 roots developed per explant and pseudobulbs with 3 cm in diameter	[53]
		Commercial formulation N.P.K (10-5-5) 2 mL L^{-1}	Vitroplants were obtained by direct organogenesis way with growth of 8.04 cm per explant	
	Seed	MS basal medium 1 gr L^{-1} activated carbon 30 gr L^{-1} sucrose 7 gr L^{-1} agar Jasmonic Acid (concentration not mentioned)	Vitroplants were obtained by direct organogenesis way with developed of 2.4 roots per explant, 1 leaf per explant and approximately 1.75 cm leaf and root growth per explant	[54]
	Roots	Vacin and Went medium modified by substituting $Fe_2(C_4H_4O_6)_3$ by 27.8 mg dm^{-3} Fe-EDTA MS micronutrients Sucrose	Vitroplants were obtained with developed of 3.75 cm root growth per explant	[55]

	Vitroplants	Vacin and Went medium Micronutrients of MS 0.01% thiamine 0.1% soy peptone 2% sucrose 0.2% phytagel	In this work ethylene production showed a decreasing trend in the first four months, presenting an initial and a final concentration of 66.11± 10.68 and 21.92±6.67 µL gr^{-1} FW h^{-1}, respectively. Likewise, an increase in ethylene production was observed at the end of the eight months (198.64±5.17), coinciding with the termination of a growth cycle	[56]
	Nodal explants	Vacin and Went medium Micronutrients of MS 0.1% activated charcoal 2% sucrose 0.7% agar Ethylene 1-MCP	For the chronic exposure to exogenous ethylene induced severe growth deterioration in young plants during the five weeks of treatment, on the contrary, the supply of 1-MPC, induced morphological effects opposite to those induced by ethylene	
Catasetum integerrimum Hook	Vitroplants	4.46 gr L^{-1} MS medium 8 gr L^{-1} agar plant 30 gr L^{-1} sucrose 3 gr L^{-1} activated carbon IAA BAP	Vitroplants were obtained with 5.73±0.45 shoots per explant and 5.84±0.48 leaves per shoot. Moreover, vitroplants developed 11.20±0.28 roots per explant and 13.20±0.28 cm root growth	Castillo-Pérez et al., 2021
	Pseudobulb	4.46 gr L^{-1} MS basal medium 8 gr L^{-1} agar plant 30 gr L^{-1} sucrose 3 gr L^{-1} activated carbon 1 mg L^{-1} IAA 1 mg L^{-1} BAP	By direct organogenesis vitroplants were obtained with 1.00±00 shoots per explant, 5.50±0.18 leaves per shoot, 4.37±0.37 roots per explant with a growth rate of 4.88±0.20 cm and a plant growth of 7.96±0.12 cm	

	Plantlet	MS basal medium (half-stregth) Sorbitol Carbon	The treatment added with 3% carbon, and 2% sorbitol presented the lowest value of growth in plantlet length (17.70±5.8). In the same way, showed the lowest shoot formation (1±00)	[51]
	Root and node	MS basal medium 3% sucrose 2.2 gr L^{-1} gelrite 2 gr L^{-1} activated carbon BAP Kinetin	Direct shoot organogenesis was observed in node explant in BAP-supplemented MS and in kinetin-supplemented MS at all concentrations tested. Indirect shoot organogenesis was observed in root explant in MS supplemented with 4.64 or 9.29 µM kinetin	
Catasetum macrocarpum Rich. ex Kunth	Seed	½ MS basal medium 0.4 mg L^{-1} tiamin 100 mg L^{-1} myo-inositol 2% sucrose BA NAA	Vitroplants were obtained with 4.1 shoots per explant and 6.1 roots per explant	[26]
	Vitroplant	First phase: Bioplant Prata® with sphagnum (1:1) Second phase: Bioplant Prata with ouro negro substrate (1:2)	The survival rates observed in acclimatization process were 93.3% for the first phase, and 96.6% for the second phase	
Catasetum pileatum Rchb. f.	Protocorm	MS basal medium 3% sucrose 0.8% Agar-agar Kinetin IBA	8.63 regenerated PLB were obtained per explant with 12.70 leaves and 7.40 average roots.	[57]

	Protocorm	MS basal medium 3% sucrose 0.8% agar 1.00 mg L^{-1} BA 0.50 mg L^{-1} NAA Colchicine	For the polyploid induction, treatment with 4.00 mg l^{-1} colchicine for 72 h was the only treatment to result in a mixoploid seedling. Moreover, developed 4.16 and 4.12 cm root growth per explant, 7.00 roots per explant, 4.58 cm leaf growth per explant and 6.66 cm leaf per explant	[58]
Catasetum schmidtianum F.E.L. Miranda & K.G. Lacerda	Protocorm	30 mg L^{-1} sucrose 2 gr L^{-1} fertilizer B and G 200 mg L^{-1} coconut water 2 g L^{-1} activated carbon 4 g L^{-1} agar 1 mg L^{-1} extract pyroligneous	By direct organogenesis vitroplants were obtained with 27.6 cm leaf growth per explant and 4.1 roots per explant	[59]
	Seed	10 mL L^{-1} Kudson C medium 30 gr L^{-1} sucrose 24 gr L^{-1} natural gelatin	By direct organogenesis vitroplants were obtained with 3 mm Protocorm growth per explant	[60]
	Vitroplants	Fertilizer B and G Coconut water Activated carbon Agar Sucrose Water Sphagnum Moss Vermiculite Carbonized rice straw Charcoal	The acclimatization treatment consists of Chile Moss + Vermiculite + Carbonized Rice Straw + Charcoal (1:1:1:1 v/v) presented the most suitable conditions for the development of the species	[61]

The conservation protocols of six different *Catasetum* species were published. Seeds and protocorms are the most widely used explants, although pseudobulbs, roots, and vitroplants have also been used. Seed germination and micropropagation for mass propagation studies are available for these species. Fernández et al. (2015) [52] used seeds from an immature capsule of *Catasetum boyi* and obtained up to 90% germination. The percentage of seed germination is low, approximately 5% of all seeds, under natural conditions [62]. Micropropagation work was also carried out for *C. gardneri* [54], *C. macrocarpum* [26] and *C. schmidtianum* [60] using seeds as explants.

There are micropropagation protocols using roots as an explant in two species of *Catasetum* orchids (*C. gardneri* and *C. integerrimum*) have worked micropropagation protocols using roots as an explant. In the case of *C. gardneri*, vitroplants were obtained with developed of 3.75 cm root growth per explant [55]. Indirect organogenesis was tested and observed in the production of *C. integerrimum* vitroplants by adding kinetin as a plant growth regulator [51]. No *Catasetum* orchid micropropagation protocol has reported leaves as an efficient explant to generate vitroplants. Castillo-Pérez et al., (2021) [39] tested this type of explants, obtaining a null response to regenerate seedlings (Fig. 3).

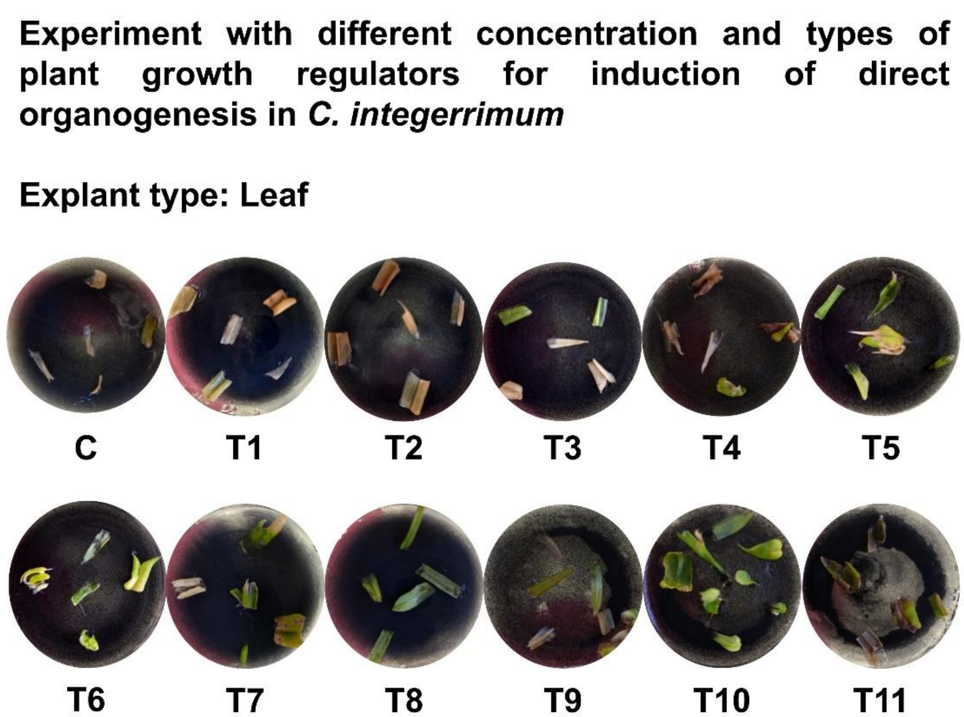

Fig. 3 Null *in vitro* response of leaves after 16 wk of culture in an experiment with different concentrations and types of plant growth regulators for induction of direct organogenesis in *C. integerrimum*

Finally, the most used culture media for the micropropagation of *Catasetum* orchids are the MS medium and the Vacin and Went medium. Furthermore, activated carbon is commonly used for the micropropagation of *Catasetum* orchids and the most frequently used carbon source is sucrose. Plant growth regulators and additives (vitamins or natural extracts) vary depending on the objective of each study (Table 4).

Future prospective and conclusions

Some *Catasetum* species showed *in vitro* anti-inflammatory, cytotoxic, and antioxidant activities. The ethnomedicinal information of these plant species was validated. However, in vivo assays and their molecular mechanism of action remains to be elucidated. Most of the secondary metabolites isolated from the *Catasetum* orchids correspond to polyphenols, and many of these compounds have previously reported anti-inflammatory and antioxidant actions. Nevertheless, some *Catasetum* orchids lack of chemical composition of their metabolites. The isolation and elucidation of the structure of new compounds obtained from the *Catasetum* genus should be carried out.

There is limited information about the obtention of new compounds from the Orchidaceae family. It is also necessary to work on the biological and ecological aspects of the *Catasetum* orchids, such as growth and climatic conditions, seasons, exposure to sunlight, altitude, and genetic composition, due these abiotic factors influence the chemical composition and the pharmacological effects of these plant species.

Biotechnological plant tissue culture techniques, including symbiotic and asymbiotic germination, clonal propagation, and direct organogenesis are available in this orchid genus. The use of biotechnological techniques can prevent and control the reduction of the pressure on wild species of this genus. In our laboratory (Environmental Science Research Laboratory - Autonomous University of San Luis Potosí, Mexico) we have worked with an efficient propagation protocol for *C. integerrimum* from pseudobulb sections and using the direct organogenesis technique [39]. Moreover, we have begun to study the production of phytochemicals produced by vitroorchids, inducing different types of stress *in vitro*, and comparing with the homologous produced by wild plants for establishing a biotechnological technique for the production of bioactive compounds. Overall, *Catasetum* orchids remain to be studied for their pharmacological, ecological, botanical, chemical, and toxicological aspects.

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CHAPTER V

Micropropagation of *C. integerrimum* Hook (Orchidaceae) through seed germination and direct shoot regeneration from pseudobulbs and roots

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Abstract

C. integerrimum is an orchid used in Mexican traditional medicine, which is over exploited due its ornamental and medicinal properties. In this scenario, conservation strategies are mandatory, on order to protect wild populations from this valuable specie. The aim of the present study was to establish a micropropagation protocol by direct organogenesis, as a tool for *C. integerrimum* production and conservation. Seeds from a mature capsule were cultivated in a Murashige and Skoog basal medium. Then, 6-month-old vitroplants were transplanted into treatments fortified with 6-bencilaminopurin (BAP) or indole-3-acetic acid (IAA) to induce plant development. Treatment fortified with 2.5 mg L^{-1} BAP in combination with 5.0 mg L^{-1} IAA developed 5.73 ± 0.45 shoots per explant and 5.84 ± 0.48 leaves per shoot. For the rooting process, the best treatment was the fortified with 2.5 mg L^{-1} IAA, which developed 11.20 ± 0.28 roots with a 13.20 ± 0.28 cm root length. *C. integerrimum* vitroplants were used as a source of leaves, roots and pseudobulbs for the induction of direct organogenesis. Leaf explants did not induce any morphogenic response. Pseudobulb explants had the best response when developing 1 shoot per explant with 5.50 ± 0.18 leaves per shoot and 4.37 ± 0.37 roots per explant with a 4.88 ± 0.20 root length. For the acclimatization stage a survival rate of more than 90% was achieved. The results obtained prove the efficacy of the micropropagation method, which contribute significantly to the conservation of the orchid under study, preserving its wild populations from indiscriminate collection.

Keywords

direct organogenesis; pseudobulb explant; root explant; *Catasetum* genus

Introduction

Orchids are one of the largest and most diverse groups among angiosperms. According to the last estimate, the Orchidaceae family includes approximately 800 genera and 25,000 species (Chase, 2015). These plants are widely distributed around the world and cultivated for their beautiful flowers. In addition to their ornamental value, some orchids are also well known for their medicinal properties, in traditional medicine (Castillo-Pérez et al. 2019). For example, *C. integerrimum* is an orchid used in some regions of Mexico for the folk treatment of tumors, abscesses, wounds, kidney diseases, and diabetes mellitus (Alcorn 1984; Alayón-Gamboa 2011; Teoh 2019).

C. integerrimum is an epiphyte orchid that reaches 70 cm in height, presents ovoid pseudobulbs, which develop leaves oblong-lanceolate and deciduous (Fig. 1A). The flowers are unisexual, dimorphic, and intensely fragrant. Male flowers are green, often suffused, and spotted with purplish red (Fig. 1B), whereas female flowers are green as well, but with the lip yellow (Fig. 1C). After being pollinated, female flowers develop an ellipsoid capsule, with six ribs, glaucous and pendulous that contain thousands or millions of tiny seeds (Fig. 1D).

This orchid is distributed on the slopes of the Gulf of Mexico and in the countries of Guatemala, Belize, Honduras, El Salvador and Nicaragua. This species grows from sea level to about 1600 m of altitude, in the ecosystems of tropical rain forest, mountain rain forest, tropical deciduous and semi-deciduous forest, warm oak forest and palm grove (Salazar et al. 1990).

The beauty and fragrance of *C. integerrimum* flowers, makes it a coveted orchid, among national and international collectors. Many orchids are threatened by overexploitation and destruction of their natural habitat, which suggest that it is urgent to adopt strategies focused on the prevention and control that allow the conservation of this species (Cheruvathur et al. 2010; Castillo-Pérez et al. 2019).

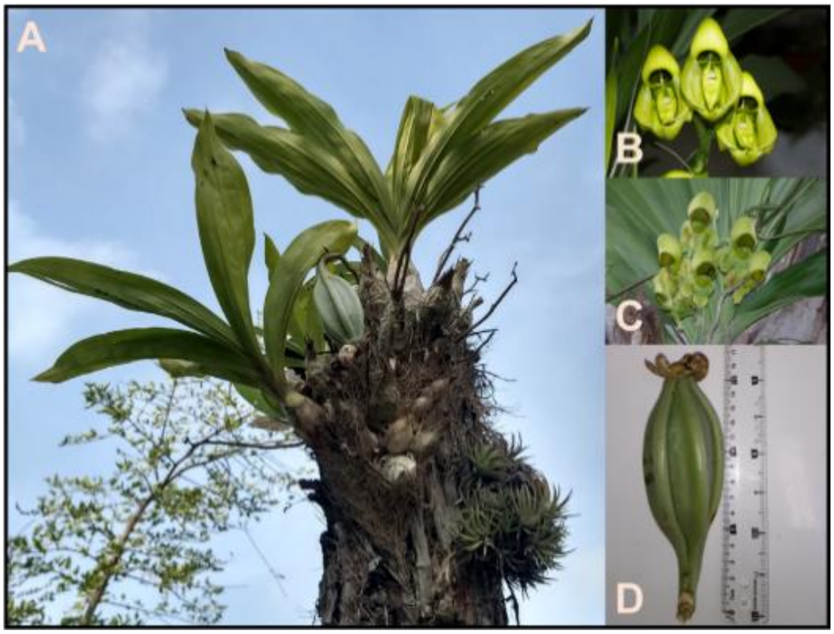

Fig. 1 *Catasetum integerrimum* (Orchidaceae). *A* Whole plant in situ; *B* Male flowers; *C* Female flowers; *D* Capsule before dehiscence.

The micropropagation of orchids by plant tissue culture is a quick and practical way to mass reproduce threatened orchid species for preventive and conservative purposes (Whiteley et al. 2016; Castillo-Pérez et al. 2021a). The *in vitro* micropropagation success depends on the aseptic culture establishment, type of explant, shoot regeneration capacity, rooting, and acclimatization. Interestingly, in recent years only three species of the genus *Catasetum* have been reported for their *in vitro* micropropagation: *C. macrocarpum* (de Melo-Ferreira et al. 2018; Moraes et al. 2020), *C. fimbriatum* (Peres and Kerbauy 1999; Silva-Maia and Pedroso-de Moraes 2017; Moraes et al. 2020) and *C. pileatum* (Zakizadeh et al. 2019). There is another report, but it does not mention which *Catasetum* species was micropropagated (Baker et al. 2014). The potential use of pseudobulb and roots sections has not been fully explored, and in some species like *C. integerrimum*, the pseudobulb and roots obtained in an early stage of plant development, could be an excellent source of explants. The establishment of a protocol by direct organogenesis will allow the continuous obtention of plant material without affecting natural populations of this orchid (Sidky 2017; Hnatuszko-Konka et al. 2019).

In this study, we established an *in vitro* micropropagation protocol for *C. integerrimum* through seed germination and we report, for the first time, the successful direct shoot organogenesis from pseudobulbs explants. This study reports a protocol large-scale and unlimited *in vitro* propagation of this orchid which is of great significance for the study of phytochemicals from this species, in addition to its conservation and commercial supply.

Materials and Methods

Plant material

Adult plants without flowers of *C. integerrimum* with development of 6 to 10 pseudobulbs and one developed capsule were collected in the community of Antiguo Tambolón, belonging to the municipality of Ciudad Valles, San Luis Potosí, Mexico (coordinates: 21º49’45.89’’ North and 98º57’28.98’’ West) at an altitude of 32 m. The seeds of the capsule were used as explants. The basal medium was used with MS vitamins (Murashige and Skoog 1962; Phyto Technology Laboratories) supplemented with 30 g L^{-1} sucrose, 3.0 g L^{-1} of activated charcoal (Karal S.A. de C.V., Leon, Mexico) and 8 g L^{-1} of agar (Phyto Technology, Shawnee Mission, KS; Plant Tissue Culture grade). The pH of the culture media was adjusted to 5.7 with NaOH 1N and/or HCl 1N and sterilized at 120°C for 20 min (1.37×10^5 Pa). The experimental units were kept in the growing room of the Research Laboratory in Environmental Science of the Universidad Autónoma de San Luis Potosí, at 25°C with a photoperiod of 16 hours of light at 45 µmol $m^{-2}s^{-1}$ and 8 hours of darkness, the light was supplied with cold white, fluorescent lamps (Phillips, Saltillo, Mexico). The conditions of the growing room remained unchanged at all stages of the experiment.

Capsule disinfection and germination protocol

Before to dehiscence, *C. integerrimum* capsule was superficially sterilized with 30% antibacterial soap for 20 minutes, $AgNO_3$ at 10% for 10 minutes, ethanol at 70% for 2 minutes, and finally sodium hypochlorite at 10% for 10 minutes. Then, inside the tissue culture hood, a transversal cut was made to the capsule and the seeds were grown to germinate in basal MS medium with 1% (w/v) of activated charcoal (Karal S.A. de C.V., Leon, Mexico) without the addition of plant growth regulators (PGR´s) and were eventually transferred to the growing room. Evaluation of the asymbiotic germination of *C. integerrimum* seeds was carried out following the protocol established by Castillo-Pérez et al. (2021b). Briefly, 10 of the sown Petri dishes were divided into 20 squares measuring 10 x 10 mm. The percentage of germinated seeds was recorded from 10 squares of each plate per day (time zero). A seed was considered germinated when the break of the seed coat was observed.

In vitro shoot multiplication

To induce the development of shoots and roots, 6-month-old *C. integerrimum* (counted from germination) vitroplants were used. For shoot induction vitroplants were grown in supplemented MS medium with two concentrations of BAP (2.5 and 5.0 mg L^{-1}; Phyto Technology Laboratories) or two concentrations of (IAA 0.5 and 1.0 mg L^{-1}; Phyto Technology Laboratories). Moreover, two BAP/IAA combinations were evaluated (BAP 2.5 mg L^{-1} + AIA 0.5 mg L^{-1} and BAP 5.0 mg L^{-1} + AIA 1.0 mg L^{-1}) and a control treatment, that consisted in just MS media without any PGR´s, was added. For each treatment, 20 vitroplants were used and transferred to the culture room. After five weeks of inoculation, the data related to the percentage of explants that responded to shoot induction, the number of days to shoot initiation, the number of shoots per explant, and the number of leaves per shoot were collected.

Rooting of *C. integerrimum* vitroplants

Shoots of *C. integerrimum*, produced under *in vitro* conditions, were used as explants in this experiment. All the explants were cultured in MS medium supplemented with different types of auxins, IAA, indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA) at 1.0, 2.5 and 5.0 mg L^{-1}, respectively, and MS auxin-free medium was used as a control treatment.

The pH of the culture media was adjusted to 5.7 with NaOH 1N and/or HCl 1N. The vitroplants were transferred to the growing room for eight weeks. The percentage of plantlets that responded to rooting and the number of days to initiate rooting were recorded in each treatment. After the evaluation period, the vitroplants were removed from the flasks and the number of roots produced under each treatment was evaluated. The length of the roots was measured starting from the vegetal neck to the apical zone of each primary and secondary roots. These measurements were made using a digital vernier inside a tissue culture hood.

Direct organogenesis from leaves, roots, and pseudobulbs

C. integerrimum vitroplants with the best response in the previous stages were used as a source of leaves, roots, and pseudobulbs for the induction of direct organogenesis. Explants of approximately 1 cm^2 were obtained by a transversal cut from each plant section. This procedure was carried out in the tissue culture hood to maintain aseptic conditions. The explants were subculture in MS medium with two concentrations of BAP (1.0 and 2.5 mg L^{-1}; Phyto Technology Laboratories) and two concentrations of IAA (0.5 and 1.0 mg L^{-1}; Phyto Technology Laboratories). PGR´s were added alone or in combination. Eight treatments with 10 explants of each plant section were established, and a control treatment without any PGR was included. The vitroplants were transferred to the culture room for 16 weeks. At the end of the exposure period, the explant response, the number of shoots, leaves, and roots per explant, root length, and plant height were recorded.

Vitroplants acclimatization

Plantlets with a height of at least 3 cm were removed from the culture jars and washed thoroughly under tap water to eliminate all media attached to the roots. For this stage, the roots were washed with distilled water to remove excess culture medium and were transplanted in a mixture of pine bark, Sphagnum peat, and Tepezil rock as a substrate, in a ratio of 3:2:1, respectively, in plastic pods 6.7 cm of diameter and placed under greenhouse conditions (average temperature 30°C, relative humidity 80-90%). The plastic pots were kept covered with transparent polybags for the first 30 days. The polybags were then gradually removed to allow the relative humidity to decrease to 50-60%. Survival was recorded monthly for 4 months.

Statistical analysis

During the processes of induction of shoots and roots, and the establishment of direct organogenesis, for each variable a variance analysis (ANOVA) of a general linear model type (MLG) pathway was performed. The assumptions of normality and homogeneity of variances were verified with the Kolmogorov-Smirnov and Levene tests, respectively. Additionally, Duncan's multiple range test (DMRT) at $p \leq 0.05$ was used to compare the mean results among the different treatments. Statistical analyses were performed using the Statistic program for Windows, version 8.0.

Results and discussion

Germination of seeds

The germination process of *C. integerrimum* seeds was quick, after 10 days a 15% germination of the total cultivated seeds was registered (Fig. 2). At this time, the vitroplants had an average 3 mm height. Several studies have confirmed that the time required for orchid seed germination after inoculation into the growing medium varies between species. For the *Catasetum* genus, only one species (*C. macrocarpum*) has been reported for the germination process and those results (15 days of germination) (de Melo-Ferreira et al. 2018) are similar to those obtained in our study (10 days).

Other orchids of different genera report the following data, *Stanhopea tigrina* germinated five days after inoculation (Castillo-Pérez et al. 2021b), seeds of *Cypripedium lentiginosum* after 45 days of *in vitro* inoculation (Jiang et al. 2017) and in the case of *Dendrophylax lindenii* germinated seven days following inoculation, but in this case mycorrhizal fungus were used (Hoang et al. 2017).

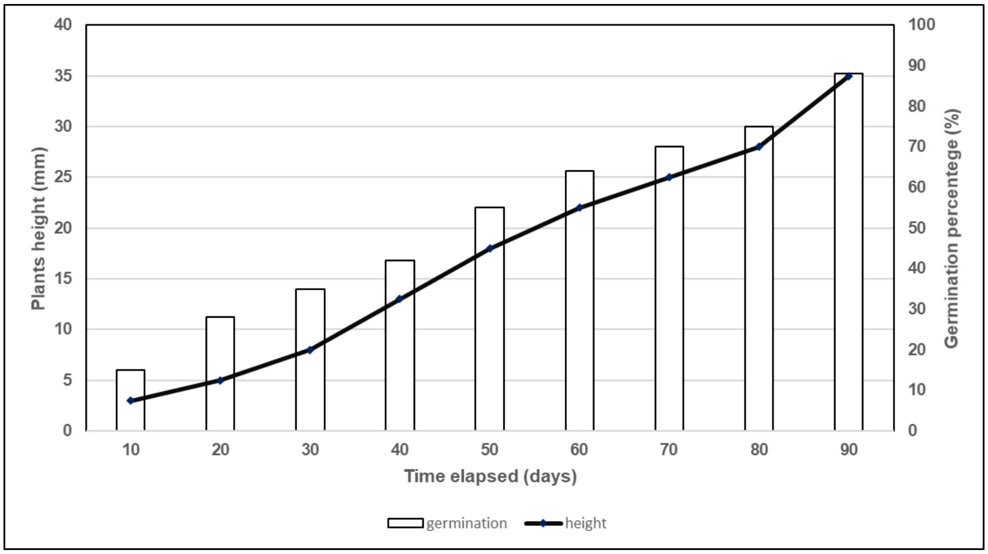

Fig. 2 Seed germination of *C. integerrimum* and seedling growth. The seeds were germinated on MS basal medium containing 1% (w/v) activated charcoal.

Concerning the morphological aspect, embryos had a completely green color after 70 days of culture and developed leaf primordia. The germination process of *C. integerrimum* seeds increased gradually by time elapsed and reached 88% after 90 days of culture. The height of the germinated seedlings increased exponentially with time too, reaching approximately 35 mm on average after 90 days (Fig. 2).

Effect of BAP and IAA on shoot development of *C. integerrimum* vitroplants

Multiple shoots of *C. integerrimum* vitroplants proliferated at all the concentrations of BAP and IAA treatments, including the control (PGR´s-free MS medium). The highest response of explants to shoots induction was recorded in T2, T4 and T6 treatments, in which 100% of the explants produced shoots (Table 1). The percentage of the response in explants was reduced in T1, T3, T5, and T7 treatments, in which 70, 85, 75, and 60% of the explants, respectively, responded to multiple shoots production.

These results of this study agree with other studies reporting the use of growth regulators such as BAP and IAA, which induce the proliferation of shoots in orchids (Paul et al. 2017; Sherif et al. 2018; Castillo-Pérez et al. 2021b). This response has not only been documented in species of this family, has also been seen in other plant species like *Musa acuminata* (Safarpour et al. 2017), *Aechmea ramosa* var. *ramosa* (Faria et al. 2018), *Eucalyptus urophylla* (Huang and Li 2020) and *Basella rubra* (Kumar and Giridhar 2021).

Table 1. Effects of different concentrations of BAP and IAA on shoot development of *C. integerrimum* vitroplants after five weeks of culture.

Treatment label	Growth regulators (mg L^{-1})		Explant responded to shoot induction (%)	Number of days to shoot initiation	Number of shoots/explant	Number of leaves/shoot
	BAP	IAA				
T1	0	0	70	43.20 ± 0.45 a	1.55 ± 0.19 c	1.50 ± 0.22 d
T2	2.5	0	100	33.17 ± 0.32 d	3.82 ± 0.27 b	2.94 ± 0.36 bc
T3	5.0	0	85	36.89 ± 0.38 b	1.68 ± 0.27 c	2.57 ± 0.35 bc
T4	0	0.5	100	32.93 ± 0.18 d	3.66 ± 0.30 b	2.40 ± 0.39 bcd
T5	0	1.0	75	35.45 ± 0.46 c	2.95 ± 0.22 b	2.31 ± 0.41 cd
T6	2.5	0.5	100	34.05 ± 0.37 d	5.73 ± 0.45 a	5.84 ± 0.48 a
T7	5.0	1.0	60	36.75 ± 0.42 b	2.08 ± 0.22 c	3.41 ± 0.46 b

Values are means ± standard error (n = 20). Means followed by the same letters in each column are not significantly different at $p < 0.05$ using Duncan's multiple range test (DMRT). BAP = 6-benzylaminopurine and IAA = indole-3-acetic acid.

The shoot proliferation began with the emergence of a new shoot primordia from the base of the vitroplant (Fig. 3A). This new shoot primordia were recorded almost at the same time and without significant ($p > 0.05$) difference in the treatments T2, T4, and T6, after 33.17 ± 0.32, 32.93 ± 0.18, and 34.05 ± 0.37 days of culturing, respectively. The initiation of shoots in the treatments T3, T5, and T7, shoots were slower compared to other treatments.

The slowest response (43.20 ± 0.45 days of culture) of explant to shoot initiation was observed in the control treatment. The results obtained with the treatments T2, T4, and T6 containg BAP (2.5 mg L^{-1}), IAA (2.5 mg L^{-1}), and the combination of BAP/IAA (2.5/0.5 mg L^{-1}) respectively, regarding shoot initiation, results are partially consistent with the reported by Bhowmik and Rahman (2020), who propagated the orchid *Aerides multiflora* and observed that the combination of BAP/AIA was the most effective, but they had to wait approximately 40 days to observe the appearance of new shoots.

In the case of the hybrid orchid Aranda Wan Chark Kuan 'Blue' x *Vanda coerulea*, it took 5.5 ± 0.3 days for the initiation of shoots using a combination of 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} IBA (Gantait and Sinniah 2012). In other family plants, the elapsed time to appreciate the shoot initiation was shorter. For example, in *Zingiber officinale* elapsed 6.87 ± 0.31 days using a treatment added with the cytokinin zeatin (Zahid et al. 2021). These examples suggest that the initiation of new shoots in plants depends on the species and the PGR´s used.

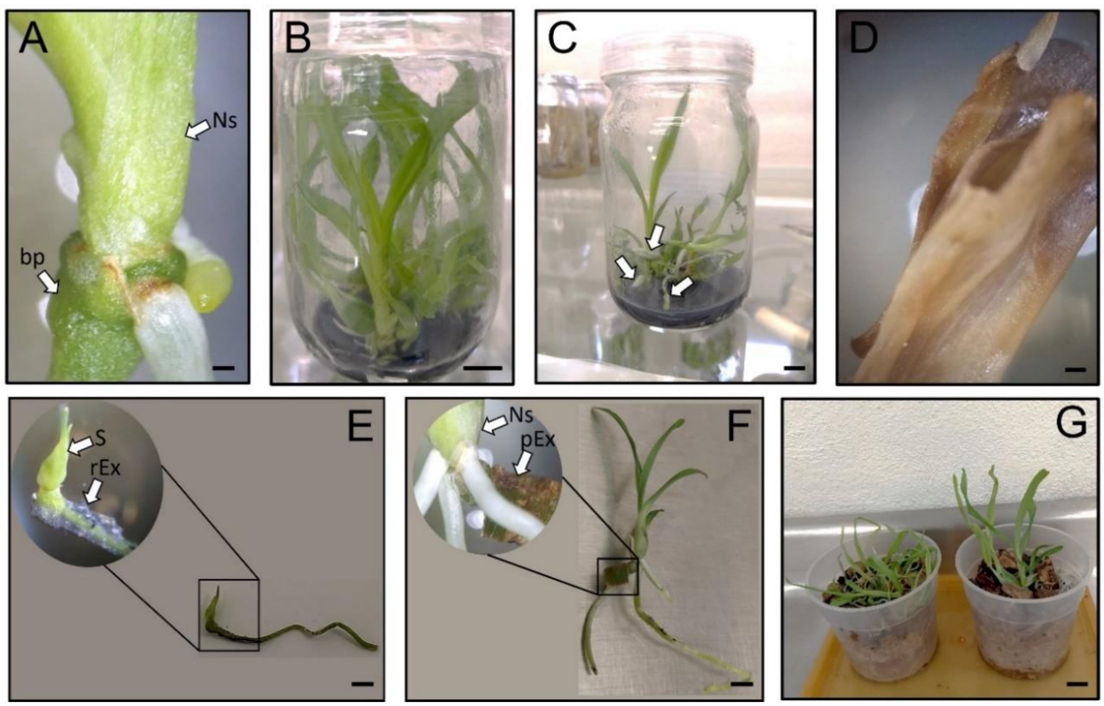

Fig. 3 *In vitro* development of *C. integerrimum*, on the modified MS medium (Murashige and Skoog 1962), fortified with several plant growth regulators. *A* emergence of shoot primordia from the base of the vitroplant; *B* Vitroplants with the best plant development grown in MS medium fortified with BAP/IAA (2.5/5.0 mg L^{-1}); *C* Vitroplants with the best root development and velamen formation (indicated with arrows), grown in MS medium fortified with 2.5 mg L^{-1} IAA; *D* Appearance of leaf explants after 45 days of culture; *E* New shoot de novo developed from root explant by direct organogenesis; *F* New shoot de novo developed from pseudobulb explant by direct organogenesis; *G* Appearance of acclimatization vitroplants formed by direct organogenesis way. Bar 1 mm (A, D) and 1 cm (B, C, E-G).

According to Sarkar and Banerjee (2020), BAP has been the most common used cytokinin for the induction and multiplication of shoot in many plant species, showing better results when added to the medium in combination with auxins like IAA, IBA, NAA, or other cytokinins. This effect was observed in this study, where the highest number of shoots per explant was registered in the treatment with the combination BAP/IAA (2.5/0.5 mg L^{-1}), in which 5.73 ± 0.45 shoots were developed, followed by the treatments with only IAA and 2.5 mg L^{-1} BAP in which there was a response without significant differences ($p > 0.05$) between these treatment groups.

The lowest number of shoots per explant was recorded in the treatments T1, T3 and T7 (Table 1). Finally, the highest number of leaves per shoot (5.84 ± 0.48) was observed too in the treatment with the combination BAP/IAA (2.5/5.0 mg L^{-1}) (Fig. 3B). The remaining treatments developed between two and three leaves per explant without significant differences ($p > 0.05$) between treatments. Other orchids such as *Tolumnia* spp. (Chookoh et al. 2019) and *Vanilla planifolia* (Carranza-Álvarez et al. 2020) have shown the same behaviour in terms of leaf development using BAP and IAA as added phytohormones.

Effect of auxins on rooting of vitroplants of *C. integerrimum*

Roots were induced in *C. integerrimum* and 100% of the explants responded to root induction in MS medium with all auxins and control (without auxin) treatments. The effect of different types and concentrations of auxins on the number of days to the first root primordia emergence was not significantly different ($p > 0.05$) in six treatments (Table 2).

The treatment added with 2.5 mg L^{-1} IAA showed the best response for the emergence of the first root primordia (4.90 ± 0.28 days) and the control treatment showed the lowest response (14.15 ± 0.26 days). Different types and concentrations of auxins caused a significant difference ($p < 0.05$) in the number and length of roots of *C. integerrimum* vitroplants.

Table 2. Effect of different types and concentrations of auxin on rooting of *in vitro* shoot of *C. integerrimum* after eight weeks of the culture.

Treatment (auxin)	Auxin concentration (mg L^{-1})	Plantlet responded to rooting (%)	Number of days to root initiation	Number of roots/explant	Root length (cm)
Control	0	100	14.15 ± 0.26 a	3.80 ± 0.25 f	6.78 ± 0.26 e
IAA	1.0	100	6.55 ± 0.24 c	6.20 ± 0.20 d	4.53 ± 0.24 f
IAA	2.5	100	4.90 ± 0.28 d	11.20 ± 0.28 a	13.20 ± 0.28 a
IAA	5.0	100	7.45 ± 0.25 b	4.10 ± 0.20 ef	4.87 ± 0.25 f
IBA	1.0	100	6.55 ± 0.31 c	7.15 ± 0.23 b	9.18 ± 0.31 c
IBA	2.5	100	7.40 ± 0.22 b	7.45 ± 0.19 b	9.21 ± 0.22 c
IBA	5.0	100	7.00 ± 0.30 bc	4.20 ± 0.22 ef	7.18 ± 0.30 e
NAA	1.0	100	7.30 ± 0.27 bc	4.55 ± 0.22 e	6.54 ± 0.27 e
NAA	2.5	100	7.85 ± 0.22 b	7.35 ± 0.20 c	8.28 ± 0.22 d
NAA	5.0	100	7.45 ± 0.29 b	9.60 ± 0.30 b	9.95 ± 0.29 b

Values are means ± standard error (n=20). Means followed by the same letters in each column are not significantly different at $p < 0.05$ using Duncan's multiple range test (DMRT). IAA = indole-3-acetic acid, NAA = 1-naphthaleneacetic acid, and IBA = indole-3-butyric acid.

The treatment with the best response in the number of roots (11.20 ± 0.28 roots/explant) and root length (13.20 ± 0.28 cm) was the one added with 2.5 mg L^{-1} IAA. Vitroplants in this treatment showed healthy roots of green color, turgid, and in some cases, the development of velamen was observed (Fig. 3C). For the number of roots, the treatments added with IAA (1.0 mg L^{-1}), IBA (5.0 mg L^{-1}), and the control treatment did not show statistical significance ($p > 0.05$) and showed the lowest response. For the root length, the treatments added with 1.0 and 5.0 mg L^{-1} IAA produced the shortest roots with 4.53 ± 0.24 and 4.87 ± 0.25 cm, respectively (Table 2).

Several studies have proved that the use of IAA promotes root induction through the elongation of cells in the meristematic regions of the stems (Bielach et al. 2017; Sharma and Zheng 2019). The induction of the root system in species of the *Catasetum* genus has been investigated using different PGR´s but never has been reported the use of IAA until this study. For example, the use of 1.0 µL L^{-1} jasmonic induced the best response for the formation of roots in *C. fimbriatum*, even this PGR helped for the development of leaves in the vitroplants (Silva-Maia and Pedroso-de Moraes 2017).

For the case of *C. macrocarpum*, de Melo-Ferreira et al. (2019) added 0.5 mg L^{-1} NAA and reported a strong formation of roots. In *C. pileatum* cv. Alba, Zakizadeh et al. (2019) used kinetin (1.0 mg L^{-1}) and indol butyric acid (0.5 mg L^{-1}), obtaining a high rooting frequency (7.40 roots per plant). Finally, Baker et al. (2014), used 0.5 mg L^{-1} of benciladenin in combination with 0.5 mg L^{-1} NAA for the obtention of 7.16 roots per plant with a 193.4 mm root length, however, these authors did not mention which *Catasetum* species was used in their study.

In other orchids of different genera, IAA concentrations that have been useful for root development are those used in the range of 0.5 to 5.0 mg L^{-1}, and sometimes combined with other auxins such as IBA or NAA (Pant and Thapa 2012; Vudala et al. 2019; Arda 2020), or more recently with organic additives (Seon et al. 2018; Carranza-Álvarez et al. 2020; Castillo-Pérez et al. 2021). The production of IAA has even been studied by adding some bacteria and fungi that could produce it naturally (Deepthi and Ray 2019; Altinkaynak and Ozkoc 2020), although this method has not been tested in plant tissue culture.

Effect of BAP and IAA on direct organogenesis using leaves, roots, and pseudobulbs as explants

The use of leaves of *C. integerrimum* vitroplants as explants for the induction of direct organogenesis was not an effective method. All leaf explants cultivated in all treatments were oxidized and died after 45 days of culture (Fig. 3D). Concerning the root explants, a morphogenic response was achieved in seven of the nine treatments tested after 16-weeks of *in vitro* culture. Any change in the explant was considered as a morphogenic response and it was not exclusive to the formation of new shoots. Some roots responded only with the elongation of the cultivated root and others formed new roots and shoots de novo (Fig. 3E). For this way, the treatments T1 and T7 were those that showed the highest response percentage (40%). In treatments T3, T4, T5, T6 and T9 a morphogenic response by 20% was achieved. Treatments T2 and T8 did not present any morphogenic response.

The highest number of shoots per explant (0.80 ± 0.20) were those developed in treatment T1 (control treatment without PGR´s). Nevertheless, for this variable the results were not statistically significant ($p > 0.05$) among the other treatments. Concerning the number of leaves per shoot, treatment T1 also showed the highest response (1.80 ± 0.58). However, this result was not significant ($p > 0.05$) when compared with treatments T3, T7, and T9, but it was significant ($p < 0.05$) with treatments T2, T4, T5, T6, and T8. The root explants of the T1 treatment were those that achieved the highest number of roots per explant (1.20 ± 0.37) with the longest length (1.15 ± 0.06 cm) and the highest total height of the new vitroplants (2.62 ± 0.46 cm). These results were statistically significant ($p < 0.05$) compared with all other treatments (Table 3). The results suggested that it not necessary the use of PGR´s for the induction of *in vitro* morphogenesis using roots, due the control showed the greatest response for plant development.

There are few reports that have documented the use of roots as explants for the development of new whole plants. In the *Ansellia africana* orchid, de novo root organogenesis was induced. However, this achievement was shown from shoot-tip and nodal explants (Vasudevan and Van Staden 2011). In the case of the *Phalaenopsis amabilis* orchid, the authors observed that the leaves and root explants registered the lowest response compared to protocorms and stem sections (Mose et al. 2017), which coincides with our research.

The pseudobulb explants showed the best development of new vitroplants. All the treatments stimulated a morphogenic response and de novo new shoots induction by direct organogenesis after 16-weeks of *in vitro* culture (Fig. 3G). Treatment T8 added with 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} IAA showed the highest response (80%), followed by treatments T3 and T4 (60%).

Table 3. Effects of BAP and IAA on direct organogenesis of *C. integerrimum* root explants after 16-weeks of culture.

Treatment label	Growth regulators (mg L^{-1})		Explant response (%)	Number of shoots/explant	Number of leaves/shoot	Number of roots/explant	Root Length (cm)	Plant height (cm)
	BAP	IAA						
T1	0	0	40	0.80 ± 0.20 a	1.80 ± 0.58 a	1.20 ± 0.37 a	1.15 ± 0.06 a	2.62 ± 0.46 a
T2	1.0	0	0	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
T3	2.5	0	20	0.40 ± 0.24 ab	0.60 ± 0.40 ab	0.40 ± 0.24 b	0.37 ± 0.21 b	1.10 ± 0.63 bc
T4	0	0.5	20	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
T5	1.0	0.5	20	0.40 ± 0.24 ab	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
T6	2.5	0.5	20	0.20 ± 0.20 ab	0.40 ± 0.40 b	0.40 ± 0.40 b	0.17 ± 0.17 b	0.60 ± 0.60 bc
T7	0	1.0	40	0.40 ± 0.24 ab	0.60 ± 0.40 ab	0.00 ± 0.00 b	0.32 ± 0.19 b	0.92 ± 0.53 bc
T8	1.0	1.0	0	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
T9	2.5	1.0	20	0.40 ± 0.24 ab	1.00 ± 0.63 ab	0.40 ± 0.40 b	0.30 ± 0.17 b	1.67 ± 0.98 ab

Values are means ± standard error (n = 10). Means followed by the same letters in each column are not significantly different at $p < 0.05$ using Duncan's multiple range test (DMRT). BAP = 6-benzylaminopurine and IAA = indole-3-acetic acid.

The lowest percentage of response was recorded in treatment T6, where only a 20% response was obtained (Table 4). The highest number of shoots per explant (1.00 ± 0.00) was obtained in treatment T8, in which each shoot developed an average of 5.50 ± 0.18 leaves. The number of shoots per explant was not significant between the treatments, as opposed to the development of new leaves per shoot (Table 4). The pseudobulb explants from treatment T8 also registered the highest number of roots (4.37 ± 0.37), with the longest length (4.88 ± 0.20 cm). The results of the two variables showed statistically significant differences ($p < 0.05$) against the other treatments. Finally, concerning the final height of the plants, in the T8 treatment, the highest average height was recorded, which was 7.96 ± 0.12 cm (Table 4).

Different explants have been used for *in vitro* orchid propagation, the most common are seeds and protocorms, but fragments of plantlets like leaves, roots, and pseudobulbs are also used (Kumar and Reddy 2011; Yeung 2017). The use of pseudobulbs as explants for the regeneration of whole plants has not been previously documented in species of the genus *Catasetum*. However, in other orchids such as *Coelogyne ovalis*, *C. cristata*, *Cymbidium finlaysonianum*, *Dendrobium palpebrae*, *Lycaste aromatica*, and *Malaxis acuminata*, efficient protocols have been established for the micropropagation process with pseudobulbs (Mata-Rosas et al. 2010; Deb and Arenmongla 2014; Islam et al. 2015; Sharma 2017; Bhowmik and Rahman 2020; Sharma 2021). In this study, the use of roots and pseudobulbs explants demonstrates the high regenerative capacity of these explants. With these results, we suggest that the morphogenetic responses obtained are dependent on the explant type and even the part of the explant that is used for *in vitro* culture.

The basal and apical sections always induced the highest level of shoot formation, but in the case of medial sections, the responses observed were very low. This effect was also observed and documented in the micropropagation of the orchid *Lycaste aromatica* (Mata-Rosas et al. 2010).

Table 4. Effects of BAP and IAA on direct organogenesis of *C. integerrimum* pseudobulbs explants after 16 wk of culture.

Treatment label	Growth regulators (mg L^{-1})		Explant response (%)	Number of shoots/explant	Number of leaves/shoot	Number of roots/explant	Root Length (cm)	Plant height (cm)
	BAP	IAA						
T1	0	0	30	0.37 ± 0.18 ab	1.75 ± 0.86 b	1.00 ± 0.50 bc	2.66 ± 0.14 c	4.00 ± 0.28 c
T2	1.0	0	40	0.37 ± 0.18 ab	1.75 ± 0.86 b	1.37 ± 0.67 bc	3.70 ± 0.50 b	6.10 ± 0.30 b
T3	2.5	0	60	0.87 ± 0.35 ab	2.50 ± 0.94 b	1.50 ± 0.46 bc	1.14 ± 0.05 e	2.74 ± 0.42 c
T4	0	0.5	60	0.87 ± 0.22 ab	2.75 ± 0.61 b	2.37 ± 0.62 b	3.35 ± 0.19 b	4.27 ± 0.32 c
T5	1.0	0.5	50	0.50 ± 0.18 ab	1.62 ± 0.62 b	1.12 ± 0.44 bc	1.55 ± 0.13 de	2.97 ± 0.76 c
T6	2.5	0.5	20	0.25 ± 0.16 b	0.87 ± 0.63 b	0.37 ± 0.26 c	2.60 ± 0.20 c	3.70 ± 0.82 c
T7	0	1.0	30	0.37 ± 0.18 ab	1.50 ± 0.75 b	1.37 ± 0.73 bc	3.33 ± 0.14 b	6.46 ± 0.72 b
T8	1.0	1.0	80	1.00 ± 0.00 a	5.50 ± 0.18 a	4.37 ± 0.37 a	4.88 ± 0.20 a	7.96 ± 0.12 a
T9	2.5	1.0	50	0.75 ± 0.25 ab	2.87 ± 0.14 b	2.12 ± 0.76 bc	2.04 ± 0.10 cd	4.06 ± 0.26 c

Values are means ± standard error (n = 10). Means followed by the same letters in each column are not significantly different at $p < 0.05$ using Duncan's multiple range test (DMRT). BAP = 6-benzylaminopurine and IAA = indole-3-acetic acid.

Finally, in the Figure 4 we propose the whole protocol for the obtention of *C. integerrimum* vitroplants by direct organogenesis, without the need to use new capsules or any plant section of a wild plant.

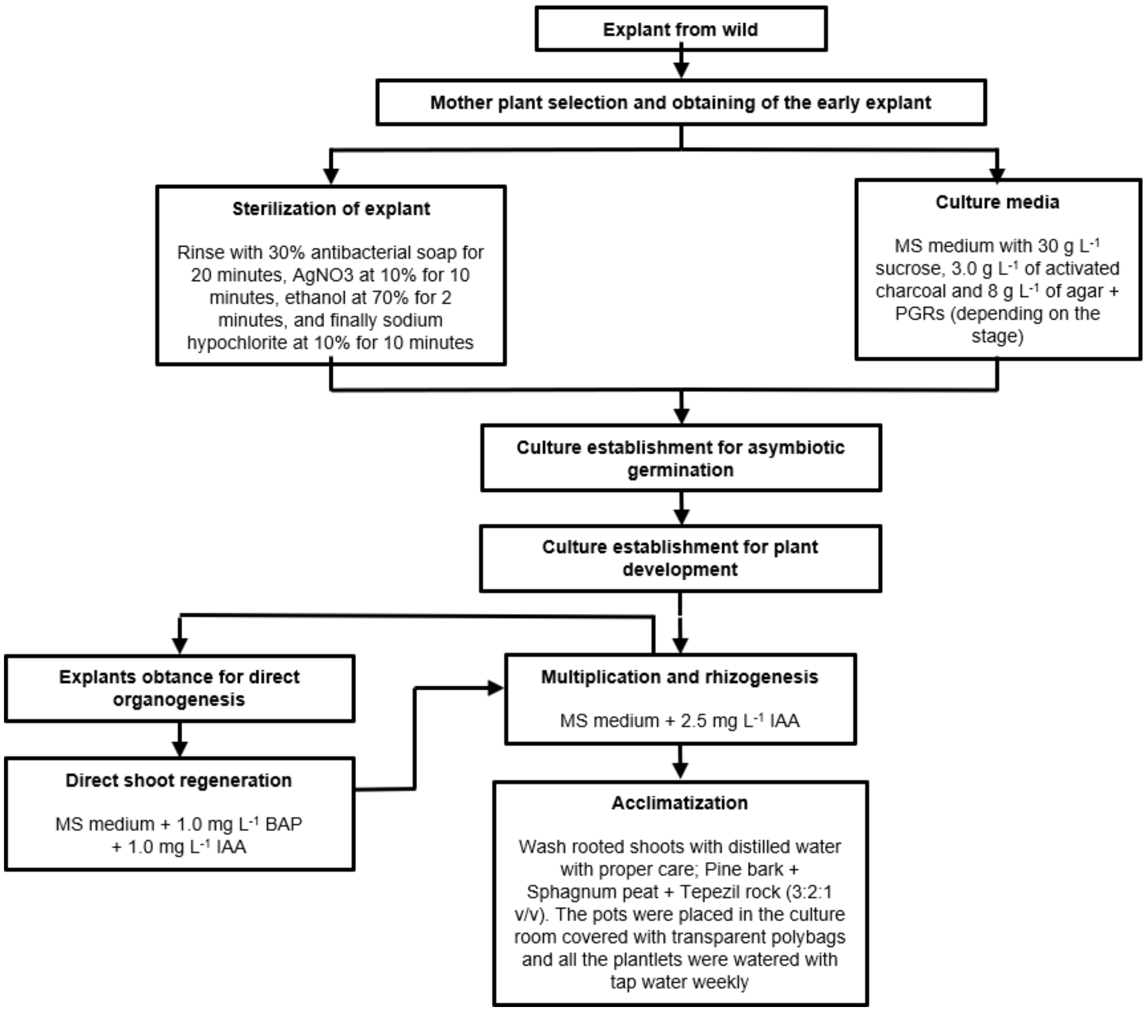

Fig. 4 Protocol for the unlimited obtention of *C. integerrimum* vitroplants by direct organogenesis.

Acclimatization of *C. integerrimum in vitro*-raised plantlets

C. integerrimum in vitro-rooted plantlets were successfully acclimatized at a 95% survival rate in a growing media mixed of pine bark, Sphagnum peat, and Tepezil rock (3:2:1) (Fig. 3I). For the study of secondary metabolites, micropropagation process and conservation efforts, the formation of new plants through direct organogenesis is preferable, since plants obtained in this way tend to maintain their genetic stability (Ghimire et al. 2012; Jung et al. 2021).

Conclusions

For micropropagation of *C. integerrimum*, the use of 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} IAA is needed for shoot production. For its root development, we suggest the addition of 5.0 mg L^{-1} of IBA or IAA. Furthermore, the use of pseudobulbs as explants added with 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} IAA results in the best method for the production of whole plantlets of *C. integerrimum* by direct organogenesis. Root explants are functional, but the response is very low and, in many cases, shoot induction is not achieved. Interestingly, it is not necessary the use of PGRs to induce a morphogenetic response using root explants. We suggest expanding the study of roots as an explant in species of the genus *Catasetum* and other orchids. Finally, *C. integerrimum* leaves are not efficient to induce a morphogenetic response. The plantlets obtained in the present study could be used either as material for further research or to satisfy horticultural demand, thus reducing pressures on wild populations. Therefore, this research represents a direct contribution to the conservation and sustainable use of this valuable natural resource.

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CHAPTER VI

Antidiarrheal action, diuretic effects, phytochemical profile and antioxidant potential of pseudobulbs of *Catasetum integerrimum* Hook (Orchidaceae)

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Abstract

Catasetum integerrimum Hook. is an epiphytic orchid distributed in tropical areas such as the Huasteca region in the state of San Luis Potosí. In several municipalities of this region, the population uses this orchid for treating kidney and gastrointestinal conditions. This research contributed to the knowledge of the medicinal properties of this orchid, evaluating and validating its pharmacological activities. An ethanolic extract of pseudobulbs (EEP) from wild orchids was obtained. Furthermore, a phytochemical profile and ABTS and DPPH tests were carried out to evaluate the antioxidant potential. Finally, to validate the medicinal potential reported by the population of Huasteca Potosina, the diuretic and antidiarrheal activity of EEP were evaluated. The phytochemical profile detected the presence of phytosterol alkaloids, terpenoids, sesquiterpene lactones, flavones, coumarins, phenolic hydroxyls, glycosides, unsaturations, sesquiterpene lactones, quinones, and saponins. Regarding the antioxidant potential, the ABTS assay found a 78% inhibition of free radicals in the presence of the sample, whereas a 52% inhibition of free radicals was obtained in the DPPH assay. EEP at 100 mg/kg did not show a diuretic effect. Concerning the antidiarrheal potential, EEP showed effectiveness by inhibiting the diarrheal process by 76%, and the ED_{50} of EEP resulted in 2 mg/Kg. Likewise, it was observed that EEP retarded the onset of diarrhea by 50%, compared to the effect caused by the reference drug loperamide. EEP also significantly ($p<0.05$) reduced gastrointestinal transit. EEP 10 mg/kg showed similar activity compared to 2.5 mg/Kg loperamide, inhibiting intestinal fluid accumulation. These results provide the first study to demonstrate the antidiarrheal potential of this orchid.

Keywords: antidiarrheal potential, medicinal orchids; medicinal plants; *Catasetum* genus, Nahuatl population

Introduction

Catasetum integerrimum (Orchidaceae), distributed on the slopes of the Gulf of Mexico, Guatemala, Belize, Honduras, El Salvador, and Nicaragua, is an epiphyte orchid that reaches 70 cm in height with a bloom time during most of the year. *C. integerrimum* commonly known in some Mexican regions as “pig's horn” or “trompa de cochino” in Spanish, is used by inhabitants of the Tenek and Nahuatl ethnic groups in the Huasteca Potosina region, Mexico to treat tumors, abscesses, wounds, kidney diseases, and diabetes mellitus (Alcorn 1984; Alayón-Gamboa 2011; Teoh 2019).

No studies have reported *in vivo* or *in vitro* biological activity of this plant species. Two Mexican species of this orchid genus have reports of pharmacological activities and phytochemical information. For instance, four chemical compounds, including the phenanthrene 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene were isolated from an ethanolic extract of the aerial parts of *C. barbatum* and tested on their anti-inflammatory and antinociceptive activity through the carrageenan-induced plantar edema test and the histamine-induced contortion tests in rats (Shimizu et al., 1988).

On the other hand, an aqueous extract prepared from *C. macroglossum* pseudobulbs showed anti-inflammatory activity on the carrageenan-induced plantar edema test in Wistar rats. These properties were attributed to the presence of flavonoids. An HPLC analysis determined the presence of phenanthrene and stilbene dihydro derivatives (Ramos et al., 2012).

Current works on *C. macroglossum* suggested the presence of phenols, flavonoids, various sugars, and some fatty acids in this plant species, and the ethanolic extract showed antioxidant activity (Molina-Sandoval, 2020; Buenaño-Morales and Santillán-Chávez, 2021). The use of *C. integerrimum* pseudobulbs in Mexican folk medicine has not been fully explored. In this study, we assessed for the first time, the antidiarrheal action, diuretic effects, and antioxidant potential of an ethanol extract of *C. integerrimum* pseudobulbs.

Materials and methods

Plant material

Pseudobulbs of *C. integerrimum* were collected in the community of Antiguo Tambolón, belonging to the municipality of Ciudad Valles, state of San Luis Potosí, Mexico (coordinates: 21° 49′ 45.89″ North and 98° 57′ 28.98″ West), at an altitude of 32 m. José García Pérez (UASLP) identified the plant material. Voucher samples (SLPM-49405) were preserved for further reference in the herbarium Isidro Palacios of the Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí (SLPM).

Preparation of the ethanol extract of *C. integerrimum pseudobulbs* (EEP)

Pseudobulbs of *C. integerrimum* were dried and ground. The powder (100 g) was extracted with absolute ethanol using the Microwave (Multiwave Pro Solv). The extract was filtered using a Whatman No.1 filter paper and concentrated in vacuo using a rotary evaporator (model B-100; Buchi AG, Flawil, Switzerland).

In vivo assays

Animals

Male BALB/c mice (25-30 g) were obtained from the University of Guanajuato animal facility. Mice were kept in cages under a controlled environment (24°C, 12 h light/dark cycle). Animals were handled following national (Official Mexican Norm NOM 062-ZOO-1999, Technical specifications for the production, care, and use of laboratory animals), and international protocols (Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals). The Research Ethics Committee of the University of Guanajuato reviewed and approved the protocol of this study (CIBIUG-P48-2022).

Diuretic activity

The methodology proposed by Hill and Randall (1976), with some modifications, was followed for this test. Mice were deprived of food for 12 hr prior to the experiment, but with access to water *ad libitum*. Animals (n = 10 per group) were orally administered with 1 ml of saline solution (vehicle group), 100 µl of EEP (100 mg/kg), or furosemide (10 mg/kg). Furosemide was used in this experiment due to its use for the treatment of edema (fluid retention; excess fluid retained in body tissues). After 15 min of drug administration, mice received 1 mL/kg physiological saline (0.9%) and were individually placed in metabolic cages, containing stainless steel sieves to retain feces. The urine was collected in gradual vials for 5 hr. Animals were restricted from food and water during the experimentation.

Antidiarrheal activity

Castor oil-induced diarrhea

Mice were deprived of food for 16 h before the induction of diarrhea. Mice received saline solution (vehicle), loperamide (2.5 mg/kg p.o.), and EEP (1, 10, 50 and 100 mg/kg p.o.). After one hour of drug administration, castor oil (4 ml/kg p.o.) was administered to each mouse through an esophageal cannula. Ricinoleic acid contained in castor oil produced irritation and inflammation of the intestinal mucosa through the release of prostaglandins, reduced fluid absorption and finally a consequent induction of diarrhea. Animals were placed individually in acrylic cylinders. The number of diarrheic feces and the onset of diarrhea were recorded for 5 h (Calignano et al., 1997). Effective dose 50 (ED_{50}) was calculated by linear regression analysis.

Small intestine transit

Mice were deprived of food for 16 h before the experiment. Mice were treated with saline solution, EEP (1–100 mg/kg), or atropine (2.5 mg/kg). After 1 h, 25 ml/kg of a suspension of 10% charcoal was administered through an esophageal cannula to each mouse in order to serve as a marker to calculate the small intestinal transit. Thirty min later, the animals were sacrificed, and the intestine was removed. The distance travelled by charcoal from the pylorus to the caecum was recorded (Meli et al., 1990).

Castor oil-induced enteropooling

In the determination of intraluminal fluid accumulation, mice were deprived of food for 16 h with free access to water. One hour before the experiment, groups of mice (n = 10) were orally administered with saline solution (vehicle), EEP (1–100 mg/kg), or loperamide (2 mg/kg). Mice were individually placed in acrylic cylinders. After 1 h of treatment, the animals were euthanized, the intestine was removed, and the intestinal content was collected and weighed (Valle et al., 2000).

Antioxidant activity

2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay

The assay was conducted as reported previously by Re (1999) with some modifications. For the 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay, 2 mL of the ABTS•+ (Sigma Aldrich) solution was taken and 20 µL of each of the extracts to be evaluated were added. Subsequently, they were mixed for 30 seconds and the absorbance at 734 nm was measured after 6 min in a UV visible light spectrophotometer (AquaMate Plus UV vis - Thermo Fisher Scientific). All measurements were performed in triplicate. The percentage of free radical capture or radical inhibition in the presence of the extract was calculated using the following formula:

$$\%\, inhibition = \frac{A° - A°f}{A°i}\, x\, 100$$

Where:

$A^{o}i$ = Initial absorbance of the radical adjusted to the desired wavelength.

$A^{o}f$ = Final absorbance of the radical plus extract.

2,2-Diphenyl-1-picrylhydrazyl assay

The assay was conducted as reported previously by Brand-Williams et al., (1995) with some modifications. For the 2,20-diphenyl-1-picrylhydrazyl (DPPH) assay, 3 mL of the DPPH• (Sigma Aldrich) radical was taken and mixed with 400 µL of the sample. Then, the reaction mixture was shaken and left in the dark at room temperature. The absorbance at 517 was measured after 20 min in a UV visible light spectrophotometer (AquaMate Plus UV vis - Thermo Fisher Scientific). All measurements were performed in triplicate. Percent free radical capture or radical inhibition in the presence of the extract was calculated using the same formula used for the ABTS Assay.

Statistical analysis

Experimental data are presented as the mean ± standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA). The significance of differences between groups was calculated using by Dunnet test. Values of $p < 0.05$ were considered significant.

Results and Discussion

The phytochemicals present in the EEP were alkaloids, phytosterols, terpenoids, sesquiterpene lactones, flavones, coumarins, phenolic hydroxyls, glycosides, unsaturations, quinones, and saponins. The methodologies of the phytochemical techniques carried out can be consulted in the Annex I of this section. These phytochemical groups have been found in other species of the Orchidaceae family, some of which are the source of important biological activities (Sut et al., 2017). However, in Mexico, this is the first study reporting the preliminary phytochemical composition of *C. integerrimum*.

Based on the discovery of a wide variety of phytochemicals, such as alkaloids, terpenoids, flavones, coumarins, and quinones, which can trap reactive oxygen species (ROS) and function as potential antioxidants, this study determined the antioxidant potential of EEP. An antioxidant compound is a substance capable of preventing the oxidation process through a neutralizing effect of free radicals. The substance that acts as an antioxidant releases electrons, which capture free radicals and maintain the stability of the organism (Gulcin, 2020).

There are several colorimetric methods for estimating the *in vitro* antioxidant capacity of plant extracts. During this research project, the *in vitro* methods ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-Diphenyl-1-

Picrylhydrazil) were used. EEP showed inhibition of free radicals by 78% (ABTS assay) at 6 minutes and 52% (DPPH assay) at 20 minutes.

In the case of the tests to validate the medicinal potential against renal diseases, the diuretic activity test was performed. This test consisted of quantifying the volume of urine produced by the mice. The results showed that did not exist diuretic effect of EEP at a dose of 100 mg/Kg (Figure 1).

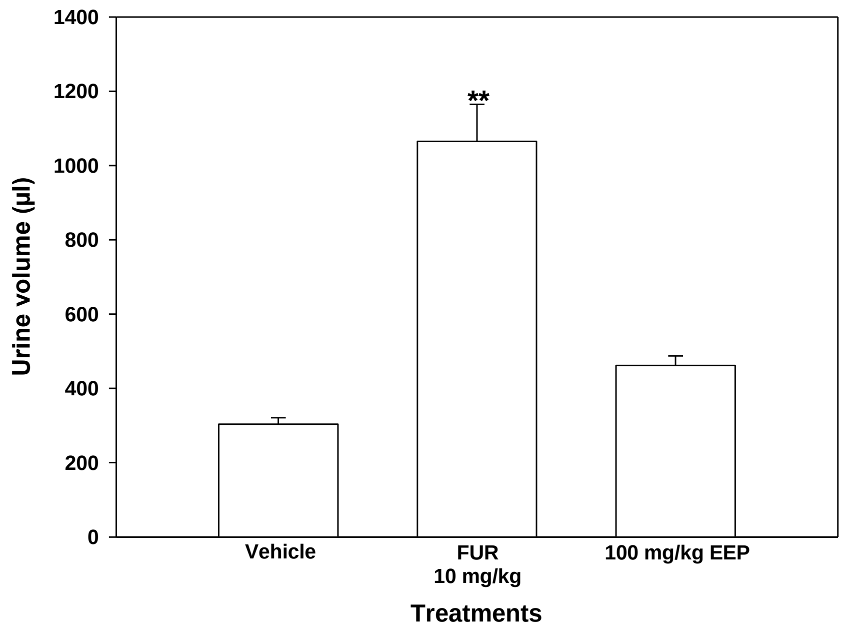

Figure 1. Diuretic activity of the EEP of *C. integerrimum*.

This finding shows that this extract cannot eliminate fluids from the body, a function fulfilled by drugs such as furosemide, used to treat high blood pressure, edema (extra fluid in the tissues), and other medical conditions (Lee et al., 2021). Even though EEP showed no diuretic effect, further tests will determine whether EEP has the potential to inhibit bacterial growth of strains causing kidney diseases, such as *Escherichia coli* and other bacteria of the genus *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Serratia* (Ahmed et al., 2019).

The four doses of EEP showed inhibition of diarrhea, with similar activity, compared to the reference drug. EEP at 1 mg/kg achieved a 40% of diarrhea inhibition, whereas 100 mg/kg EEP produced a very similar effect to that obtained with loperamide, inhibiting diarrheal events by approximately 80% (Figure 2).

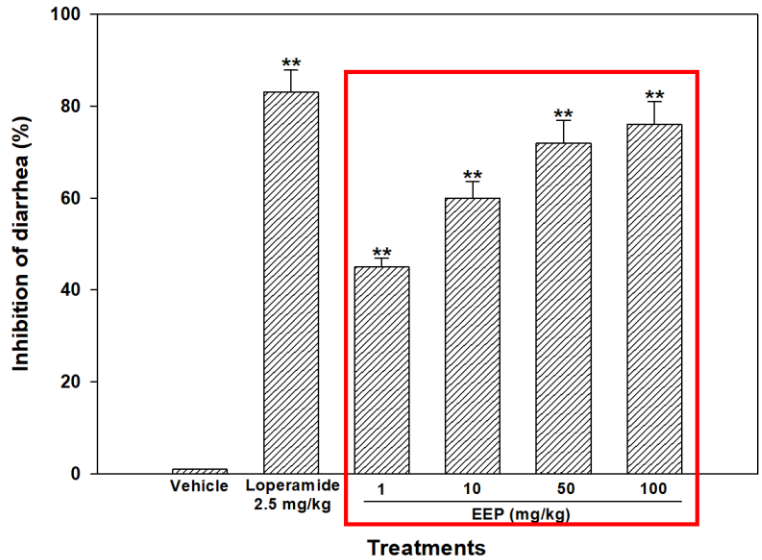

Figure 2. Percentage inhibition of diarrhea induced by castor oil.

Regarding the onset of diarrhea, EEP showed a delay in the onset of diarrhea without a dose-dependent effect, this activity was not comparable to that shown by loperamide (Figure 3).

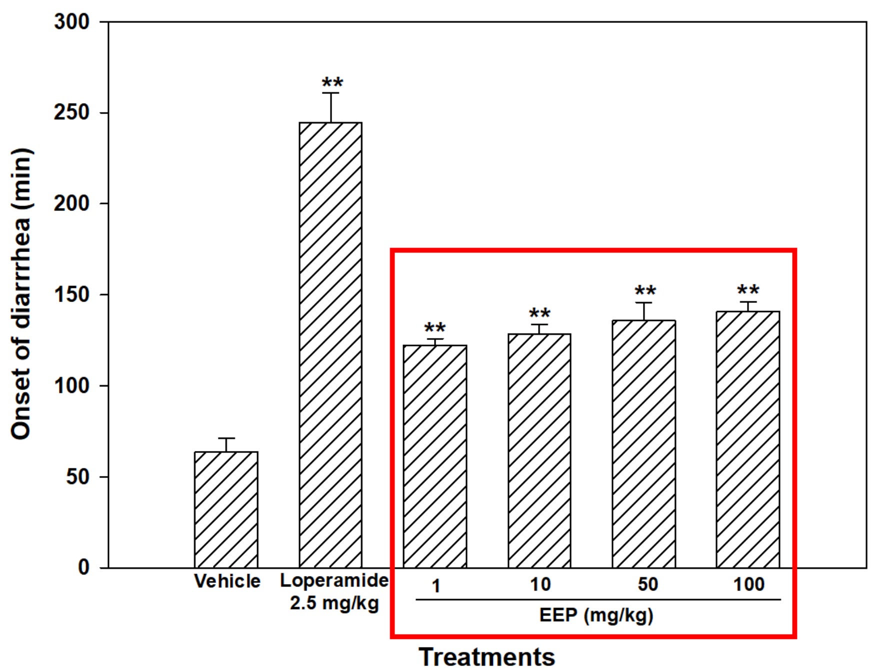

Figure 3. Onset of diarrhea induced by castor oil.

The results showed that the three doses of EEP inhibited intestinal transit. However, as seen in Figure 4, 100 mg/Kg EPP produced half of the effect (42%), compared to the effect manifested by the reference drug (atropine, 78%). These results could explain the use of this plant in traditional medicine to control gastrointestinal diseases since EEP inhibited the movement of diarrheal feces in the intestine. Some studies report amounts of inhibition of intestinal transit of only 20%, even using purified molecules such as diterpene tilifodiolide (Alba-Betancourt et al., 2019).

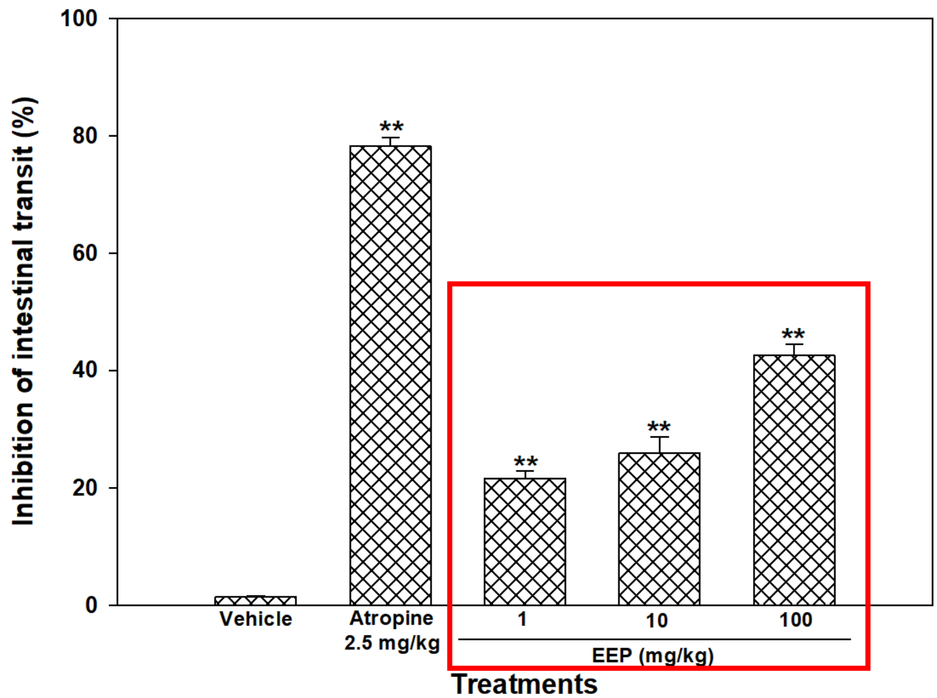

Figure 4. Percentage inhibition of intestinal transit.

Finally, the percentage of inhibition of intestinal fluid accumulation was recorded. In this parameter, the ability of the EEP to prevent fluid accumulation in the intestines, a process that frequently occurs in diarrheal events, was evaluated. The results showed that all three doses of EEP prevented fluid accumulation. EEP 1 mg/kg showed a similar effect to the reference drug (loperamide), achieving approximately 24% inhibition of accumulated intestinal fluid. However, when the EEP dose was increased, the pharmacological effect decreased (Figure 5).

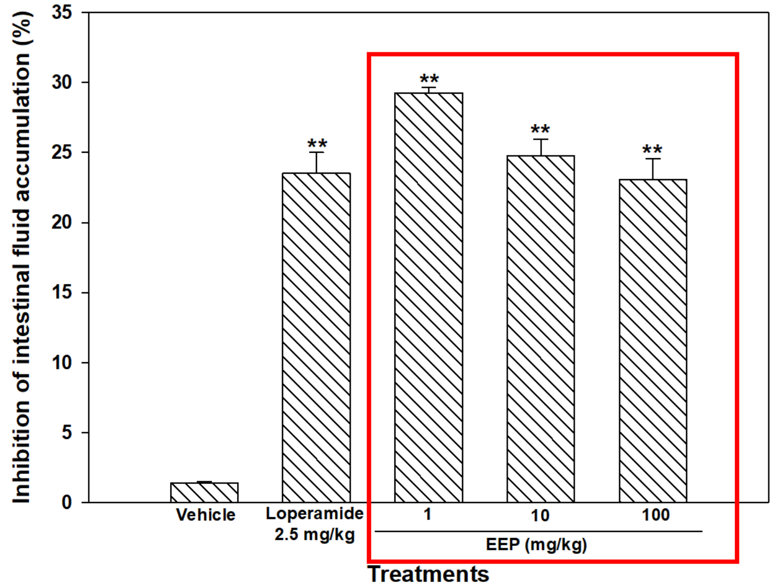

Figure 5. Percentage inhibition of intestinal transit.

The mean effective dose (DE_{50}) was also determined, a parameter that indicates the dose at which 50% of a population presents the expected effect. When carrying out the regression analysis with the concentrations and data obtained with the percentage of inhibition of diarrhea, a DE_{50} of 2 mg/Kg of EEP was obtained to observe the antidiarrheal effect (Figure 6).

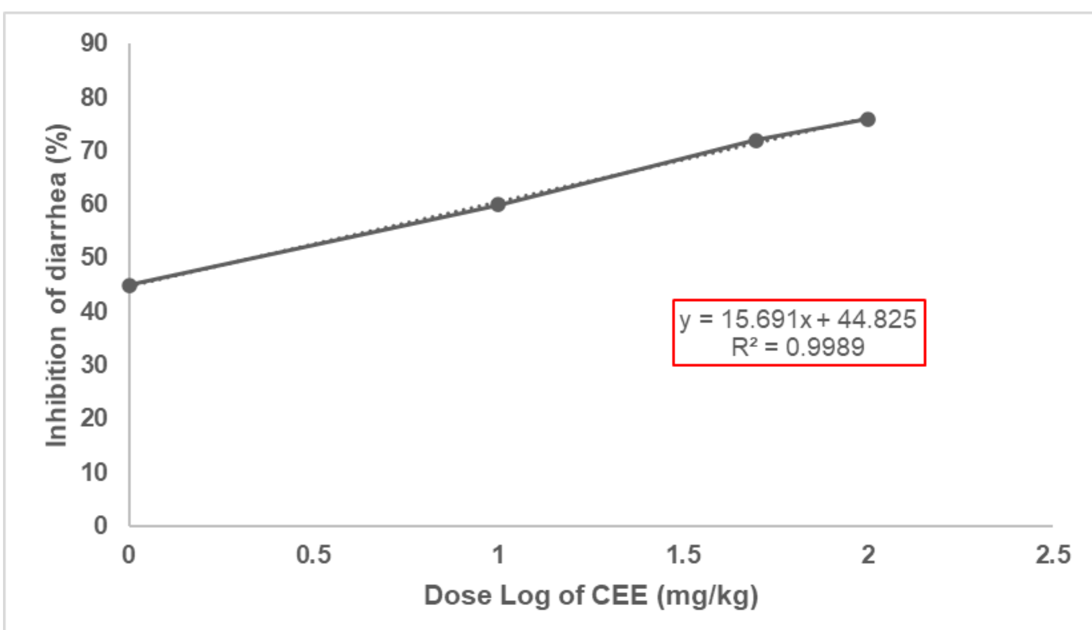

Figure 6. Determination of the ED_{50} of the EEP of *C. integerrimum*.

However, it is important carry out new experiments with smaller doses to show the effect caused more efficiently. This result is interpreted with the fact that only 2 mg of EEP are necessary to observe an effect in half the population. Interestingly, it is one of the highest ED_{50} in terms of medicinal plant analysis that has been carried out in our research team.

Conclusion

These results showed the huge potential of *C. integerrimum* as a plant with antidiarrheal properties. However, this study must be complemented with more pharmacological tests that contribute to understanding and characterizing the bioactive compounds that this plant can provide, with a perspective to developing possible new drugs.

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Annex I

Determination of phytochemical compounds

For the phytochemical analysis, micro-scale qualitative techniques were used, which made it possible to determine if there is a presence (+) or absence (-) of secondary metabolites, through colorimetric and precipitation reactions given by specific compounds.

Determination of alkaloids

Mayer's test: 200 µL of the extract were placed in a test tube, later 3 drops of Mayer's reagent were added through the walls. The presence of a creamy-white precipitate indicates a positive test. Distilled water was used as a negative control and caffeine as a positive control.

Wagner's test: 200 µL of the extract were placed in a test tube, later 3 drops of Wagner's reagent were added through the walls. A reddish-brown precipitate confirms the test as positive. Distilled water was used as a negative control and caffeine as a positive control.

Determination of sterols

Liebermann-Burchard reaction: 200 µL of the extract were dissolved in a test tube together with 200 µL of chloroform. From the previous solution, 200 µL were taken and mixed with 200 µL of acetic anhydrous and 200 µL of concentrated H_2SO_4. A series of color changes shows the presence of phytosterols. A blue-green ring indicates the presence of terpenoids. Distilled water was used as a negative control and almond oil as a positive control.

Determination of unsaturations

A 2% solution of $KMnO_4$ in water was added dropwise to a test tube with 200 µL of the extract. Discoloration of the solution or formation of a brown precipitate indicates the formation of manganese dioxide (positive reaction). Distilled water was used as a negative control and olive oil as a positive control.

Determination of sesquiterpenlactones

Baljet test: In this test, the mixture of two solutions in equal volumes was used. Solution A: 0.5 g of picric acid was placed in 50 mL of ethanol. Solution B: 5 g of NaOH in 50 mL of water was added. For the test, 200 µL of the extract and four drops of the reagent were added. The test is positive if the mixture turns orange or dark red. Distilled water was used as a negative control and celery as a positive control.

Determination of flavonoids

H_2SO_4 test: 200 µL of the extract were placed in a test tube, then 200 µL of concentrated sulfuric acid were added. The test was positive when yellow colorations were observed for flavones and flavanols; orange-cherry for flavones; red-bluish for chalcones and red-purple for quinones. Distilled water was used as a negative control and guava as a positive control.

Determination of saponins

Salkowski test: In a test tube, 200 µL of the extract were dissolved together with 200 µL of chloroform and 200 µL of concentrated H_2SO_4. The test was positive if a yellow or reddish-brown color developed. Distilled water was used as a negative control and cassava as a positive control.

Shinoda test: Magnesium filings were added to a test tube with 200 µL of extract, followed by drops of concentrated HCl added to the walls. The test was positive with the appearance of orange, red, pink, pink-blue to violet colors. Distilled water was used as a negative control and cassava as a positive control.

Determination of coumarins

NaOH test: 200 µL of the extract were placed in a test tube and 200 µL of 10% NaOH solution was added. The test was positive when a yellow color appeared that disappeared when acidifying with 200 µL of HCl. Distilled water was used as a negative control and cinnamon as a positive control.

Determination of phenolic hydroxyls

$FeCl_3$ test: 200 µL of the extract were placed in a test tube and drops of 5% $FeCl_3$ were added. The test was positive with the appearance of a red, blue-violet or green precipitate.

Determination of glycosides

Keller-Killiani test: 0.5 g of the extract was mixed with 5 mL of distilled water in a test tube. Subsequently, 2 mL of glacial acetic acid were added with a few drops of ferric chloride, followed by 1 mL of H_2SO_4. The formation of a brown ring at the interface indicated a positive result for cardiac glycosides.

CHAPTER VII

General discussion and conclusions

The Orchidaceae family is one of the most diverse and evolved families in the plant kingdom. Currently, the number of species is more than 35,000 worldwide. These species are distributed on all continents except for Antarctica and the most arid deserts. The region on the planet with the greatest distribution of orchids is the tropics, and the preferred ecosystems for orchids to thrive are cloud forests, tropical jungles, and boreal paramos. These species are valued as ornamental plants due to their rarity, color, perfume, and shapes. The fruits of orchids are edible, and some other orchids are valued for their medicinal potential.

An example of a medicinal orchid is *C. integerrimum*, an epiphytic orchid distributed in the low forests of the Huasteca Potosina. This species was studied during this research project, due to the medicinal potential reported by the Nahuatl ethnic group in this region. The Nahuatl population of several municipalities affirms that this orchid is used to treat and alleviate kidney and gastrointestinal diseases and diabetes mellitus.

Orchids have a wide distribution in Mexico, constitute a high degree of endemism, and are also one of the most vulnerable and threatened families in the country. These data were evidenced in Chapter I through a systematic review of the endemic orchids of Mexico. For this reason, it is needed to implement propagation methods using plant biotechnology as an environmental prevention and control alternative for the conservation of these species. Other works have already been successfully worked on in other parts of the world and with different plant species this information is mentioned in the review about the biotechnological use of medicinal plants in Chapter II.

The development of this work presents a comprehensive solution to three socio-environmental problems: 1) the conservation of the traditional medicine heritage in the Huasteca Potosina region, which has gradually been lost, taking with it a large amount of valuable information on medicinal plants with a huge potential for possible bioactive compounds, 2) the establishment of an *in vitro* micropropagation protocol by direct organogenesis way, which allows obtaining an unlimited production of useful vitroplants for physical, chemical and biological studies, without the disadvantage of affecting wild populations. Furthermore, this biotechnological protocol can probably be replicated for other plant species, and 3) the scientific validation of the antidiarrheal medicinal potential conferred by the Nahuatl population of the Huasteca Potosina to this orchid.

The solution propose for these environmental problems, provides valuable and necessary information for the knowledge of medicinal plants, due in Mexico only 5% of the plants to which an empirical medicinal property is conferred have been confirmed. Currently, there was no data on the number of medicinal orchids distributed in the country, which became evident with the creation of the first review on this subject, described in Chapter III.

Ones of the Mexican orchids with medicinal potential are those of the *Catasetum* genus. A genus little studied worldwide because it lacks ornamental, nutritional, and medicinal importance. In this sense, the contribution of the review published in Chapter IV provides the first knowledge on the therapeutic properties, biotechnological works, and ecological relationships focusing specifically on this genus.

Chapter V of this research, the possibility of unlimited production of *C. integerrimum* vitroplants was confirmed by an *in vitro* micropropagation biotechnological protocol. This technology is an advantage when studying bioactive compounds from plants that have conservation problems, as in the case of orchids. However, in this part of the project, it is necessary to mention that the hypothesis raised at the beginning of the investigation, which established that vitroplants could produce the same or more phytochemicals than wild plants, is rejected. Phytochemical compounds in vitroplants are produced in less diversity than in wild plants.

With the contribution developed in Chapter VI, it was found that the ethanolic extract of *C. integerrimum* pseudobulbs (EEP) has an important antioxidant capacity and the presence of a complex diversity of phytochemicals. These phytochemicals could confer medicinal potential to this orchid. According to the reported medicinal properties, EEP does not have a diuretic effect. Nevertheless, this result does not imply that it can be used to control kidney infections caused by microorganisms. On the other hand, the antidiarrheal potential in this orchid is highly effective. The calculated ED_{50} was 2 mg/Kg, and it was found that EEP can inhibit diarrhea mainly through intestinal fluid accumulation, in addition to delaying the onset of diarrheal processes.

Consequently, the analysis of the medicinal potential of EEP is promising and it opens new ways of investigation and application as a source of bioactive compounds. Furthermore, it is possible to develop future drugs. In addition, it validates the use of this plant as alternative medicine in the Huasteca Potosina communities.

Finally, the results obtained allowed us to satisfactorily the specific objectives and the general objective of this project. The comparison between the production of phytochemicals in vitroplants and wild plants was an achievement. Moreover, the biological and medicinal potential of *C. integerrimum* was scientifically validated.

Overall conclusions

The results of this research lead to four overall main conclusions:

1. Orchids are plants studied from ecological, ornamental, and nutritional perspectives and some species are for their medicinal properties. In Mexico, 64 species of orchids are considered by several ethnic groups as medicinal plants. However, their information is empirical, so it is necessary to carry out more scientific investigations to validate this information.
2. It is necessary to develop some intervention projects in communities that promote the appropriation of this ethnopharmacological knowledge.
3. Techniques based on plant biotechnology are useful for preventing and controlling resources for threatened plant species. The establishment of *in vitro* protocols produces enough plant material that serves as a source of raw material for the creation of extracts that allow chemical, biological,

biochemical, phytochemical, and pharmacological studies. However, the diversity of phytochemicals is not greater than those produced in wild plants.

4. The ED_{50} of the ethanolic extract of *C. integerrimum* pseudobulbs is 2 mg/Kg. This dose inhibits and delays diarrheal processes. However, to reduce fluid accumulation and intestinal transit. The phytochemicals contained in this plant have antidiarrheal action and may be useful for treating gastrointestinal diseases.

Recommendations and future prospects

Recommendations and perspectives for the future work are listed below:

- To realize more fieldwork in other municipalities of the Huasteca Potosina where *C. integerrimum* is distributed, and more medicinal uses can be registered. Furthermore, it is important to work on the abundance of this species to update its distribution status.
- To formulate and apply an intervention project in communities with extensive knowledge of traditional medicine to identify and preserve this ancestral knowledge.
- To realize ecological and biochemical studies to determine if the diversity of phytochemicals produced in each plant depends on ecological aspects, such as height, temperature, host phorophyte, and relationships with other organisms.
- To add antioxidants in micropropagation procedures by indirect organogenesis way to evaluate the phytochemicals production in the friable callus stage.
- To evaluate the *in vitro* micropropagation process by indirect organogenesis way, in a semi-solid medium and using temporary immersion bioreactors.
- To induce different types of physical, chemical, and biological stress in vitroplants to evaluate the phytochemicals production.
- To evaluate the antimicrobial activity of EEP from *C. integerrimum* against strains that cause kidney and gastrointestinal infections.

- To evaluate the hypoglycemic effect of the EEP of *C. integerrimum*.
- To characterize the EEP of *C. integerrimum* to know the molecule(s) that could be causing the antidiarrheal effect.
- To scale up with the tests of antidiarrheal effect with the perspective of manufacturing a new drug.

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REVIEW

The endemic orchids of Mexico: a review

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Abstract
Orchidaceae is the most extensive family of flowering plants. There are approximately 30,000 - 35,000 species, belonging to 850 genera, dispersed in almost all the ecosystems on earth, except in polar zones and extreme deserts. In Mexico, there are more than 1,200 species distribuited in approximately 170 genera, of which 40% are endemic to the country. In this review, aspects related to geographical distribution, ecology, conservation, biotechnological applications and uses of orchids growing in Mexico are discussed. The geographical zones where their ecological niches are located are described and the zones that belong to priority conservation areas in Mexico are highlighted. In addition, all works on Mexican orchids related to micropropagation, medicinal uses, beneficial applications for humans, and microorganism-host interaction are discussed. In summary, this study provides perspectives for new studies and research focused on the use, propagation and conservation of Mexican endemic orchids.

Keywords Mexican orchids · conservation · ecology · endemic species

Article published in the JCR International Journal "Biologia"

In Vitro Cellular & Developmental Biology - Plant (2022) 58:279–289
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PLANT TISSUE CULTURE

Micropropagation of *Catasetum integerrimum* Hook (Orchidaceae) through seed germination and direct shoot regeneration from pseudobulbs and roots

Luis J. Castillo-Pérez[1] · Angel Josabad Alonso-Castro[2] · Javier Fortanelli-Martínez[3] · Candy Carranza-Álvarez[4]

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Abstract
Catasetum integerrimum is an orchid used in Mexican traditional medicine, which is overexploited due to its ornamental and medicinal properties. In this scenario, conservation strategies are mandatory to protect wild populations from this valuable orchid. The aim of the present study was to establish a micropropagation protocol by direct organogenesis, as a tool for *Catasetum integerrimum* production and conservation. Seeds from a mature capsule were cultivated in a Murashige and Skoog basal medium. Then, 6-mo-old vitroplants were transplanted into treatments fortified with 6-bencilaminopurin (BAP) or indole-3-acetic acid (IAA) to induce plant development. Treatment fortified with 2.5 mg L^{-1} BAP in combination with 5.0 mg L^{-1} IAA developed 5.73 ± 0.45 shoots per explant and 5.84 ± 0.48 leaves per shoot. For the rooting process, the best treatment was 2.5 mg L^{-1} IAA, which developed 11.20 ± 0.28 roots with a 13.20 ± 0.28-cm root length. *C. integerrimum* vitroplants were used as a source of leaves, roots, and pseudobulbs for the induction of direct organogenesis. Leaf explants did not induce any morphogenic response. Pseudobulb explants had the best response when developing 1 shoot per explant with 5.50 ± 0.18 leaves per shoot and 4.37 ± 0.37 roots per explant with a 4.88 ± 0.20-cm root length. For the acclimatization stage, a survival rate of over 90% was achieved. The results obtained prove the efficacy of the micropropagation method, which contributes significantly to the conservation of this orchid, preserving its wild populations from indiscriminate collection.

Keywords Direct organogenesis · Pseudobulb explant · Root explant · *Catasetum* genus

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ACADEMIC PRESS

Phytomedicine
A Treasure of Pharmacologically Active Products from Plants
2021, Pages 35-58

Chapter 2 - Biotechnological approaches for conservation of medicinal plants

Luis Jesús Castillo-Pérez [a], Angel Josabad Alonso-Castro [b], Javier Fortanelli-Martínez [c], Candy Carranza-Álvarez [d]

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Chapter published in the book “Phytomedicine. A treasure of pharmacologically products from plants” from the Academic Press International Editorial

Re: Status Chapter

jentsung
Para: LUIS JESUS CASTILLO PEREZ
Mar 13/12/2022 08:00 PM

Dear Colleague,

Your chapter has been delivered to the publisher and it still needs months to finalize the editorial work.
Anyway, I'll keep you updated on the status.

All the best,

Prof. Jen-Tsung Chen
Department of Life Sciences, National University of Kaohsiung, Taiwan
-----Original message-----
From:LUIS JESUS CASTILLO PEREZ<jesus.perez@uaslp.mx>
To:jentsung<jentsung@nuk.edu.tw>
Date: Tue, 13 Dec 2022 22:02:14
Subject: Status Chapter
Dear Professor Jen-Tsung Chen

I am sending this email to find out the current status of the chapter that we sent for the book "Advances in Orchid Biology, Biotechnology and Omics".

We will be attentive for your response.

All the best.

Luis J. Castillo-Pérez
Professor at UASLP-MX

Chapter sent for publication in the book "Advances in Orchid Biology, Biotechnology and Omics" from Springer International Editorial

Recibido: 04.03.2019 | Aceptado: 03.04.2019

Palabras clave: Conservación ambiental, ecología de orquídeas, interacciones ecológicas, orquídeas mexicanas y redes ambientales.

¿Cómo crecen y se relacionan las orquídeas?

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Dissemination science article published in the Journal "Universitarios Potosinos", edited by the Universidad Autónoma de San Luis Potosí

Certificate of Participation

LatinXChem

This is to certify that Luis Jesús Castillo-Pérez, Ángel Josabad Alonso-Castro, Javier Fortanelli-Martínez, and Candy Carranza-Álvarez participated in the #LatinXChem Twitter Conference 2020 on Sept 7th 2020 with the poster entitled "Efecto de dos reguladores de crecimiento vegetal sobre la morfogénesis in vitro de Catasetum integerrimum (Orchidaceae)".

Dr. Gabriel Merino
Organizer

Dr. Miguel Ángel Méndez
Organizer

Dra. Luciana G. de Oliveira
Organizer

Dissemination of research advances at the "LatinXChem 2020 International Congress"

Dissemination of research advances at the "1st Virtual Forum for Scientific Dissemination", organized by the Graduate Program in Biosciences of the Universidad de Guanajuato.

Dissemination of research advances at the "LatinXChem 2021 International Congress"


Asociación Mexicana de Orquideología, A.C.

50 Aniversario

Otorga la presente constancia a:

M.C. Luis Jesús Castillo Pérez y Dra. Candy Carranza Álvarez

Por presentar la ponencia de:

"Conservación y estudios fitoquímicos de orquídeas de la Huasteca Potosina"

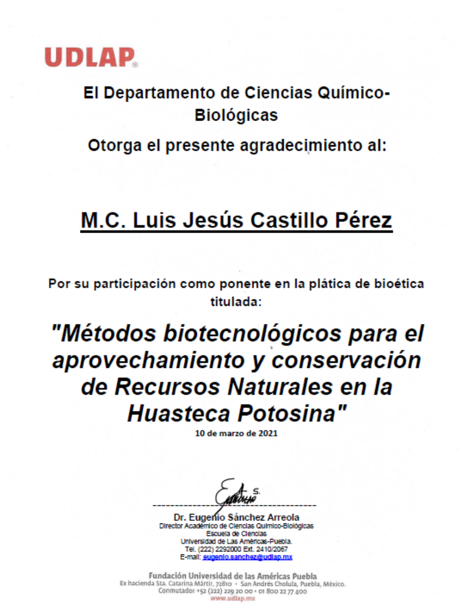
Presentada durante el evento académico de la Exposición de Otoño de Orquídeas 2021

Realizado del 14 al 17 de octubre de 2021 en la Ciudad de México

Presidente:
Dra. Adriana Becerril Montes

Coordinador programa académico:
Dr. Eduardo A. Pérez García

Dissemination of research advances at the academic event of the "Fall Orchid Exhibition 2021", organized by the Asociación Mexicana de Orquideología A.C.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El Departamento de Ciencias Químico-Biológicas

Otorga el presente agradecimiento al:

M.C. Luis Jesús Castillo Pérez

Por su participación como ponente en la plática de bioética titulada:

"Métodos biotecnológicos para el aprovechamiento y conservación de Recursos Naturales en la Huasteca Potosina"

10 de marzo de 2021

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