Centro de Investigación en Alimentación y Desarrollo, A.C.

TAXONOMÍA Y ACTIVIDAD ANTIPROLIFERATIVA DE AISLADOS DE *Ganoderma*DE SONORA: MICELIO *VS* CUERPOS FRUCTÍFEROS

Por:

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TESIS APROBADA POR LA:

COORDINACIÓN DE TECNOLOGÍA DE ALIMENTOS DE ORIGEN VEGETAL

Como requisito parcial para obtener el grado de

DOCTOR EN CIENCIAS

Hermosillo, Sonora Octubre 2016

APROBACIÓN

Los miembros del comité designado para la revisión de la tesis de Damian López Peña, la han encontrado satisfactoria y recomiendan que sea aceptada como requisito parcial para obtener el grado de Doctor en Ciencias.

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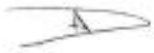

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Dr. Pablo Wong González
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AGRADECIMIENTOS

A todos los ciudadanos contribuyentes y a CONACYT que distribuye las becas a los estudiantes de posgrado

Al CIAD, por abrirme sus puertas para realizar mis postgrados

A mi familia: Ana Peña, Jorge López, Manuel López y David Haney

A mi director de tesis: Dr. Martín Esqueda Valle por su confianza y apoyo

A mis asesores de tesis: Dra. Mayra de la Torre, Dr. Jesús Hernández, Dra. Mary Islas, Dra. Carmen Contreras, Dr. Valenzuela por el apoyo brindado y por sus críticas constructivas hacia este trabajo de investigación

A mis amigos y colegas: Heriberto Torres Moreno, Guadalupe López Robles, Edgar Rascón, Alexel Urgara, Mónica Reséndiz, Eduardo Hernández, Idaly Morales, Alberto Jiménez, Aldo Gutiérrez Saldaña, Rafa Coronado, Georgina Vargas Rosales, María del Carmen Granados Nevares, Nico, Jorge Mercado, Karla Martínez Robinson, Antonio Orozco Avítia, Javier Ojeda, Jesús Manuel García Robles, Elizabeth Peralta, Alberto Sánchez, Francisco Soto, Alfonso Sánchez por el apoyo técnico brindado

A mis amigos: Esmeralda, Eric, Guerra, Gonzalo, Tania, Hery, Max, Raúl, Wenses, Javier, Rubén, Norberto, Tito, Manuel, Fabiola (Chichi), Beto, Idaly, Jorge, Josué, Lupita, Nico, Pame, por todos esos buenos momentos

Un agradecimiento especial a la Dra. Ana Ramos Valdivia, por su apoyo y por transferir su conocimiento sin pedir nada a cambio, una gran revolucionaria

Un agradecimiento muy especial para: Dr. Ramón E. Robles Zepeda por su apoyo con los experimentos en la parte de bioactividad y a todos los integrantes del Laboratorio de Productos Naturales de la UNISON, así mismo a todos los integrantes del laboratorio de Inmunología de la UNISON

A todo el personal de la Coordinación de Programas Académicos: Argelia Marín Pacheco, Héctor Galindo Murrieta, Verónica Araiza Sánchez, Laura Elizabeth García Cruz, Norma Angélica García Sánchez, Ana Aurora Vidal Martínez, Alfonso Sesma Coronado y María Alejandra Córdova Moreno por el apoyo técnico brindado

A don Gerardo Reyna por el apoyo técnico brindado, por todos los raites, las pláticas y por la bibliografía proporcionada

A la Dra. Rosina Cabrera Ruiz, por el apoyo técnico brindado

AGRADECIMIENTOS (continuación)

A la Dra. Verónica Mata por su apoyo con equipo de laboratorio

A la M.C. María del Refugio Robles Burgueño por el apoyo técnico brindado

Gracias a la Dra. Irasema Vargas Arispuru por el apoyo técnico brindado y por prestarnos equipo para cromatografía en capa fina

Al Dr. Marcos Lizárraga, por haberme formado como biólogo y micólogo y por haberme brindado todo su apoyo cuando lo he necesitado, también por ser un amigo leal y una de las personas que me enseñó el valor de la ética profesional

A la Dra. Helvia Pelayo, por todo el apoyo brindado al salir de la carrera y por brindarme toda esa enseñanza en mi formación como biólogo, una de las mejores profesoras que he tenido en toda mi vida

DEDICATORIA

Dedico este trabajo de investigación a las tres personas más importantes en mi vida:

A mí querida madre, Ana Margarita Peña Juárez por todo el apoyo que me has brindado durante toda mi vida, por enseñarme a pensar y tomar decisiones por mí mismo, por enseñarme que el tiempo de todos es igual de valioso, por enseñarme que la vida no tiene nada que ver con las irrealidades que las personas piensan, por enseñarme que nunca se debe de perder la dignidad, por enseñarme que los sueños es lo único importante en esta vida, POR ENSEÑARME LA IMPORTANCIA DE LA LIBERTAD DE EXPRESIÓN… siempre en las buenas y en las malas.

A mi carnal Jorge López Peña, por el apoyo completamente incondicional que me has brindado y por meter las manos al fuego por mí, por ser mi guardaespaldas, por ese espíritu de lucha que siempre me reanimó, por todos los viajes juntos, físicos y mentales…
de los pocos verdaderamente revolucionarios que conozco carnal, viva la REVOLUCIÓN!!, que ya está prácticamente encima de nosotros, que se salve quien esté preparado ;)

A la mujer más maravillosa de este planeta, Esmeralda Heredia, por mostrarme tu fortaleza, por tu presencia, por tu ausencia, por tus ojos y tus miradas, por el infinito en el que me sumerjo tan solo con pensar en ti, por los maravillosos momentos que pasamos juntos, por confiar en mí, por el tiempo que compartes conmigo, por tus comidas, por las noches y los días, por las risas y los enojos, por ser tú!!!,siempre tú!!... mi alma gemela… por todas las vidas

CONTENIDO

RESUMEN

Algunas especies de *Ganoderma*, principalmente *G. lucidum* se han utilizado en la medicina tradicional asiática por más de dos milenios, como tónico medicinal, tanto cuerpos fructíferos como micelio pulverizados. El consumo de este hongo se recomienda para tratar distintos padecimientos, entre estos el cáncer. Algunas especies del género sintetizan triterpenoides con actividad antiproliferativa. *Ganoderma* presenta una distribución cosmopolita, con registros en zonas templadas, tropicales y desérticas. La especie más estudiada y la única que se cultiva a nivel mundial es *G. lucidum*, no obstante existen más de 219 en el género y muchas de ellas aún no han sido estudiadas. En este trabajo se realizaron exploraciones a distintos puntos de Sonora, con el fin de aislar cepas de *Ganoderma* e identificarlas taxonómicamente. Se estudiaron ejemplares de ocho especies: *G. applanatum*, *G. curtisii*, *G. lobatum*, *G. oerstedii*, *G. sessile*, *G. sessiliforme*, *G. subincrustatum* y *G. weberianum*. Este es el primer reporte de *G. lobatum* y *G. oerstedii* en Sonora, y de *G. sessile* y *G. weberianum* en México. Además, se estudió la variabilidad morfológica de una cepa de *G. subincrustatum*, comparando basidiomas silvestres y cultivados. El estudio mostró que los caracteres propuestos para la delimitación de las especies del género (tipo de contexto e incrustaciones, forma de los elementos de la cutícula y esporas) son constantes entre los distintos ejemplares estudiados. También se evaluó la actividad antiproliferativa de extractos de dos fases de desarrollo (micelio dicariótico y cuerpos fructíferos) de aislados de *G. subincrustatum* y *G. weberianum* de Sonora, sobre distintas líneas celulares. Se encontró que los extractos de cuerpos fructíferos presentan mayor actividad que los de micelio. Mediante cromatografía en una columna con sílica se obtuvieron 26 sub-fracciones del extracto de cuerpos fructíferos de *G. subincrustatum* (escogido por su alta actividad), de las cuales las fracciones F7 y F15 presentaron actividad antiproliferativa en la línea A549 (IC_{50}: 37.9 y 41.9 µg/mL, respectivamente). Sin embargo, el extracto completo tuvo mayor actividad (IC_{50}:<25 µg/mL) sobre todas las líneas evaluadas (HeLa, Raw 264.7, A549 y L-929), lo cual indica que existen otros compuestos bioactivos en el extracto.

Palabras clave: Taxonomía, corología, cultivo sólido y líquido, triterpenos bioactivos

ABSTRACT

Some species of *Ganoderma*, mainly *G. lucidum* have been used in traditional Asian medicine during more than two millennia. Both fruiting bodies and mycelia work for treatment of various ailments, including cancer. Some species of this genus synthetize lanostane-type triterpenoids with antiproliferative activity. *Ganoderma*is a cosmopolite genera, its range extends across temperate, tropical and desert areas. There are more than 219 taxa, but the only species cultivated and the most studied worldwide is *G. lucidum*. In this work, different areas of Sonora State were explored to collect fruiting bodies. Specimens of *G. applanatum*, *G. curtisii*, *G. lobatum*, *G. oerstedii*, *G. sessile*, *G. sessiliforme*, *G. subincrustatum* and *G. weberianum* were found. *Ganoderma lobatum* and *G. oerstedii* were not previously reported in Sonora, and this is the first record of *G. sessile* and *G. weberianum* in Mexico. Additionally, morphologic variability of a strain of *G. subincrustatum* from a peach orchard was studied, by comparing wild and cultivated fruiting bodies. The shape of cuticle elements, the context type and incrustations and spores of wild and cultivated fruiting bodies of a specimen of *G. subincrustatum* isolated from a peach orchard remained constant. We addressed the activity of extracts from dikaryotic mycelia and fruiting bodies of *G. subincrustatum* and *G. weberianum* specimens on several cell lines. We found the fruiting bodies extracts had higher activity than mycelia extracts. After the separation of the extracts in a silica gel column chromatography 26 sub-fractions were obtain. The fractions F7 and F15 of the fruiting bodies of *G. subincrustatum* had antiproliferative activity. However, the whole extract had the highest antiproliferative activity on all the cell lines (HeLa, Raw 264.7, A549 and L-929).This result suggested there are other bioactive compounds in the extract.

Key words: Taxonomy, chorology, solid and liquid cultivation, bioactive triterpenes

INTRODUCCIÓN GENERAL

Distintas especies del género *Ganoderma* principalmente *G. lucidum*, se han utilizado en la medicina tradicional asiática por más de dos milenios, como tónico medicinal en infusiones, tanto carpóforos como micelio pulverizados (Stamets, 2000). Este hongo es recomendado para incrementar la actividad inmune y tratar la hipertensión, artritis, asma, anorexia, gastritis, hemorroides, hepatitis, problemas vasculares, cáncer y otros padecimientos (Xu *et al*., 2010; Trigos *et al*., 2011; Wang *et al*., 2012). Actualmente se conoce que *Ganoderma* es responsable de la biosíntesis de una gama de moléculas bioactivas, destacando la proteína LZ-8 de *G. lucidum*, la cual tiene actividad inmunomoduladora y distintas lectinas que presentan actividad mitogénica (Paterson, 2006). Las moléculas bioactivas presentes en este género que son más estudiadas actualmente son los polisacáridos, los cuales muestran principalmente actividad antioxidante e inmunomoduladora (Trigos y Suárez, 2011) y los triterpenoides derivados del lanosterol como ácidos ganodéricos (AG) y moléculas relacionadas que exhiben una potente actividad antimicrobiana, anticancerígena, antiviral (Xu *et al*., 2010) e inmunomoduladora (Wang *et al*., 2007).

Debido a estas características se comercializan distintos productos de *Ganoderma*, los cuales generan una derrama de 5-6 billones de dólares a nivel mundial (Trigos y Suárez, 2011). Para cubrir dicha demanda se cultiva *G. lucidum* en medio sólido para obtener fructificaciones, no obstante se requieren de 3 a 5 meses para completar un ciclo de producción (Stamets, 2000). Recientemente la técnica de cultivo sumergido de hongos medicinales ha tomado gran auge, ya que se pueden dirigir los procesos de fermentación hacia la obtención de productos particulares, tener uniformidad entre lotes y reducir los tiempos de producción. Actualmente se conocen algunos elicitores que potencian la producción de polisacáridos y AG en cultivo sumergido de *G. lucidum* (Ren *et al*., 2010; Zhu *et al*., 2008).Los genes relacionados con la síntesis de triterpenoides en *Ganoderma*

se expresan constitutivamente, con una mayor expresión en la fase de formación de primordio, lo cual se correlaciona con un mayor contenido de triterpenoides en esta etapa de su ciclo de vida (Chen *et al*., 2012).

Se ha observado actividad antiproliferativa de extractos crudos y moléculas aisladas de distintas especies de *Ganoderma*. Extractos etanólicos obtenidos de distintas especies de *Ganoderma* mostraron actividad antiproliferativa, v.g. el extracto de *G. lucidum* mostró la mayor actividad inhibitoria sobre una línea celular de leucemia (HL-60), asimismo se observó que el extracto es capaz de inducir apoptosis temprana en 12.7% con un tratamiento de 125 µg/mL a 48 h (Zhou *et al*., 2006). Además, el AG DM presenta actividad antiproliferativa sobre una línea celular de cáncer de próstata (PC-3) con IC_{50}: 40 µM, además se conoce que esta actividad está relacionada con la interacción de esta molécula con componentes de los microtúbulos (Liu *et al*., 2012). También se ha observado que el AG DM inhibe en *ca.* 50% de la viabilidad de una línea de cáncer de mama (MCF-7) a 100 µM (Wu *et al*., 2012). Adicionalmente, se ha registrado actividad antiproliferativa del AG T sobre una línea de cáncer de pulmón (95-D), con IC_{50}: 25 µg/mL, la cual está relacionada con la inducción de apoptosis mediada por mitocondria. Dicha actividad fue de tipo selectiva sobre las líneas cancerosas, ya que los IC_{50} para líneas no cancerosas oscilaron alrededor de 150 µg/mL (Tang *et al*., 2006).Por la actividad biológica mencionada anteriormente, existe investigación acerca de la biosíntesis de estos compuestos en *Ganoderma*.

La biosíntesis de triterpenoides en *Ganoderma* ocurre mediante la ruta del mevalonato. En eucariotas superiores se conoce que la hidroxi-3-metilglutaril-CoA reductasa cataliza la síntesis de mevalonato; posteriormente la escualeno sintasa cataliza la formación de escualeno a partir de dos moléculas de farnesil difosfato; luego la lanosterol sintasa cicla 2,3-oxidoescualeno para formar lanosterol, el cual es el esqueleto de anillos de tipo lanostano de los triterpenoides de *Ganoderma* (Zhang *et al*., 2010). Finalmente ocurren transformaciones secundarias como alquilación, oxidación, reducción y conjugación, las cuales confieren las características distintivas (Shi *et al*., 2010). Estudios recientes indican que la biosíntesis de estos compuestos en micelio secundario en cultivo sumergido está relacionada tanto con estrés biótico (Zhu *et al*., 2008) como abiótico

(Zhang *et al*., 2010) y depende de la fase del ciclo de vida en que se encuentra el organismo (Shi *et al*., 2010).

Existen diferencias entre los compuestos producidos en la fase de micelio dicariótico *vs* basidiomas en *G. lucidum*. Los principales triterpenoides del micelio secundario son compuestos sustituidos en posición 3α, mientras que en cuerpos fructíferos son mayoritariamente compuestos 3-ceto o con sustituciones en posición 3β (Xu et al., 2010). Estos ligeros cambios estereoquímicos producto de las diferencias en la posición de los grupos de sustitución podrían relacionarse con su bioactividad.

A nivel mundial existen 219 especies, de las cuales a finales del siglo pasado se habían citado al menos 16 en México (Mendoza *et al*., 2011; Torres-Torres *et al*., 2015). *Ganoderma lucidum* es la especie más citada a nivel mundial, debido principalmente a determinaciones incorrectas de distintos taxones que principalmente presentan con píleo laqueado (Wang *et al*., 2012). Estudios recientes demuestran que ejemplares registrados como "*G. lucidum*" nativos de China corresponden a *G. sichuanense*. Al parecer *G. lucidum sensu stricto* se encuentra restringida al Continente Europeo (Cao *et al*., 2012; Wang *et al*., 2012). Mendoza *et al.* (2011) realizaron un estudio taxonómico de *G. oerstedii* en México y encontraron que comúnmente se había determinado como *G. lucidum*. Torres-Torres *et al*. (2015) registraron *G. curtisii*, *G. oerstedii*, *G. sessile, G. sessiliforme, G. subincrustatum* y *G. zonatum* en México, las cuales son afines con *G. lucidum*. El género se distribuye en Sonora (Esqueda *et al*., 2010), por lo que surge la inquietud de aprovechar y estudiar los recursos naturales de la zona, ya que las especies de *Ganoderma* distribuidas en este estado podrían tener potencial farmacológico.

Por lo cual surgen las preguntas: ¿Qué especies de *Ganoderma* se encuentran en Sonora?, ¿Producen metabolitos con actividad antiproliferativa? Por lo que el objetivo de este trabajo fue evaluar la actividad antiproliferativa de extractos obtenidos de dos fases desarrollo de cepas de *Ganoderma* spp. aisladas en Sonora, sobre distintas líneas celulares.

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INTEGRACIÓN DEL TRABAJO DE INVESTIGACIÓN

La información obtenida en este estudio se presenta en forma de capítulos con formato de artículos de investigación, con la secuencia programada para responder a las preguntas planteadas y contrastar la hipótesis.

Capítulo I: Current Advances in the Study of Bioactive Triterpenoids in *Ganoderma* Species (Damian López-Peña, Rigoberto Gaitán-Hernández, Georgina Vargas, Martín Esqueda). En este capítulo se hace una recopilación del conocimiento actual de la diversidad, cultivo, biosíntesis, actividad biológica e identidad de triterpenoides de *Ganoderma* spp. y se hace énfasis en la falta de investigación sobre algunas especies del género. También se discuten las carencias y fortalezas de la información publicada por distintos autores. Con respecto a la actividad antiproliferativa de triterpenoides provenientes de distintas especies de *Ganoderma*, los estudios no incluyen líneas celulares no cancerosas para contrastar dicha actividad.

Además en esta revisión bibliográfica se encontró que la mayoría de la información concerniente a cultivo y metabolitos son en la especie "*G. lucidum*", no obstante existen estudios en los que se comprueba que la mayoría de las cepas cultivadas en China, donde se tiene la mayor tradición en el consumo de este hongo corresponden a *G. sichuanense*, la cual está morfológicamente relacionada con *G. lucidum*. Al parecer la distribución de *G. lucidum sensu stricto* está restringida al continente europeo.*"Ganoderma lucidum"* es la especie más estudiada del género, no obstante existen más de 220 descritas. Por la información recopilada en este manuscrito surgen las preguntas: ¿Qué especies de *Ganoderma* se encuentran en Sonora?, ¿Producen metabolitos con actividad antiproliferativa?

Capítulo II: Diversidad y distribución de *Ganoderma* (Polyporales: Ganodermataceae) en Sonora, México (Damian López-Peña, Aldo Gutiérrez, Eduardo Hernández-Navarro,

Ricardo Valenzuela y Martín Esqueda). El género *Ganoderma* presenta una distribución cosmopolita, encontrándose principalmente en zonas templadas, tropicales y subtropicales. En Sonora se encuentra distribuido este género, no obstante existen zonas potenciales para la su distribución las cuales no habían sido exploradas. Es por ello que en este capítulo se informa acerca de la distribución y diversidad de *Ganoderma* en parte de Sonora. Se realizaron exploraciones en distintos puntos del estado con el objetivo de encontrar ejemplares del género e identificarlos taxonómicamente. Se estudiaron ejemplares pertenecientes a siete especies, de los cuales *G. lobatum* y *G. oerstedii* se encontraron por primera vez en Sonora, mientras que *G. sessile* y *G. weberianum* para México. El género presenta una amplia distribución en Sonora, en donde se encuentra en bosque de encino, encino-pino, pino-encino, encino abierto y en zonas xerófilas con mezquital. *Ganoderma oerstedii* se encontró creciendo sobre la base de *Steneocereus thurberi*, lo cual lo hace el primer registro sobre una cactácea. Es necesario realizar exploraciones en zonas de Sonora que no han sido estudiadas para conocer la distribución real del género en el estado.

Capítulo III: Morphological Characteristics of Wild and Cultivated Specimens of a *Ganoderma subincrustatum* Murrill Isolated from Sonora, Mexico (López-Peña D, Morales-Estrada I, Hernández-Navarro E, Gutiérrez A and Esqueda M.). El género *Ganoderma* presenta una gran plasticidad morfológica y con ello, uno de los géneros más complicados de Polyporales, taxonómicamente hablando. *Ganoderma subincrustatum* es una especie poco estudiada a nivel mundial, de la cual existen registros en Jamaica, Argentina y México. Con el objetivo de evaluar los caracteres propuestos para la circunscripción de las especies del género (tipo de contexto, incrustaciones resinosas, células de la cutícula y forma de esporas), en este capítulo se caracterizan morfológicamente un ejemplar silvestre y cultivados de un aislado de *G. subincrustatum* de Sonora, para conocer la variabilidad morfológica de dicha especie en respuesta a las condiciones ambientales. Adicionalmente se evaluaron dos condiciones de luz (350 y 3500-4000 lux) en el desarrollo de los cuerpos fructíferos.

El tamaño, forma y color de los cuerpos fructíferos varía significativamente entre el ejemplar silvestre y los cultivados, asimismo entre los desarrollados bajo los dos niveles de iluminación. Los ejemplares sometidos a 350 lux se desarrollaron adecuadamente, mientras que los desarrollados con 3500-4000 lux tomaron una forma alargada sin un desarrollo adecuado del píleo y tubos, y consecuentemente no hubo desarrollo de esporas. El tipo de contexto se conserva relativamente homogéneo entre todos los ejemplares, con incrustaciones y bandas resinosas a través de éste. Las células de la cutícula con forma clavada y ramificaciones laterales y/o apicales se conservan entre los distintos ejemplares. Asimismo la forma de las esporas y la disposición sublibre de los pilares interparietales se conservan entre todos los ejemplares analizados. Por lo anterior se concluye que los caracteres propuestos para la delimitación de las especies en *Ganoderma* se conservan constantes entre un ejemplar silvestre y cultivados de un aislado de *G. subincrustatum* de Sonora. Además los niveles de iluminación influyeron sobre la forma, tamaño y color de los basidiomas, no obstante los caracteres de importancia taxonómica se mantienen. La información generada en los capítulos II y III sirvió como base para seleccionar las cepas con las que se continuó trabajando en este estudio.

Capítulo IV: Antiproliferative activity of *Ganoderma subincrustatum* and *G. weberianum* from Sonora, Mexico: Mycelia *vs* fruiting bodies(López-Peña Damian, Torres-Moreno Heriberto, Robles-Zepeda Ramon E., Esqueda Martín). Distintas especies de *Ganoderma* sintetizan triterpenoides con actividad antiproliferativa. Debido a que es más rápido obtener micelio secundario que fructificaciones, la tendencia en el cultivo de especies de *Ganoderma* para obtener sus productos es mediante la fermentación líquida. Sin embargo se conoce que en *G. lucidum* existen diferencias entre los compuestos producidos en la fase de micelio dicariótico con respecto a la fase de cuerpos fructíferos, además los transcritos correspondientes a algunos genes relacionados con la ruta biosintética se acumulan en la fase de formación de cuerpos fructíferos, lo cual corresponde con la acumulación de triterpenoides en esta fase. *Ganoderma subincrustatum* y *G. weberianum* son especies distribuidas en Sonora, de las cuales se desconoce su perfil de triterpenoides y bioactividad. Es por ello que en este

capítulo se evaluó la actividad antiproliferativa de dos fases de desarrollo (micelio secundario *vs* cuerpos fructíferos) de aislados de *G. subincrustatum* y *G. weberianum* sobre distintas líneas celulares cancerosas (HeLa, Raw 264.7, A549) y una línea no cancerosa (L-929) para conocerla fase con mayor actividad biológica.

Los extractos obtenidos de fructificaciones presentaron mayor actividad antiproliferativa que los obtenidos del micelio secundario; además generan vesículas y detrito celular en todas las líneas. El extracto de cuerpos fructíferos de *G. subincrustatum* presentó la mayor actividad antiproliferativa sobre todas las líneas evaluadas (IC_{50}: <25µg/mL), por lo que la actividad fue de tipo no selectiva sobre las líneas cancerosas. Este extracto se seleccionó para ser fraccionado por cromatografía en columna con sílica para conocer si los compuestos bioactivos se pueden obtener en una fracción específica. De las 26 sub-fracciones obtenidas, F7 y F15 mostraron actividad antiproliferativa (IC_{50}: 37.9 y 41.9 µg/mL respectivamente). No obstante la actividad del extracto crudo de *G. subincrustatum* fue mayor, lo que indica que además de los compuestos bioactivos presentes en estas fracciones, existen otros que ejercen actividad aditiva o sinérgica. Es necesario realizar estudios de citometría de flujo para conocer si la actividad observada se debe a la inducción de apoptosis.

HIPÓTESIS

Extractos obtenidos en la fase de cuerpos fructíferos de aislados sonorenses de *Ganoderma* spp. presentan mayor actividad antiproliferativa con respecto a los extractos de la fase de micelio dicariótico.

OBJETIVO GENERAL

Evaluar la actividad antiproliferativa de extractos obtenidos de micelio dicariótico y cuerpos fructíferos de aislados sonorenses de *Ganoderma* spp. sobre distintas líneas celulares, para conocer en cual fase de desarrollo se presenta mayor actividad antiproliferativa.

OBJETIVOS PARTICULARES

1. Obtener aislados de *Ganoderma* en Sonora, colectar los cuerpos fructíferos e identificarlos taxonómicamente.
2. Producir micelio de aislados seleccionados de *Ganoderma* en cultivo sumergido, obtener extractos ricos en triterpenoides y caracterizarlos por cromatografía en capa fina (TLC).
3. Evaluar la actividad antiproliferativa de extractos obtenidos de micelio secundario y cuerpos fructíferos de los aislados seleccionados.
4. Fraccionar el extracto que presente mayor actividad antiproliferativa y evaluar las fracciones sobre la línea celular A549 (cáncer de pulmón).
5. Caracterizar mediante TLC las fracciones que presenten mayor actividad antiproliferativa sobre la línea celular A549.

Capítulo I

Current Advances in the Study of Bioactive Triterpenoids in *Ganoderma* Species

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Artículo de revisión

Enviado a la revista International Journal of Medicinal Mushrooms

Current Advances in the Study of Bioactive Triterpenoids in *Ganoderma* Species

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Bioactive triterpenoids of *Ganoderma*

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Abstract: Some *Ganoderma* species, mainly *G. lucidum*, have been used in traditional Asian medicine for more than two millennia because of the health benefits they offer. Currently, it is known that *Ganoderma* express several biologically active compounds, among which ganoderic acids—highly oxygenated lanostane-type triterpenes with differences in their substitution groups and bioactivity—stand out. More than 200 related molecules have been discovered, some specific to certain species and others shared among them. Because many taxa have not yet been studied, new molecules may still be discovered. Several studies have focused on basidiome production in solid culture; however, the current trend is towards liquid *G. lucidum* cultivation to obtain these metabolites. *G. lucidum* is the only species commercially cultivated worldwide, though there are more than 200 species, several of which have great pharmacological potential. The taxonomy of the genus is complicated and ambiguous determinations are common; and some dogma regarding *G. lucidum* was even broken when it was demonstrated that the distribution of *G. lucidum* does not include the Asian continent, where China has the greatest tradition in the consumption of *Ganoderma* species. This review addresses the knowledge of bioactive triterpenoids and their production in *Ganoderma*, and highlights the lack of information regarding other species with strong pharmacological potential.

Key words: Biodiversity, medicinal mushrooms, bioactive compounds, solid and submerged cultivation.

Introduction

Some species of *Ganoderma*, mainly *G. lucidum*, have been used in traditional Asian medicine for more than two millennia, consumed as medicinal tonic tea, powdered

fruiting bodies and mycelia (Stamets, 2000). The consumption of this species is recommended to increase the immunological activity and to treat hypertension, arthritis, asthma, anorexia, gastritis, hemorrhoids, hepatitis, vascular problems, cancer and other illnesses (Wang *et al*., 2007; Xu et al., 2010; Trigos and Suárez, 2011). Over the last three decades this knowledge has been evaluated scientifically. It is currently known that *Ganoderma* is responsible for the biosynthesis of a wide gamma of bioactive molecules, particularly *G. lucidum* LZ-8 protein, which has immunomodulatory activity, and different lectins that exhibit mitogenic activity (Paterson, 2006). The bioactive molecules found in this genus that have received the most attention by many research groups are polysaccharides, which show mainly antioxidant and immunomodulatory activity (Trigos and Suárez, 2011) and lanostane-type triterpenoids such as ganoderic acids (GA) and related molecules exhibiting antimicrobial, anticancer, antiviral (Xu*et al*., 2010) and immunomodulatory activity (Wang *et al*., 2007).

Due to these characteristics there are several *Ganoderma* products on the market and these have an economic impact of 5.6 billion dollars worldwide (Trigos and Suarez, 2011). To reach this high demand *G. lucidum* is mainly grown on a solid medium for fruiting bodies production; however, this technique requires 3-5 months to complete a production cycle (Stamets, 2000). Recently the technique of submerged culture of medicinal mushrooms has become increasingly popular as the fermentation process can be biodirected towards obtaining particular products, with uniformity between batches and reduced production time. Knowledge of the submerged cultivation of *G. lucidum* has grown to the point where elicitors that enhance polysaccharide and GA production have now been identified (Zhu *et al*., 2008; Ren *et al*., 2010). However, *G. lucidum* is

currently the only species cultivated commercially worldwide, though there are more than 200 in this genus and several with great pharmacological potential (Paterson, 2006). Globally there are 219 species, of which 18are recorded from México (Torres-Torres *et al*., 2015; López-Peña *et al*., 2016). *Ganoderma lucidum* is the most cited species worldwide, mainly due to incorrect determinations of different taxa with laccate pileus (Wang *et al*., 2012). In this review the issues of diversity, cultivation, biosynthesis, biological activity and identity of triterpenoids of *Ganoderma* spp. are addressed, emphasizing the lack of research on other species of this genus, mainly those with pharmacological potential.

Taxonomy and distribution

Ganoderma P. Karst. belongs to Ganodermataceae (Basidiomycota: Polyporales), which is distributed throughout almost the entire world. More than 219 species have been recorded, 65 of them proposed as synonyms (Moncalvo and Ryvarden, 1997). By the end of the last century, at least 18 species had been reported for Mexico (Torres-Torres *et al*., 2015; López-Peña *et al*., 2016). For several years it has been noted that the taxonomy of *Ganoderma* is complex due to morphological similarities between species, thus circumscription is frequently difficult and controversial (Steyaert, 1978). The type species is *G. lucidum*, the most popular due to its pharmacological potential and it is related as a morpho-species to *G. oregonense* Murrill, *G. tsugae* Murrill, *G. pfeifferi* Bres., *G. curtisii* (Berk.) Murrill, *G. resinaceum* Boud. (Moncalvo *et al*., 1995), *G. oerstedii* Torrend (Mendoza *et al*., 2011), *G. colossus* (Fr.) C.F. Baker, *G. zonatum* Murrill, *G. meredithiae* Adask. & Gilb. (Adaskaveg and Gilbertson, 1989), among others with which it is commonly confused.

Recent work mentions the importance of *pileipellis* components and resinous incrustations in the context as constant taxonomic characters, and discriminative among certain species (Torres-Torres *et al*., 2015). Additionally, the importance of supporting morphological studies with molecular markers, for an integral characterization of the species and strains have been mentioned (Mendoza *et al*., 2011). Currently, the molecular analysis of basidiomycetes focuses on rDNA sequences, specifically internal transcribed spacers (ITS 1 and ITS 2), as this region is considered the fingerprint of most fungal groups (Moncalvo *et al*., 1995). In some genera, however, this approach does not provide enough information to explain phylogenetic relationships among some species, and this occurs in *Ganoderma*.

Another marker assessed is the mitochondrial small subunit ribosomal DNA (mtSSU), which can be useful to delimit species at the sub- and infrageneric levels in *Ganoderma* (Hong *et al*., 2002) because it presents both, conserved and variable domains with more informative sites than ITS does. Douanla-Meli and Langer (2009) obtained 2.5 times more informative sites and a better phylogenetic resolution using ITS than they did with mtSSU. This discrepancy could be due to the quantity of the sequences utilized in the two studies. Additionally, the *rpb2* gene, ITS and intergenic spacer sequences have been used to clarify the taxonomic status of what is referred to as *G. lucidum* (see below) cultivated in China (Wang *et al*., 2012), as has the *tef1-α* gene to establish a new species (Cao *et al*., 2012), with good results. Likewise, subunit 6 of ATP synthase (*atp6*) and β-tubulin have been studied (Malarvizhi, 2014). Regardless of the resolution capacity of the different markers evaluated by several authors, it is necessary to obtain the sequences of most species for more robust phylogenetic analyses.

Recent studies show that specimens recorded as *G. lucidum* natives from China are actually *G. sichuanense* J.D. Zhao & X.Q. Zhang; apparently *G. lucidum sensu stricto* is restricted to the European Continent (Cao *et al*., 2012; Wang *et al*., 2012). Mendoza *et al*. (2011) did a taxonomic study of *G. oerstedii* in Mexico, finding that this taxon is commonly recorded as *G. lucidum*. Torres-Torres *et al*. (2015) recorded *G. curtisii, G. oerstedii, G. sessile, G. sessiliforme, G. subincrustatum* and *G. zonatum* in Mexico, which are closely related to *G. lucidum sensu stricto*. This suggests that different species of *Ganoderma* could have great pharmacological and biotechnological potential.

Due to the abovementioned taxonomic challenges, the following questions arise: Which species have the metabolites with biological activity reported for *G. lucidum*? Is *G. lucidum* the only species that is globally marketed? The name *G. lucidum sensu lato* will be used the rest of this manuscript.

Triterpenoid biosynthesis and biotechnological processes

From several low polarity fractions obtained from the mycelia, spores and fruiting bodies of *G. lucidum*, more than 200 different lanostane-type triterpenes with biological activity have been isolated, including GA, ganoderiols, lucidones, lucidenic acids and ganolucidic acids, which differ mainly in their functional groups and their position (Adamec *et al*., 2009; Trigos and Suárez, 2011; Xia *et al*., 2014). Triterpenoid biosynthesis in *Ganoderma* occurs via the mevalonate pathway (MVP) (Zhang *et al*., 2010).

The MVP involves 6 enzyme reactions to produce isopentenyl pyrophosphate (IPP), and IPP D-MAPP isomerases are then responsible of the interconversion of IPP to a more reactive isomer, dimethylallyl pyrophosphate. Of these enzymatic steps hydroxy-3-metylglutaryl-CoA reductase (*HMGR*) catalyzes the synthesis of mevalonate, which is

an enzyme associated with the endoplasmic reticulum, and this is the rate limiting step of the pathway (Shang *et al*., 2008; Chang *et al*., 2013). Then squalene synthase (*SQS*) catalyzes the formation of squalene (30 carbons), from two molecules of farnesyl diphosphate (15 carbons); lanosterol synthase then catalyzes the cyclization of 2,3-oxidosqualene to form lanosterol, which is the skeleton of triterpenoids in *Ganoderma* (Zhang *et al*., 2010). Finally there are secondary transformations, such as alkylation, oxidation, reduction and conjugation, which confer unique characteristics on the different molecules (Shi *et al*., 2010). Recent studies indicate that the biosynthesis of these kinds of molecules in secondary mycelium by liquid fermentation is related to biotic (Zhu *et al*., 2008) and abiotic stress (Zhang *et al*., 2010); likewise, the development phase of the organism is important for its production (Shi *et al*., 2010).

The genes involved in triterpenoid biosynthesis in *Ganoderma* express constitutively, with an increase in primordia development, which is correlated with a greatest content of triterpenoids in this phase of its lifecycle (Chen *et al*., 2012). Nevertheless, efforts are being made to produce these compounds by the submerged cultivation of secondary mycelium. Production time and control of fermentation factors are two important advantages of this technique over solid cultivation (Tang and Zhong, 2002; Xu *et al*. 2008; Tang *et al*., 2011). However, solid culture still prevails and most of the *Ganoderma* products marketed are based on pulverized fruiting bodies or their extracts.

Ganoderma is a white rot fungi that degrades cellulose, hemicellulose and lignin, thus fruiting body production on solid media is the conventional way of obtaining its products. Oak (*Quercus* spp.) wood chips are mainly used for its cultivation; however, in recent years the research on alternative substrates like agro-industrial, agricultural and forestry waste for its cultivation , including composted food waste, has increased (Eun-

Young and Johng-Hwa, 2012; Zhou *et al*., 2012), with reports of varying yield depending on the substrate utilized. Peksen and Yakupoglu (2009) obtained a biological efficiency (BE) of 39.6% with 80:20 ratio of *Carpinus betulus* L. wood chips and tea waste, using a strain of *G. lucidum*. The same year, a BE of *ca.* 20% was recorded using tulip tree (*Liriodendron tulipifera* L.) wood supplemented with corn flour and molasses (a by-product of refining sugar cane) (Erkel, 2009). Rolim *et al*. (2013) reported a BE of 0-64% with 6 formulations based mainly on elephant grass (*Pennisetum purpureum* Schumacher).

Ayala and Lizárraga (1993) recorded a BE of 5.2 and 2.1% with *G. lucidum*, cultivated on maize stalks and grape pomace, respectively. In another study, BE values of 8.5, 7.8 and 5% were obtained, using maguey tequila bagasse enriched with cottonseed hulls, bagasse supplemented with peanut shells, and pure bagasse, respectively (Soto-Velazco *et al*., 2005). Two strains of *G. lucidum* cultivated on different agro-industrial wastes had a BE of 6.9-8.2%, using combinations of maize stalks, bean pods, coffee pulp, wheat bran, $Ca(OH)_2$ and $CaSO_4$ as substrate (Bernabé-González *et al*., 2015). Time is a limiting factor in solid fermentation to produce fruiting bodies, which take at least 3-5 months (Stamets, 2000). Therefore, the submerged cultivation of medicinal mushrooms has become a very important technique, because it offers inter-batch homogeneity, less time is required, and fermentation factors can be controlled for the specific production of bioactive compounds (Chang *et al*., 2006).

Because polysaccharides and GA are the most important metabolites of *Ganoderma*, most of the research is focused on increasing yield and productivity by submerged cultivation (Xu *et al*., 2010). *G. lucidum* is the main cultivated species, in which distinct strategies have been employed, such as the optimization of carbon and nitrogen sources

to produce GA and biomass, using response surface methodology. With the optimized media, biomass, GA production, and GA productivity values of 21.53 g/L, 496 mg/L and 110.2 mg/L d^{-1}were obtained, respectively (Xu *et al*., 2008). With pH, dissolved oxygen tension and/or lactose feeding manipulation during the fermentation process for the simultaneous production of GA, exo- and intrapolysaccharides, up to 754.6 mg/L, 3.54 and *ca*. 4.8 g/L, at 12, 18 and 14 d of fermentation were recorded, respectively (Tang *et al*., 2009). The authors did not mention the number of replicates nor data variability.

Another strategy in *G. lucidum* has been the overexpression of genes involved in GA biosynthesis by submerged cultivation, with promising results. Xu *et al*. (2012) overexpressed an N-terminally truncated *HMGR* gene, while Zhou *et al*. (2014) overexpressed a *SQS* gene. Overexpression of these genes increased total GA production, while *SQS* overexpression specifically enhanced GA: T, S, Mk and Me, to which, distinct well-documented bioactivities are attributed (Tang *et al*., 2006; Wang *et al*., 2007; Liu *et al*., 2012). In these studies, an increase in the production of squalene and lanosterol, which are immediate precursors of GA, was also observed. In recent years, research focused on *G. lucidum* metabolites production has advanced to the point where different elicitors that enhance its production by submerged cultivation are now known (Zhu *et al*., 2008; Liang *et al*., 2010; Ren *et al*., 2010).

Although *G. lucidum* is the only species cultivated worldwide, there are few isolated reports of liquid culture of other species of *Ganoderma*. In *G. applanatum*, it was observed that temperature, the initial concentration of the carbon source, C/N ratio and fermentation time are the main factors influencing biomass, endo- and exopolysaccharide production (Lee *et al*., 2007). In liquid-cultured *G. tsugae* mycelium,

cube root and Luedekin-Piret mathematical models explain biomass and exopolysaccharide production, respectively (Narkprasom *et al*., 2012).Nevertheless these studies did not measure GA production. Isaka *et al*. (2013) grew *G. orbiforme* in liquid media to obtain triterpenoids, which were characterized by NMR to elucidate their molecular structure.

***Ganoderma* triterpenoids: production, bioactivity and identity**

To obtain and quantify GA, the method most employed is liquid-liquid extraction. This involves a first extraction step with 95 or 50% ethanol from biomass (Xu *et al*., 2008), the extract is then dried and partitioned between chloroform and water. The bottom chloroform layer is recovered and washed with a 5% $NaHCO_3$ solution. Acid triterpenoids remain in the aqueous layer, while neutral components stay in the chloroform layer (Li *et al*., 2012). Subsequently, 2 N HCl is added to adjust the pH (below 3) of the $NaHCO_3$ phase and this is then once again extracted with chloroform. The chloroform is then removed by evaporation at 40 °C and GA is dissolved in absolute ethanol, and its absorbency measured at 245 nm (Ren *et al*., 2010). Thus, several steps are involved in this technique, yield is *ca.* 5 % and only acid triterpenoids are considered. In addition, there has been at least one study about using adsorption resins to recover GA (Li *et al*., 2012). There is no information about the characterization of this technique, and papers about the liquid fermentation processes do not provide evidence that such absorbance corresponds only to acid triterpenoids. This represents a window of opportunity for future research.

There are differences between the compounds produced in the mycelial phase *vs*. basidiomata in *G. lucidum*. The major triterpenoids from secondary mycelia and fruiting bodies are 3α *vs*. mainly 3keto or 3β substituted compounds, respectively (Xu *et al*.,

2010). These subtle stereochemical changes resulting mainly from differences in the position of their functional groups, could be related to their bioactivity. Thus, liquid cultivation technique for the production of bioactive compounds with a reduction of production time and/or control of fermentation factors, as an alternative to solid cultivation, seems questionable. Therefore, the development of metabolomic studies in *Ganoderma* species at different development stages is necessary to use these natural resources in a systematic way. With the exception of Zhou *et al*. (2014), who enhance the production of specific AG with well documented bioactivity, most of the studies on GA and polysaccharide production, provide no evidence of the bioactivity of the metabolites produced.

The GA of *G. lucidum* have been evaluated on the proliferation of different types of cell lines, highlighting lung cancer (Tang *et al*., 2006), HeLa cell line (the oldest corresponding to cervical cancer) (Xu *et al*., 2010) and hepatomas. Regarding isolated molecules, GA "X" inhibits the growth of the cell line HuH-7 (human hepatocarcinoma), by inducing apoptosis with inhibition of topoisomerases I and IIα (Li *et al*., 2005). GA "T" inhibits the growth of 95-D cells (lung cancer) by mitochondria-mediated apoptosis, with an IC_{50} (50% reduction in cell viability) of *ca*. 25 µg/mL (Tang *et al*., 2006). GA "Me" increases the activity of natural killer cells and the expression of IL-2 and INF-γ*in vivo* (Wang *et al*., 2007), and also induces cell cycle arrest in the G_1 phase in cells with p53 - / -, while in cells with p53 + / + cell arrest occurs in the transition G_1/S phase (Chen and Zhong, 2009). In addition, GA "DM" inhibits 50% of PC-3 cell viability (IC_{50}) (prostate cancer) to 40 µM, while replacing the carboxyl group with a methyl ester at the C-26 position increases the molecule's bioactivity, with an IC_{50} of 3µM.

It was also found that this molecule is able to interact with α and β tubulin (Liu *et al*., 2012). This suggests that these molecules have potential as a treatment for various types of cancer, by different routes of action. However, as mentioned above, it is necessary to evaluate a noncancerous cell line to define its selectivity, yet this has not been done in most studies. There is extensive information on the bioactive compounds of *G. lucidum* but information about the metabolites and low polarity fractions of other *Ganoderma* species with biological activity is limited.

Other species of *Ganoderma* with biological activity

Some low polarity fractions of *G. atrum* basidiomata have shown antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Proteusbacillus vulgaris.* The minimum inhibitory concentrations (MIC) and bactericides (MBC) ranged from 1.5-6.2 and 3.1-25 mg/mL, respectively, depending on the organisms evaluated. Also these fractions showed good antioxidant capacity. Among the compounds present in these extracts were GA "T", "C", "K" and "C2", determined by HPLC-MS (Li *et al*., 2012). Some mycelium essential oils of *G. japonicum* also exhibit antimicrobial activity against various pathogenic bacteria with MIC and MBC of 1-4.1 and 1-8.2 mg/mL respectively, with variations between organisms studied (Liu *et al*., 2009). It has been observed that basidiomata ethanolic extracts of *G. amboinense*, *G. oerstedii* and *G. resinaceum* inhibit the growth of HL-60 cells (promielitic cells of human leukemia) in different percentages at 125µg/mL. The anti-proliferative activity was by apoptosis induction; however, selectivity using a non-cancerous cell line was not evaluated (Zhou *et al*., 2006).

Additionally, fruiting body triterpenoids from *G. tsugae* have been found to exhibit anti-inflammatory activity. These compounds may have great therapeutic value as anti-

inflammatories and for protection from UV light (Ko*et al*., 2008). It has also been observed that a methanolic extract of this species has a high degree of anti-proliferative activity against a cell line of colorectal cancer (Colo250), via cell cycle arrest at the G_2/M phase, as demonstrated *in vitro* and *in vivo* (Hsu *et al*., 2008). From the secondary mycelium of *G. orbiforme* grown in liquid medium an epimer of ganoderic acid "T" was obtained, which showed high degree of activity against *Mycobacterium tuberculosis*, with an MIC of 1.3 µM (Isaka *et al*., 2013).

Ganoderma applanatum has different sterols and a lanostanoid with antibiotic activity against gram positive bacteria. In addition, some applanoxidic acids isolated from non-polar fractions exhibited activity against skin tumors in mice (Trigos and Suarez, 2011). Lee *et al*. (2006) isolated eight compounds from nonpolar fractions of *G. applanatum*, including ergosterol peroxide which exhibited a potent inhibitory activity of aldose reductase (IC_{50}: 15.4 µg/mL), enzyme associated with the generation of diabetes mellitus. Seven triterpenoids known as colosolactones "A", "B", "C", "D", "E", "F" and "G" with moderate cytotoxicity against L-929, K-562 and HeLa cells, and IC_{50} values of 15-35 mg/mL have been isolated from *G. colossus* (Trigos and Suárez, 2011). These authors also mentioned the presence of bioactive compounds in low polarity extracts from *G. australe, G. concinnum, G. fornicatum, G. orbiforme, G. sinense and G. pfeifferi*. The metabolites extracted from these species are mainly obtained from wild fruiting bodies, because most of them are not cultivated. Some of the triterpenoids recorded from several species of *Ganoderma* are listed in **Table 1.**

Of these triterpenoids, GA “V” is shared between *G. lucidum* and *G. orbiforme* (Toth *et al*., 1983), while GA “A”, “B”, “C” and “D” are shared between *G. lucidum* and *G. tsugae* (Chen *et al*., 1999). The most studied species is *G. lucidum*, from which more

than 200 triterpenoids have been reported (no included in Table 1), followed by *G. applanatum* and *G. sinense* (Xia *et al*., 2014).

Future perspectives

One of the next challenges is to clarify the identity of *Ganoderma* species to achieve the rational and systematic use of these as a natural resource. Taxonomists must unify their morphological criteria to delimit the species of *Ganoderma*. Despite the advances made using molecular markers, it is necessary to obtain sequences of neotypes from most species agreed upon by taxonomists to realize robust studies, and to also look for other markers to clarify their phylogenetic relationships. With the information generated so far it is possible to rename many of the strains and collections recorded as *G. lucidum* worldwide.

There are numerous studies on the solid and liquid cultivation of *Ganoderma*, with the aim of obtaining fruiting bodies, mycelium or specific metabolites, but few provide information about the bioactivity or functionality of the products. In studies about enhancing polysaccharide and GA production, the bioactivity of the products is not indicated and bioactivity is not attributed to any particular metabolite. This raises the question, Why enhance the production of metabolites whose bioactivity is unknown? Moreover, it is necessary to characterize the different phases of the lifecycle (secondary mycelia and basidiomata) of isolated strains of this fungus because together, biotic and abiotic factors combined with species variability, play a major role on synthesis of bioactive compounds.

Solid and liquid cultivation techniques to obtain fruiting bodies and secondary mycelium are not necessary mutually exclusive. Basidiomata production seems to be a good way to mitigate the environmental impact generated by the accumulation of agro-industrial by-

products, to generate biomass with a high market value and thus, increase commercial activity in certain areas. Besides, certain bioactive compounds are only produced at this stage of the fungus' lifecycle. On the other hand, submerged cultivation to produce secondary mycelium and specific metabolites seems to be suitable for establishing controlled processes and to obtain high quality products, with uniformity between batches. There has been a breakthrough in the understanding of GA biosynthesis in *G. lucidum*. However, it is necessary to generate basic information on the regulation of this pathway to develop new processes to produce bioactive compounds.

Although there is extensive information about the biological activity of GA isolated mainly from *G. lucidum*, it is still necessary to investigate the selectivity of these compounds, especially with respect to their anti-proliferative activity, to elucidate their mechanism of action and possible synergism. Only a few studies have included noncancerous cell lines to assay this bioactivity and, using flow cytometry, to determine whether the activity is due to apoptosis induction. Also, it is essential to dismiss any toxicity of these compounds by *in vitro* and/or *in vivo* studies, to generate information on other species to take advantage of fungal resources from particular areas, and to break the dogma that *G. lucidum* is the only one species with this potential in *Ganoderma*.

Acknowledgement

We want to thank Gerardo Reyna Cañez for his valuable technical support in the search for bibliographic information, important and essential to carry out this manuscript.

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Table 1. Some triterpenoids recorded from *Ganoderma* spp. (*G. lucidum* is excluded).

Source	Compound
G. amboinense	Lanosta-7,9(11),24-trien-3β, 15α, 22-triacetoxy-26-oic acid
	Ganoderic acid X
G. applanatum	Ganoderic acid AP_2, AP_3; Methyl ganoderenate AP, D, H, I
	Ganoderenic acid F, G; Furanoganoderic acid
	Applanoxidic acid A, B, C, D, E, F, G, H
	23-Dihydroganoderic acid I, N; 23-Dihydroganoderenic acid D
G. australe	Austrolactone; Australic acid
G. carnosum	Carnosodione
G. cochlear	Fornicatin G, H
G. colossus	Colossolactone A, B, C, D, E, F, III, IV, V, VI, VII, VIII
	Ganorbiformin A
G. concinnum	5α-Lanosta-7,9(11),24-triene-15α-26-dihydroxy-3-one
	8α, 9α-Epoxy-4,4,14α-trimethyl-3,7,11,15,20-pentaoxo-5α-pregnane
G. fornicatum	Fornicatin A, B, C
G. orbiforme	Ganoderic acid V; Ganorbiformin B, C, D, E, F, G
G. pfeifferi	Lucialdehyde D; Ganoderone A, C

G. resinaceum	3-Oxo-5α-lanosta-8,24-dien-21-oic acid 3-Epipachymic acid
G. sinense	Ganoderic acid GS-1, GS-2, GS-3, Jc; Ganolucidic acid γa; Ganosinensin A, B, C; Sinensoic acid; Ganosinoside A; Ganoderiol J; Ganodermatetraol; Ganolactone B; Lucidenol; Ganolucidate F Methyl ganosinensate A; Ganosinensic acid A; Ganosinensic acid B
G. tsugae	Tsugarioside A, B; Tsugaric acid A, B, C; Lucidone A
G. zonatum	Lanosta-7,9(11),24-trien-3-one15,26-dihydroxy; Ganoderic acid Y Lanosta-7,9(11),24-trien-26-oic,3-hydroxy

Source: Xia *et al.*[59]

Capítulo II

Diversidad y distribución de *Ganoderma* (Polyporales: Ganodermataceae) en Sonora, México.

Damian López-Peña, Aldo Gutiérrez, Eduardo Hernández-Navarro, Ricardo Valenzuela y Martín Esqueda. *Artículo original*

Botanical Sciences

Diversidad y distribución de *Ganoderma* (Polyporales: Ganodermataceae) en Sonora, México

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Botanical Sciences
94 (2): 431-439, 2016

DOI: 10.17129/botsci.463

Resumen

Se estudiaron siete especies de *Ganoderma*: *G. applanatum*, *G. curtisii*, *G. lobatum*, *G. oerstedii*, *G. sessile*, *G. sessiliforme* y *G. weberianum*. De ellas, *G. lobatum* y *G. oerstedii* son nuevos registros para Sonora, mientras que *G. sessile* y *G. weberianum* para México. El género tiene una amplia distribución en Sonora, en donde se encuentra en bosque de encino, encino-pino, pino-encino, encino abierto e incluso en zonas xerófilas con mezquital. *Ganoderma oerstedii* se encontró en la base de *Stenocereus thurberi*, que lo hace el primer registro sobre una cactácea.

Palabras clave: corología, hongos poliporoides, sierra Sonorense, taxonomía.

Diversity and distribution of *Ganoderma* (Polyporales: Ganodermataceae) from Sonora, Mexico

Abstract

Seven species of *Ganoderma*: *G. applanatum*, *G. curtisii*, *G. lobatum*, *G oerstedii*, *G. sessile*, *G. sessiliforme*, and *G. weberianum* are discussed. *Ganoderma lobatum* and *G. oerstedii* are new records for Sonora, while *G. sessile* and *G. weberianum* for Mexico. The genus has a wide distribution in Sonora, being found in oak, oak-pine, pine-oak, open oak forests, also in xerophytic vegetation like mesquite scrub. *Ganoderma oerstedii* was founded on *Stenocereus thurberi*, being its first record on a living Cactaceae.

Key words: chorology, polyporoid fungi, Sonoran sierra, taxonomy.

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Ganoderma P. Karst. (Polyporales: Ganodermataceae) es un género con más de 200 especies descritas, pero muchas son sinónimas (Moncalvo y Ryvarden, 1997; IFP, 2014). Se reconocen dos subgéneros con base en características del píleo (Gottlieb y Wright, 1999a, b): *Elfvingia* (P. Karst.) Imazeki (especies con superficie del píleo opaca) y *Ganoderma* (con superficie brillante o laqueada). Por su complejidad y variabilidad, el género ha generado confusiones taxonómicas con determinaciones incorrectas. Estudios recientes indican la importancia del contexto y elementos de la cutícula como caracteres decisivos en la delimitación de las especies (Gottlieb y Wrigth, 1999a, b; Torres-Torres y Guzmán-Dávalos, 2005; Torres-Torres *et al.*, 2012).

En México se han registrado alrededor de 16 especies (Mendoza *et al.*, 2011), de las cuales *Ganoderma adspersum*, *G. applanatum*, *G. lucidum* y *G. sessiliforme* están citados para Sonora (Esqueda *et al.*, 2010). El género es frecuentemente observado en regiones tropicales, subtropicales y templadas (Gilbertson y Ryvarden, 1986; Zhao, 1989; Torres-Torres *et al.*, 2012). En Sonora se conoce su distribución en la zona urbana de Hermosillo, Reserva Forestal Nacional y Refugio de Fauna Silvestre Ajos-Bavispe (bosque de pino-encino, bosque de galería y matorral desértico micrófilo) y Reserva de la Biosfera Sierra de Álamos-Río Cuchujaqui (selva baja caducifolia) (Esqueda *et al.*, 2010). Sin embargo, existen zonas poco exploradas, como la Sierra de Yécora (bosque de pino-encino y encino-pino), Sierra de Mazatán (bosque de encino, matorral subtropical y mezquital) y Sierra de San Javier (bosque de encino abierto y matorral subtropical), en donde se hizo el presente estudio.

Materiales y métodos

Se realizaron exploraciones en la Sierra de Yécora (municipio de Yécora), Sierra de San Javier (municipio de San Javier) y Sierra de Mazatán (colindancia de los municipios de Ures, Mazatán y Villa Pesqueira) durante 2012 y 2013, en donde se realizaron 17 recolecciones de *Ganoderma*. Para el estudio morfológico de los ejemplares se siguieron las metodologías propuestas por Furtado (1965), Largent *et al.* (1977), Bazzalo y Wrigth (1982), Gottlieb y Wright (1999a, b), Ryvarden (2000) y Torres-Torres *et al.* (2012). Los principales caracteres macroscópicos estudiados fueron dureza y grosor de la cutícula del píleo, tipo de contexto (homogéneo, relativamente homogéneo o dúplex), presencia de incrustaciones o bandas resinosas y la forma y tamaño de los basidiomas.

La descripción de los colores de los basidiomas se realizó de acuerdo a Kornerup y Wanscher (1978); aunque el código sólo se indica en los nuevos registros para México. Se hicieron preparaciones en solución KOH al 5 % para la caracterización de estructuras microscópicas. Estas fueron el tamaño y forma de las basidiosporas, disposición de los pilares interparietales de las basidiosporas; forma, tamaño e incrustaciones de los elementos de la cutícula; diámetro de los poros y el grosor del disepimento. También se consideró la reacción de esporas y elementos de la cutícula con el reactivo de Melzer y la presencia de clamidosporas. Se midieron al menos 30 esporas de cada ejemplar, para calcular el coeficiente Q (largo/ancho). Los nombres de las especies y autores están basados en la base de datos Index Fungorum (IFP, 2014). Se generó un mapa de distribución con base en los registros anteriores y el presente trabajo. Los especímenes se depositaron en la colección de hongos "Dr. Martín Esqueda Valle" del herbario de la Universidad Estatal de Sonora (UES).

Taxonomía

Ganoderma applanatum (Pers.) Pat., Hyménomyc. Eur. (Paris): 143 (1887) (Figura 1A-C).
Basidioma de 67–225 × 105–165 × 33–83 mm, perenne, sésil, suberoso a leñoso. Píleo semicircular a ligeramente lobado o irregular; superficie opaca, sulcada concéntricamente, zonada, marrón, en ocasiones cubierta con esporas color óxido. Cutícula de hasta 0.48 mm de grosor. Elementos de la cutícula de 30–45 × 5.6–10 μm, claviformes a digitiformes, en ocasiones con protuberancias laterales y ramificaciones, marrón-rojizo a amarillo-dorado, mezclados con hifas esqueléticas. Basidiosporas de 8–9 × 5.2–6.2 μm, Q = 1.45–1.53, elipsoides, marrón claro, exosporio con pilares interparietales libres.

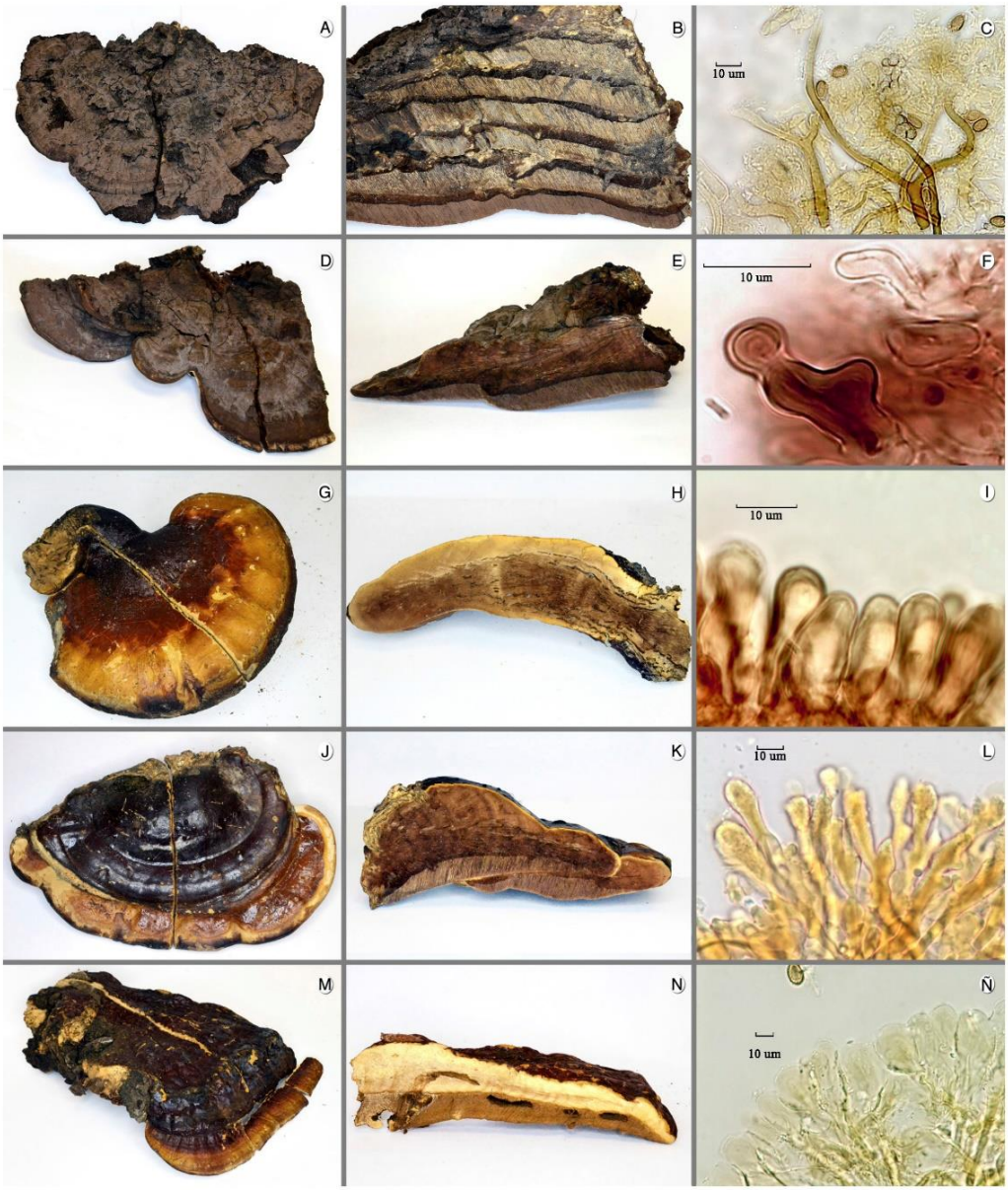

Figura 1. *Ganoderma applanatum, G. curtisii, G. lobatum, G. oerstedii* y *G. sessiliforme*. *G. applanatum*: **A)** basidioma, **B)** contexto, C) cutícula; *G. lobatum*: **D)** basidioma, **E)** contexto, **F)** cutícula; *G. curtisii*: **G)** basidioma, **H)** contexto, **I)** cutícula; *G. oerstedii*: **J)** basidioma, contexto, L) cutícula. *G. sessiliforme* **M)** basidioma, N) contexto, **Ñ)** cutícula.

Material estudiado. Municipio de Yécora, Sierra de Yécora, E. Hernández, A. Gutiérrez y D. López, 28-09-2012, UES 10420, 10421.
Comentarios. La formación de capas del contexto y de los tubos por las temporadas de desarrollo está acorde con lo descrito para especies perennes. Esta especie puede confundirse con *Ganoderma australe* (Fr.) Pat., pero se diferencia por la cutícula más delgada de < 0.5 mm y las basidiosporas más pequeñas de 9–13.5 × 6–10 μm en *G. australe*. La especie en discusión fue recolectada en bosque de encino-pino sobre la base de *Quercus* sp.

Ganoderma curtisii (Berk.) Murrill, N. Amer. Fl. (New York) **9**(2): 120 (1908) (Figura 1G-I).
Basidioma de 22–71 × 17–57 × 10–20 mm, anual, estipitado, suberoso a fibroso. Píleo reniforme a semicircular; superficie brillante, laqueada, carácter que se pierde con el tiempo, quedando irregularmente con zonas opacas; anaranjada a amarilla con tonalidades marrón-rojizas. Estípite de 40–54 × 10–24 mm, lateral, aplanado a cilíndrico, brillante, con tonalidades marrón rojizo. Contexto dúplex, azonado, con 2–4 bandas resinosas, completas o intermitentes a través de todo el contexto. Elementos de la cutícula de 29–47 × 7.3–15.5 μm, claviformes, sin incrustaciones, en ocasiones con protuberancias y ramificaciones laterales. Basidiosporas de (7.3–) 7.7–9 × 4.5–5.6 μm, Q = 1.44–1.76, elipsoides a oblongas, con pilares interparietales sublibres.
Material estudiado. Municipio de San Javier, Sierra de San Javier, A. Gutiérrez, E. Hernández, y D. López, 27-09-2012, UES 10402. Municipio de Ures, Sierra de Mazatán, C. Trujillo, E. Hernández, A. Gutiérrez, A. Jiménez y D. López, 18-09-2013, UES 10403.
Comentarios. *Ganoderma curtisii* es una de las especies con mayor variabilidad morfológica, macro y microscópica dentro del género según Torres-Torres y Guzmán-Dávalos (2005), quienes registraron basidiosporas de (9.2–) 10.4–12.8 (–13.6) × 5.6–8 μm en ejemplares mexicanos de Jalisco, Hidalgo y Morelos, mientras que Zhao (1989) de 8.7–11.3 × 5.2–6.9 (8) μm en ejemplares de China. Steyaert (1980) observó esporas de 8.5–10 (–12) × 5.5–6.2 (–7) μm. Lo anterior sugiere que se puede tratar de un complejo de especies. *Ganoderma curtisii* se recolectó en bosque de encino en una zona conocida como Isla de Montaña, sobre madera muerta de *Quercus* sp. y en bosque de encino abierto sobre raíz de *Quercus* sp.

Ganoderma lobatum (Schwein.) G.F. Atk., Annls. mycol. **6**(3): 190 (1908) (Figura 1D-F).
Basidioma de 36–155 × 55–125 × 26–44 mm, perenne, sésil, en ocasiones subestipitado, suberoso a leñoso. Píleo dimidiado a lobulado, ocasionalmente semicircular; superficie opaca; marrón a marrón-grisáceo. Elementos de la cutícula de 34–40 × 4.4–10 μm, claviformes a digitiformes, de pared gruesa, marrón a marrón-rojizo. Basidiosporas de 8.3–9.8 (10.8) × 5.3–6.3 μm, Q = 1.44–1.81, elipsoides a oblongas, con pilares interparietales libres.
Material estudiado. Municipio de Yécora, Sierra de Yécora, E. Hernández, A. Gutiérrez y D. López, 28-09-2012, UES 10422, 10423, 10424.
Comentarios. Steyaert (1980) consideró a *Ganoderma lobatum* dentro del antiguo subgénero *Anamixoderma*, caracterizado por presentar elementos de la cutícula claviformes, mezclados con terminaciones hifales, lo cual se observó en los ejemplares revisados. Este es el primer registro de *G. lobatum* para Sonora. Crece en bosque de encino-pino sobre la base de *Quercus* sp.

Ganoderma oerstedii (Fr.) Torrend, Bull. Torrey bot. Club **29**: 606 (1902) (Figura 1J-L).
Basidioma de 105–133 × 85–100 × 28–31 mm, perenne, sésil, fuertemente adherido, leñoso. Píleo flabeliforme-semicircular a irregular; superficie glabra, lisa a irregular, con una capa de laca brillante, la cual puede perderse con el tiempo, sulcada concéntricamente; marrón-rojizo oscuro en casi toda la superficie, con tonalidades anaranjadas hacia el margen. Contexto zonado, con bandas e incrustaciones resinosas conspicuas. Elementos de la cutícula de 40–67.7 × 7.7–12 μm, claviformes, con ramificaciones y protuberancias tanto laterales como apicales, amarillo dorado. Basidiosporas de (7.4–) 8.3–10 × 5.1–6.3 (–6.5) μm, Q = 1.42–1.67, elipsoides a oblongas, con pilares interparietales parcialmente anastomosados.
Material estudiado. Municipio de Ures, Sierra de Mazatán, A. Gutiérrez, E. Hernández, R. Maldonado y D. López, 10-09-2012, UES 10419.
Comentarios. Las basidiosporas de esta especie varían según la referencia bibliográfica. Ste-

yaert (1980) registró basidiosporas de 9–10.5 (–12.5) × 6–7.7 (–9) μm, mientras que Mendoza *et al.* (2011) de (10–) 11–13 (–14) × 8–10 μm. Estos últimos autores citaron *Ganoderma oerstedii* de áreas tropicales y subtropicales de Chiapas, Morelos, Sinaloa y Veracruz. Los ejemplares de Sonora provienen de mezquital, en una zona donde se registran temperaturas mayores a 40 °C. Se recolectó sobre la base de *Stenocereus thurberi*. Éste es el primer registro de la especie desarrollándose sobre una cactácea y la primera cita para Sonora.

Ganoderma sessile Murrill, Bull. Torrey bot. Club. **29**: 604. (1902) (Fig. 2A-D).
Basidioma de 50 × 50 × 21 mm, anual, sésil, solitario, de consistencia fibrosa a suberosa. Píleo circular-flabeliforme, ligeramente convexo, conchado, dimidiado; superficie laqueada, con una franja delgada hacia el margen sin laca, corrugada, sulcada concéntricamente, cubierta frecuentemente por una fina capa de basidiosporas anaranjado-parduzco (7C4); marrón rojizo (8E8) a marrón oscuro (7F8); margen estéril, agudo, liso, blanquecino en fresco, marrón amarillento (5D8) a marrón (5F8) en seco. Contexto de hasta 12 mm de grosor, dúplex, zonado, suberoso a esponjoso, con algunos depósitos resinosos dispersos difíciles de observar, la porción superior anaranjado pálido (5A3), la inferior marrón (5F7). Tubos de hasta 9 mm de longitud, anaranjado tenue (5A4) a anaranjado grisáceo (5B4) cerca de la superficie, ligeramente estratificados. Poros de 4–5 por mm, circulares a angulados, con borde irregular, blancos, se manchan de color marrón al maltratarlos o tocarlos, al igual que todas las especies del género, con una porción marrón (5F7) hacia el margen, de 169–304 μm diámetro; disepimento de 21.4–59.6 μm de grosor. Sistema hifal trimítico. Hifas generativas de la trama del contexto no observadas; hifas esqueléticas de 2.2–7.7 μm diámetro, de pared gruesa a sólida, arboriformes y aciculiformes, hialinas a amarillo pálido en la porción superior, marrón pálido a marrón en la porción inferior; hifas conectivas de 1.1–3.1 μm diámetro, fuertemente ramificadas, hialinas. Elementos de la cutícula de 33.7–53 × 8.2–14.9 μm, cilíndricos a fuertemente claviformes, ocasionalmente con algunas protuberancias laterales, anaranjado pálido; hifas generativas de 1.8–3.9 μm diámetro, con fíbulas, amarillo pálido; hifas esqueléticas de 1.8–2.8 μm diámetro, con paredes gruesas, marrón-amarillento, hifas conectivas de 1.1–2.1 μm diámetro, altamente ramificadas, pared gruesa a sólida, amarillo pálido. Basiodiosporas de 9.6–10.5 × 5.8–6.8 μm, Q= 1.44–1.72, elipsoides a oblongas, con ápice truncado, perisporio perforado, exosporio con pilares interparietales sublibres y delgados. Basidios no observados.
Material estudiado. Municipio de Ures, Sierra de Mazatán, A. Gutiérrez, E. Hernández, R. Maldonado y D. López, 10-09-2012, UES 10405.
Comentarios. Esta especie se caracteriza por su contexto dúplex, zonado, con depósitos resinosos, aunque difíciles de observar. Elementos de la cutícula fuertemente claviformes, generalmente enteros o con una protuberancia lateral. Moncalvo y Ryvarden (1997) y Ryvarden (2000) consideraron *Ganoderma sessile* como sinónimo de *G. resinaceum* Boud., no obstante Gottlieb y Wrigth (1999a) manejan estas especies como especies independientes, diferenciándolas microscópicamente por la ornamentación de las esporas y el tamaño de los pilares interparietales. Estos autores mencionaron que el holotipo de *G. resinaceum* se encontraba en mal estado, aceptando la posición de Murrill (1902), quien indicó esporas de 9–11 × 6–8 μm, lo cual corresponde con el material estudiado. Por otra parte el tipo de *G. sessile* en NY parece tener problemas de identidad, como lo hicieron ver Moncalvo y Ryvarden (1997). Indudablemente *G. sessile* necesita de más estudios, sobre todo del tipo. Guzmán (1977, 1978) citó esta especie del centro de México, pero Mendoza *et al.* (2011) mencionaron que las colecciones de Guzmán corresponden a *G. oerstedii*. De esta manera, ésta es la primera cita de *G. sessile* para México. En Sonora se recolectó en un bosque de encino, en la base de *Quercus* sp.

Ganoderma sessiliforme Murrill, Bull. New York Bot. Gard. **8**: 149 (1912) (Figura 1M-Ñ).
Basidioma de 44–65 × 58–151 × 30 mm, anual, sésil a subestipitado. Píleo flabeliforme a conchado. Contexto claro sin bandas resinosas, homogéneo a relativamente homogéneo, azonado. Elementos de la cutícula de 31–53 × 7.5–14.3 μm, claviformes, enteros a ocasionalmente con una protuberancia lateral, las cuales reaccionan con el reactivo de Melzer, tornándose negras inmediatamente. Basidiosporas de 8.8–11.1 (–11.5) × 6.2–7.2 (–7.6) μm, Q = 1.38–1.75, elipsoides a oblongas, exosporio con pilares interparietales sublibres a libres.

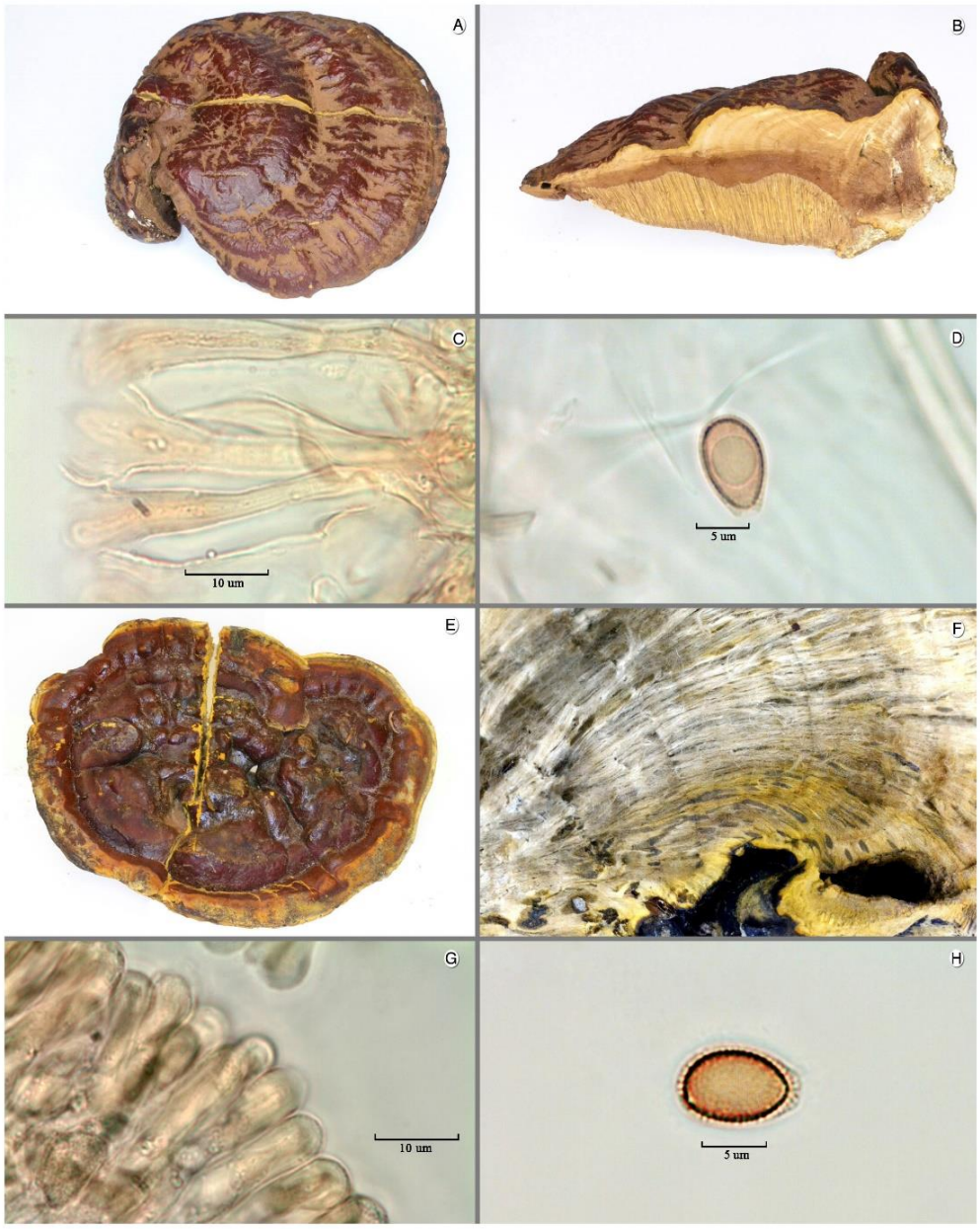

Figura 2. *Ganoderma sessile* y *G. weberianum*. *G. sessile*: **A)** basidioma, **B)** contexto, **C)** cutícula, **D)** Espora; *G. weberianum*: **E)** basidioma, **F)** contexto, **G)** cutícula, **H)** espora.

Material estudiado. Municipio de Ures, Sierra de Mazatán, A. Gutiérrez, E. Hernández, R. Maldonado y D. López, 10-09-2012, UES 10407, 10408.
Comentarios. Las características distintivas de esta especie son su contexto claro, sin bandas resinosas, azonado, el cual es más grueso en la base, adelgazándose hacia el margen. Esta es una especie poco común. Fue registrada de Brasil (Torres-Torres *et al.*, 2012), Argentina (Gottlieb y Wright, 1999a) y México (Raymundo *et al.*, 2013). Esta es la segunda cita para Sonora, la primera fue por los últimos autores. Los ejemplares se recolectaron en bosque de encino sobre la base de *Quercus* sp.

Ganoderma weberianum (Bres. & Henn. ex Sacc.) Steyaert, Persoonia **7**(1): 79 (1972) (Figura 2E-H)
Basidioma de 45–200 × 30–185 × 20–52 mm, sésil a subestipitado cuando crece en el suelo sobre raíces, anual, solitario a gregario, de consistencia fibrosa a suberosa. Píleo semicircular a flabeliforme, aplanado a ligeramente convexo; superficie glabra, cubierta por una capa de laca brillante, con zonas opacas, abollada a irregular, ligeramente sulcada concéntricamente; marrón-rojizo (8F8) en la base o en el centro cuando subestipitada, amarillo-parduzco (5C7) a blanco-anaranjado (5A2) hacia el margen, el cual es estéril, obtuso a agudo, blanco-anaranjado (5A2) en fresco, marrón (5F8) a negro cuando seco. Contexto de hasta 30 mm de grosor, relativamente homogéneo, zonado, con incrustaciones resinosas difíciles de observar, más conspicuas en la base, ausentes en algunos ejemplares, blanco anaranjado (5A2) a anaranjado pálido (5A3). Tubos de hasta 18 mm de largo, marrón-amarillento (5D5). Poros de 3–6 por mm, redondeados a angulados, concoloros con el margen, el cual es irregular, con zonas marrón cuando seco, de 122–287 μm diámetro; disepimento de 25–94 μm. Sistema hifal dimítico. Hifas generativas de la trama del contexto no observadas; hifas esqueléticas de 2.1–9.7 μm diámetro, de pared gruesa a sólidas, sin septos, arboriformes y aciculiformes, amarillo pálido. Elementos de la cutícula de 33.8–50.6 × 6.5–12.2 μm, cilíndricos a claviformes, con una o dos protuberancias o ramificaciones laterales a enteros, con gránulos en el ápice, amarillos; hifas generativas de 3.1–5.6 μm diámetro, pared delgada, con fíbulas, hialinas; hifas esqueléticas de 1–5.7 μm diámetro, pared gruesa a sólida, amarillo oro; hifas conectivas de 1–2.1 μm diámetro, ramificadas, pared gruesa a sólida, amarillenta a hialina. Basidiosporas de 7.4–10.3 × 5.3–6.7 (–7.1) μm, Q = 1.22–1.66, generalmente elipsoides, algunas ampliamente elipsoides u oblongas, ápice truncado, perisporio perforado, exosporio con pilares interparietales sublibres a libres, delgados, marrón-amarillento. Basidios no observados.
Material estudiado. Municipio de Ures, Sierra de Mazatán, A. Gutiérrez, E. Hernández, R. Maldonado y D. López, 10-09-2012, UES 10415, 10417; C. Trujillo, E. Hernández, A. Gutiérrez y D. López, 27-09-2013, UES 10410, 10411, 10412, 10416.
Comentarios. Los caracteres distintivos de esta especie son el contexto zonado, claro, con incrustaciones resinosas en ocasiones difíciles de observar (ausentes en algunos de los ejemplares revisados), elementos de la cutícula cilíndricos a ligeramente claviformes, con incrustaciones apicales y esporas relativamente pequeñas. Pan y Dai (2001) mencionaron la presencia de clamidosporas en basidiomas de esta especie, lo cual no se observó en los ejemplares de Sonora. Este es el primer registro de *G. weberianum* para México. Recolectada en bosque de encino en la Isla de Montaña, en la base de un *Quercus* sp.

Distribución de Ganoderma *en Sonora*. Con base en los estudios previos (Esqueda *et al.*, 2010; Raymundo *et al.*, 2013) y en el actual, considerando todas las especies de *Ganoderma* registradas en Sonora, *G. curtisii* presenta la distribución conocida más amplia en el estado. Se encuentra en tres localidades: Reserva Forestal Nacional y Refugio de Fauna Silvestre Ajos-Bavispe (RFAB), Sierra de Mazatán y Sierra de San Javier; seguida por *G. applanatum* en la Sierra de Yécora y la zona urbana de Hermosillo; *G. coffeatum* (citada por Esqueda *et al.*, 2010) en RFAB y Reserva de la Biosfera Sierra de Álamos-Río Cuchujaqui (RBAC); *G. lucidum* (Curtis) P. Karst. (citada por Esqueda *et al.*, 2010) en RFAB y RBAC; *G. sessiliforme* (presente estudio y citada por Raymundo *et al.*, 2013) en Sierra de Mazatán y Tuape. Con una localidad, *G. adspersum* (citada por Esqueda *et al.*, 2010) en RFAB, *G. lobatum* en Sierra de Yécora, mientras que *G. oerstedii*, *G. sessile* y *G. weberianum* en la Sierra de Mazatán. De las diez especies de

Ganoderma conocidas en Sonora, cinco se encuentran distribuidas en la Sierra de Mazatán, por lo que es una de las zonas con mayor diversidad de *Ganoderma* en la entidad.

Discusión

Algunas de las especies determinadas en este estudio presentan la medida de las basidiosporas diferente a lo que han registrado otros autores, como se ha comentado. Esto puede deberse a la gran variabilidad que existe o a la falta de más observaciones o malas determinaciones, lo cual podría aclararse con el estudio de los tipos. *Ganoderma oerstedii* se encontró en mezquital, en una zona con temperaturas mayores a 40 °C, lo que muestra el grado de adaptación de esta especie. Las especies de la Sierra de Mazatán están en una isla ecológica rodeada por áreas xerófilas. Aquí se presentan distintos tipos de vegetación como mezquital en el pie de la montaña, seguido de matorral subtropical y bosque de encino en la parte alta (Piña-Páez *et al.*, 2013). En esta área se encontró el 50 % de las especies conocidas de *Ganoderma* para Sonora. Este comportamiento podría deberse a que los encinos están afectados por la epífita *Tillandsia recurvata*, la cual compite por la luz con el encino. Dicha condición está capitalizada por distintas especies de *Ganoderma*. Sería conveniente monitorear mejor la zona para entender los cambios ecológicos que ocurren. Por otra parte, es interesante observar que en Sonora se distribuyen cerca del 50 % de las especies de *Ganoderma* conocidas en México, lo cual se puede deber a la intensidad de las exploraciones (p. ej. Esqueda *et al.*, 2010) y a la diversidad de ecosistemas presentes en este estado.

Existen dudas sobre la distribución de *Ganoderma lucidum* en el Continente Americano y se especula que la especie está restringida a Europa, como lo han hecho ver Moncalvo *et al.* (1995) y Moncalvo y Ryvarden (1997). Los registros de *G. lucidum* en Sonora por Esqueda *et al.* (2010), deberán revisarse detalladamente para corroborar su identidad. Por otro lado, debido a la complejidad del género y a su amplia distribución en México, es necesario realizar una monografía a nivel nacional.

Agradecimientos

Los autores agradecen a Georgina Vargas por su apoyo técnico, así como a Alberto Jiménez, Carolina Trujillo y Rafael Maldonado por su apoyo en los muestreos. Asimismo a los revisores asignados de este manuscrito por sus valiosas observaciones.

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Recibido:
28 de noviembre de 2014

Aceptado:
26 de enero de 2015

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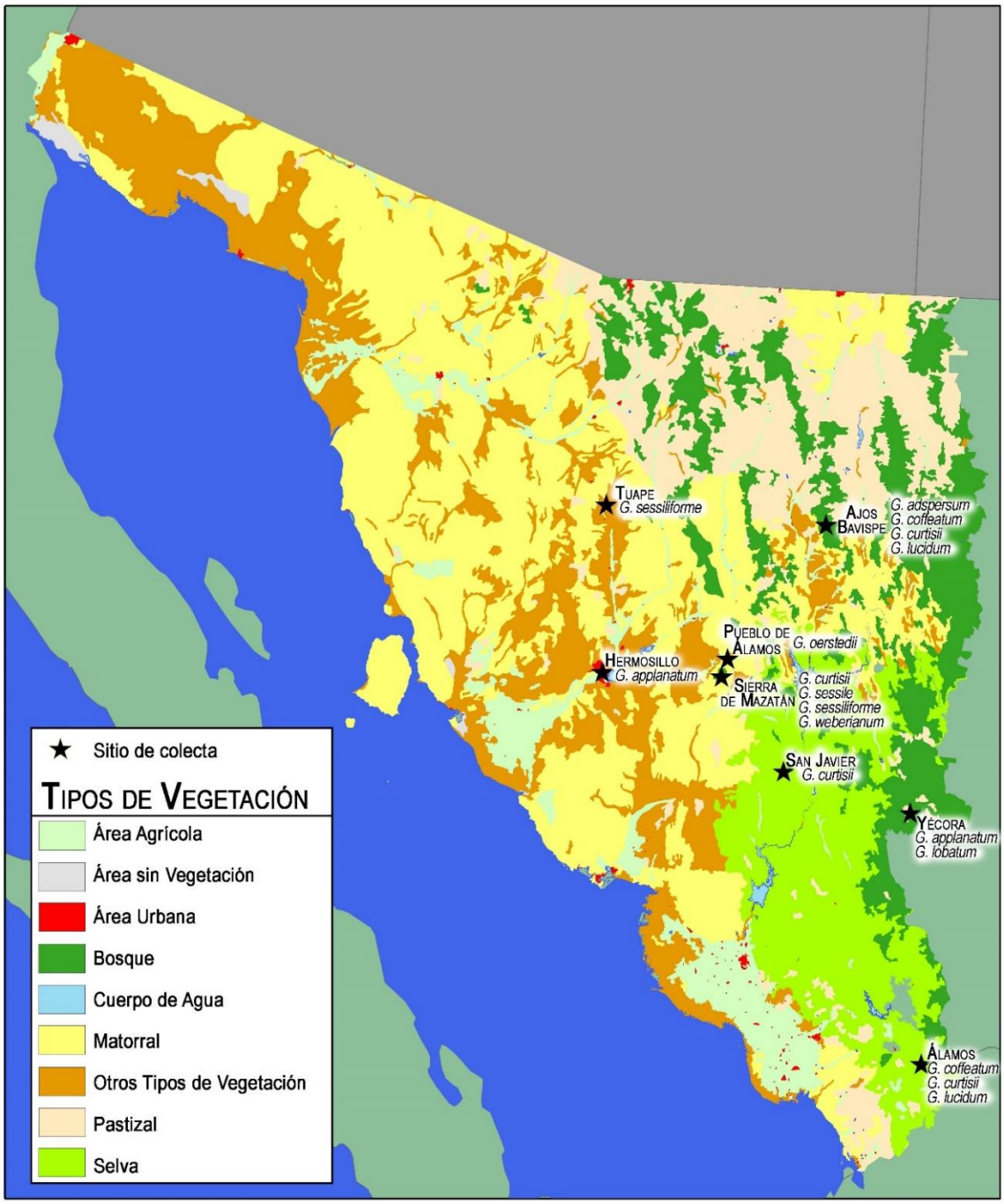
Material complementario



Distribución de *Ganoderma* en Sonora

Capítulo III

Morphological Characteristics of Wild and Cultivated specimens of a *Ganoderma subincrustatum* isolated from Sonora, Mexico

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En preparación para ser enviado a la revista Botanical Sciences

Morphological Characteristics of Wild and Cultivated Specimens of a *Ganoderma subincrustatum* isolated from Sonora, Mexico

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Author Contributions

Damian López-Peña made most of the taxonomical descriptions and wrote the manuscript.

Idaly Morales-Estrada cultured the fungus for production of fruiting bodies.

Crystal Samaniego-Rubiano examined cultivated specimens.

Eduardo Hernández-Navarro collaborated with the edition of the manuscript.

Aldo Gutiérrez edited the figures.

Martín Esqueda collaborated with the edition of the manuscript.

Abstract

Ganoderma is a complex genus whit a high morphological variability. Context type, resinous bands and incrustations, shape and size of pileipellis cells and basidiospores are the major characters to delimit species within the genus. *Ganoderma subincrustatum* is a common parasite and saprophytic species, and its circumscription is doubtful for some authors. The aim of this work was to study morphological variability of wild and cultivated specimens of a *G. subincrustatum* strain from a peach orchard in La Costa de

Hermosillo, Sonora, Mexico. The strain was cultivated under high and low illumination conditions (350 and 3500-4000 lux). Basidiomata color, size and shape were different between wild and cultivated specimens, as well as between basidiocarps cultivated under high and low lux condition. High lux condition caused stipe elongation (antler shape fruiting bodies), whereas under low lux condition normal basidiocarps were developed. Wild and cultivated specimens presented the same type of context, with two resinous bands. Shape of pileipellis cells and basidiospores seems to be constant characters, nevertheless spore were largest in wild specimen. This is the first record of this species for Sonora.

Key words: taxonomy, circumscription, context type, pileipellis cells, basidiospores.

Resumen

Ganoderma es un género complejo con alta variabilidad morfológica. Los caracteres más importantes para la delimitación de las especies de éste género son el tipo de contexto, presencia o ausencia de incrustaciones o bandas resinosas, y la forma y tamaño de células de la cutícula y basidiosporas. *Ganoderma subincrustatum* es una especie parásita y saprófita común y su circunscripción es dudosa para algunos autores. El objetivo de este trabajo fue estudiar la variabilidad morfológica de una cepa de *G. subincrustatum* aislada de una huerta de durazno en La Costa de Hermosillo, Sonora, México, mediante la comparación de especímenes silvestres y cultivados. Adicionalmente se evaluaron dos condiciones de desarrollo de los basidiomas cultivados (iluminación alta *vs* baja). Se encontró una alta variabilidad de forma, tamaño y color entre el basidioma silvestre y los cultivados, incluso entre los especímenes cultivados (iluminación alta y baja). La aplicación de 3500-4000 lux causó elongación del estípite (cuerpos fructíferos con forma de asta), mientras que la condición de 350 lux provocó un

desarrollo adecuado de los basidiocarpos. Tanto el ejemplar silvestre como los cultivados, presentaron el mismo tipo de contexto, con dos bandas resinosas. La forma de las células de la cutícula y basidiosporas parecen ser caracteres constantes, no obstante el tamaño de las basidosporas varió ligeramente (más largas en el espécimen silvestre). Este es el primer registro de esta especie para el estado de Sonora.

Palabras clave: taxonomía, circunscripción, tipo de contexto, células de la cutícula, basidiosporas.

Ganoderma P. Karst. (Polyporales: Ganodermataceae) is a genus with a cosmopolitan distribution, recorded from tropical, temperate, desert-like areas, and farmlands (Gottlieb & Wright 1999, Torres-Torres *et al*. 2012, López-Peña *et al*. 2016). Morphological plasticity makes *Ganoderma* one of the most complex genus in Polyporales; a sign of this are the 290 published names, many of these considered synonyms (Ryvarden 2000). Therefore, different molecular markers for species delimitation have been assessed (Douanla-Meli & Langer 2009, Wang *et al*. 2012). The taxonomy of *Ganoderma* is usually based on macro and micro- morphological characters, the most important are shape and size of spores and pileipellis cells (Steyaert 1972; Gottlieb & Wright 1999; and Ryvarden 2000). Recently, resinous incrustations and context type were used to discriminate some species since they also are constant characters (Torres-Torres et al. 2012). A rather homogeneous context and resinous bands and incrustations are characteristics of *G. subincrustatum* Murrill, a scarcely cited species, recorded from Argentina, Jamaica and Mexico (Torres-Torres et al. 2015). The aim of this study was to compare the morphological characteristics of wild and cultivated specimens of a *G. subincrustatum* strain isolated from a peach orchard in La costa de Hermosillo, Sonora,

Mexico, and to assess the effect of light intensity on fruiting bodies development, to know if taxonomical characters remain the same between them.

Materials & Methods

Collection and strain isolation. In September 2012,*ex professo* explorations in a peach orchard in La costa de Hermosillo were made. Sections of hymenophore were placed in Petri dishes with malt extract agar (MEA), supplemented with chloramphenicol (0.5 g/L) and benomyl (22 mg/L). After mycelium development, portions of mycelium were transferred to MEA without supplements. Mycelia-samples from this second culture were examined to find clamp connections (Largent et al. 1977), with an Olympus BX51 microscope.

Culture conditions. Ganoderma subincrustatum strain was maintained on MEA Petri dishes. Inoculum preparation was carried out using wheat grain. Grain was soaked for 24 h in distilled water to reach 60% humidity, later sterilized at 121°C for 1.5 h. After temperature was cooled down, wheat grain was inoculated with portions of the MEA culture. Wood wastes were hydrated for 12 h, drained and sterilized in polypropylene bags for 1.5 h at 121°C. The bags were inoculated with 5% (w/w) of wheat grain spawn and incubated in a dark room at a controlled temperature of 25±1°C. Basidiocarps development were performed under two conditions, the former was maintained at 25±1°C, 85-90% of relative humidity (RH) and 350 lux (low light condition). The second was 27±1°C, 90-100% RH and 3500-4000 lux (high light condition), photoperiod for both treatments was 12 h (Stamets, 2000). Basidiocarps were harvest when growth stopped, whit two developmental shapes: antler shape (3500-4000 lux treatment), and when pileus was fully developed (350 lux treatment), to study context characteristics and pileipellis elements.

Morphological characterization. Thin sections of basidiomes were mounted with 10% KOH and analyzed using an Olympus BX51, with an Infinity Analyze 2 integrated camera for microscopic descriptions. Macro and micro- morphological features were considered for this study.

Descriptions were made according to Gottlieb & Wright (1999), and Torres-Torres and Guzmán-Dávalos (2012). Basidiospore shape was expressed according to Q ratio (length/width) of at least 20 randomly selected spores (Largent et al. 1977). Terminology for descriptions was taken from Torres-Torres and Guzmán-Dávalos (2012). Context types are homogeneous, not completely homogeneous, and duplex. Also resinous incrustations in the context and pileipellis cells were considered. Color descriptions were made according to Kornerup & Wanscher (1963).

Results

We isolated a strain of *G. subincrustatum* on a living peach (*Prunus persica*) tree in an orchard at La costa de Hermosillo, and it was directly performed taking sections of the basidiome (hymenophore) into Petri dishes with MEA supplemented whit antibiotics. A large quantity of clamps was founded in the isolated mycelia, which serve as indicative of good isolation, because clamps are basidiomycete's specific structures. We also collect the basidiome for morphological characterization.

Ganoderma subincrustatum Murrill, North American Flora 9:122. 1908.

Wild specimen description (Figure 1).

Basidiome 270 x 120 x 70 mm, annual, substipitate to sessile, dimidiate, fibrous to spongy. Pileus semicircular-flabelliform; surface glabrous, smooth, slightly dented and corrugated, bright to dull, with semi-concentric furrows, more conspicuous to the margin, cuticle that can detach, covered by a brown (6E8) basidiospore layer; light

orange (5A5), reddish-golden (6C7), deep orange (6A8), light brown (6D8), reddish-brown (8E6), dark brown (8F8) to photo brown (9F8), almost black in some zones, with deep yellow (4A8) furrows due to cuticle detaching; margin sterile, obtuse, smooth, with groove zones, pale yellow (4A3), with yellowish-orange (4A7) zones, greyish yellow (4C7) after contact. Context up to 22 mm thick, fibrous-spongy, relatively homogenous, with a deep yellow (4A8) band under the cuticle, light orange (5A5) to brown (5F7) toward the tubes, concentrically zonate, with 2 resinous bands, the upper thickened, both intermittent, ¾ the length of the context, interrupted near the margin. Tubes up to 14 mm long, brown (5E8), orange white (5A2) towards the pores surface, unstratified. Pores 3-5 per mm, pale yellow (4A3), greyish-yellow (4C7) after contact, angular to rounded, with irregular edge, 133-171 µm diameter. Stipe 50-75 x 29-52 mm, lateral, smooth, shiny to dull, photo brown (9F8), flattened to cylindrical, solid, fibrous to spongy, concentrically zonate. Hyphal system trimitic. Generative hyphae simples, 1.6-7.2 µm diam, septate, fibulate, hyaline to pale yellow, hard to observe, generally collapsed; skeletal hyphae arboriform to non-branched, 1.6-7.2 µm diam, solid to thick-walled, light brown; connective hyphae branched, 1.6-3.2 µm diam, thick-walled, hyaline to pale yellow. Pileipellis a crustohymeniderm, cells 31.2-90.4 x 8-12 µm, narrow clavate to clavate, generally with one to three protuberance or branches, solid to tick walled, sometimes multistratified, pale yellow to deep yellow. Basidiospores (8) 9.6-11.2 (-12.8) x 5.6-8 µm, Q= 1.49-1.64, ellipsoids to oblong, apex truncate, with apical germ pore, exosporium with subfree to partially anastomosed interwalled pillars, reddish-brown. Specimen examined. La costa de Hermosillo, Sonora, Mexico. Mayra de la Torre, UES, 10500.

Cultivated specimens description (Figure 2).

Basidiomata 59-84 x 39-75 x 6-19 mm, substipitate to sessile, dimidiate, fibrous to spongy. Pileus semicircular-flabelliform, some specimens with antler form (high light condition); surface glabrous, granular, mainly dull with few shiny zones, some specimens mainly shiny, dark-brown (8F8), brown (6D8), orange (5A6) in the middle to yellowish-brown (5D8) near the base; margin sterile, obtuse, smooth, orange white (5A2). Context 4-13 mm thick, relatively homogeneous, corky to fibrous, slightly zonate, with one or two resinous or colored bands ¾ the length of the context, interrupted before the margin, the lower portion dark brown (6F6) to golden brown (5D7), upper portion orange (5A6), brownish-orange (5C6) to light orange (5D6). Tubes 2-8 mm long, greyish-brown (5E4), unstratified. Pores 3-5 per mm, white in fresh. Pileipellis a crustohymeniderm, cells 42.33-58.85 x 5.36-9.61 µm, narrow clavate to clavate, with one to three lateral or apical ramifications or protuberances, with apical incrustations, light yellow to deep yellow. Basidiospores 7.71-9.97 (-10.59) x 5.78-7.7 µm, Q= 1.21-1.47, ellipsoids to broadly ellipsoids, with subfree to partially anastomosed interwalled pillars, reddish-brown. Characters not mentioned are de same than for wild specimen.

Specimens examined. UES, 10501, 10502, 10503, 10504.

Discussion

Most of the products from La costa de Hermosillo are produced with exportation purposes, and annually large losses are caused by *Ganoderma* infections in those fields, mainly of peach fruits. *G. subincrustatum* is a parasite and saprophytic species recorded mainly from tropical and subtropical areas, even in *Pinus*-*Quercus* forest (Torres-Torres et al. 2015). Size, color and shape variability of basidiomata was observed between wild and cultivated specimens, also among distinct cultivated basidiocarps. Wild basidiome

presents a larger size than cultivated, which could be attributed to different environmental conditions. Some specimens under high light conditions were developed like an antler form, and others with long stipe but tubes and basidiospores developed. Stamets (2000) mention that under low light conditions, stipe elongation becomes slow and the mycelium enters into the pilei development period in *G. lucidum*, thus longer stipes in cultivated specimens could be attributed to illumination condition. Context type remains similar between wild and cultivated basidiomata, even those harvested in antler developmental stage (Figures 1b, 2b). Which remains as significant character in *G. subincrustatum*.

Shape of pileipellis cells and spores seems to be a constant character in *G. subincrustatum*. In both cases, cells were clavate, with two or three lateral or apical branches or protuberances, light to deep yellow, some of them with apical incrustations (Figures 1c, 2c). A slight basidiospores variability between wild and cultivated specimen was observed. Wild basidiome presents larger basidiospores, mostly ellipsoids, some oblong (Figure 1d,e) while cultivated basidiomata presents mostly ellipsoids, some broadly ellipsoids basidiospores (Figure 2d,e), all of them with subfree to partially anastomosed interwalled pillars. This is the first record of the species for Sonora State.

According with Torres-Torres et al. (2015), *G. subincrustatum* presents a perennial growth, but Gottlieb & Wright (1999) did not mentioned that character. In this study all basidiomata were harvested in their first developmental stage, even the wild specimen. Bazzalo & Wrigth (1982), and Ryvarden (2000) considered *G. subincrustatum* as a synonym of *G. resinaceum*, but Gottlieb & Wright (1999) contemplated it as independent species, with pileipellis cells of 33-45 x 8-10 µm, and spore size range of (9) 10-12 (13) x 6-9, they also consider *G. applanatum* as synonym of *G. resinaceum*. In

addition, Torres-Torres et al. (2015) maintain *G. subincrustatum* as independent species, and report pilleipelis cells of 32-80 (-96) x 5.5-14.5 µm, and a spore size range of 9.6-12.4 x 7.2-8.4 µm. In this work we recorded smaller basidiospores, which probably can be attributed to environmental conditions, however the shape remains equal.

Temperature in that zone in September 2012, when the wild basidiome was developing fluctuated between 24-37°C, and relative humidity was 24-89% (Weather Underground, 2016). It is important to mention that those fields are under constant irrigation, thus moisture in that microenvironment is maintained high. To our knowledge, there are no studies focused on the characterization of wild and cultivated specimens of *Ganoderma*, which contributes to the circumscription of the species. Due to the intrinsic species variability recorded for *Ganoderma* spp. worldwide, it is necessary to do harder work on its taxonomy, and to find molecular markers to support morphological data, and to demonstrate *G. subincrustatum* is a real independent species.

Acknowledgments

We want to thank Biol. Georgina Vargas and Alfonso Coronado Sesma for their technical support. We want to thank Ph.D. Mayra de la Torre for wild basidiome collection, very important for this study. We also want to thank Ph.D. Rigoberto Gaitán-Hernández for the donated cultivated specimens.

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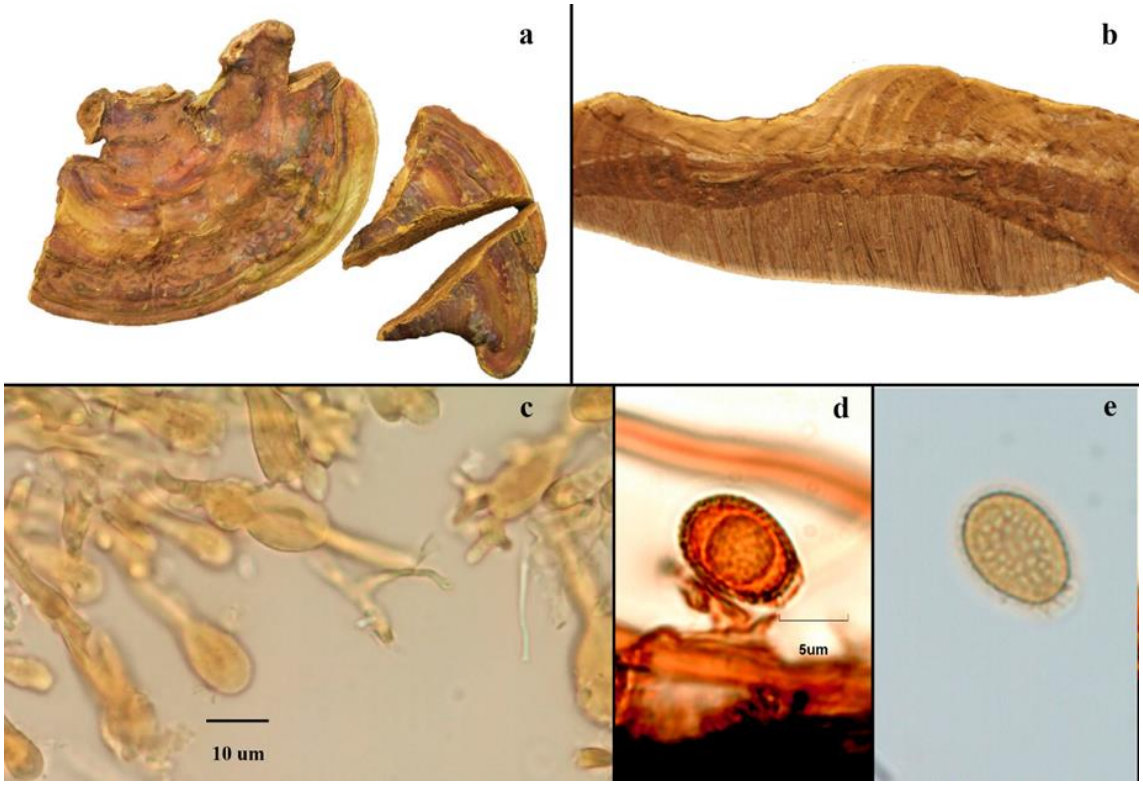
Figure 1. Morphological characteristics of wild specimen of *G. subincrustatum*.a: basidiome, b: context, c: cuticle elements, d, e: spores.

Figure 2. Morphological characteristics of cultivated specimens of *G. subincrustatum*.a: basidiomata, b: contexts, c: cuticle elements, d,e: spores.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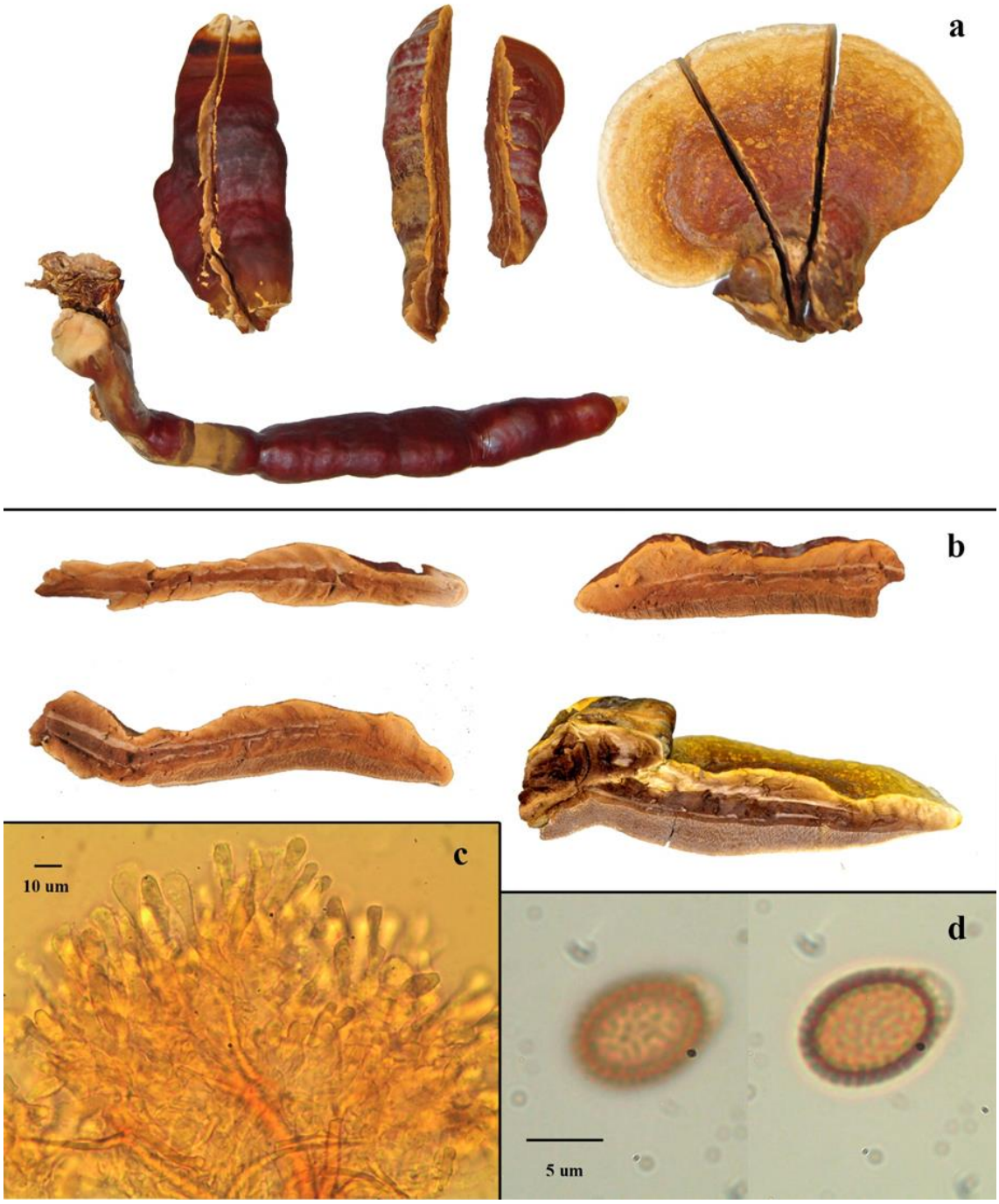
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Capítulo IV

Antiproliferative activity of *Ganoderma subincrustatum* and *G. weberianum* isolated from Sonora, Mexico: Mycelia *vs* fruiting bodies

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Artículo en preparación para enviarse a la revista Journal of Natural Medicines

Antiproliferative activity of *Ganoderma subincrustatum* and *G. weberianum* isolated from Sonora, Mexico: Mycelia *vs* fruiting bodies

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Abstract

Several species of *Ganoderma* synthetize triterpenoids with antiproliferative activity. In this work, chloroform extracts from two developmental stages (mycelia and fruiting bodies) of *G. subincrustatum* and *G. weberianum* isolated from Sonora were studied. Extracts from fruiting bodies exhibited higher activity than mycelial extracts, this could be due to differences in metabolites produced between developmental stages. Both extracts induced vesicle and cellular debris formation, in all cell lines. Bioactivity was non-selective on cancerous cell lines. *G. subincrustatum* fruiting bodies extract presented the higher activity against all cell lines (A549, HeLa, Raw, L-929), thus, this was selected to be subjected to column chromatography. Two sub-fractions exhibited bioactivity (IC_{50} of 37.9 and 41.9 µg/mL, respectively) against A549 cell line, nevertheless, crude chloroform extract showed higher activity (<25 µg/mL). More research in necessary in order to know whether antiproliferative activity is due to apoptosis induction, besides, to know the mechanisms of the non-selective activity.

Key words: *Ganoderma* triterpenes**,** anti-cancer drugs, fungal extracts, cancer cell lines.

Introduction

Some species of *Ganoderma*, mainly *G. lucidum* have been used in traditional Asian medicine for more than two millennia (Wagner *et al*., 2003), to increase the immunological activity and to treat hypertension, arthritis, asthma, anorexia, hepatitis,

cancer and other illnesses (Paterson, 2006).Cancer is one of the most important diseases worldwide, therefore, the search for new compounds that suppress the growth of cancer cells is a growing area (Reuter *et al*., 2008; Liu *et al*., 2012).*Ganoderma* presents a cosmopolite distribution, comprising more than 250 species, many of them unstudied. It is well known that several *Ganoderma* species synthetize biologically active compounds, mainly lanostane type triterpenes, which exhibit antiproliferative activity against different cell lines (Adamec *et al*., 2009; Trigos & Suárez, 2011; Xia *et al*., 2014).

Because fruiting bodies production is a time-consuming activity to obtain products from *Ganoderma* (3-5 months), the production of mycelia by liquid culture seems to be a promising technique, because it offers inter-batch homogeneity, lees time is required and fermentation factors can be bio-directed to the production of bioactive compounds (Chang *et al*., 2006). Nevertheless, it is known in *G. lucidum* that expression of genes involved on the synthesis of these kind of compounds increase in primordia development, which is correlated with a greatest triterpene contents in this phase of its lifecycle (Chen *et al*., 2012). Additionally, it has been reported that secondary mycelia contain mainly 3α substituted compounds, whereas fruiting bodies presents mainly 3keto or 3β substituted compounds (Xu *et al*., 2010), which could be related with their bioactivity.

Ganoderma subincrustatum and *G. weberianum* are distributed in Sonora, Mexico. These are two poorly studied species, of which, metabolite profile and bioactivity are unknown to date. In order to take advantage and to study of our own natural resources, antiproliferative activity of chloroform extracts from two developmental stages of *G. subincrustatum* and *G. weberianum* isolated was studied.

Materials and methods

Fungal material and fermentation conditions

Ganoderma subincrustatum strain was isolated from a farmland in La costa de Hermosillo fields, Sonora, while *G. weberianum* strain was isolated from an oak tree (*Quercus* sp.) in La sierra huerfana, Ures, Sonora, Mexico. Both were authenticated by

the first author of this manuscript (López-Peña *et al*., 2016). Strains are deposited in the Fungi strain collection of the Institute of Ecology (Xalapa, Mexico), registered as 21-BH (*G. weberianum*) and A-BH (*G. subincrustatum*). The strains were maintained on malt extract agar (MEA) in Petri dishes. Liquid culture media composition for mycelia production was as follows: 16 g/L of glucose, 2.93 g/L of peptone, 21 g/L of corn flour and 7 g/L of soybean protein powder (Xu *et la*., 2008). 1 L Erlenmeyer flasks with 400 mL of culture media were inoculated with 16 agar discs (1 cm diam.) each one, the same for both strains. Liquid fermentation conditions were maintained at 28±1°C, shaking of 120 rpm, pH 5.5, 13 d, in darkness. Mycelia were recovered by centrifugation and washed with distilled water. Fruiting bodies production was performed using oak wood ships. Sterilized substrate was inoculated with spawn (wheat grain) (5% w/w) previously prepared. Incubation was conducted at 25±1°C in the dark. Conditions for fruiting bodies development were maintained at 25±1°C, 85-90% of relative humidity, and 350 lux (Gaitán-Hernández *et al*., 2006). Fruiting bodies were harvested when pilei were fully developed or when it has stopped growing. Mycelia and fruiting bodies were freeze dried and stored in a dry place until use.

Extract preparation

Mycelia were milled whit an electric blender to obtain a powder, fruiting bodies were first passed through a hand mill, and subsequently milled whit an electric blender. For extracts preparation, dried biomass (mycelia and fruiting bodies) (1 g) were extracted with 95% (v/v) ethanol (30 mL), by sonication for 45 min (Ultra Sonic Cleaner, Branson 2210). Biomass was removed by centrifugation and filtration, then the extract was concentrated under reduced pressure conditions (BÜCHI Rotavapor, RE 121). Afterwards, the dried residues were suspended in distilled water and then extracted with chloroform, the chloroform layer was recovered (Xu *et al*., 2008) and dried at 30°C to evaluate bioactivity. Extracts were stored at -20°C until use. The codes for the extracts are G.sM (*G. subincrustatum* mycelial extract), G.sFB (*G. subincrustatum* fruiting bodies extract), G.wM (*G. weberianum* mycelial extract), G.wFB (*G. weberianum* fruiting bodies extract). All extracts were qualitatively characterized by thin layer chromatography (TLC).

Cell culture

Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 5% heat-inactivated fetal bovine serum and penicillin-streptomycin (100 U/mL) in 25 cm^2 culture dishes. Cultures were maintained in an incubator Isoterm (Fisher Scientific, USA) with 5% of CO_2, 37°C and 95% relative humidity. Cancer cell lines HeLa (human cervical carcinoma), A549 (human lung carcinoma), and normal cell line L-929 (subcutaneous connective tissue) were purchased from the American Type Culture Collection (ATCC, Rockville, MD). RAW 264.7 (macrophages transformed by virus *Albeson leukemia*) lines were kindly provided by Dr. Emil R. Unanue (Department of Pathology and Immunology, Washington University in St. Louis, MO) (Torres-Moreno *et al*., 2015).

Antiproliferative activity screening

Antiproliferative activity was performed by MTT assay (Cell proliferation Kit I, Roche), according to Müller *et al*. (2006), with some modifications. Cells suspensions (200,000 cell/mL) were placed in 96 ELISA plates (Costar, Corning, N.Y. USA) and incubated for 24 h. Afterwards, extracts were dissolved in DMSO and re-dissolved in DMEM to give a final DMSO concentration of 0.25% (which show no interference with the assays). Challenges with the evaluated extracts were done for 48 h. After treatments, 10 µL of MTT solution were added to each well and incubated for 4 h in a humidified atmosphere at 37°C according to the manufacturer's instructions. Later, 100 µL of the solubilization buffer were added into the wells and incubated overnight and then absorbances were read at 570 and 655 nm in a microplate reader. Antiproliferative activity was evaluated by the mitochondrial-dependent conversion of MTT to purple formazan crystals by metabolic active cells (expressed as IC_{50} values, based on three independent experiments).

Bioguided fractionation

2.2 g of selected *Ganoderma* chloroform extract was subjected to column chromatography on silica (4 x 50 cm), eluted with EtOAc-MeOH (*v*/*v*, 100:0, 95:5,

90:10, 85:15, 80:20, 50:50, 0:100). Three hundred thirty three fractions (10 mL) were collected and analyzed by TLC. Twenty six (F1 to F26)sub-fractions were grouped according to TLC patterns. Anysaldehide was used as develop reagent. Also, TLC plates were visualized at 254 and 366 nm with an UV light lamp.F1 (8.2 mg), F2 (83.1 mg), F3 (13.4 mg), F4 (27.8 mg), F5 (323.8 mg), F6 (93.3 mg), F7 (114.2 mg), F8 (6.2 mg), F9 (37 mg), F10 (12.7 mg), F11 (34.3 mg), F12 (22.6 mg), F13 (61.7 mg), F14 (8.5 mg), F15 (39.2 mg), F16 (11.8 mg), F17 (44.3 mg), F18 (18.7 mg), F19 (14.3 mg), F20 (7.8 mg), F21 (9.3 mg), F22 (17.4 mg), F23 (18.2), F24 (30.8 mg), F25 (55 mg), F26 (337. 8 mg). Antiproliferative activity of all fractions were tested against A549 cell line.

Statistical analysis

One way analysis of variance was applied to antiproliferative activity data (F7 and F15 activity). Also Tukey-Kramer test was performed in order to compare treatments with their respective control ($p \leq 0.05$). Data are presented as mean ± standard deviation.

Results and discussion

Some species of *Ganoderma* presents antiproliferative activity, attributed mainly to lanostane-type triterpenes present in mycelia and fruiting bodies. Selected culture media for mycelia production allowed the production of a high amount of triterpenoids in *G. lucidum* (Xu et al., 2008), nevertheless, each strain and species respond in a particular way to a certain media composition.

Antiproliferative activity of Ganoderma strains

In order to compare the effect on the growth of tumor cells of two developmental stages (mycelia and fruiting bodies) of *G. subincrustatum* and *G. weberianum*, antiproliferative activity against several cell lines was performed by MTT assay. Extracts from fruiting bodies (G.sFB and G.wFB) showed greater bioactivity than mycelial extracts, on all evaluated cell lines. Likewise, G.sFB extract exhibited the greatest inhibitory activity against HeLa, RAW 264.7 and A549 cancer cell lines, with IC_{50} values of <25 µg/mL..According to the established by the National Cancer Institute, G.wFB is considered cytotoxic against the evaluated cell lines, because IC_{50} values are under 30

µg/mL(Zapata et al., 2009).The lower activity was observed in G.wM (>100 µg/mL against all cell lines). L-929 control cell line was highly affected by both fruiting bodies extracts, thus antiproliferative activity of G.sFB and G.wFB was non-selective on cancer cell lines (Table 1). Also, after treatment, G.sFB and G.wFB were able to induce vesicle formation in all cell lines, being more evident in A549 cell line (Figure 1).

Table 1. Antiproliferative activity of *Ganoderma* spp. strains against several cell lines: Mycelia *vs* fruiting bodies

	Cell Lines IC_{50} (µg/mL)			
Extracts	**HeLa**	**RAW 264.7**	**A549**	**L-929**
G.sM	ND	81.9±4.3	ND	ND
G.sFB	<25	<25	<25	<25
G.wM	ND	ND	ND	ND
G.wFB	57.7±6.8	<25	42.8±5.5	<25

IC_{50} represented as mean ± standard deviation. Extract codes: G.sM (*G. subincrustatum* mycelia), G.sFB (*G. subincrustatum* fruiting bodies), G.wM (*G. weberianum* mycelia) and G.wFB (*G. weberianum* fruiting bodies). ND: not determined to 100 µg/mL.

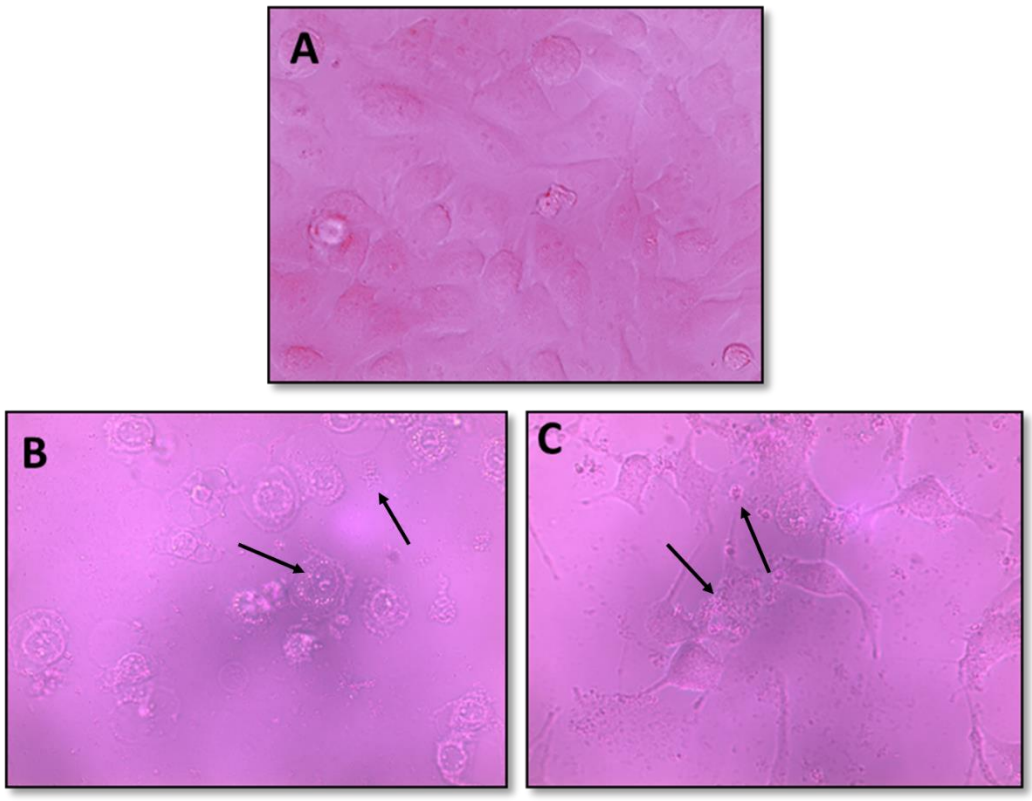

Figure 1. A549 cell morphology. A: Control, B: G.wFB (100µg/mL), C: G.sFB (100µg/mL). 40X magnification. Arrows indicate vesicle and debris formation.

TLC analysis showed a greater amount and intensity of bands that absorb at 254nm in extracts from fruiting bodies compared with mycelial extracts (data not shown). This corresponds with a higher activity against all evaluated cell lines in extracts from this developmental stage. Therefore, this could be attributed to a higher production of bioactive compounds in fruiting bodies developmental stage (Chen *et al*., 2012). Lanostane-type triterpenes of *Ganoderma* absorb at that wavelength, due to the presence of α,β-unsaturated carbonyl groups (Wagner *et al*., 2003).Liu *et al*. (2012) demonstrated that carboxylic group of ganoderic acid DM binds with α and β-tubulin in PC-3 cells (human prostate cancer) through an amidation reaction. In order to know if acid components were present in fruiting bodies extracts, a purification step to obtain acid triterpenes was assay and monitored by TLC (Xu *et al*., 2008). TLC runs showed that acid components were present in both extracts (data not showed). A large number of triterpenes from *Ganoderma* presents a carboxylic group at C-26, thus, some of the acid molecules present in both extracts could interact with this microtubules components in all cell lines, which could be related with the non-selective activity of the extracts.

Searching for bioactive fractions

Due to the high activity showed, we selected and subjected G.sFB extract to column chromatography. F7 and F15 sub-fractions showed the greatest bioactivity against A549 cell line, with IC_{50}values of 37.9 ± 6.27 and 41.9 ± 4.9 µg/mL, respectively. Both sub-fractions exhibited similar activity, and treatments at 12.5-50µg/mL were statistically different whit their respective control ($p \leq 0.05$) (Figure2). Nevertheless, IC_{50} of these were higher than crude chloroform extract, which suggest that more than one molecule is responsible for the bioactivity and presents a synergic or additive effect. Some isolated triterpenes from *Ganoderma* presents IC_{50} between 25-150 µg/mL on different cell lines, in addition, ganoderic acids T, S and Mf can induce mitochondria mediated apoptosis (Tang *et al*., 2006; Liu & Zhong, 2011). A pool of several bioactive molecules in certain extracts could result in a high activity, because components may exert bioactivity through distinct mechanism of action. Moreover additional research is necessary in order to know whether antiproliferaive activity is due to apoptosis induction, besides, to know the reason of the non-selective activity.

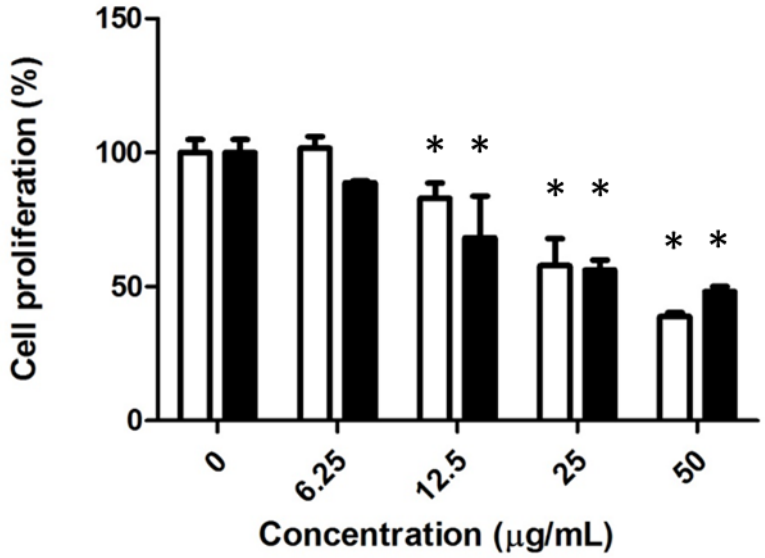

Figure 2. Antiproliferative activity of F7 (white bars) and F15 (black bars) against A549 cell line. Asterisk represents significant differences between treatment and their respective control ($p \leq 0.05$).

Acknowledgments

We want to thank M.Sc. Idaly Morales Estrada and Ph.D. Rigoberto Gaitán Hernández for providing the fruiting bodies for this study.

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CONCLUSIONES

En Sonora se distribuyen la mitad de las especies de *Ganoderma* citadas para México, probablemente por la diversidad de ecosistemas presentes en el estado. Las especies encontradas *G. lobatum*, *G. oerstedii* y *G. subincrustatum*, creciendo sobre *Quercus* sp., *Stenocereus thurberi* y *Prunus persica* respectivamente, se registran por primera vez para Sonora, mientras que *G. sessile* y *G. weberianum* (sobre *Quercus* sp.) para México. Debido a que existen diversas zonas de Sonora por explorar, es factible aún encontrar otras especies. Los caracteres determinantes para los taxones de *Ganoderma* se mantienen constantes en una cepa cultivada de *G. subincrustatum*, independientemente de la intensidad de luz en la que se desarrollaron los cuerpos fructíferos.

La hipótesis planteada originalmente fue que extractos obtenidos en la fase de cuerpos fructíferos de los aislados sonorenses de *Ganoderma* spp. presentaban mayor actividad antiproliferativa con respecto a los extractos de la fase de micelio dicariótico. Dicha hipótesis se comprobó para *G. weberianum* y *G. subincrustatum*, ya que los extractos obtenidos de fructificaciones de estas especies presentaron mayor actividad antiproliferativa que los obtenidos del micelio dicariótico, lo cual corresponde con una mayor cantidad de compuestos, según los análisis por TLC.

El extracto clorofórmico de *G. subincrustatum* presentó una variedad de compuestos bioactivos con actividad aditiva o sinérgica, los cuales no pudieron ser obtenidos en una sola fracción cromatográfica. Es necesario evaluar estos extractos sobre otras líneas celulares, principalmente sobre líneas no cancerosas humanas para conocer la selectividad real del extracto. Este estudio coadyuvó al conocimiento de la distribución del género en Sonora y el potencial de estas especies para la obtención de micofármacos, ya que en principio tienen actividad antiproliferativa sobre todas las líneas evaluadas.