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

CARACTERIZACIÓN Y EVALUACIÓN DE SAPONINAS DE *Yucca baccata* CONTRA TROFOZOÍTOS DE *Giardia intestinalis in vitro*

Por:

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RESUMEN

La giardiosis humana es un problema de salud pública mundial, incluyendo a México. Se ha estimado que alrededor de 200 millones de personas son infectadas por giardiosis anualmente. En México puede existir una prevalencia del 7 hasta el 68%, aun cuando existe una campaña nacional de desparasitación desde 1993. Los tratamientos de elección pueden ser costosos y pueden acompañarse de efectos secundarios, principalmente en la población infantil. En este contexto la investigación en química de productos naturales es una alternativa viable para la búsqueda de compuestos bioactivos con propiedades antiparasitarias. Recientemente se ha informado que los extractos de la planta *Yucca schidigera* nativa del suroeste de Estados Unidos posee actividad anti-*Giardia*. Por otro lado, el desierto sonorense tiene una flora endémica poco investigada en este contexto. *Yucca baccata* se encuentra distribuida en el desierto de Sonora pero se desconoce si presenta actividad como la exhibida por *Yucca schidigera.* Estudios previos realizados por nuestro grupo, han señalado que los extractos butanólicos de *Y. baccata* Torr., tienen actividad contra *Giardia intestinalis* en jerbos infectados. El objetivo de este trabajo fue aislar y caracterizar las saponinas del tallo de *Y. baccata* y determinar su actividad anti-*giardia in vitro*. Los resultados de resonancia magnética de protón (RMN^{1}H) de los extractos butanólicos obtenidos a partir de 680 g de harina del tallo de *Y. baccata* (EEYB), han revelado la presencia de saponinas. El EEYB fue sometido a cromatografía en columna utilizando sílica gel 60, 230-400 mesh y una fase móvil AcOEt-MeOH-H_2O, aislando a una saponina esteroidal tipo espiroestano (SESE), la cual se caracterizó mediante RMN de ^{1}H, ^{13}C, y experimentación de una y dos dimensiones HSQC, HMBC. El análisis mediante HR-ESI-MS mostró un ion con m/z de 739.4232 para $C_{39}H_{63}O_{13}$. La actividad antiparasitaria de SESE de *Y. baccata* fue evaluada *in vitro* sobre la proliferación de trofozoítos *de G. intestinalis* a diferentes concentraciones (μM). El metronidazol fue utilizado como control positivo (referencia). La actividad antiparasitaria fue evaluada 48 horas después de

añadir el tratamiento. La saponina SESE de *Y. baccata* mostró una IC50 de 2.12 µM contra *G. intestinalis* (P<0.001). En conclusión, la actividad anti-*giardia* de la saponina SESE sugiere su utilidad potencial como desparasitante. Se recomiendan estudios adicionales sobre la propiedad anti-*giardia* de la saponina de *Y. baccata* para elucidar su mecanismo de acción, y determinar sus efectos de toxicidad.

Palabras clave: *Giardia intestinalis*, anti-*giardia*, *Yucca baccata*, saponinas

ABSTRACT

Human giardiosis is a global public health problem, including in Mexico. It has been estimated that about 200 million people are infected with Giardiosis annually. Its prevalence in Mexico is high (68%), even though a national deworming campaign is active since 1993. The treatments of choice may be costly and may be accompanied by side effects, mainly in the childhood population. In this context the research on natural products chemistry is a viable alternative for the search of bioactive compounds with antiparasitic properties. It has recently been reported that extracts from the *Yucca schidigera*, a plant native of southwestern United States shows anti-*Giardia* activity. On the other hand, the Sonoran desert has an endemic flora little investigated about this context. *Yucca baccata* is widely spread in the Sonora desert and the investigation about its properties remains to be carried out. Previous studies conducted by our research team in our have revealed that the butanolic extracts of *Y. baccata Torr*., exhibit activity against *Giardia intestinalis* in infected gerbils. The aim of this work was to isolate and to characterize the saponins of the *Y. baccata*'s *stem* and to determine its anti-*giardia* activity *in vitro*. The proton magnetic resonance (NMR^{1}H) results of the butanolic extracts obtained from 680 g of *Y. baccata* starch flour (EEYB), provided evidence of the presence of saponins.The EEYB was subjected to column chromatography using silica gel 60, 230-400 mesh and a mobile phase AcOEt-MeOH-H2O, isolating a spiroestane-like steroidal saponin (SESE), which was characterized by ^{1}H, ^{13}C NMR, and one and two-dimensional HSQC experiments, HMBC, and the HR-ESI-MS analysis showed an ion with m/z of 739.4232 for $C_{39}H_{63}O_{13}$. The antiparasitic activity of *Y. baccata* SESE was evaluated *in vitro* on the proliferation of *Giardia intestinalis* trophozoites at different concentrations (µM). Metronidazole was used as a positive control. The anti-*giardia* activity was evaluated forty eight hours after addition of the treatment. *Y. baccata* SESE saponin showed an IC 50 of 2.12 µM against *Giardia intestinalis* (P <0.001). In conclusion, the anti-*giardia* activity of SESE saponin suggested its usefulness as

potential deworming. Further studies on the property of the anti-giardia effect of *Y. baccata* saponin are recommended to elucidate its mechanism of action, as well as to determine its effects of toxicity.

Keywords: *Giardia intestinalis*, anti-*giardia*, *Yucca baccata*, saponin

SINOPSIS

Las parasitosis intestinales son un problema de salud pública a nivel mundial, pero la población infantil es particularmente la más afectada (Okyay et al., 2004; Nxasana et al., 2013). Esto es grave para nuestro país ya que en su cuadro clínico, las parasitosis no solo ocasionan dolores de estómago, diarreas, vómito o malestar en general; sino que pueden inducir problemas nutricionales graves (Escobedo et al., 2010). Esto tiene como consecuencias pérdida de peso en adultos y baja talla en niños (Quihui et al., 2003). Hacemos resaltar que es un problema de salud pública pues aunque no es causa de mortalidad, si es motivo de ausentismo y baja de rendimiento escolar, aumento de gastos médicos (hospitalización y medicinas) y análisis de laboratorios.

Una de las parasitosis intestinales de mayor distribución en el mundo es la giardiosis, causada por *Giardia intestinalis*. Su frecuencia anual era estimada en un billón de casos con una prevalencia global del 30% (Upcroft et al., 2001; Gupta et al., 2004). En algunas regiones de México, la prevalencia de giardiosis llega hasta un 68% (Ponce et al., 2002; Secretaría de Salud, CENAVECE), y específicamente en Sonora, presenta una alta prevalencia pudiendo afectar hasta un 40% de su población general. Sonora está listado como uno de los 10 estados con mayor incidencia a nivel nacional (SSA, 2013). Además, *Giardia* se le asocia con tasas altas de morbilidad infantil, especialmente en niños de 1-4 años. Estos datos son muy preocupantes y esto nos motiva a realizar investigaciones que ayuden a combatir parasitosis.

Giardia intestinalis se encuentra frecuentemente en perros, gatos, animales de crianza y en humanos. Se transmite por ruta fecal-oral y comidas o agua contaminadas. Los quistes son resistentes a la cloración y puede vivir por largos períodos de tiempo en aguas frías (DeRegnier et al., 1989. Existen seis especies de

Giardia, como por ejemplo *G. muris* que afecta a roedores y *G. agilis* a anfibios, entre otras. La especie de *Giardia* de nuestro interés es *G. intestinalis*, que afecta principalmente a los mamíferos, incluyendo al hombre (Bénéré et al., 2012). *Giardia intestinalis*, es un parásito unicelular, eucariótico, que se encuentra clasificada dentro del orden Diplomonadida, caracterizada principalmente por poseer flagelos que le permiten su movimiento. En su ciclo de vida podemos encontrarlo como quiste (forma infectante) y trofozoíto (forma móvil) (Figura 1).

El quiste, es la manera por la cual nos infectamos, y puede transmitirse por el consumo de alimentos y agua contaminados, o vía fecal-oral. Es la forma más resistente del parásito, ya que puede permanecer en el ambiente en condiciones adversas, como sobrevivir en temperaturas frías de hasta 4°C por 3 meses (Faubert, 2000) ya que tiene una pared quística la cual lo hace resistente (4 nucleos en estado maduro).

El trofozoíto que es la forma en la cual se encuentra en el hospedero infectado , tiene una simetría bilateral, es piriforme, y tiene 4 pares de flagelos (anterior, posterior, caudal y ventral), 2 núcleos y en su parte anterior tiene un disco ventral el cual le permite aparentemente adherirse en las paredes del intestino del hospedero (Tay, 1994; Campaniti, 2002). El ciclo de vida de *Giardia intestinalis* empieza en el hospedero cuando se ingiere el quiste. Después de un par de horas, ya que ha pasado por el pH ácido del estómago y se expone al pH básico del intestino delgado proximal, se abre el quiste. El trofozoíto se une al epitelio intestinal y se multiplica asexualmente por fisión binaria en el revestimiento luminal (Faust et al., 1961). Después de la multiplicación, los trofozoítos pasan a la parte terminal del intestino delgado y forman nuevos quistes que se excretan en las heces. Los quistes sobreviven en el medio ambiente, para llegar a otros hospederos susceptibles y causar nuevas infecciones (Sulaiman, 2004; Adam, 2001) (Figura 2).

La giardiosis es una enfermedad multifactorial que incluye diversos mecanismos de acción (Ortega-Pierres et al. 2009). Estudios diversos nos han demostrado varios

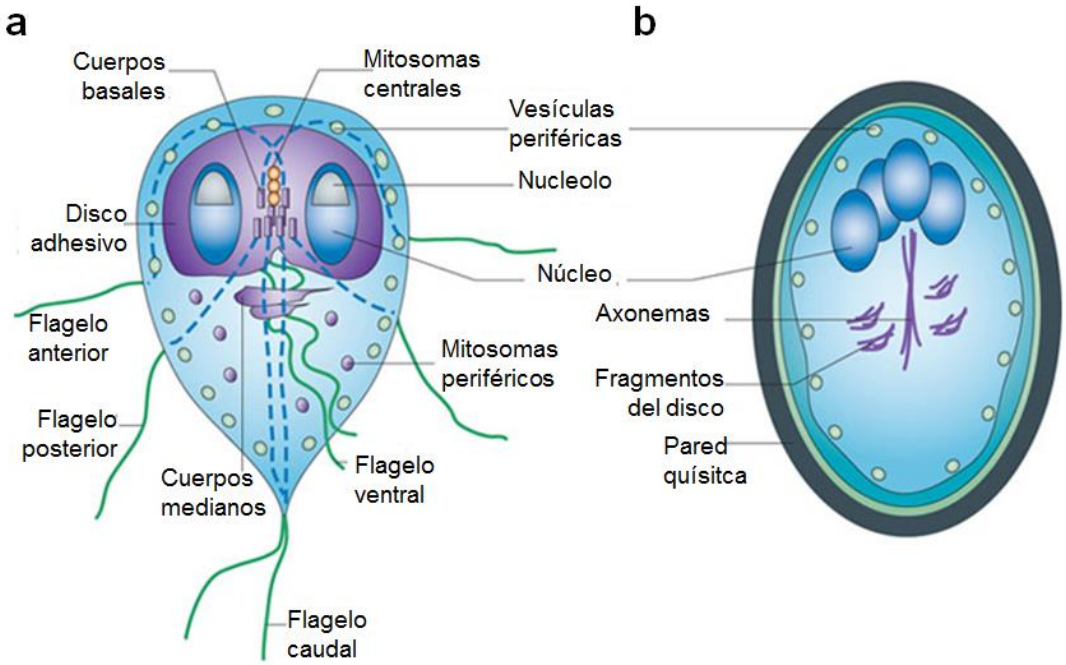

Figura 1. *Giardia* en su forma de trofozoíto y quiste. **a |** El trofozoíto de *Giardia* mide 12–15 µm de largo y 5–9 µm de ancho. Aquí se muestra el trofozoíto desde una vista dorsal. Presenta ocho flagelos organizados en cuatro pares: flagelos anteriores, flagelos ventrales, flagelos posteriores y flagelos caudales; las líneas punteadas indican estructuras internas. Los cuerpos basales son los sitios de donde se originan los flagelos. Los cuerpos medianos son un estructura microtubular cuya función se desconoce. El disco adhesivo es una estructura de adherencia, grande y rígida, compuesta de microtúbulos. Hay varios mitosomas centrales y periféricos en la célula. Las vesículas periféricas son vesículas tipo-lisosomas que se encuentran debajo de la membrana plasmáticas a través de toda la célula. **b |** En el quiste, la pared quística y la capa interna, consistente en dos membranas, protegen al parásito. Los quistes de *Giardia* son de forma ovalada, no móviles, y miden 8–12 µm de largo por 7–10 µm de ancho. La capa externa del quiste tiene un grosor de 0.3–0.5 µm, y se compone de una red de filamentos que van de 7 a 20 nm de diámetro. Esta pared se compone principalmente de N-acetilgalactosamina y tres proteínas de pared quística diferentes (CWP1, CWP2 y CWP3). El disco adhesivo y los flagelos se desmontan y almacenan en el parásito. El quiste tiene cuatro núcleos tetraploides. Ankarklev et al., 2010.

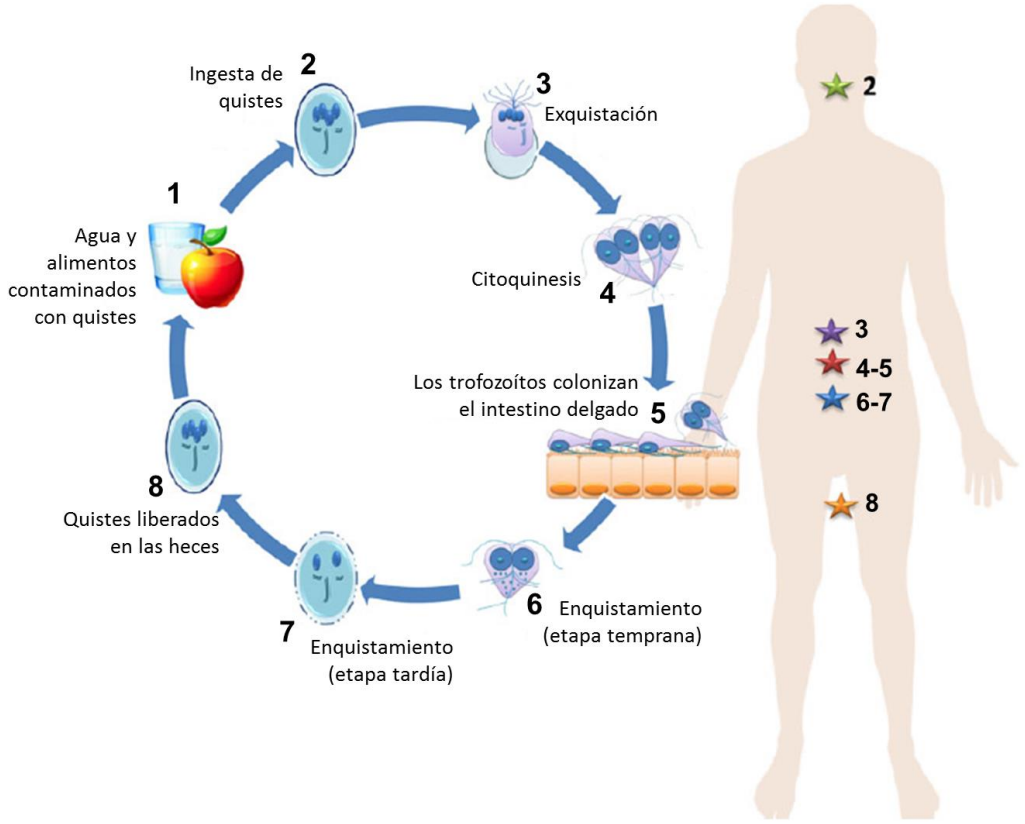

Figura 2. Ciclo de vida de *Giardia*. La ruta de transmisión de la giardiosis es la fecal-oral. La infección inicia con la ingesta de quistes en agua o alimentos contaminados (1 y 2). La exposición al ambiente ácido en el estómago induce el proceso de exquistación (3). Cada quiste produce dos trofozoítos (4). Los trofozoítos colonizan y se replican en el intestino delgado, pudiéndose adherir epitelio intestinal (5). A medida que los trofozoítos viajan a través del intestino, el ambiente bajo en colesterol, alto en bilis y ligeramente alcalino, puede inducir una etapa temprana de enquistamiento, en la cual los trofozoítos adquieren una forma redondeada y proteínas específicas de enquistamiento son transportadas hacia la superficie celular mediante vesículas para formar la pared del quiste (6). El disco adhesivo se desmonta y la célula se somete a replicación de ADN para obtener una célula que contiene dos núcleos (4N cada uno). Durante la etapa final de enquistamiento, el núcleo se divide, dando lugar a cuatro núcleos (2N cada uno), y el DNA se duplica de nuevo para generar un quiste maduro con cuatro núcleos (16N) (7). Los quistes son liberados en las heces permitiendo de esta forma la finalización del ciclo de transmisión al infectar un nuevo hospedero (8). Adam, 2001; López-Romero et al., 2015.

de estos caminos que sigue *Giardia* para afectar al hospedero. La explicación más aceptada es el acortamiento de las microvellosidades, inhibición, insuficiencia y disminución de las enzimas que se encuentran en el borde de cepillo del intestino

Además, esta la competencia por productos del hospedero como, esteroles, colesterol y fosfolípidos, ya que *Giardia* no sintetiza este tipo de compuestos. Otro, es el factor mecánico que influye alterando las funciones de la barrera intestinal. Finalmente el factor de la respuesta inmune local y la producción de anticuerpos anti-*G.intestinalis*.

Aunque puede actuar de diferentes maneras para afectar al hospedero, se sabe que la giardiosis es una enfermedad autolimitada, ya que el hospedero tarde o temprano tiene la capacidad de defenderse eficazmente contra *Giardia*. Esto va a depender de varios factores como la edad, y estado inmunológico y nutricional del hospedero.

Dentro de la sintomatología que produce la giardiosis están desde los síntomas más comunes como diarrea crónica, dolor de cabeza y malestar en general; hasta los más severos, principalmente en niños que no son atendidos oportunamente, como malabsorción de nutrientes y desnutrición (estado crónico). Los síntomas por la infección con *Giardia* varían de persona a persona en relación a la intensidad de la infección, la cantidad de quistes ingeridos, la cepa del parásito y el estado inmunológico y nutricional del paciente.

Una de las características de la giardiosis es que la persona puede estar infectada con el parásito *y* no presentar ningún síntoma. En los pacientes con cuadro sintomatológico, las heces pueden ser mucosas, pastosas o líquidas, fétidas y espumosas, la diarrea es constante en algunos casos, mientras en otros no (Sing, et al., 2000). La fase aguda de la giardiosis dura 3 ó 4 días y en raras ocasiones existen otras complicaciones como urticaria, rinitis e insomnio (Heuman et al., 1997). La mayoría de las personas se recupera totalmente después del tratamiento, pero algunos sufren de malestares estomacales o diarreas que pueden durar dos años o más. En casos muy graves existe una mala absorción de nutrientes y desnutrición (Adam, 1991) cuando *Giardia* se adhiere en las células del duodeno y causa una obstrucción mecánica de la superficie de absorción del intestino. Este es el

mecanismo posible, que tiene una relación sinérgica con el desbalance de la flora intestinal (Woolcott et al., 2003). En la giardiosis hay un intercambio acelerado de la mucosa intestinal afectada ya que hay una atrofia de las vellosidades. Algunos autores opinan que la pérdida epitelial y el desprendimiento de enterocitos son las causas de las diarreas (Buret et al., 1990). También se ha observado que los niveles de hemoglobina, vitaminas y parámetros antropométricos son afectados (Barreras y Ontiveros, 2002). En general las infecciones intestinales pueden afectar el estado nutricio de la persona. En niños y personas desnutridas, la infección puede volverse crónica y provocar diarreas graves, pérdida de peso y talla en los niños y una mala absorción de nutrientes. Esta mala absorción de nutrientes puede ser en general, o específicamente de algún nutrimento, ya sea vitaminas (como B9 y B12), lactosa, almidón, entre otros (Ortega et al., 1997). En nuestro estado, datos publicados por la Secretaría de Salud Pública en el 2012, nos indicaban que Sonora tenía una incidencia del 78.54%, donde la población de 1-4 años era la más afectada. Esto aumentó en un 53% comparado con los datos publicados en el 2011. Datos más recientes publicaban en el 2014 que Sonora se encontraba en el 7mo lugar en incidencia de giardiosis en niños de 1-4 años. Es difícil encontrar varios estudios que evalúen de una misma manera la eficacia de los medicamentos usados en el tratamiento contra la giardiosis. Actualmente no existe un fármaco ideal con el que se logre un porcentaje alto de curación con pocos efectos secundarios (Gardner, 2001). Anteriormente esta infección era tratada con productos a base de arsénico, mercurio y bismuto. Gracias al descubrimiento de la quinacrina en los años treinta, este medicamento fue usado como antigiardiásico con una eficacia del 90% (Tracy, 1996). Sin embargo, los efectos secundarios incluían vómitos, nauseas, coloración amarilla de la piel, cefalea y malestar general. Como consecuencia se presentó una disminución drástica de su empleo y su producción. Hay distintos tratamientos que tratan de brindar los mejores resultados en el paciente, pero todos en mayor o menor proporción causan efectos secundarios indeseables.

La furazolidona, ha sido un producto efectivo contra la giardiosis. Estudios realizados en la década de los ochenta demostraron que las tasas de curación alcanzadas con este compuesto oscilaban entre 80% y 90%. Además se sugirió su

uso en la población infantil por sus bajos efectos indeseables y por su fácil disponibilidad en suspensión.

Los nitroimidazoles son las drogas más usadas, con tasas elevadas de curación para la infección con *Giardia*. Dentro de ellos el metronidazol ha sido el más estudiado y sus tasas de curación oscilan entre 60% y 100%. Sin embargo, hay evidencia de efectos adversos con su uso entre ellos; sabor metálico, dolor de cabeza, náuseas y toxicidad del sistema nervioso central. Secnidazol y tinidazol son otros nitroimidazoles muy utilizados. El poder emplearlos en una sola dosis y la poca probabilidad de aparición de efectos secundarios han determinado un incremento gradual en su uso en los últimos años (Gardener y Hill, 2001). Los benzimidazoles se han usado con relativa frecuencia (Al-Wailli, 1992). De ellos, el albendazol, es el que ha proporcionado los mejores resultados tanto en estudios *in vitro*, como en ensayos clínicos (Hill, 1993).

Por otro lado, aparentemente no hay diferencias en los tratamientos contra giardiosis entre nitromidazoles, furazolidona y bencimidazoles; En un estudio se evaluó la eficacia de albendazol, tinidazol y cloroquina en 165 niños con giardiosis. El porciento de curación de la cloroquina fue de 86% y de tinidazol 91% (Escobedo et al., 2008). Ambos superaron la efectividad mostrada por el albendazol. Otro estudio realizado en Cuba en 100 niños de 7 a 12 años comparó mebendazol contra metronidazol y no encontró diferencias entre los tratamientos. En cambio en lo que respecta a los efectos secundarios, no se encontraron en el grupo tratado con mebendazol, pero en el grupo tratado con metronidazol hubo efectos secundarios hasta en un 24%, tales como nauseas, anorexia y sabor metálico en la boca (Sadjjadi et al., 2001). El albendazol es el medicamento usado por la campaña de desparasitación nacional desde 1993. Este medicamento es administrado en 1 toma sencilla (400 mg/día) 2 veces al año (Septiembre y Marzo). Estudios realizados por Quihui y Morales en el 2013, han indicado que a pesar del tratamiento administrado por la campaña, la prevalencia de giardiosis puede suparar aun el 20%. .

Los antiparasitarios usados actualmente pueden representar un problema económico, sanitario y social. La mayoría de ellos han mostrado efectos tóxicos tanto *in vitro* como *in vivo* en modelos animales (Mudry, et al., 1994).

Es necesario el uso de fármacos en donde los efectos adversos sean mínimos, para prevenir y tratar infecciones parasitarias. Desafortunadamente los fármacos están perdiendo utilidad, debido a la presencia de tolerancia y resistencia por algunas especies parasitarias (*G. intestinalis*, *Coccidia* spp., *Entamoeba* spp. *Plasmodium* spp.) y esto es crítico ya que a nivel mundial el número de personas expuestas a este tipo de infecciones se encuentra en aumento. No existen medicamentos realmente innocuos y que puedan ser consumidos sin secuelas. Esto ha exigido la investigación y el control clínico que permitan la disponibilidad de antiparasitarios útiles y de mayor inocuidad así como la identificación de agentes tóxicos en vigencia en algunas regiones del mundo. Por esta razón se propone utilizar otras alternativas para la curación de la giardiosis. La OMS en el 2013 publicaba que el 80% de la población en el mundo sigue utilizando la medicina tradicional para combatir diferentes tipos de enfermedades. Las plantas son fuente potencial de metabolitos primarios, (aminoácidos, carbohidratos y ácidos grasos) metabolitos secundarios cuyas propiedades curativas (anticancerígenos, antimicrobianos, antiinflamatorios, y antiparasitarios) pueden ser aplicadas en beneficio del hombre (Singh et al., 2003). Estas sustancias que no parecen participar directamente en el desarrollo, simplemente aportan a la planta que las produce una ventaja para responder a estímulos del entorno (Quiroga et al., 2004). Además, estos metabolitos naturales tienen potencial para usarse en la prevención de algunas enfermedades (Ferguson, 2001).

Yucca es una planta que pertenece a la familia *Agavaceae*, del genero *Sarcocarpa*, la cual posee hojas en forma de espadas y racimos de flores blancas. Su hábitat va desde el norte de las grandes llanuras a través de los bosques y el trópico seco de México, hasta el suroeste de Estados Unidos. *Yucca baccata* (Figura 3) es una de las 10 especies que se encuentran en la región del Desierto de Sonora, (Mielke, 1993; Dimmitt, 2000). De las raíces de algunas especies de *Yucca* se han obtenido compuestos químicos útiles para hacer jabón, y agentes espumantes para bebidas. También se ha observado que tienen efectos terapéuticos que son atribuídos a las saponinas; metabolitos secundarios que producen algunas plantas

Figura 3. *Yucca baccata* en la zona aledaña a Cananea, Sonora. Pertenece a la familia *Agavaceae*, del genero *Sarcocarpa*, posee hojas en forma de espadas y racimos de flores blancas. Su hábitat va desde el norte de las grandes llanuras a través de los bosques y el trópico seco de México, hasta el suroeste de Estados Unidos. *Yucca baccata* es una de las 10 especies que se encuentran en la región del Desierto de Sonora. Mielke, 1993; Dimmitt, 2000.

desérticas (Piacente et al., 2005) y las cuales se encuentran en mayor concentración en las plantas de *Yucca* spp.

Las saponinas (Figura 4) son detergentes naturales encontrados en muchas plantas que contienen compuestos solubles en agua y en grasa. Están formadas por una aglicona y uno o más monosacáridos. Existen dos tipos de saponinas, las saponinas esteroidales y las saponinas triterpenoides.

Uno de los posibles mecanismos de acción por el cual las saponinas puedan actuar contra de los diferentes tipos de células es la inducción de formación de poros con la consecuente lisis celular. Otros posibles mecanismos podrían ser la inducción de apoptosis o la inhibición de enzimas.

Se sabe que los extractos de *Yucca* en sus diferentes variedades, tienen actividad bactericida, antimicrobiana, citotóxica, hemolítica, hipocolesterolemica y parasitaria. Todos estos estudios se han realizado ya sea utilizando diferentes extractos de la planta o bien compuestos puros (pocos estudios), como en este caso algún tipo de saponina.

Ya que las plantas medicinales se han utilizado desde tiempos muy remotos como alternativa para tratar enfermedades, principalmente en los países en desarrollo, en este artículo se discute a profundidad información reciente sobre la medicina tradicional. Por esta razón, en el capítulo I del presente trabajo se presenta un artículo de revisión donde se exploran las diversas actividades biológicas que pueden presentar las distintas variedades de las plantas de *Yucca* spp., en sus extractos o compuestos puros. .

Estudios diversos han mostrado que las diferentes partes de esta planta (tallo, hojas y raíces), tienen compuestos con uso potencial en diferentes sectores de la salud. Aproximadamente existen 50 especies del género *Yucca* (familia Agavaceae) y estudios filogenéticos han demostrado que algunas variedades de la clase *Sarcocarpa* pueden encontrarse tanto en el noroeste de México como en el sur de Estados Unidos. Entre las especies más estudiadas podemos encontrar a *Yucca schidigera* y entre la menos estudiada a *Yucca baccata* Entre las actividades biológicas más evaluadas en *Yucca*, se encuentra la actividad citotóxica, donde las saponinas ya aisladas y purificadas, se han probado sobre diferentes tipos de líneas

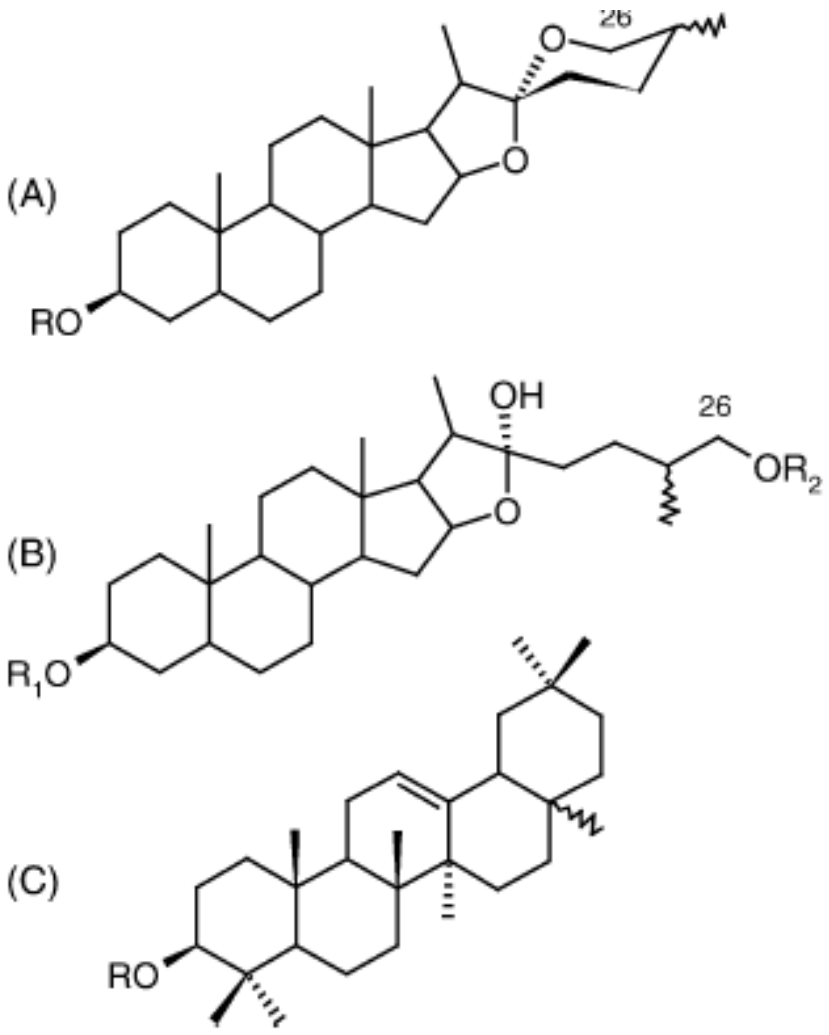

Figura 4. Estructura general de los diferentes tipos de Saponinas. Son detergentes naturales encontrados en muchas plantas que contienen compuestos solubles en agua y en grasa. Están formadas por una aglicona y uno o más monosacáridos, unidos mediante un enlace glicosídico. (A) Saponina Esteroidal Espirostano, son glicósidos esteroides con un núcleo espirostano, compuestas por 27 átomos de carbono.y derivadas del Espirostano (B) Saponina Esteroidal Furostano, son glicósidos esteroides compuestas por 27 átomos de carbono y derivadas del Furostanol (C) Saponina Triterpenoide, son triterpenos que pertenecen al grupo de saponinas compuestas. Están compuestos en una configuración de cuatro o cinco anillos, de 30 átomos de carbono. Hostettman y Marston, 1995.

celulares cancerígenas, HCT116 (colon), MCF7 (mama), HepG2 (hígado) y A549 (pulmón). Se observó un efecto inhibitorio con algunas de las saponinas aisladas y probadas, concluyéndose que la actividad citotóxica de las saponinas podría depender de su estructura. Otra de las actividades más estudiadas de los extractos y saponinas de *Yucca*, es la actividad hipocolesterolémica. Sin embargo, estudios sobre su actividad antiparasitaria son limitados. Por otro lado, hay muy pocos estudios que han evaluado la capacidad antiparasitaria de *Yucca*, especialmente de *Yucca baccata* contra *Giardia intestinalis*. En el capítulo II se presenta un artículo original cuyo objetivo fue evaluar la actividad anti-*Giardia* de extractos de *Yucca baccata.*

Se usó un modelo experimental de giardiosis en jerbos (*Meriones unguiculatus*) infectados oralmente. 42 jerbos (hembras), se asignaron aleatoriamente en 5 grupos tratados con extractos de *Yucca baccata* en concentraciones de 24,4 mg / ml (grupo 1), 12,2 mg / ml (grupo 2) y 6,1 mg / ml (grupo 3), un control positivo tratado con 2 mg / ml de Metronidazol o referencia (grupo 4) y un control negativo o con PBS (grupo 5). El tratamiento se administró una vez al día intragástricamente durante 3 días. Nueve jerbos de los diferentes grupos tratados murieron durante el curso del estudio. El día 10 después de la infección, los jerbos fueron sacrificados y los trofozoítos de duodeno y yeyuno fueron cuantificados. Se llegó a la conclusión de que los extractos de yuca reducen, aunque no significativamente, los trofozoitos presentes en duodeno. La única concentración que redujo significativamente los recuentos de trofozoitos en el segmento proximal del intestino delgado fue de 24,4 mg/ml y fueron similares a los resultados obtenidos por el desparasitante comercial metronidazol (Quihui et al., 2014).

Actualmente solo existe otro estudio donde se evalúa la actividad anti-*Giardia*, que estudio el efecto que tienen los extractos de otra especie de *Yucca schidigera,* utilizando como modelos experimentales jerbos y corderos. Mc Allister y colaboradores en el 2001. Jerbos infectados con *Giardia* fueron tratados oralmente con un extracto de butanol o polvo de yuca en su dieta. Al final del ensayo se observó que el extracto de butanol tuvo mayor actividad que el polvo de *Yucca* en base a la excreción fecal de quistes. Se realizó un ensayo *in vitro* usando un extracto

butanólico de 1500µg/mL y como control metronidazol a 40µg/mL. Ambos tuvieron la misma eficacia al reducir hasta en un 50% la población de trofozoítos en la placa. En los jerbos tratados, los trofozoítos fueron parcialmente eliminados del duodeno y yeyuno, lo que sugiere que es difícil eliminar *Giardia* de estas regiones. Sin embargo, los trofozoitos fueron eliminados por completo del íleon (McAllister, 2001). Estos datos son comparables con aquellos observados en nuestro estudio.

Hay pocos trabajos que informan sobre la actividad antiparasitaria en general, utilizando *Yucca*. Uno de los más recientes, es el realizado por Rambozzi y colaboradores en el 2011, quienes investigaron la efectividad de las saponinas del extracto de *Y. schidigera* en terneros naturalmente infectados con coccidiosis. Este parásito (Coccidia) infecta seres humanos y animales, en particular los destinados a la producción. Fue un ensayo de seguimiento de 75 días. Un total de 27 terneros fueron asignados aleatoriamente a tres grupos. El primer grupo o control positivo recibió una ración que contenía monensina (Rumensin® 100 Premix, Elanco Animal Health, Greenfield, IN, EE.UU.-140 mg / animal / día) un aditivo en los alimentos para proteger a los terneros contra las infecciones parasitarias (Bittar et al. Al, 2002); el segundo grupo recibió una ración con extracto de saponina de *Yucca schidigera* (15 g / animal / día); y el tercer grupo fue el control negativo o no medicado. El día 15, la excreción de ooquistes fue significativamente menor en los grupos tratados con el extracto de *Yucca* y monensina en comparación con el grupo no tratado ($p = 0,014$ y 0,017, respectivamente). Después del día 30 en adelante no se encontró ninguna diferencia en todos los grupos. Sin embargo, debe enfatizarse que no se encontró diferencia entre los grupos de monensina y extracto de yucca a lo largo del estudio. En conclusión, se sugiere que las saponinas de *Y. schidigera* administradas por vía oral muestran una actividad similar a la monensina anticoccidial.

Como podemos observar, en la mayoría de los trabajos donde se evalúa la actividad antiparasitaria, se han utilizado solo extractos de la planta, más no un compuesto purificado, que finalmente es lo que se requiere para identificar al compuesto que presenta tal actividad y continuar con futuras investigaciones. Por esta razón en el capìtulo III se plantea la caracterización y evaluación de las

saponinas presentes en *Yucca baccata* contra trofozoìtos de *Giardia intestinalis* in vitro.

La hipótesis del presente estudio establece que las saponinas presentes en el extracto de *Yucca baccata* son los compuestos responsables de poseer actividad antigiardiosis in vitro.

Se aislaron y caracterizaron las saponinas del tallo de *Y. baccata*. Los extractos butanólicos se analizaron por cromatografía en columna (fase estacionaria sílica gel 60, 230-400 mesh; fase móvil AcOEt-MeOH-H_2O) y sus saponinas fueron aisladas. La caracterización se realizó mediante RMN (^1H y ^{13}C en una y dos dimensiones). Se obtuvo una saponina esteroidal del tipo spiro estanol (SESE), y se evaluó su efecto sobre el crecimiento de trofozoítos de *Giardia intestinalis* expuestos a distintas concentraciones de SESE *in vitro*. Los resultados indicaron que la saponina SESE de *Y. baccata* mostró una IC50 de 2.12 µM contra *G. intestinalis* ($P<0.001$).

Estudios adicionales son requeridos para identificar las concentraciones de dosis específicas de saponinas de *Yucca* que no produzcan efectos tóxicos, efectos secundarios mínimos y costos razonables en su fabricación y administración, además de delucidar el mecanismo por el cual nuestra saponina aislada puede inducir daño al protozoario patógeno. Estos objetivos deben ser considerados en el desarrollo de estudios para que las saponinas ya aisladas y purificadas de *Yucca baccata* puedan ser una alternativa terapéutica contra giardiosis no solo humana sin también animal.

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CAPÍTULO I

Artículo:

Different biological properties of saponins of *Yucca* spp.: A recent review

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Abstract. The activities of some species of *Yucca* have been extensively investigated. Studies have revealed that the different parts of this plant have properties to be potentially applied in the area of public health. It has been recognized that about 50 species of the genus *Yucca* (*Agavaceae* family) and phylogenetic studies have shown that some varieties of *Sarcocarpa* class can be found both in northwestern Mexico and southern United States. *Yucca schidigera* has been the most widely studied of *Yucca* species and only limited information can be found on the biological activities of the extracts of *Yucca gloriosa*, *Yucca desmentiana*, *Yucca glauca*, *Yucca aloifolia* and *Yucca baccata*. One of most investigated biological activities of *Yucca* has been the reduction of ammonia concentration from feces of field animals. Successful results have been observed and commercial patents already exist to treat this problem. The hypocholesterolemic activity has been also investigated and the involved mechanism has been partially elucidated. On the other hand, there are some studies on cytotoxic activity in vitro and results against different cancerous cell lines have been satisfactory. The present review from the year 2006 up to date contains information about the biological and pharmacological properties of the extracts; and their diverse isolated and characterized compounds of species of *Yucca* using different solvents. This review will provide information on unexplored research areas on *Yucca* species.

Keywords: *Agavaceae*, *Yucca*, extracts, biological activity.

Introduction

Medicinal plants are used as an alternative therapy to treat different diseases especially in developing countries[1]. Plants have been used by ancient civilizations like Chinese, Greek, Chinese, Mayans and Aztecs to cure their diseases, and their knowledge of plant's properties has been transmitted from generation to generation creating a "traditional healing system" Based on this, traditional medicine is an important source of information for health systems worldwide [2]. Even with the advent and progress of the Pharmaceutical Chemistry for the development of new drugs, "traditional" medicine remains as a good choice to the population to solve its health problems [3]. In 2002, the World Health Organization estimated that around 80% of the population worldwide used traditional [4]. Also, it was published that nearly 6% of the plants in the world had biological activity and only 15% of them has been phytochemically studied [5]. Indigenous healers or people who have used plants for years have information on medicinal properties of the plants, but risk to loss this information is high . Due to this, the needing to gather and to perpetuate this knowledge is required [6] since most of this information is based on beliefs or experiences of the people [7]. Sustained use of natural products in health practice may provide an idea of their effectiveness and how they are capable of identifying new areas of research to lead the search for new drugs [8]. The responsible compounds for these activities in plants are their secondary metabolites that are thought to have arisen through evolution nearby 400 million years ago, when the plants had to protect themselves against bacteria, insects and herbivorous animals. All plants have different secondary metabolites which are responsible for their anticancer, antimicrobial, antifungal, and antiparasitic activities. Plants belonging to the *Yucca* spp. family have been investigated because of their various biological activities associated to their secondary metabolites particularly saponins. They usually grow in the arid southwestern United States and northern Mexico and fifty of them are known for their medicinal properties and their roots and leaves

have been the most used parts to treat different diseases [9]. Nowadays, the extracts of different species of *Yucca* are used to combat or to cure arthritis, and respiratory and intestinal disorders. Leaves and stems, rhizomes, flowers and seeds of *Yucca* have bioactive compounds of therapeutic relevance. Steroid saponins are glycosides with a polar (glycosides) and a nonpolar (sapogenin) molecular structures. Saponins can form stable foams in aqueous solution and they also show hypocholesterolemic and hemolytic properties. The compounds of *Yucca* has been considered GRAS (Generally Recognized as Safe), which means that additive of this plant can be tested as safe for human consumption [10].

Biological activities

Cytotoxic activity

Saponins of leaves of *Y desmentiana* has been isolated and characterized by mass spectrometry and NMR unidimensional. (25*R*)-3β-hydroxy-5α-spirostan-3-*O*-β-D-xylopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (desmettianoside C), it was the structure of the new saponin identified. The anti-cancer activity of the isolated saponins was assessed on various cell lines, such as: HCT116 (colon), MCF7 (breast), HepG2 (liver), and A549 (lung). The Saponin 3, smilagenin, 3-*O*- [β-D-glucopyranosyl-(1→2)-*O*-β-D-galactopyranoside] was isolated from *Yucca gloriosa* [11] and *Y. elephantipes* [12], and Saponin 4, Tiogenina, (25*R*)-5α- spirostan- 3β -ol) isolated by Mahato et al in 1982 [13]. The IC50 (concentration inhibiting cells 50% compared to untreated cells) of these four saponins was assessed in an *in vitro* assay using MTT reagent. During this assay, Doxorubicin was included as control positive, a current drug used against cancer. The saponins furostano 1 and 2 showed no cytotoxic activity, but the saponins spirostane 3 and 4 inhibited the tumor cells, in a dose dependent. For example: the IC50 HCT116 cells of Saponin 4 was 2.4 µM compared to 4.4 µM of Saponin 3. However, the greatest inhibitory effect of

Saponin 4 was on HepG2 cells (IC50 1.1 µM) compared to Saponin 3 (IC50 3.5 µM), and twice more active than Doxorubicin. Based on this finding, it was concluded that cytotoxic activity of the saponins may depends on its structure. In this case, the Saponin 4 has larger number of sugars than Saponin 3 [14]. In another study, 6 steroidal glycosides and 14 known compounds were isolated from *Y. glauca* roots and their structures were determined by dimensional NMR. Cytotoxic activity was assessed against HL-60 human leukemia cells and A549 human lung adenocarcinoma using MTT reagent. Four spirostane glycosides and three furostano had cytotoxic activity against those two cell lines. 5b-spirostanol glycoside (1, 6, and 9-12) and furostanol glycoside-5b (13-18 and 20) had IC50 values of 2.5 and 17.8 µM respectively against the HL-60 cells [15].

Activity hypocholesterolemic

The *Y. schidigera*'s powder is used as animal feed additive and its effect has been widely investigated. A 10-week trial in 24 lambs (Akkaraman) of approximately 2.5 months of age found no difference in some blood parameters (P> 0.05) among the control group (basal diet), a *Y. schidigera* powder I (YSPI) (basal diet +100 ppm powder) group, and an YSPII (basal diet + 200 ppm powder) group. However, the cholesterol, HDL and LDL serum levels decreased significantly (P <0.05) in the YSPII group [16]. On the other hand, in a 60-day follow up study in 15 males (Swiss albino mice) per group, revealed that a high-fat-diet significantly increased the plasma levels of glucose, cholesterol, triglycerides and LDL. However, in another study, leptin levels decreased significantly in mice receiving both a high fat diet (gastric tube) and *Y. schidigera* extracts [17] probably as a result of saponin thermogenesis [18]. This extract contains mostly steroidal saponins and phenolic substances [19]. It has been shown in this case, that saponins aid to the nutrition process by regulating the lipid metabolism [20]. Due to this, *Y. schidigera* may be used to prevent malnutrition, particularly, within the area of lipid disorders.

Hemolytic activity

Hemolytic activity of extracts of *Yucca* has been investigated. A study used extract concentrations from 5 to 666 µg/ml of *Quillaja*, *soy* and *yucca*. Trials in 96-well plates were performed using 1% suspension of chicken blood with water as positive control and chicken blood with phosphate buffered saline as negative control. It was observed that both *soy* and *yucca* extracts caused no hemolysis at the tested concentrations [21].

Additive

Recently, Alagawany et al., 2016, tested *Y. schidigera* extracts (above 100 mg/kg) as an additive feed in chicken from 36 to 52 weeks of age. The higher amount of additive feed the higher number of eggs laid and body mass. In addition a positive effect on the production of immunoglobulin was observed (IgG) [22].

Ammonia reduction

Cattle breeding poultry or any animal can produce high waste material such as urine and stool containing over 160 chemicals that are not fully known. These generate gases polluting the environment that eventually affect the life-quality of both animals and man [23]. Recent research based on the development of effective methods in reducing odors from animal production technologies is known such as the chemical oxidation that is an effective and stable treatment. However, these methods have some disadvantages such as high costs spent on the operation of equipment. An alternative to this proposal, can be the biological methods based on using microorganisms capable of reducing concentrations of volatile compounds present in the fecal waste of farm animals [24]. This can be a more advantageous alternative, since the odor can be attacked by different routes. For this reason current investigations are focused on the development of

biological preparations containing both microorganisms and plant extracts, or even enzymes and proteins, which together can fight this problem avoiding pollution of the environment and damage to our health. For this reason, extracts from *Y. schidigera* are widely used in the reduction and control of ammonia concentration in the feces of different types of animals such as cattle, birds, cats, rabbits, and dogs [25]. The effect of *Yucca* extracts in ammonia removal from feces has been examined. Reductions up to 58-73% in the concentration of odoriferous compounds have been estimated in relation to the extract concentration provided [26]. In another study, by Dos Reis and collaborators in 2016, 20 adult dogs of breed "Beagles" were subjected to a diet enriched with 25 to 34% crude protein combined with four different concentrations of the extract of *Y. schidigera* (0, 250, 500 and 750 mg/kg). As a result, the overall fecal odor was significantly reduced ($P < 0.05$) when 500 mg/kg of extract with 34% crude protein was used in the diet. In addition, the concentrations of 250 and 500 mg/kg reduced the intestinal gas ammonia. Also, only the inclusion of 750 mg/kg of extract was associated to the increased mean corpuscular hemoglobin concentration (MCH).

Regardless of protein content, the *yucca* extracts can be capable of reducing the fecal ammonia concentration. However, a range to be provided should be delimited as *Yucca* extracts in higher doses may cause adverse effects [27]. Some theories have raised to justify the mechanism by which *Yucca* extract can reduce odor and fecal ammonia. It has been hypothesized that a component in the extract inhibits the urease enzyme activity or probably is a result of the removal of a union of ammonia with a compound of the extract [28; 29].

Antiparasitic activity

Now it has been recognized that extracts of different species of *Yucca* have some efficacy against parasites but published information is very limited. Rambozzi et al., (2011) investigated the effectiveness of saponins of *Y. schidigera* extract in calves naturally infected with coccidiosis. This parasite

(*Coccidia*) can infect humans and animals, particularly those destined for production [30]. It was a 75-day follow up trial. A total of 27 calves were randomly assigned to three groups. The first group or positive control was fed with monensin (Rumensin® 100 Premix, Elanco Animal Health, Greenfield, IN, USA-140 mg /animal/day) that is an additive in food to protect calves against parasitic infections; the second group with saponin extract of *Yucca schidigera* (15g/animal/day); and the third group was the negative control or not medicated. Fecal material was taken, weighted, analyzed and recorded on days 0, 15, 30, 45 and 75. On day 15, oocyst excretion was significantly lower in the groups treated with the extract of *Yucca* and monensin compared to the untreated group ($p = 0.014$ and 0.017, respectively). After day 30 onwards no difference was found among all groups, however, it should be emphasized that no difference was found between the monensin and *Yucca* extract groups throughout the study. In conclusion, it was suggested that *Y. schidigera* saponins administered orally show similar activity to monensin anticoccidial. In another recent study by Quihui and Leon et al (2014), the antigiardia activity of *Y. baccata* was assessed in forty-two gerbils (females) orally infected with *Giardia intestinalis*. They were randomly assigned into 5 groups treated with extracts in concentrations of 24.4 mg/mL (group 1), 12.2 mg/ml (group 2), and 6.1 mg/ml (group 3), a positive control with 2 mg/ml of metronidazole (group 4) and a negative control with PBS (group 5). Treatment was administered once daily intragastrically for 3 days. Nine gerbils died during the study course. On day10 post-infection, the gerbils were euthanized and trophozoites were quantified. *Yucca* extracts reduced, although not significantly, the trophozoites present in the duodenum. The concentration of 24.4 mg/mL significantly reduced the trophozoite-counts in the proximal segment of the small intestine and they were similar to those by the metronidazole [31].

Fungicidal activity

The fungicidal effect of *Y. schidigera* extracts on sorghum seeds has also been investigated. The aqueous extract at concentrations of 2.5 and 10% that significantly reduced the incidence of *Leptosphaeria sacchari* (syn. *Phoma sorghina*), *Fusarium* spp., *Cladosporium* spp, and *Cochliobolus lunatus* (syn. *Curvularia lunata*). The effect of *Yucca* extracts was greater than that produced by the commercial fungicide fludioxonill and dependent on the extract concentration. In addition, the appearance and growth of seedlings were evaluated showing a significant improvement in the extract-treated seedlings. The study concluded that the aqueous *Yucca* extract may be used as fungicide for seed sorghum [32]. One mechanism proposed by Simmons et al, 1996 to explain the observed antifungal activity is that likely the saponins inhibit the sterol biosynthesis inducing cell death; or saponins may interact with the membrane components steroidal fungi forming pores leading to cell death.

In another study, five concentrations of *Y. schidigera* (0.1%, 0.5%, 1.0%, 2.0%, and 4.0% (w/v) extracts were tested against common phytopathogenic fungi (*Verticillium dahliae, Alternaria solani, Colletotrichum Coccodes, Pythium ultimum* and *Fusarium oxysporum*). This was an *in vitro* assay where the inhibitory effect of the concentrations reducing the diameter of the fungal colony by 50% (DRC50) was measured and compared to the negative control. A significant growth inhibition (54.1-100%) was observed in all fungi cultures tested. This work concluded that *Yucca* can play an important role in the control of fungi in crops and they can provide an alternative solution to replace the chemical-based fungicides [33]. The effect of the *Y. schidigera* extract on the control and infection process of the fungus *Venturia inaequalis*, causal agent of "apple scab", has also been evaluated. In trials with apple seedlings, a significant reduction of signs caused by this fungus was observed in the fruit and leaves and they were similar to those observed by chemical treatment [34].

Other activities

A 50-day follow up study by Štochmal'ová et al., 2015, revealed that *Y. schidigera* might show fertility activity. A standard diet for rabbits was added a *Y. schidigera* powder as a supplement at a dose of 5 g/100 kg diet (group 1) and 20 g/100 kg of feed (group 2). The conception rate was higher in the group two (100%) than in the one (82.4%) and the control groups (47.1%). On the other hand, the rate of female rabbits who gave birth was also significantly higher in the groups treated with *Yucca* extract (70.6% and 100% for the groups 1 and 2 respectively) in comphe control group (41.2%) [35]. However, no difference was found in the number of rabbits born alive and dead between the control and treated groups. The explanation proposed is that *Yucca* may favors the release of progesterone at doses of 1 mg/mL, but not at doses of 10 and 100 µg/mL. This effect may be due to *Yucca* extracts promotes the cholesterol metabolism that is a precursor of steroid hormones. Some studies have suggested that *Yucca* extracts can stimulate the release of eggs [36]. *Yucca* has also shown an inhibitory effect on NFkB transcription factor implicated in the control of ovarian cells [37]. On the other hand, schistosomiasis (bilharzia) is a parasitic disease that occurs in tropical areas worldwide. Millions of people have been infected and in 2016 the number rose to more than 61.6 million people [38]. Aquatic snails of the genus *Biomphalaria* are the intermediate host of the parasite. There are some synthetic molluscicides, but only niclosamida is recommended by WHO, and therefore new alternatives based on natural products are still searched. It is known that extracts from *Y. desmettiana* show molluscicidal activity. Diab et al in 2012, isolated and characterized two new steroidal saponins to which they named desmettianosides A and B, with six and five sugar units respectively. These new saponins were tested against *Biomphalaria alexandrina* snails at concentrations ranging from 5 to 200 mg/L and LC100 was achieved with 6 mg/L of saponin A and 11 mg/L with saponin B respectively [39]. The mechanism of action remains unknown but it is hypothesized that the molluscicidal activity is dependent on the structure of each

saponin, such as the number of sugar residues and their sequences. On the other hand, based on the recent concern about environmental protection, studies of development of new products for making edible films and coatings polymers are now promoted. It is recognized that one of the disadvantages of these films is their instability when exposed to moisture changes as they are hydrophilic in nature [40]. Soy protein is one of the most investigated because of both its biodegradability and structural properties that ease the film preparation [41]. In this case virgin coconut oil was used to improve and to stabilize the hydrophobic properties of the films. Coconut oil is stable to oxidation as it has low levels of unsaturated fatty acids (only 10%) [42]. Lipids can also be incorporated into the films through the formation of emulsions, wherein the lipid compound is dispersed with the aid of surfactants [43]. *Y. schidigera* extract has been incorporated as natural surfactant to further improve the properties of the films. Capine et al (2016) analyzed the morphology of these films after *Yucca* extract incorporation and significant changes were observed [44]. Application of *Yucca* extracts in less than 2:10 proportion, the films showed greater flexibility and lower permeability related to the good dispersion of coconut oil in the matrix. The saponins of *Y. schidigera* have also been evaluated on some bacteria living in the rumen of cows. An *in vitro* assay, ruminal fluid was collected in Jersey cows. It was observed that *Yucca* saponins significantly increased the abundance of *R. flavefaciens*, *Prevotella*, and *F. succinogenes*. It was concluded that saponins can modulate the rumen microbial populations in a dose-dependent manner [45].

Yucca has also been investigated to determine the protective effect against oxidative damage caused by acute intoxication with nitrites. A bioassay was performed with male rats (180-250 g) which were assigned randomly into three groups with 12 rats each. One group of healthy rats (control), one group intoxicated with nitrite induced subcutaneously (60 mg / kg Sodium Nitrite), and finally another group intoxicated with nitrite and treated with *Y. schidigera* extracts The *Yucca* treated group was fed with commercial feed plus *Yucca* powder (Sarsaponin 30) (100 ppm) during four weeks.

The tissues analyzed were kidney, lung and liver. Histopathology and clinical biochemistry were performed. Acute nitrite intoxication induced an accumulation of MetHb in blood. However, in the *Yucca* group a significant decrease in malondialdehyde and methemoglobin in blood was observed ($p < 0.01$). These results suggested that supplementation with *Y. schidigera* has direct antioxidant properties, since the concentration of malondialdehyde (MDA) was reduced. This effect may be due to *Yucca*'s ability to capture secondary reactive radicals, preventing the formation of hydrogen peroxide in normal metabolic activity. Additional studies are required to understand this activity in detail [46].

Conclusion

This review is intended to analyze the most important recent studies focused to investigate the biological and functional activities of multiple compounds from different species of *Yucca* (Table 1). Some supplements of *Yucca* with different biological activities tested "*in vitro*" studies have already begun marketed. It is extremely important to give emphasis to the investigation of active compounds present in significant amounts in *Yucca* spp. such as saponins that can provide a major potential of practical use in the area of public health. Additional studies in animals and humans are required to uncover the potential use of those compounds for man's benefit.

Table 1. Summary of the various biological properties of *Yucca* spp.

Specie	Biological activity	Compounds	Reference
Y desmentiana	Cytotoxic activity: anti-cancer activity were assessed on various cell lines, such as: HCT116 (colon), MCF7 (breast), HepG2 (liver), and A549 (lung).	Saponins	13
Y. glauca	Cytotoxic activity was assessed against HL-60 human leukemia cells and A549 human lung adenocarcinoma	Steroidal glycosides	15
Y. schidigera	Activity hypocholesterolemic	Powder	16
Y. schidigera	Activity hypocholesterolemic	Extract	17
Y. schidigera	Hemolytic activity	Extract	21
Y. schidigera	Additive	Extract	22
Y. schidigera	Reduction and control of ammonia concentration in the feces	Extract	26
Y. schidigera	Reduction and control of ammonia concentration in the feces	Extract	27
Y. schidigera	Antiparasitic activity	Extract	30
Y. schidigera	Antiparasitic activity	Extract	31
Y. schidigera	Fungicidal activity	Extract	34
Y. schidigera	Promoter of fertility	Extract	35
Y. schidigera	Molluscicide activity	Extract	39
Y. schidigera	Film formation	Extract	44
Y. schidigera	Fungicidal activity	Saponins	45
Y. schidigera	Antioxidant activity	Extract	46

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Research Article

Marked Antigiardial Activity of *Yucca baccata* Extracts: A Potential Natural Alternative for Treating Protozoan Infections

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Human Giardiosis is a public health problem in Mexico, where the national prevalence was estimated to be up to 68%. Misuse of antiprotozoal drugs may result in low effectiveness and undesirable side effects. Research on natural products is a good strategy for discovering more effective antiparasitic compounds. This study evaluated the antigiardial activity of extracts of *Yucca baccata*, which is native to northwestern Mexico. Forty-two gerbils (females) were weighed and orally inoculated with 5×10^6 *Giardia* trophozoites. Two gerbils were selected at random to confirm infection. Forty living gerbils were randomly allocated into 5 treatment groups (8 per group). Gerbils were randomly assigned to be treated with 24.4 mg/mL, 12.2 mg/mL, and 6.1 mg/mL of extracts, metronidazole (2 mg/mL) or PBS, which were intragastrically administered once per day for 3 days. Nine gerbils died during the study course. On day 10 postinfection, gerbils were euthanized and trophozoites were quantified. *Yucca* extracts reduced, albeit not significantly, the trophozoite counts in the duodenum segment. Only the high-extract concentration significantly reduced the trophozoite counts in the proximal segment and it was similar to that of metronidazole. Extracts of *Y. baccata* may represent an effective and natural therapeutic alternative for human giardiosis.

1. Introduction

One of the most important intestinal protozoans responsible for human infections worldwide is *Giardia duodenalis*. In 2000, it was estimated that 200 million people in Asia, Africa, and Latin America showed symptomatic giardiosis and 500,000 new cases were diagnosed annually [1]. Later estimations showed that it could be responsible for one billion cases annually and is accompanied by an overall worldwide prevalence of 30% [2]. Acute symptoms include

diarrhea, gas, abdominal cramps, nausea or vomiting, and dehydration [3, 4]. In some areas of Mexico, the prevalence of giardiosis can reach up to 68% [5] and now *G. duodenalis* is the most important protozoan parasite causing human intestinal infection in Northwestern Mexico [6]. Currently, this infection is treated with conventional and effective drugs (quinacrine, nitroimidazoles, and nitrofurans) [7]; however, people reject them because of their undesirable side effects [8]. Otherwise, a meta-analysis review [9] concluded that safety, effectiveness, and low cost make of albendazole an alternative and/or replacement for the metronidazole in the treatment of giardiosis in humans. However, the development of resistant strains due to overuse of any drug and the possibility of finding a drug to be used in shorter treatment times than those of the conventional drugs are some reasons for considering alternative therapies. One of them being investigated for the treatment of giardiosis consists of new antigiardialcompounds with different structures and mechanisms of action compared to conventional drugs. Since ancient times, people have used plants to treat common infectious diseases, and some of them have been integrated as a cure for diverse pathologies [10]. *Yucca* was one of the plants used as an important natural resource by Indians of the American Southwest, Mexico, and Latin America for thousands of years against arthritis, fever, headaches, ulcers, and appendicitis [11]. Extracts of *Yucca* contain a variety of compounds such as saponins, alkaloids, tannin, terpenoids, and reducing sugars [12]. Saponins of *Yucca schidigera,* a plant of the family Agavaceae and native to the southwestern United States and Baja California in Mexico [13], have been investigated as defaunation agents. In addition, extracts of *Y. schidigera* have been shown to reduce the rumen protozoal population in vitro [14] and in vivo [15]. Based on that information, the present study was conducted to investigate whether extracts of another species, *Y. baccata,* which is native to the desert on the Mexican side (Northwestern Mexico), possess antigiardial activity and to consider it as a potential agent for controlling giardiosis could be potential agents for controlling giardiosis.

2. Materials and Methods

2.1. Plant Material. Samples of *Yucca baccata* were collected at the Rancho El Aribabi located in northeastern Sonora, Mexico. The samples were placed in bags and transported to the herbarium of the University of Sonora (Herbarium USON) for their taxonomic authentication. The material used in this study was backed up with a specimen of reference (USON 18607, J. Sánchez 2011-095) that is currently deposited in the USON collection.

2.2. Yucca baccata Extract Preparation. Once the *Yucca* stem was dried under sunny conditions (25°C–35°C), it was pulverized using a Wiley Mill (Thomas-Wiley Mill, Model 4, Laboratory Mill, USA) to obtain a coarse powder (2 mm particle size). The preparation of the extracts was performed based on the method described by Newbold et al. [16] for *Sesbania sesban*. An aqueous suspension of the pulverized material of the stem of *Y. baccata* (33 g/L) was left overnight at room temperature (25°C–30°C). The aqueous material was filtered and shaken in equal volumes of *n*-butanol for 30 minutes at room temperature. Extractions were repeated with *n*-butanol. The resulting organic layers from each extraction process were pooled and dried using a rotavapor (ROTAVAPOR BUCHI 461 Water Bath, Pace Analytical Services, Minneapolis, MN, USA) at 40°C, and the residue was weighed and resuspended in PBS at a 1 : 15 ratio. The solution was clarified by filtration through preweighed Whatman no. 1 filter paper. Solid residues were scraped from the filter paper and washed three times with *n*-butanol, and the liquid extractant was rotoevaporated. The residue was weighed and stored at 4°C in 15 mL centrifuge tubes prior to use in the preparation of extracts with 3 different concentrations before their administration to the treatment groups.

2.3. Animals. Forty-two female Mongolian gerbils of the strain *Meriones unguiculatus*, aged 6–12 weeks old and weighing 41 g–64 g, from the Animal Bioterium Center of the National Autonomous University of Mexico (acronym in Spanish UNAM), were used in this study. We decided to work with this number of gerbils on the basis of our previous findings with *Y. schidigera* [17]. The gerbil is susceptible to *G. duodenalis* infection [18].

2.4. Ethical Considerations. This study was approved by the ethical committee of the Centro de Investigación en Alimentación y Desarrollo A.C. (Centre of Research in Food and Development) to be carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Mexican Official Regulations [19] and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources [20]. All surgery was performed under chloroform anesthesia, and efforts to minimize animal suffering were made during the study course.

2.5. Bioterium Area Conditions. The bioterium was sanitized with sodium 10% hypochlorite. The temperature was maintained between 20°C and 26°C (mean 22°C ± 1°C) and humidity between 40% and 70%; there were 10 to 14 air-room changes per h and 12-12 h-light/dark cycles. The gerbils were housed in individual sanitized stainless-steel cages (60 × 40 × 30) and bedded on sterilized sawdust that was replaced daily. Cages were arranged on racks and their sanitation was continuously monitored. Biohazard bags [21] were used to autoclave infective biological materials at the end of the study (urine, feces, and carcass) [22].

2.6. Maintenance Diet and Supplied Water. Gerbils were given a commercial diet for a 20-day period based on their daily nutritional requirements. It was composed of casein, corn oil, fiber, mineral mixture, zinc gluconate, vitamins mixture, choline, corn starch, and sucrose [23]. Both diet and

commercial pure water were supplied *ad libitum* to the gerbils throughout the entire study.

2.7. Culture, Preparation, and Administration of G. duodenalis Inoculum. The *Giardia* isolate used in this study was *G. duodenalis* strain GS/M83-H7. *Giardia* trophozoites culture (1×10^6 trophozoites/mL, 250 μL) was added in 15 mL glass screw-capped test tubes containing 7 mL of TYI-S-33 medium supplemented with 10% bovine serum. Subculturing was performed every 72–96 h by incubation in an anaerobic chamber (18% CO_2 atmosphere) at 37°C for 24 h [24]. After incubation, the plates were placed on ice and rocked gently (20 min) to suspend nonadherent trophozoites, and the liquid was quickly discharged to avoid contamination using a purifier [25]. Trophozoites were washed 3 times in PBS pH 7.2 (GIBCO PBS pH 7.2, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA) by centrifuging at 800 g for 10 min at 4°C. The pellet was resuspended in 500 μL of PBS and further diluted 1 : 15 with PBS and 1 : 2 with 0.4% Trypan Blue (Solution Trypan Blue, SIGMA Aldrich Company, ST Louis MO, USA) for microscopic analysis and trophozoite counting in a Neubauer chamber at 40x magnification. Washed pellets were adjusted to a concentration of 5×10^6 trophozoites in 200 μL. Experimental infections with *Giardia* GS/M-83-H7 were established by orogastric inoculation with 5×10^6 trophozoites suspended in 500 μL of PBS [18].

2.8. Bioassay. Previous to the bioassay, the 42 female gerbils were weighed and gastric inoculation was performed with *G. duodenalis* trophozoites. A couple of inoculated gerbils were randomly selected, anaesthetized with chloroform, and euthanized for examination to confirm establishment of the infection via trophozoite detection in the duodenum and the proximal intestine at day 6 of postinfection [18]. Forty gerbils were randomly allocated into 5 treatment groups (8 per group). The treatments were randomly assigned to the 5 groups, and they comprised 0.5 mL oral doses of PBS (negative control), metronidazole (2 mg/mL in PBS or 1 mg per dose, positive control), or 1 of 3 different *Y. baccata* extracts with different concentrations (24.4 mg/mL, 12.2 mg/mL, and 6.1 mg/mL). Treatments were administered once daily for 3 days from day 7 of postinfection to day 9 of postinfection, and the weight of each gerbil was recorded again before euthanasia. After asepsis, 5 cm each of the proximal (midsection) and duodenum segments were removed and cut from fixed locations along the intestine length. Each segment was slit longitudinally and washed with PBS (shaking at 130 rpm) and the washing solutions were transferred to 3 mL vials and kept at 4°C until we counted the trophozoites. After homogenization, the solutions were transferred to conical tubes and centrifuged at 800 g at 4°C for 10 min. The supernatant was discharged and sediment was homogenized. An aliquot of the sediment (0.5 μL) was diluted 1 : 20 with PBS, and 0.5 μL of the dilution was placed in a Neubauer chamber. Trophozoites on each 1 mm^2 square (4 in total) were microscopically counted at 40x magnification (Microscope Olympus, CKX41, Center Valley, PA, USA). The total number of trophozoites per mL was calculated as follows: (Number of trophozoites/4 squares) × (500/100) × 10000 [26].

2.9. Study Design. This was a ten-day bioassay with a completely randomized design, in which the treatments consisted of different concentrations of *Yucca* extract and the experimental units were the gerbil groups infected with 5×10^6 trophozoites of the *G. intestinalis* clone GS/M-83-H7. The assignment of gerbils to each experimental unit (groups) and the assignment of the treatments to each experimental unit (groups) were completely random (Figure 1).

2.10. Statistical Analysis. Descriptive statistics were generated for the weight and trophozoite count data from the study gerbils. The Wilcoxon matched-pairs signed rank test was used to compare the change of weight in the gerbil groups between the baseline and day 10 of postinfection. The effect of the 3 different concentrations of *Yucca's* extracts on *G. intestinalis* trophozoites were measured based on the geometric median of the active trophozoite counts observed in the intestinal tissue of the *Giardia* infected gerbils in each experimental unit after treatment, and the significance of the differences among log-transformed geometric medians was calculated with the nonparametric Kruskal-Wallis one-way and Tukey-Kramer (post hoc) tests. The Mann Whitney *U* test was used to test the difference in the geometric mean trophozoite counts between those from the duodenum and those from the proximal segments of the intestine of the infected gerbils. All analyses were performed using the STATA (Stata Corp LP, 4905 Lakeway Drive, College Station, TX 77845, USA), and statistical significance was established at $P \leq 0.05$.

3. Results and Discussion

This study investigated the activity of extracts of *Y. baccata* from the desert area of Northwestern Mexico against *G. duodenalis*. Our study gerbils were 6–12 weeks of age and weighed 52 to 56 grams. This weight and age combination is proper for bioassays in gerbils to test their susceptibility to *Giardia* infection. McAllister et al. [17] successfully developed bioassays using gerbils that were 6–9 weeks of age and weighing 50 to 60 g. In this study, infection with *G. duodenalis* in Mongolian gerbils (*Meriones unguiculatus*) was established by orogastric inoculation with 5×10^6 trophozoites/500 μL of PBS, and the test confirming infection in 2 gerbils at day 6 of postinfection revealed median trophozoite counts of 120,000 trophozoites/mL PBS and 85,000 trophozoites/mL PBS in the duodenum and the proximal segments, respectively. McAllister et al. [17] used 2×10^5 *Giardia* trophozoites in 0.5 mL of PBS to infect, by orogastric inoculation, the same strain of gerbils as those used in this study. They found that, at day 6 after inoculation, the trophozoite counts tended to be higher in the duodenum than those in the jejunum or ileum. In this study, the trophozoite counts always tended to be higher in the duodenum than those in the proximal intestine of the infected gerbils ($P \geq 0.05$) (data not shown). Most likely, the different configuration of the intestinal

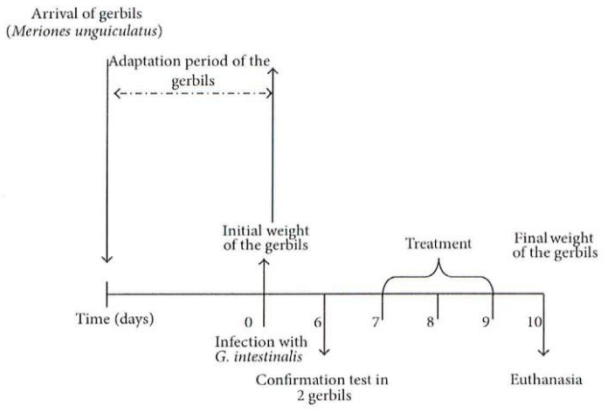

FIGURE 1: Design of the bioassay based on five treatments and 42 gerbils to test the antigiardial activity of the *Y. baccata* extracts.

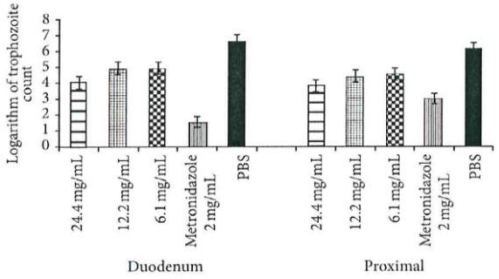

FIGURE 2: Effect of the extracts with 3 different concentrations of extracts per dose of *Y. baccata*, of metronidazole, and phosphate buffered saline (PBS) on the trophozoite counts in the duodenum and proximal of *Giardia* infected Mongolian gerbils.

membrane at the bottom of the villus and the villus : crypt ratios play an important role in trophozoite attachment in the different sections of the small intestine sections [27]. The median weights at preinfection (baseline) and at day 10 of postinfection for 31 gerbils randomly allocated to 5 experimental groups are shown in Table 1. Over the course of the study, each experimental group showed weight loss, but it was not significant (Table 1). Bénéré et al. [28] published that the overall weight gain in 24 gerbils was significantly lower for 16 *Giardia* infected gerbils (0.491 ± 0.0167 g) than 8 gerbils free of *Giardia intestinalis* (0.769 ± 0.059 g) at day 18 of postinfection. It is well recognized that giardiosis may be asymptomatic or associated with acute and chronic diarrhea. *Giardia* trophozoites may cause intestinal lesions, leading to nutrient malabsorption that may explain the weight loss in infected humans [29] or animals [30]. Most likely, this 10-day bioassay was not sufficiently long to observe differences in the pre- and postinfection gerbil weights. In addition, the orogastric administration of the 24.4 mg/mL, 12.2 mg/mL, and 6.1 mg/mL butanol extracts did not significantly reduce the trophozoite counts in the duodenum segment of the infected gerbils compared to the untreated group at day 10 of postinfection. In addition, no difference was observed in the trophozoite count reductions among the 3 extract-treated groups ($P \geq 0.05$) (Figure 2).

In contrast, the high concentration extract (24.4 mg/mL) alone significantly reduced ($P < 0.05$) the trophozoite counts in the proximal segment of the infected gerbils compared to the untreated infected gerbils (Figure 2). There was no difference in the trophozoite count reductions between the group treated with the high extract concentration and the group treated with metronidazole ($P > 0.05$). The trophozoite count was significantly lower for the high concentration extract ($P < 0.05$) than that for the medium and low extract concentrations (Figure 2). The geometric mean of the trophozoite counts remained unchanged in the untreated infected gerbils at day 10 of postinfection in both the duodenum and proximal segments (Figure 2). In contrast, metronidazole significantly reduced the trophozoite counts in both the upper and lower intestine of the infected gerbils ($P = 001$) (Figure 1). McAllister et al. [17] found that the oral administration of 6.1 mg/mL of *schidigera* extracts using the same treatment regime as that used in the present study significantly reduced the number of trophozoites in the lower region (jejunum and ileum) but not the number in the duodenum of the infected gerbils. Those authors explained that the extract's compounds may form complexes with bile salts released in the duodenum [17, 31, 32], reducing their antigiardial properties in the upper regions of the small intestine. However, only the higher extract concentration was capable of significantly reducing the trophozoite counts in the lower region. Gerbils exhibited toxic reactions to the different administered concentrations in this study. Three gerbils per group that received *Yucca* extracts died. McAllister et al. [17] found that a higher extract concentration than the

Table 1: Median weight of 35 gerbils allocated in 5 treatment groups at preinfection and day 10 of postinfection during the bioassay.

Butanol extract	n	Weight at preinfection (g) Median (SE)	Weight at day 10 of postinfection (g) Median (SE)	n	ΔWeight (g)	P^*
24.4 mg/mL of extract[&]	5	52.7 (1.44)	50.3 (1.84)	5	−2.4	0.293
12.2 mg/mL of extract[&]	5	48.7 (3.1)	47.5 (3.87)	5	−1.2	0.403
6.1 mg/mL of extract[&]	5	56.6 (1.2)	55.6 (1.18)	5	−1.0	0.400
Metronidazole	8	56.0 (2.71)	55.3 (2.62)	8	−0.7	0.636
Untreated	8	52.6 (2.25)	49.9 (2.21)	8	−2.7	0.372

(SE): (Standard error); P: * Wilcoxon matched-paired-sum test; Significance at $P \leq 0.05$.
[&] 3 gerbils died per extract-treated group during the bioassay course.

concentrations in this study (50 mg of butanol extract in eight doses over the 3 day period) had a negative impact on the overall health of the gerbils. Although metronidazole failed to eradicate the trophozoite populations, it was more effective at eliminating trophozoites from the proximal segment than from the duodenum segment of the intestine.

4. Conclusion

The butanol extracts of *Y. baccata* exhibited antigiardial activity by reducing, although not significantly, the trophozoite counts in the duodenum, and the higher extract concentration showed a similar antigiardial activity to that of metronidazole in the proximal segment. The effective antigiardial activity in the proximal segment and the toxic effects observed in the gerbils in this study have encouraged us to study the chemical characterization, mechanism of action, and degree of toxicity of the components with antigiardial properties and to compare their antigiardial efficacy against *Y. schidigera* under similar laboratory conditions. Extracts of *Y. baccata* may represent an effective and economical alternative to treat intestinal parasitism in humans.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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CAPÍTULO III

Artículo:

Isolation and characterization of saponins from stem of *Yucca baccata* and antigiardiosis assessment *in vitro*

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En revisión interna

Isolation and characterization of saponins from stem of *Yucca baccata* and antigiardiosis assessment *in vitro*

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Abstract

Ethnopharmacological relevance: Human giardiosis is a major public health problem in Mexico, especially among children; with a general prevalence that can reach up to 68%. It has been estimated that about 200 million people in the world can have giardiosis. Conventional drug therapies against *Giardia* are aggressive, particularly in children and currently cases of resistance have been detected. Therefore, new treatments using natural products that are effective and cause minimal side effects remain searched. Previous studies have revealed that butanolic extracts of *Yucca baccata* Torr., can show anti-*giardial* activity in infected gerbils.

Material and methods: Saponins were isolated from the ethanolic (70%) and butanolic extracts of stem of *Y. baccata* by column chromatography (stationary phase silica gel 60 230-400 mesh; mobile phase of ascending polarity AcOEt-MeOH-H^2O). These were characterized by one and two dimensions nuclear magnetic resonance (^1H and ^{13}C). An inoculum of 10^5 trophozoites of *G. intestinalis* GS/M-83-H7 in TYI-S-33 growth medium was used. Then, different concentrations of saponin (SESE) (from 0.2-to 34 µM) were tested. After 48 hours exposure, the trophozoites viability was analyzed using light microscopy.

Results: A pure saponin, steroid type (SESE) was obtained, which is attached to two glucose in its glycosidic part. Its chemical formula: $C_{39}H_{64}O_{13}$ and has an exact mass of 740.4347. The saponin isolated and characterized from the stem of *Yucca baccata*, showed an IC 50 of 2.12 µM against *G. intestinalis* (P <0.001).

Conclusion: This study provided information about the ability of some plants may have in the treatment of giardiosis. Saponin (SESE) isolated stem *Yucca baccata* is a potential compound to inhibit the *in vitro* growth of this parasite. More research will be needed to develop new products against *Giardia*, and especially to analyze cytotoxicity and to determine the appropriate concentrations of this ~~new~~ saponin.

Keywords: *Giardia lamblia, Yucca baccata*, saponin, *in vitro*, anti-giardia

Introduction

Giardia intestinalis (*G. intestinalis*) is one of the most common intestinal protozoan parasite infection worldwide in humans and mammals. Approximately 280 million people have symptoms caused by *Giardia* annually being childrenthe group at higher risk of infection and re-infection (Feng and Xiao, 2011). Giardiosis symptoms are diarrhea, abdominal pain and general discomfort (Adam, 2001) but in severe cases may occur nutrients malabsorption and weight loss (Halliez and Buret, 2013). Choice treatment for giardiosis includes one or more drugs, mostly, of the nitroimidazoles family. Adverse effects produced by drugs and cases of resistance have been seen in *Giardia intestinalis* (Tejman-Yarden et al., 2011). However, adverse reactions such as nausea, diarrhea, headache, seizures, and dizziness can be associated with those drugs (Campaniti and Monteiro-Leal, 2002). This has conducted to the search of new, effective, safe and inexpensive therapeutic agents with minimal or no side effects not only to replace conventional drugs in the usual therapeutic scheme but also to avoid parasite drug resistance (Hernández and Hernández, 2009; Quihui-Cota et al., 2014). Plants are important sources of bioactive products, considering their wide variety and complexity of metabolites with potential therapeutic properties (Oliveira et al., 2011). The genus *Yucca* of the family *Agavaceae* is a desert plant that grows natively in northern Mexico and southwestern United States. *Yucca baccata* Torr., is widely distributed in Sonora. Now, two types of saponins are recognized; the triterpenoids and steroidal saponins which are secondary metabolites found in greater proportion in the genus *Yucca*, and to which some biological activities have been attributed (Varanda, 1984; Korchowiec et al., 2015).

Saponins have been classified into two types based on the nature of their aglycone: Steroidal and Triterpenoid saponins (Brunéton, 1995). Steroidal saponins consist of a 27 carbons skeleton, which generally comprises a six-ring structure. Triterpenoid saponins have a skeleton of 30 carbons with a five-ring structure (Sparg et al, 2004).

Biological activity of saponins has been extensively studied and for example they can exhibit antitumor activity (Eskander et al., 2013), anti-arthritic activity (Cheeke et al., 2006) and antiparasitic activity among others (Ibrahim et al., 2013). Another study showed that the steroidal glycosides from the *Yucca´*s stem induced apoptosis in cells from individuals with leukemia (Yokosuka et al., 2014). Previous studies have found that *Y. baccata* butanol extracts can reduce *G. intestinalis* trophozoites counts in gerbil proximal duodenum (Quihui-Cota and Leon et al., 2014). Theantigiardial activity and toxic effects of the *Y. baccata* extracts observed in gerbils, encouraged us to characterize the saponins of *Y. baccata* and to study their antigiardial activity in vitro.The furostanol type saponins have been found in *Y. gloriosa* and around 21 saponins have been identified in *Y. shidigera* (Skhirtladze et al., 2011; Oleszek et al., 2001a; Oleszek et al., 2001b). However, no studies on saponins of *Y. baccata* have been published.

Materials and Methods

Plant Material

The stems of *Y. baccata* Torr., were collected in "The Aribabi" ranch (coordinates 30 ° 53'35.8"N 110 ° 42'30.0"O), in April 2013 (springtime), at an average environmental temperature of 23.6 °C.

Samples were taken to the University of Sonora herbarium for taxonomic authentication.The stems were cut into 2 cm wide slices and subjected to a drying process by exposing them to sunlight for 30 days. The dried plant

material was pulverized into 2 mm particle size (Whiley Mill Model Thomas-4 Laboratory Mill USA).

Extraction and Isolation

The method by Newbold was used for extract preparation. The dust of stem of *Y. baccata* (680 g) was suspended in water (33 g / L) and kept under constant stirring for 3 days at temperature from 25-to 30 °C. Next, the suspension was centrifuged at 1500 g., for 10 min (4 °C). Once filtered (type of filters) the stirred slurry was separated into equal volumes of hexane, ethanol, methanol and butanol for 1 day at room temperature. Then each mixture was separated and concentrated using a rotary evaporator (Rotavapor Buchi B-491, Buchi Laboratoriums, Switzerland) coupled to a vacuum pump at 40 °C (Welch 1376N-01 Vaccum Technology). Finally, the extracted solid was weighed and stored dry at room temperature (25 °C).

The extracts were subjected to a chromatographic column and eluted with a polarity upward gradient in ethyl acetate (ACOEt), methanol (MeOH) and water. Fractions (40 mL) were collected and analyzed by NMR. Saponin fractions (8, SESE) were purified again by column chromatography. The successive fractions (15 mL) were collected and mixed based on their chemical composition and after taken to dryness. All fractions were analyzed to determine their structure by NMR.

General Experimental Procedures

NMR spectra were recorded on Agilent Technologies NMR spectrometer 400 MHz. The experiments were carried out in 1 ml of DMSO-d6 and / or CD3OD for ^{1}H and ^{13}C, 1D and 2D at 25-40 °C. Column chromatography was performed

using silica gel 60 (230-400) and eluting with a mobile phase of ACEOt-MeOH-H_2O (75.5: 20: 4.5, v / v / v, respectively). Thin layer chromatography was performed on pre-coated silica gel F254 aluminum plates (coating thickness 0.2 mm, Merck) in a glass chamber. The mobile phase was ethyl acetate, methanol and distilled water in 9: 3: 1.5 proportions, respectively. The developing solution composition was 85 ml methanol, 10 ml glacial acetic acid, 5 mL concentrated sulfuric acid and 0.5 ml p-anisaldehyde. The mobile phase was added to a sealed chamber and saturation was led for 30 minutes. The base was marked at 0.5 cm from the edge; then 0.5 cm width lanes were marked and samples were added. The plate was placed into the chamber and it was allowed to run for 10 minutes. At the end, the plate was sprayed with the developing solution and baked for 8 min at 100 °C for color generation. The steroidal saponins were revealed green with anisaldehyde reagent/sulfuric acid (Hostettmann and Marston, 1995). After monitoring the different extracts by TLC, saponin isolation from the ethanolic and butanolic extracts was carried out by column chromatography.

Culture of G. intestinalis

The*Giardia* isolate used in this study was *G. intestinalis* strain GS/M83-H7, obtained from The American Collection, Rockville, MD, USA. The trophozoites in tubes with 20 ml of TYI-S-33 medium, were incubated in an anaerobic chamber (18% CO_2 atmosphere) at 37°C for 72 h. After incubation, the tubes were placed on ice and rocked gently (4°C /20 min) to suspend nonadherent trophozoites, and the liquid was immediately discharged using a purifier to avoid contamination. Trophozoites were washed in triplicate with PBS pH 7.2 (GIBCO PBS pH 7.2, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA) and then centrifuged at 800g for 10 minutes at 4 ° C. Finally trophozoites were counted using a chamber Neubauer at 40x magnification. Washed pellets were adjusted to a concentration of 5×10^4 trophozoites suspended in 150 µL of TYI-S-33 medium (Diamond et al., 1978; Keister, 1983).

Growth inhibition and viability assay

Saponin activity (SESE) was evaluated by inhibiting the *G. intestinalis* growth *in vitro*. A stock solution of 200 ug / mL, dissolved in DMSO and TYI-S-33 was prepared. It was then diluted three times with an initial concentration of 25 µg / ml of which serial dilutions were made up to 0.18 µg / ml.

Then, $5x10^4$ trophozoites were added to each test tube and after an incubation at 37 ° C for 24 hours the treatment was applied. Metronidazole was used as a positive control using the same range of concentrations afore mentioned, and untreated tubes as negative control.

After 48 hours, the trophozoites counts in each tube were performedt for viability. Detachment of trophozoites was done by placing the tubes on ice water bath (4 ° C) for 20 minutes. The trophozoites washed in triplicate with PBS, were centrifuged for 10 minutes at 800g. The cell pellet was re-suspended and the total number of cells per tube using a hemacytometer were counted.

Data Analyses

The *in vitro* assay was performed in triplicate. Values were expressed as mean ± standard deviation (SD). The analysis was 3x8 factorial arrangement, where the factors were the concentrations and the variables the treatments. The comparison of means was made by Tukey Kramer, establishing a statistical significance at $p \leq 0.05$. All analysis were performed using he statistical package NCSS 2007 (NCSS Statistical Software, Kaysville, UT, USA).

Results and Discussion

The first chromatographic step of the butanol extract of the stem of *Y. baccata* was performed using a column chromatography. This yield 37 fractions (called F1, F2, F3, F4 ... F36, F37) which were subsequently analyzed by both thin

layer chromatography and NMR. Anisaldehyde-sulfuric acid (ASAR) is a developer reagent for natural products and can exhibit different colors depending on the type of compound with which is reacting. The development is promoted by heat and the colors observed are temperature dependent (Jork et al., 1990). The butanol extract of stem of the *Y. baccata* showed a series of bands which were eluted mostly with high polarity solvents. During the development, *Y. baccata* steroidal saponins were revealed in olive green. TLC analysis showed that the most components F1 through F21 were a mixture of saponins and free carbohydrates, except F8, that showed to be a pure compound. The components of F22 to F37 were light brown to dark brown, with the most polar compounds in the extract, confirming the presence of free carbohydrates by NMR.

The most abundant saponin isolated from the stem of *Y. baccata* was named SESE. Resonance spectrum ^{1}H and ^{13}C are shown in Fig. 1 and Fig. 2 respectively.

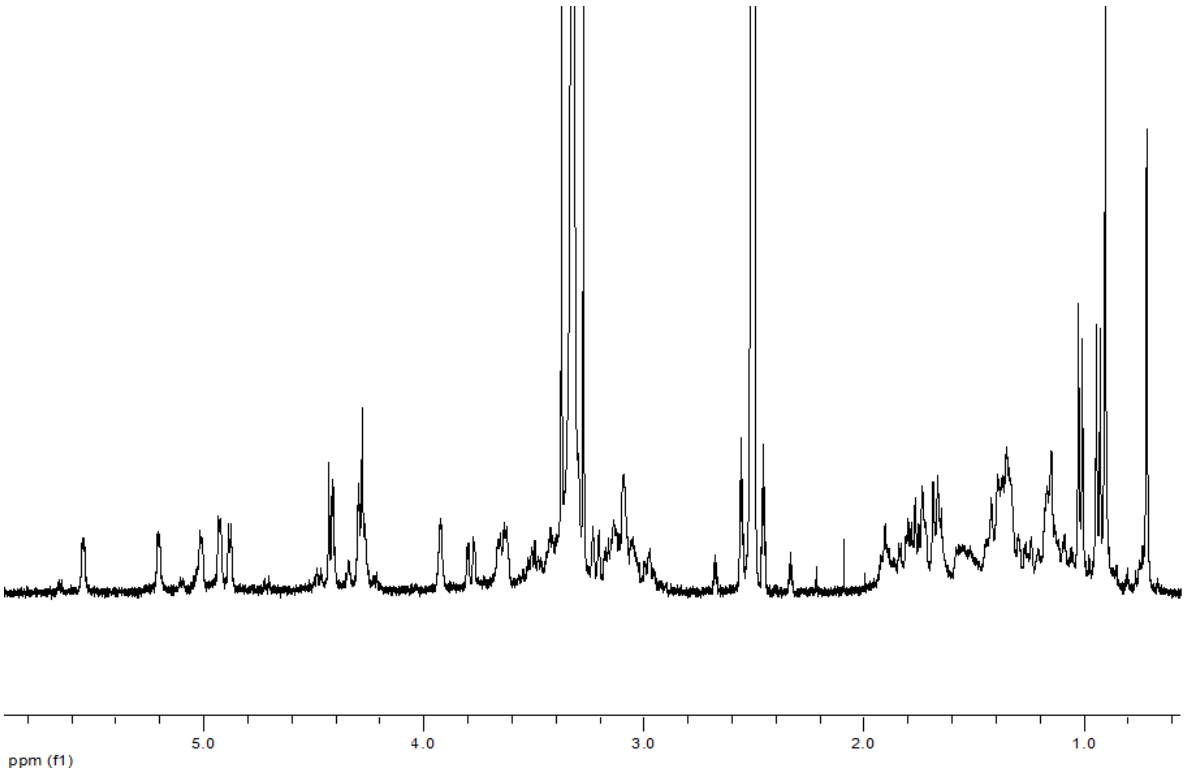


Fig 1. ^{1}H NMR for SESE in CD_3OD (δ in ppm).

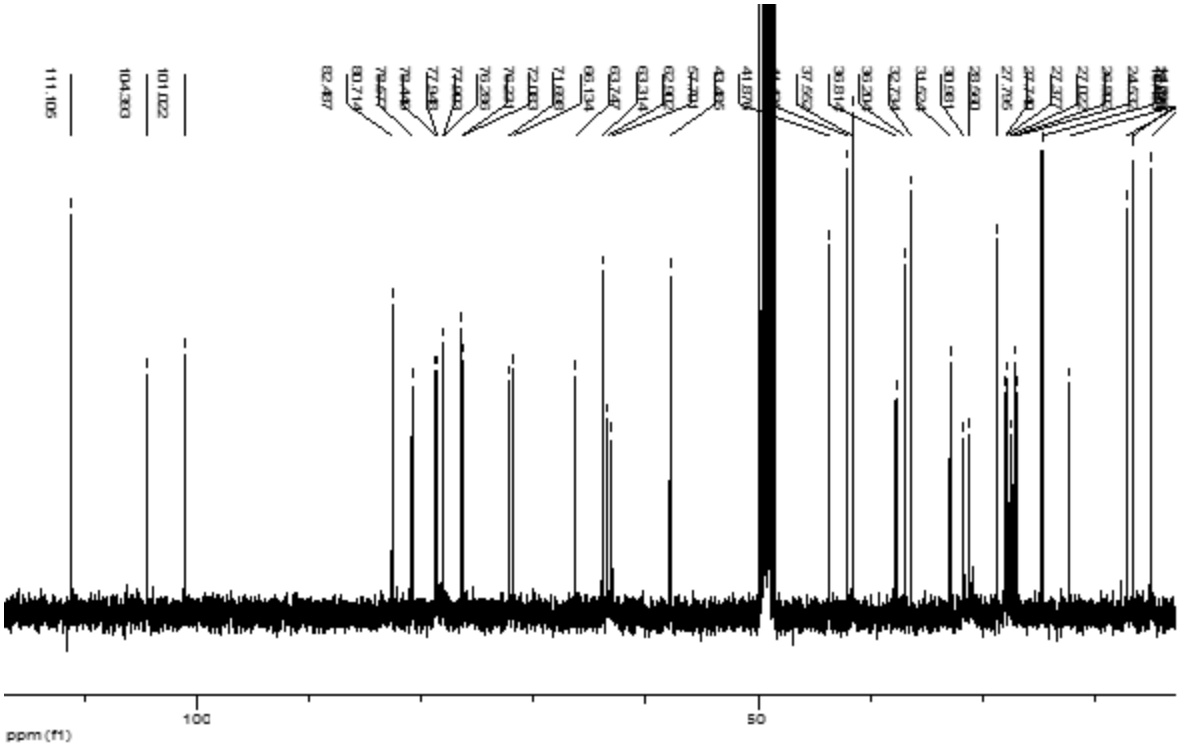


Fig 2. ^{13}C NMR for F8 in CD_3OD (δ in ppm).

In the proton spectrum (Fig 1) a high-field signals corresponding to the aglycone in the proton spectrum, two singlets and two doublets in the region from 0 to 1.00 ppm were observed. This showed the presence of four methyl groups in the structure.

Figure 2, in the region after 1.00 ppm shows the anomeric protons of the two monosaccharides bound to the aglycone.

The downfield ^{13}C NMR main spectrum signals were 101.02, 104.39, and 111.10 ppm. The first two corresponded to the anomeric carbons of the glycosides and the signal 111.10 ppm corresponded to C-22, which is the junction between E and F rings directly bonded to two oxygen atoms. This showed the presence of a saponin spirostane with 27 carbons, with 2 monosaccharides linked to the aglycone (11 carbons). ^{13}C displacement are shown in Table 1.

In a study conducted in *Yucca elephantipes*, the butanol extraction column chromatography and thin layer chromatography were performed. Preliminary analysis showed a positive reaction (green) with anisaldehyde reagent. The

structures were elucidated by NMR, producing 6 new steroidal saponins with a spirostan skeleton. In our case the analysis of the purified saponin revealed a spirostane steroidal saponin (Zhang et al., 2008).

Similarly, in April 2006 the furostano type saponins were the first isolated from *Yucca gloriosa*. It confirmed that saponins are the main compounds in *Yucca* genus of *Agavaceae* family (Skhirtladze et al., 2006). Up to date, all species of the genus *Yucca* contain steroidal saponins (Oleszek et al., 2001a; Oleszek et al., 2001b).

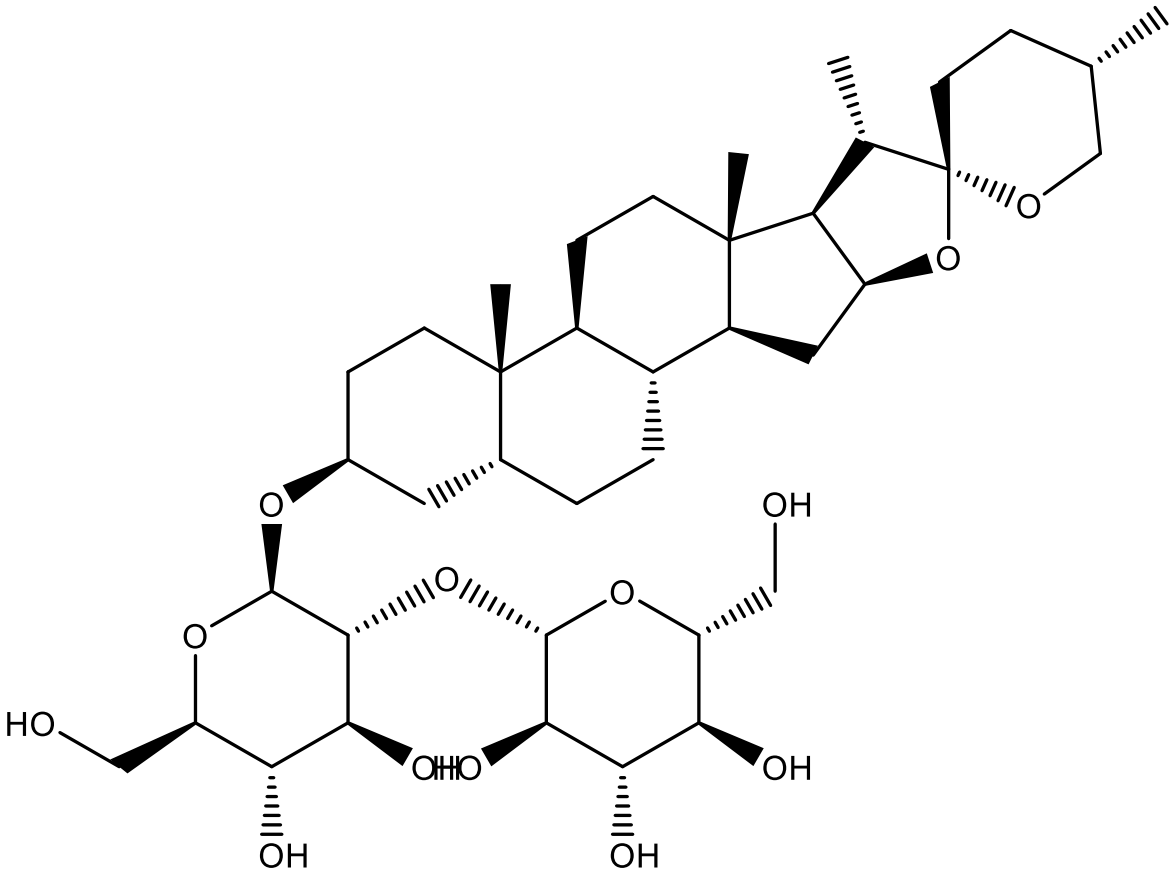
At present published studies on *Y. baccata* saponins structural analysis are very limited. . So far, based on the structures analysis, the unequivocal chemical structure isolated from the stem of *Y. baccata* is shown below (Fig. 3) which can be described as a spirostane steroidal saponin with two carbohydrate residues of the extract of the *Yucca baccata*'s steam.

Table 1

^{13}C NMR Spectropic data for the saponin SESE isolated from the stem of *Y. baccata* 400 Mhz. (^{13}C), CD3OD, δ (ppm).

Carbons	F8 δ	Carbons	F8 δ	Carbons	F8 δ
1	14.79	**14**	32.73	**27**	72.08
2	16.45	**15**	36.20	**28**	76.23
3	17.01	**16**	36.81	**29**	76.28
4	22.08	**17**	37.55	**30**	77.90
5	24.53	**18**	41.43	**31**	77.95
6	26.80	**19**	41.87	**32**	78.45
7	27.02	**20**	43.48	**33**	78.58
8	27.37	**21**	57.70	**34**	80.71
9	27.74	**22**	62.90	**35**	82.48
10	27.80	**23**	63.31	**36**	101.02
11	28.56	**24**	63.74	**37**	104.39

12	31.00	**25**	66.13	**38**	111.10
13	31.52	**26**	71.69		



Chemical Formula: $C_{39}H_{64}O_{13}$
Exact Mass: 740.4347

Fig 3. Suggested chemical skeleton of steroidal saponin spirostane; **25(S)-Schidigera-saponin.**

Anti-*Giardia* activity

The anti-giardia *in vitro* activity of SESE by testing different concentrations was carried out. Metronidazole was used as positive control. The results are shown below (Fig. 4) (Table 2).

The minimal inhibitory concentration for saponin SESE was 2.12 µM. No differences was found when comparing the highest concentration of saponin (34 µM) used against the Metronidazole concentration.

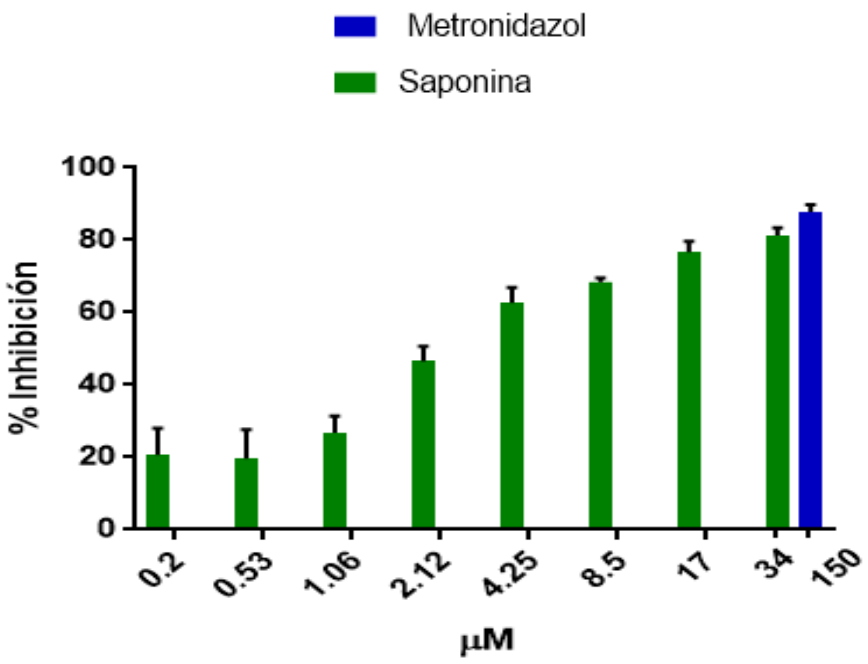


Fig 4. The activity of *Y. baccata* SESE on the proliferation of *G. intestinalis* trophozoites at different concentrations (µM) was assessed *in vitro*. Metronidazole was used as a Positive Control. The antiparasitic activity was evaluated 48 hours after addition of the treatment. The overall average of 6 independent trials with 4 replicates is observed. The values are presented in means ± SD and compared by ANOVA, Tukey Kramer, with a significant difference between treatments compared to Control (+) ($p < 0.05$).

Table 2

Inhibition of *G. intestinalis* growth by Saponin (SESE) over 24 h exposure. Trophozoites of *G. intestinalis* were incubated in the presence of different concentrations (25 – 0.18 µg/mL).

Concentrations (µg/mL)	**% Inhibition METRONIDAZOL**	**% Inhibition SAPONIN (SESE)**
25	**91**	**81**
12.5	**86**	**82**
6.25	**85**	**75**
3.125	**75**	**44**
1.5	**53**	**42**
0.75	**33**	**16**
0.37	**22**	**3**
0.18	**14**	**7**

Conclusion

A completely pure saponin was isolated from the *Y. baccata's* stem extract. Its yield was 0.259 g per 680 g of extract used. This corresponded to 0.04% of the total dry weight of the *Y. baccata*'s stem.

The structure of the purified and characterized saponin was as follows: $C_{39}H_{64}O_{13}$, with an exact mass of 740.4347.

The *in vitro* assessment showed that the IC50 for the pure saponin (SESE) was 2.12 μM and the highest concentration of the saponin used (34 μM) showed the same anti-*Giardia* activity than the Metronidazole.

It is recognized that Metronidazole, is one of the first choice drugs used against giardiosis. Additional both *in vitro* and *in vivo* studies are required to continue isolating and characterizing other saponins of the extracts of *Y. baccata*, in order to determine their appropriate therapeutic and toxic concentrations.

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