ISSN 0717 7917
www.blacpma.cl

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas

BLACPMA

Volumen 6, Número 3

Con el auspicio de

SOCIEDAD LATINOAMERICANA DE FITOQUÍMICA

Registrada en INDEX COPERNICUS®, LATINDEX®, IMBIOMED®

OBJETIVOS DEL BOLETÍN

Estimular a los grupos de trabajo existentes en Latinoamérica, sean investigadores, productores, funcionarios o simplemente interesados en las plantas medicinales y aromáticas, poniendo a su disposición este Boletín para la difusión y la divulgación de sus investigaciones y de las actividades que en general desarrollen en torno a plantas.

Ser una herramienta de difusión para la Sociedad Latinoamericana de Fitoquímica, principalmente, y de otras sociedades y agrupaciones que se sientan representadas por este Boletín.

Constituir un nexo entre los profesionales de habla hispana, francesa, portuguesa e inglesa de la región, relacionados con el tema central del Boletín

El BOLETÍN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS (BLACPMA), ISSN 0717 7917, es una publicación científica electrónica bimensual dirigida a diversos profesionales y técnicos vinculados al campo de las plantas medicinales y aromáticas. BLACPMA es una entidad sin ánimo de lucro. Aunque auspiciada por la Sociedad Fitoquimica Latinoamericana (SLF), este boletín no es propiedad de Club o Asociación alguna. Ni BLACPMA ni la SLF son responsables en ningún momento de las opiniones vertidas en sus páginas, que son responsabilidad única de sus respectivos autores. Todo el material gráfico ha sido creado de manera genuina o bien remitido por sus autores con el permiso de éstos. Todas las marcas y logos referidos en estas páginas son propiedad de sus respectivos autores o empresas. En Chile, 1 de Enero de 2007.

BLACPMA WEB Site:

www.blacpma.cl

Envio de trabajos Online a nuestra editorial (Online Submission)
Blacpma_editorial@hotmail.com

BLACPMA esta Indexada por:

Index Copernicus
http://www.indexcopernicus.com

(Impact Factor 3.73)

IMBIOMED
http://www.imbiomed.com.mx

LATINDEX
http://www.latindex.unam.mx

Instrucciones para los autores

El BOLETÍN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS (BLACPMA), ISSN 0717 7917, es una publicación científica electrónica bimensual dirigida a diversos profesionales y técnicos vinculados al campo de las plantas medicinales y aromáticas y en general dedicados a los productos naturales de interés medicinal o nutraceutico. Se aceptarán trabajos relacionados con las áreas que cubre el Boletín y que son: agronomía, antropología y etnobotánica, aplicaciones industriales, botánica, calidad y normalización, ecología y biodiversidad, economía y mercado, farmacología, fitoquímica, farmacognosia, legislación, informaciones y difusión de eventos, cursos, premios, reglamentaciones, noticias, cuestiones de mercado, ponencias, bibliografía, o cualquier otro tipo de material que se crea importante comunicar.

Se podrán presentar trabajos de **revisión** y de **investigación científica original**, c**omunicaciones cortas**, así como **ensayos** y escritos para **debate** escritos en idioma español, inglés, portugués o francés de libre extensión siempre que razonablemente se ajuste al objetivo del trabajo. Los anuncios, noticias y otros no deberán exceder la cuartilla. En todos los casos están incluidas las tablas.

Los trabajos serán presentados en lenguaje de Microsoft Word (versión 3.1 o superior, con letra Times New Roman número 11) y enviados por correo electrónico a la siguiente dirección: blacpma_editorial@hotmail.com o en su lugar por correo aéreo en disquete de 3.5 pulgadas a: Lic. José Luis Martínez, Editor, Casilla de Correos 70036, Santiago 7, Chile.

Los trabajos se acompañarán de una relación de los correos electrónicos y/o direcciones postales de todos los autores. El autor principal se responsabilizará de la conformidad de cada uno de ellos con su publicación en BLACPMA, así como de cualquier problema surgido por la autoría y/o originalidad del trabajo.

Una vez recibidos, los trabajos se enviarán a dos evaluadores que decidirán su aprobación o rechazo.

Los trabajos se dividirán en Introducción, Materiales y Métodos, Resultados, Discusión y Conclusiones y Bibliografía. En cualquiera de las modalidades en la cual se presenten los trabajos, en la primera página deberá aparecer: Título del trabajo (en español e inglés), autores, institución a la cual pertenecen los autores, dirección del autor principal y correo electrónico. Deberá aparecer además un resumen en español e inglés de no más de 100 palabras, un título corto y un máximo de 6 palabras clave. Los números de las tablas y las figuras deben ser arábigos.

Las citas en el texto deberán incluir apellido del autor y año, separados por coma y colocados entre paréntesis (ejemplo: Bruneton, 1995); si hay más de una trabajo del mismo autor, se separarán por comas (ejemplo: Bruneton, 1987, 1995, 2001). Si hay dos autores se citarán separados por "y" o su equivalente, respetando el idioma original de la fuente. Si hay más de dos autores, sólo se citará el primero seguido de la expresión *et al.* destacada en itálica (ejemplo: Dixon *et al.*, 1999), en tanto que en la bibliografía deberán figurar todos los autores. Si hay varios trabajos de un mismo autor y año, se citará con una letra en secuencia adosada al año (ejemplo: Mayer *et al.* 1987a, 1987b). Si un trabajo no tiene autor, se lo citará como Anónimo seguido de la fecha de publicación. Si hubiera más de una cita de este tipo en el mismo año, se adosará una letra correlativamente (Anónimo, 2002a, Anónimo, 2002b).

La bibliografía incluirá sólo las referencias citadas en el texto, ordenadas alfabéticamente por el apellido del primer autor, sin número que lo anteceda y sin sangría. Apellido/s del autor seguido de las iniciales del nombre sin puntos ni separación entre ellas.

El nombre de la revista se colocará abreviado según normativa ISO y en itálica de acuerdo con el Botanico Periodicum Huntianum, (disponible solamente en edición impresa) o con el mas conveniente Pubmed Journals Database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Journal , ISO abbreviation), que ofrece la posibilidad de chequear online el nombre y abreviatura (en ambos sentidos) de un enorme número de revistas. Por ultimo se citará el volumen de la publicación, mejor si seguido del número entre paréntesis, dos puntos y el número de página desde x hasta y, sin espacios entre medio.

Las citas de libros deben explicitar que páginas fueron consultadas y el año de edición (presten atención a no equivocar el año de la primera edición con el de la edición que están Uds. consultando).

No se admitirán citas incompletas y todo defecto será motivo de retraso del artículo hasta su corrección acuerdo a estas normas.

Autoridad Final de La Editorial

Los editores se reservan el derecho de corregir o alterar los manuscritos enviados para publicación en BLACPMA. Este procedimiento se ejecutara en los casos en los que los manuscritos no se avengan a estándares científicos generalmente aceptados o si el contenido es innecesariamente largo o poco claro. Estas alteraciones pueden ser requeridas directamente al autor y en todo caso retrasaran la publicación del manuscrito.

Gracias de antemano por sus contribuciones

El comité editorial de BLACPMA

MODELOS

Publicaciones periódicas

Soto H, Rovirosa J, San Martín A, Argandoña V. 1994. Metabolitos secundarios de *Dictyota crenulata. Bol. Soc. Chil. Quím.* 39(3):173–178.

Libros

Durand E, Miranda M, Cuellar A.1986. Manual de prácticas de laboratorio de Farmacognosia. Ed. Pueblo y Educación, La Habana, Cuba, pp. 90, 120-121.

Capítulos de libros editados

Lopes de Almeida JM. 2000. Formulación farmacéutica de productos fitoterapéuticos, pp. 113-124. En Sharapin N: Fundamentos de tecnología de productos fitoterapéuticos. Ed. CAB y CYTED, Bogotá, Colombia.

Tesis (aceptable sólo si no hay fuente alternativa)

González de Cid D. 2000. Estudio de cianobacterias con efectos nocivos (deletéreos y tóxicos) en ambientes acuáticos de la provincia de San Luís. Tesis Doctoral, Universidad Nacional de San Luís, Argentina, pp. 234, 245-244.

Comunicaciones a Congresos

Si no hay libro oficial de abstracts:

Novak A, Pardo de Santayana M, Prieto JM. 2006. Antioxidant activity and fingerprinting of Spanish *Bupleurum* species used as anti-inflammatory remedies. Comunicación a la British Pharmaceutical Conference 2006 (Royal Pharmaceutical Society of Great Britain, Manchester, UK, 4-6 Septiembre).

Si hay libro oficial de abstracts:

Novak A, Pardo de Santayana M, Prieto JM. 2006. Antioxidant activity and fingerprinting of Spanish *Bupleurum* species used as anti-inflammatory remedies. Resúmenes de la British Pharmaceutical Conference 2006 (Royal Pharmaceutical Society of Great Britain, Manchester, UK, 4-6 September) p. 23.

Si los resúmenes fueron a su vez publicados en una revista se menciona SÓLO a la revista como si fuera un artículo más.

Novak A, Pardo de Santayana M, Prieto JM. 2006. Antioxidant activity and fingerprinting of Spanish *Bupleurum* species used as anti-inflammatory remedies. *J. Pharm. Pharmacol.* 58(Suppl. 1):82.

Recursos electrónicos

Nota: Si hay que partir alguna dirección se recomienda hacerlo después de una barra inclinada

ATENCION: hoy existen muchos otros tipos de dominios que no son http. Por ejemplo los hay https o ftp. Igualmente existen muchos dominios que no son www, sino www2 u otros. Por tanto preste atención a la dirección completa y no asuma que por defecto van a ser http o www.

Duncan R. 2000. Nano-sized particles as "nanomedicines". http://www.mhra.gov.uk/home/idcplg?IdcService=GET_FILE&dDocName=con2022821&RevisionSelectionMethod=Latest . [Consultada el 6 de Octubre de 2006].

En caso de no haber un autor, o cuando no hay un responsable principal, se toma la institución responsable como equivalente al autor, y en el texto se cita (CNN, 2000).

CNN. Cuba's health care manages despite embargo. http://www.cnn.com/TRANSCRIPTS/0108/18/yh.00.html [Consultada el 5 octubre de 2006].

Boletines o revistas on-line con ISSN, la fuente debe ser citada como cualquier otra revista.

Prieto JM. 2005. El Bálsamo de Fierabrás. *BLACPMA* 4(3):48-51.

Importante NOTA sobre la citación de páginas Web

En estos días se esta comprobando el creciente ABUSO de la citación de paginas Web para avalar afirmaciones científicas hechas por los autores. Resulta muy peligroso para su credibilidad como autor, y para la credibilidad de este Boletín, citar información obtenida en páginas Web que no tengan ninguna entidad científicamente reconocida que se haga responsable de la susodicha información. Las páginas Web "anónimas" Solo deben ser usadas en casos muy justificados y ante la absoluta ausencia de ninguna otra fuente primaria científicamente reconocida. El Comité Editorial de esta revista realizará todo esfuerzo para eliminar el recurso fácil a páginas Web pseudo-científicas y desde luego los autores deben en todo caso dar una explicación de porque han recurrido a este tipo de fuentes. Todo abuso será motivo de rechazo para publicación, incluso si este ya fue (erróneamente) aceptado por los revisores. Si se trata de boletines o revistas on-line con ISSN, la fuente debe ser citada como cualquier otra revista.

Editorial

BLACPMA ha iniciado un camino que lo esta elevando a un sitio privilegiado en el ámbito de las publicaciones científicas en el área de las plantas medicinales. Pensar que todo nació hace más de 6 años con la idea de que fuera un boletín principalmente de carácter noticioso.

El proceso de transformación comenzado en enero de 2005 con el cambio cualitativo en estética y contenidos cristalizo en Noviembre de 2006 durante FAPRONATURA. En Varadero (Cuba) tuvo lugar el primer Simposio de BLACPMA gracias al decisivo apoyo de los dos personajes más destacados de la Farmacología de productos naturales cubana que generosamente colocaron en el centro de la atención a BLACPMA. Ellos son los profesores Rene Delgado y Gabino Garrido. En la semana que pasamos en la isla se efectuaron diversas reuniones formales e informales que permitieron dar un mejor ordenamiento al boletín, racionalizar el flujo de trabajo del comité editorial para absorber mas artículos por mes, y establecer objetivos a uno, tres y cinco años asi como reforzar la comunicación interna.

Para conseguir todo ello, la parrilla directiva se modifico por primera vez en los siguientes cargos: José María Prieto (Universidad de Londres) acompañará como Editor Jefe a José Luis Martínez. Salieron del cargo de Editor el Dr. Jorge Rodríguez (Cuba) al que el comité reunido le quiso reconocer su aportación fundamental como co-fundador del boletín estableciendo el susodicho cargo en la relación del comité al lado de José Luis Martínez. Se nombró como nuevo Editor Ejecutivo al Dr. Gabino Garrido (Cuba) y se creo un nutrido equipo de co-editores que pueda absorber mas trabajo y a la vez asegurar un rápido proceso editorial. Esto se consiguió con la entrada de los doctores Patricia Arenas (Argentina), Carla Delporte (Chile) y Damaris Silveira (Brasil), que acompañaran a los ya anteriormente presentes Dres. Arnaldo Bandoni (Argentina), Patrick Moyna (Uruguay) y Francisco Morón (Cuba), saliendo de este grupo la Dra. María Engracia Medina (Nicaragua).

Por primera vez se designan coordinadores de publicación, que serán lo encargados de coordinar los artículos recibidos para velar por la presteza y corrección del proceso editorial. Así cada coordinador se comunica con el autor del artículo y los revisores –o *referees*- En forma auxiliar Claudio Laurido (Chile) se unió a los co-editores en esta tarea. La idea es que los artículos sean revisados rápidamente -idealmente en un plazo máximo de 10 días- y de esta manera que el autor sepa la evaluación de su artículo en menos de dos semanas.

Como todos los Mayos de cada año, se procedió a la remodelación del listado de miembros del Comité Editorial. Esta vez se hizo previa encuesta a todos ellos para pedir sus opiniones y sugerencias, así como compromisos de trabajo. Como resultado entran a formar parte del Comité los doctores Peter Taylor (Venezuela), Jen Yen Sung (Taiwán), Rocio Alarcón (Reino Unido), Nilka Torres (Panama) y Armando Herrera (México), que sustituyen a un número similar de antiguos miembros.

BLACPMA, que como decimos nació simplemente como boletín de noticias, empezó a atraer la publicación de trabajos originales y de revisión desde muy temprano. El resultado es que acumula ya un importante capital de trabajos publicados, cerca de 70 a final de este año. Lo que mas importancia tiene no es sin embargo el número sino la increíble heterogeneidad de su procedencia. BLACPMA a tenido un gran éxito fuera de lo que seria su nicho natural, Latinoamérica, y se ha convertido de facto en una revista apreciada en los cinco continentes. Trabajos de Japón, Malasia, Pakistán, Ghana, Camerún. Sudáfrica, Estados Unidos, Italia, Reino Unido han ido apareciendo en sus paginas.

Desde la reunión de Varadero, hemos trabajando febrilmente para darle un nuevo impulso a BLACPMA: el resultado es la transformaron del boletín en una verdadera revista científica, con un incremento del 200% en su contenido. Este nuevo proyecto será presentado durante la segunda reunión del Comité editorial BLACPMA a celebrar en conjunto con SILAE en La Plata (Argentina) en Septiembre próximo en donde esperamos poder contar con un importante numero de miembros presentes y así obtener su apoyo explicito para sostener este proyecto nacido un día de mayo durante un paseo por el Cerro San Cristóbal en Santiago de Chile.

Sin mas, les dejamos disfrutar del nuevo BLACPMA, y como siempre, esperamos sus contribuciones.

Ldo. José Luis Martínez
Dr. José Maria Prieto

Editores Jefes de BLACPMA

Derechos de Publicación © 2007 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 6 (3), 48 - 51
BLACPMA ISSN 0717 7917

Ensayo/Debate

La Medicina Tradicional y Natural en el Sistema de Salud Pública Cubano

[Natural and Traditional Medicine in the Cuban National Health System]

Grisel DEL TORO GARCÍA*, Yoagne María TRAPERO QUINTANA*

Departamento de Biofísica, Centro de Biofísica Médica, Universidad de Oriente; Patricio Lumumba s/n, 90500, Santiago de Cuba, Cuba.

*Contacto: hubert.delestre@accs.co.cu , ymtquin@infomed.sld.cu

Recibido 13 Marzo 2007. Aceptado 5 Agosto 2007.

Abstract

A bibliographical study is presented about the incorporation of the traditional and natural medicine to the medical practice in the Cuban public health system. The modernization of the health system in the contemporary societies promoted the study and use of therapeutic methods based on scientific knowledge and in high medical technologies. In Cuba the national program to development and generalization of traditional and natural medicine emerged. The bioethical approach of its incorporation to the modern public health system pursue the public benefit, equal justice and self-reliance. Stimulating the study and use of natural and traditional medicine is fair since it extends the possibilities of treatment at a massive level.

Keywords: Natural remedies; traditional medicine, national health system, integration.

Resumen

Se presenta una revisión bibliográfica sobre la incorporación de la medicina tradicional y natural a la práctica médica en el sistema de salud pública cubano. La modernización del sistema de salud en las sociedades contemporáneas promovió el estudio y uso de métodos terapéuticos, basados en conocimientos científicos y altas tecnologías. En Cuba surge el programa nacional para el desarrollo y generalización de la medicina tradicional y natural, cuyo enfoque bioético considera la beneficencia, la no maleficencia, la justicia y la autonomía. La estimulación del estudio y uso de la medicina tradicional y natural amplía las posibilidades de tratamiento a nivel masivo.

Palabras clave: medicina natural, medicina tradicional, sistema nacional de salud; integración.

La medicina tradicional y natural (MTN) comprende conocimientos y técnicas basados en teorías, creencias y experiencias de diferentes culturas; utilizados para mantener la salud, prevenir, diagnosticar o tratar enfermedades físicas y mentales. Conforma el acervo cultural de la humanidad ya que sus procedimientos varían de una región a otra, recibiendo la influencia de factores culturales, históricos y personales (MINSAP, 1999; Torres y Quintana, 2004). La teoría y aplicación de la MTN difieren de las de la medicina convencional. La utilización a lo largo del tiempo de sus procedimientos y la experiencia transmitida de generación en generación, han demostrado su inocuidad y eficacia (Freyre, 1998).

La concepción orientalista de la medicina como ciencia ha evolucionado durante milenios y sus resultados, en cuanto a promoción de salud, prevención de enfermedades, curación y rehabilitación de la salud del hombre son relevantes. De otra parte, la concepción occidentalista ha constituido un baluarte dentro de la medicina cubana. En el desarrollo de la medicina como ciencia, tanto en Cuba como en otros países, se fueron incorporando conocimientos y prácticas, cuyos resultados fueron valiosos en el perfeccionamiento del Sistema Nacional de Salud (Castiñeiras, 1994; MINSAP, 1999).

Una de las tendencias actuales de la medicina ha sido incorporar la MTN a la práctica profesional, no como un método alternativo motivado por causas

económicas, sino como una disciplina científica que se debe estudiar, perfeccionar y desarrollar permanentemente, por sus ventajas éticas y científicas; aún cuando se superen las desigualdades entre los pueblos pobres y los grandes monopolios farmacéuticos (MINSAP, 1999). Además, porque constituye un medio de recuperación del acervo cultural de los pueblos, en peligro de desaparecer ante el avance de la "medicina moderna" (Torres y Quintana , 2004).

La generalización del estudio y puesta en práctica de los métodos y técnicas de la MTN ponen en manos de los trabajadores de la salud nuevos recursos terapéuticos basados en ambas concepciones de la ciencia médica, que posibilitarían la solución de muchos problemas actuales del sistema de salud.

Se realizó un muestreo bibliográfico de publicaciones cubanas en temas de MTN para resumir algunas de las ideas relacionadas con el conflicto entre lo moderno y lo tradicional en la medicina; algunos de los factores que estimularon la reincorporación del saber tradicional y natural en la medicina moderna cubana, así como sus proyecciones, resultados y aspectos bioéticos relacionados.

A partir del siglo XVI la competencia entre la ciencia y la filosofía occidentales antiguas y la nueva filosofía científica no respetó la autonomía de las comunidades colonizadas. La supresión violenta de la MTN interrumpió el desarrollo de la cultura cubana aborigen, acontecimiento histórico que condicionó el arraigo en nuestra isla de tradiciones populares y ancestrales de Europa, Asia y África (Freyre, 1998).

La modernización de la salud pública influyó directamente en contra de la MTN, la cual había sido identificada como seudocientífica y rechazada por los sistemas institucionalizados de salud pública de las sociedades contemporáneas (Freyre, 1998). El desarrollo de las ciencias fomentado por la observación, la experimentación y la tecnología moderna no toleraba el saber no convencional.

Un conflicto ciencia-tradición en la medicina, al convivir lo viejo y lo nuevo, lo tradicional y lo moderno; el cual tiende a resolverse en favor de la medicina convencional pues la seguridad y la eficacia del tratamiento con medicina tradicional, pudiera poner en riesgo la salud del paciente. Prejuicios tradicionalistas y populares que asociaron la superioridad de la medicina moderna científica con sus fundamentos racionales empírico-teóricos, y con su real eficacia terapéutica (Freyre, 1998).

El triunfo de la Revolución Cubana abrió posibilidades para el desarrollo de la MTN en relación con el protagonismo político de las masas populares del campo y la ciudad, y con la promoción, por parte del gobierno, de un sistema de salud pública que satisficiera las demandas médicas y sanitarias de carácter masivo (Castellanos, 1987; Freyre, 1998).

Aunque en Cuba, la ética médica asociada a los sistemas modernos de salud pública se basó fundamentalmente en bases científicas (Alonso y Smith, 1989), se conformó una tradición propia en el uso de plantas medicinales expresada en la persona del ilustre sabio *Juan Tomás Roig Mesa* (Roig, 1974); lo cual no sucedió con las técnicas de origen asiático. La homeopatía fue introducida en la primera mitad del siglo XIX con gran auge en el siglo pasado; destacándose los doctores *Juan José Hevia, Adolfo de Varona y José Joaquín Navarro*, entre otros. Sin embargo, la falta de escuela, de instituciones oficiales y la influencia de la Industria Médico Farmacéutica Norteamericana hicieron que disminuyera el auge de esta terapéutica en el país. No fue hasta 1992 que se abrieron nuevamente las puertas a esta disciplina (MINSAP, 1999; Laza *et al*., 2002).

La crítica a los dogmatismos en todas las esferas de la sociedad y el cambio de actitud oficial hacia los creyentes influyeron en favor de la medicina no convencional, observándose cierto cambio de actitud a finales de la década de los 80 (Castellanos, 1987). La crisis económica de la década de los 90 asociada al derrumbe del campo socialista y al recrudecimiento del bloqueo norteamericano a la isla, promovieron su incorporación como un complemento del desarrollo de la medicina moderna (MINSAP, 1999).

En el año 1991 se inició en el país un Programa de Plantas Medicinales que incluía el uso científico de plantas medicinales conocidas y su elaboración por la industria farmacéutica, la determinación de complejos fitoterapéuticos contenidos en las plantas medicinales de uso popular, sus efectos terapéuticos, los ensayos clínicos imprescindibles y la generalización consecuente de los resultados más importantes (Orta y Pascual, 1998; MINSAP, 1999). Se introdujo el saber tradicional en la atención primaria y dentro de ésta, en el sistema del médico de la familia (MINSAP 1992a, 1992b, 1993; Jardines, 1995).

Los valiosos resultados de este plan, así como la experiencia y desarrollo inicial alcanzados en el campo de la MTN en el mundo propiciaron, en 1999, la implementación de un Programa para el Desarrollo

y Generalización de la Medicina Tradicional y Natural (Acosta, 1995). El mismo dotaría al sistema de salud de un instrumento de trabajo para el desarrollo de un subsistema de atención médica dirigido a lograr la introducción y generalización, en todo el país, de la MTN (MINSAP, 1999).

El enfoque bioético de la incorporación de la MTN al sistema moderno de salud pública considera la beneficencia, la no maleficencia, la justicia y la autonomía (Alonso y Smith, 1989; Lolas, 1993, Pellegrino, 1995). En consecuencia con estos principios el estado cubano estimula la incorporación de la medicina tradicional, siempre y cuando sea comprobada científicamente su eficacia y sea practicada por especialistas competentes (Strauss, 1987; CITMA, 1995; MINSAP, 1999).

Al exigirse validación científica se produce una situación de desventaja o de competencia desleal en detrimento de la tradición, a sabiendas de que la eficacia del fármaco o tratamiento tradicional depende de factores no susceptibles de explicación científica convencional (Strauss, 1987; Freyre, 1998). Debido a su utilización actualmente masiva, el estudio y la validación científica de las tácticas no convencionales es vital para potenciar la efectividad; por lo cual la investigación en estos recursos representa una línea importante de desarrollo de la medicina moderna (Acosta *et al.* 2000a, 2000b; Rodríguez *et al*. 2000; Rivera *et al.* 2004).

Tanto la terapia tradicional como la convencional poseen eficacia relativa. La racionalidad moral de un tratamiento tradicional hay que evaluarla considerando por igual todos los principios bioéticos. Las metodologías de investigación y evaluación de la MTN deben garantizar la inocuidad y la eficacia de las medicinas herbarias y las terapias basadas en procedimientos tradicionales. (Freyre, 1998; WHO, 1992; 2002).

El sistema moderno de salud pública cubano tiende a la incorporación de prácticas médicas tradicionales que antes habían sido rechazadas, marginadas e inventariadas como seudocientíficas o no científicas, en el contexto de los procesos de colonización y modernización. Como resultado, se abre la posibilidad de cooperación, intercomplementación y competencia leal entre ciencia y tradición en el sistema institucionalizado de salud pública (MINSAP 1992c, 1999; Freyre 1998; Francia y Alea, 2003).

La MTN en Cuba se convirtió en una especialidad de perfil amplio, con enfoque integrador y holístico de los problemas de salud, prevención de enfermedades, diagnóstico, tratamiento y rehabilitación de los pacientes, propios de la Medicina Tradicional Asiática, de la Medicina Natural y el empleo de procederes terapéuticos de especialidades como la Medicina Física y la Rehabilitación.

El estudio científico de la MTN es importante para elevar su eficacia, considerando que han cambiado sus destinatarios, como las nuevas enfermedades, nuevos estilos de vida, nuevas formas de pensar, nuevas creencias o nuevas apreciaciones morales.

El estudio de los sistemas de medicina tradicional aplicados en regiones de diferentes continentes, contribuye al aprovechamiento práctico de estos conocimientos y al enriquecimiento cultural de cada etnia, sociedad o civilización, además de enriquecer el potencial de las fuentes para la obtención de medicamentos.

BIBLIOGRAFÍA

Acosta L. 1995. *Proporciónese salud. Cultive plantas medi*cinales. Ed. Científico-Técnica, La Habana, Cuba, pp. 227.

Acosta L, Fuentes V y Rodríguez C. 2000a. Investigaciones agrícolas en especies de uso frecuente en la medicina tradicional. IV. Llantén menor. *Plantago lanc*eolata L. *Rev. Cubana Plant. Med.* 5(3):84-86.

Acosta L, Fuentes VR, Martín G y Rodríguez CA. 2000a. Investigaciones agrícolas en especies de uso frecuente en la medicina tradicional. I. Llantén (*Plantago major L.*) *Rev. Cubana Plant. Med.* 5(1):6-9.

Alonso D y Smith Vvon. 1989. *Ética y deontología médica. Texto básico.* Ed. Pueblo y Educación, La Habana, Cuba, pp. 96.

Castellanos I. 1987. *Las plantas medic*inales. *El militante comunista*. Ed. Política, La Habana, Cuba, pp. 84-89.

Castiñeiras R. 1994. Redimensionamiento del Sistema Nacional de Salud. Comunicación al Taller Nacional *Estrategia de desarrollo de la economía de la salud en Cuba* (Santiago de Cuba, Cuba).

CITMA. 1995. *Código sobre la ética profesional de los trabajadores de las ciencias.* Academia de Ciencias de Cuba. Ciudad de La Habana, Cuba, pp.1-10.

Francia ME y Alea B. 2003. Alternativas no farmacológicas en pacientes comunitarios. *Rev. Cubana Med. Gen. Integr.* 19(4):100-107.

Freyre EF. 1998. Tendencias y controversias en la incorporación de la medicina natural y tradicional al sistema moderno de salud pública, pp. 278-287. En: Acosta S: *Bioética desde una perspectiva cubana.* 2da ed. Centro "Félix Varela", Ed. MINBAS, La Habana, Cuba.

Jardines JB. 1995. Cuba: El reto de la atención primaria y la eficiencia en salud. *Educación Médica Superior*. 9(1-2):3-13.

Laza D, Rodríguez I, Sardiña G. 2002. La homeopatía en el tratamiento del cáncer. Análisis de información. *Rev. Cubana Plant. Med.* 7(1):6-13.

Lolas SF. 1993. La medicina como invención narrativa. *Boletín de la Oficina Sanitaria Panamericana*. 114(1):49-56.

MINSAP. 1992a. *Guía terapéutica dispensarial de fitofármacos y apifármacos*. Ed. Ciencias Médicas, La Habana, Cuba, pp. 181.

MINSAP. 1992b. *El plan del médico de familia en Cuba*. Ed. Ciencias Médicas, La Habana, Cuba, pp. 46.

MINSAP. 1992c. *Objetivos, propósitos y directrices para incrementar la salud pública cubana*. Ministerio de Salud Pública de Cuba, La Habana, Cuba, pp. 25-35.

MINSAP. 1993. *Plantas Medicinales. FITOMED II.* Ed. Ciencias Médicas, La Habana, Cuba, pp. 117.

MINSAP. 1999. *Programa nacional de medicina tradicional y natural.* Ministerio de Salud Pública de Cuba. 1ra ed. La Habana, Cuba: 1-98.

Orta SD y Pascual MA. 1998. La investigación clínica en seres humanos en Cuba, pp. 112-127. En Acosta S: *Bioética desde una perspectiva cubana*. 2da ed. Centro "Félix Varela", Ed. MINBAS, La Habana, Cuba.

Pellegrino DE. 1995. Metamorfosis de la ética médica. Una mirada retrospectiva a los últimos 30 años. *Cuadernos del Programa Regional de Bioética*. OPS-OMS, Santiago de Chile, (1): 19-34.

Rivera MM, Hechevarría I, Carballo C y Reyes M. 2004. Posibilidades de control de enfermedades a partir de productos naturales y controles biológicos en las plantas medicinales. *Rev. Cubana Plant. Med.* 9(3):70-75.

Rodríguez CA, Acosta L, Fuentes V y Martín G. 2000. Investigaciones agrícolas en especies de uso frecuente en la medicina tradicional. II. Mejorana *Origanum majorana* I. *Rev. Cubana Plant. Med.* 5(3):87-90

Roig JT. 1974. *Plantas medicinales, aromáticas o venenosas de Cuba*. Ed. Científico-Técnica, La Habana, Cuba, pp. 1125.

Strauss L. 1987. *Antropología estructural*. Ed. Ciencias Sociales, La Habana, Cuba, pp. 3.

Torres IB y Quintana IJ. 2004. Análisis comparativo sobre el empleo de plantas medicinales en la medicina tradicional de Cuba e Islas Canarias. *Rev. Cubana Plant. Med.* 9(1):9-14.

WHO. 1992. Métodos de Control de calidad de plantas medicinales. WHO/PHARM, Ginebra, pp. 559.

WHO. 2002. Pautas generales para las metodologías de investigación y evaluación de la medicina tradicional. WHO/EDM/TRM/1.

Derechos de Publicación © 2007 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 6 (3), 52 - 60
BLACPMA ISSN 0717 7917

Revisión/Review

Pharmacognosy and pharmacology of Haruan (*Channa striatus*), a medicinal fish with wound healing properties

[Farmacognosia y farmacología de Haruan (*Channa striatus*), un pez medicinal con propiedades cicatrizantes]

Abdul Manan MAT JAIS

Department of Biomedical Products, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Contact: ammatjais@yahoo.com

Received 20 May 2007. Accepted 30 June 2007.

Abstract

Haruan *(Channa striatus*) is an indigenous fresh water carnivorous air breathing fish species, widely distributed in Malaysia. This white boneless meaty and tender taste edible fish is both a popular food choice and a natural remedy in traditional medicine, particularly popular among post-operative patients to induce wound healing. Other pharmacological activities include anti-microbial, anti-inflammatory, cell proliferation, induction of platelet aggregation and anti-nociceptive properties of the mucus. Both wild and cultured Haruan are equally effective, thus ensuring a sustainable source for this popular medicine. Its chemical composition includes high levels of essential amino acids and a good profile of fatty acids that could directly improve tissue growth and wound healing but ongoing efforts are pursuing the isolation of other bioactive compound(s) present in the mucus. As a consequence, it is a promising candidate for future nutraceutical and pharmaceutical products. Our group is now working to commercialize Haruan-based biomedical products, and clinical trials are already ongoing. I am hoping here to highlight how drug discovery and commercialization of biomedical products from natural sources also apply for animal drugs and not only for plant based remedies.

Keywords: Haruan, Channa striatus, wound healing, antinociceptive activity,antibiotic activity, animal drug.

Resumen

Haruan (*Channa striatus*) es un pez carnívoro, capaz de respirar aire libre, indígena de Malasia. Su carne blanca y sin espinas posee un delicado gusto que la ha hecho una opción gastronómica muy popular pero también esta considerada como un remedio natural entre pacientes post-operativos para ayudar en el proceso de cicatrización. Otras propiedades farmacológicas incluyen una potente actividad antinociceptiva del mucus que recubre las escamas de este pez así como prometedoras actividades antimicrobianas, antifúngicas y anticoagulantes. Tanto el pez criado en condiciones salvajes como el cultivado poseen las mismas características organolépticas y medicinales. Y por tanto constituye una prometedora y sostenible fuente para esta medicina tradicional. Su composición química incluye elevados niveles de aminoácidos y ácidos grasos esenciales que influencian positivamente el proceso de cicatrización pero actualmente se trabaja intensamente en la caracterización de otros bioactivos presentes sobre todo en el mucus. Todo esto o convierte en un prometedor candidato tanto como nutracéutico como producto netamente farmacéutico. Nuestro grupo trabaja intensamente en el desarrollo y comercialización de productos basados en Haruan y los ensayos clínicos ya han comenzado. Con esta revisión del estado de conocimientos acerca de esta particular droga animal espero ilustrar como el desarrollo y comercialización de producto biomédicos a partir de fuentes naturales también se puede aplicar a las drogas animales y no solo a las vegetales.

Palabras clave: Haruan, Channa striatus, actividad cicatrizante, actividad antinociceptiva, actividad antibiotica, droga animal.

INTRODUCTION

Nature has been and still is a continuous source of medicinal products. When saying this, many scientists might only think at plants as source of medicinal bioactive. Actually animals are a yet poorly explored source for medicines despite they are well known ingredients for many popular medicines some of them recognized by current and/or past pharmacopoeias around the world. Geckos, frogs and other various insects are used in many Asitic Materia Medica; Spanish flies and leeches were listed for a long time in Western Pharmacopoeias, and maggots has been recently listed in the US Pharmacopoeia (Root-Berstein and Root Bernstein, 1999; Rubin, 2004). In the last decades a great attention has been paid to marine animals mainly sponges, but non-marine animal drugs are still largely neglected by researchers as a source of medicines possibly because they pose serious problems including complex chemical matrixes, poor yield on bioactive, ethical problems, protection by the authorities and sometimes difficulty in finding a reliable and sustainable supply.

Malaysia is, beyond any doubt, endowed with lavish flora and fauna species providing to its inhabitants with a unique source of balanced foods as well as medicinal products. Local medicinal plants have been a very popular health choice among Malaysians but the traditional knowledge also includes the medicinal use of animal drugs, such a fishes, insects and others.

Freshwater animals, fishes in particular, are long associated with daily life in our country and some of them has become legendary in various different ways. Haruan, *Channa striatus* (Figure 1**)** a local freshwater species, belonging to the family Channidae, is known and widely consumed throughout the nation not only as a food, but also as a remedy for wound healing. This knowledge have been kept thanks to our mid-wives who constantly are promoting Haruan for wound healing despite the lack of a proper scientific basis of the claimed. People in China, Indochina, Thailand, Singapore, Indonesia, Philippines and India consumed and believed in the biomedical properties of the fish. Obviously, the Channidae are well distributed within this region namely China, Taiwan, Indochina, Thailand, Philippines, Indonesia and India (Ling, 1977; Mat Jais, 1991; Mohsin and Ambak, 1983; Wee, 1982). There are thirty identified species around the world, and eight were reported found in Malaysia (Inger and Kong, 1962; Mohsin and Ambak, 1983; Wee, 1982). Among them is Haruan *C. striatus*, commonly found in rice fields and the surrounding areas, as well as various natural and man made water body. As a carnivorous fish, Haruan is considered as a pest and therefore it has not been listed as a priority to the farming activity in Malaysia.

However, many other countries in the region, Thailand, Indochina, Indonesia, The Philippines, China and India are having extensive breeding programs and it is among the most popular table fish even in seafood restaurants (Mat Jais, 1991; 1997). This fish is also one of the sort about freshwater for *Ee Sang* during the Chinese New Year, and many believe that Haruan contains all the essential elements to maintain a good health and helps to recover the lost energy after long illness (Mat Jais, 1997; Mat Jais, *et al*., 1994; 1998a).

Figure 1. Haruan (*Channa striatus*).

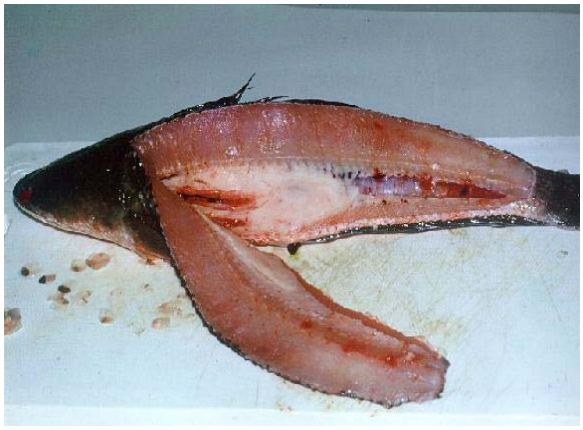
PHARMACOGNOSY OF HARUAN

Taxonomy and distribution

Haruan (*Channa striatus*, Channidae) is a tropical, fresh water, carnivorous, air breathing fish species, indigenous to Malaysia widely distributed within the country. Other members of the same family Channidae are also found in neighbouring countries in the region. Studies on the genetic variability of Haruan's mitochondrial DNA revealed that *C. striatus* of two stocks, and being present in Malaysia for more than 600,000 years thus proving that the fish is a truly Malaysian indigenous species (Kajima *et al.*, 1994; Kumar, 1995).

The traditional drug is the boneless meat (Figure 2). The mucus is also medicinal, and its production is enhanced by cold stress (Mat Jais *et al.*, 1998b).

Figure 2. The boneless meat of Haruan (*Channa striatus*) is a popular Malaysian traditional medicine used for wound healing.



Biology of Haruan

Despite of being a carnivorous, *C. striatus* is not a good swimmer but with a fast flip action is quite able to catch prey, and as an air breather the fish needs to surface for air. Therefore, *C. striatus* prefers slow running or stagnant, shallow not more than 2 meters, with aquatic plants and some dead logs to hide or to hunt. However Haruan has been also found in waters up to 12 meters deep and 4 to 80 meters width. Most of its natural habitats are remote but some are in closed proximity to human settlements. Ponds, small lakes, agriculture canals, small rivers, rice fields and similar water catchments seem the most ideal habitats, but Haruan has been also found in various unexpected places such as rivers with salinity about 10 ppt and higher ground with water temperature around 20^{o}C. The pH of the habitat was 4.30 to 7.90, temperature was between 20.70 to 26.40^{o}C, conductivity between 0.10 to 1.30 mScm^{-1}, turbidity between 2 to 268 ppm and dissolved oxygen between 1.20 to 6.10 ppm. As for Malaysia, a tropical country, the weather is constantly hot and humid, and no significantly different throughout the year (Ling, 1977; Minear and Keith, 1982; Mohsin and Ambak, 1983). Similarly, the water parameters seem to have minimal changes, within a day, weeks and months. There were hardly any changes even after a heavy storm, and any torrential effects will be buffered by in and out flow of the water. Although Malaysia had never experienced such a drastic weather change in the last 20 years, La Nina and El Nino are now provoking an increment in haze phenomena. It was a real problem in August to October 1998 when the mist prevented the sunray from reaching the ground and inhibited the normal aquatic rat of photosynthesis thus affecting the whole ecosystem (Mat Jais, 2000).

Other chemical parameters of the Haruan's habitat are chlorine (0.20 to 0.40 mgl^{-1}), ammonia (0.60 to 3.00 gml^{-1}), nitrate between (0.50 to 6.00 mgl^{-1}), phosphate (0.50 to 0.80 mgl^{-1}), sulphate (2.00 to 13.00 mgl^{-1}), calcium (3.40 to 19.60 ppm), magnesium (1.70 to 25.10 ppm) and sodium (2.37 to 9.81 ppm). This is a definitely a good quality and non-polluted environment. Although, many believes that Haruan is a hardy fish and certainly could tolerate to some extend any deterioration of the quality, it is very sensitive to contamination of its habitat and developed an unique physiological adaptation namely the ability to move from pond to pond by crossing on land in order to find suitable and clean water (Mat Jais, 1991). Similarly, Haruan is also known to bore into mud during dry spell and only out when situation granted. Subsequently, we studied the requirements of the soil but readings were inconsistent and the range was big: calcium, magnesium and sodium composition in the soil, were 1.70 to 16.80 ppm, 0.28 to 29.32 ppm and 2.67 to 34.60 ppm, respectively. This was probably due to prblems with the handling and storage of the samples.

Relatively, the environmental condition and physiology of all the stations in this study could be categorized as clean and coincidently these localities were away from those identified 25 polluted rivers in Malaysia (Mat Jais, 2000).

Chemistry of Haruan

As a food, this white boneless meaty tender taste Haruan is a good source of protein (78.32 $\pm$ 0.23 %) low in lipids (2.08 $\pm$ 0.08 %) and 0.265 $\pm$ 0.013 mg Vitamin A per total lipid. The lipids were further categorized into phospholipid, partial glyceride, cholesterol, fatty alcohol, triglyceride and cholesterol ester, and as expected, a carnivorous *C. striatus* should contained. It also has a high content of arachidonic acid (AA) 20:4ω6, and docosahexaenoic acid (DHA) 22:6ω3 (Zuraini, *et al*., 2006).

The roe, roe refers to the gonads of fish in the pre-spawning season, are used as food and in many countries are considered a prized delicacy. Its fatty acid composition has been established and is qualitative and quantitative different from this of fillets. Moreover, the particular process of roe extracts (freeze-dried extracts or raw extracts) actually affects their qualitative composition (see Table 1).

Table 1. Qualitative comparison of the Fatty acid composition of freeze-dried roe extract (FD Roe), non freeze-dried roe extract (Roe), mucus extract and fillet from Haruan, *Channa striatus*

Fatty acid	FD Roe[1]	Roe[1]	Mucus[1]	Fillet[2]
14:0 (myristic)	-	-	-	-[a]
15:1 (pentadecenoic)	+	+	-	-
16:0 (palmitic)	-	-	+	+
16:1 (palmitoleic)	+	+	-	+
17:0 (margaric)	+	+	-	-
18:0 (stearic)	-	-	+	+
18:1 (oleic)	+	+	+	+
18:2 n-6 (linoleic)	+	+	+	+
18:3 n-3 (linolenic)	+	+	+	+
20:0 (arachidic)	-	+	+	-[a]
20:1 (gadoleic)	+	-	+	-
20:4 n-6 (arachidonic)	+	+	+	+
20:5 (Eicosapentanoic acid)	-	-	-	-[a]
21:0 (heneicosanoic)	-	+	+	-
22:1 (erucic)	+	+	+	-
22:6 (Decosahexanoic acid)	-	-	-	+
22:6 n-3 (docosahexaenoic)	+	+	+	-
24:0 (lignoceric)	-	-	+	-
24:1 (nervonic)	-	-	+	-

[1]According Mat Jais *et al.* (1998b); [2]According Zuraini *et al.* (2006);
[a] Detected only in *C. micropeltes;* Note: *C. lucius* fillets have the same FA qualitative composition.

Furthermore, Haruan possess a good profile of dietary minerals such as magnesium, copper, calcium, manganese, iron, and zinc. Nickel and lead are also naturally occurring minerals in Haruan but there are well below toxic levels to humans (Heimann, 1982; Jaafar, 1985; Lands, 1986; Liu, *et al*., 2001; Mat Jais, 1997).

Finally, the mucus covering the fish has been also studied due to its antinociceptive/anaesthetic activities. The composition of Haruan mucus consists of about 95% water, glycoprotein as a major organic component and fatty acids which composition is qualitatively different from both fillets, or the roe (Mat Jais, *et al.*, 1998) (see Table 1).

PHARMACOLOGICAL PROPERTIES OF HARUAN

Skin diseases

Malaysians live in a humid warm tropical throughout the year and it is estimated that a 15 to 25 % of the population, i.e. no less than 2 millions people, are suffering some sort of skin related problems. Infant, children, young, grown-up teenager, young adults and senior citizens all are having rashes, hormonal-imbalance induced skin problems, acne, pimples, allergy, psoriasis, sclerosis, infection and all kind of skin problems. Haruan is very useful in these conditions and help patients to ease them as well as supporting the maintenance of a healthy skin of any consumer. Basically its action is due to its content on docosahexaenoic acid (DHA) 22:6ω3. This essential fatty acid has been fully identified as a nutraceutical with clinical value in the treatment of (Mat Jais, *et al*., 1998a; Mori, *et al*., 1999). The recognition that AA metabolism is altered in psoriasis and other skin diseases prompted attempts to inhibit the generation of proinflammatory lipoxygenase products, LTB_4 and 12-hydroxyeicosatetraenoic acid (12-HETE), which are markedly elevated in the psoriatic lesions. In particular LTB4 is related with both the onset and maintenance of chronic topical inflammatory conditions (Rao *et al.*, 1994) When humans ingest fish or fish oil, the EPA and docosahexaenoic acid (DHA) from fish or fish oil lead to modulate prostaglandin metabolism and decrease the symptoms of such disorders (Simopoulos, 2002).

Wound healing

The popular, age-old indication of Haruan is wound healing. Nowadays, this is fully exploited in post-operative patients especially caesarian mothers and injuries due to road accidents thanks to the Malaysian midwives and nurses, who constantly and aggressively promote Haruan consumption for wound healing. Besides the rich content in essential amino acids and fatty acids ongoing research is focusing in the characterization of other metabolites possibly related with this activity. This will be a challenging task as wound healing is an extremely complex process involving a series of reactions and interactions among cells and mediators. Each year, new mediators are discovered and our understanding of inflammatory mediators and cellular interactions grows. The skin itself is a complex tissue that becomes infiltrated with pro-inflammatory cells during wound repair. Nowadays, targets for new drugs to be used in wound healing comprises metalloproteinases, leukotrienes, nuclear transcription factors and nuclear receptors. During repair, many different matrix metalloproteinases are produced by multiple cell types residing in various compartment within the wound environment. This diversity of enzymes, coupled with discreet cellular expression, implies that different matrix metalloproteinases serve different functions, acting on a variety of substrates, during wound

healing (Page-McCaw *et al.*, 2007; Parks, 1999). During wound-healing, cells are required to migrate rapidly into the wound site via a proteolytically generated pathway in the provisional matrix, to produce new extracellular matrix, and, subsequently, to remodel the newly formed tissue matrix during the maturation phase. There is now substantial experimental evidence that blocking matrix metalloproteinases will prevent or seriously delay wound-healing (Steffensen *et al*., 2001). Experimental studies established the dogma that inflammation is essential to the establishment of cutaneous homeostasis following injury and, in recent years, information about specific subsets of inflammatory cell lineages and the cytokine network orchestrating inflammation associated with tissue repair has increased. Recently, this dogma has been challenged, and reports have raised questions on the validity of the essential prerequisite of inflammation for efficient tissue repair. (Eming *et al*., 2007). More recently Peroxisome Proliferator-Activated Receptor (PPAR) dysfunction has been implicated in the manifestation of many diseases and illnesses, ranging from obesity to cancer. The alpha, beta, and gamma isotypes of peroxisome proliferator-activated receptor (PPAR) are expressed in the mouse epidermis during fetal development but they disappear progressively from the interfollicular epithelium after birth. Interestingly, PPARalpha and beta expression is reactivated in the adult epidermis after various stimuli -such as tetradecanoylphorbol acetate topical application, hair plucking, or skin wound healing resulting in keratinocyte proliferation and differentiation. The inflammatory reaction associated with a skin injury activates the keratinocytes at the edges of the wound. This activation involves PPARbeta, whose expression and activity as transcription factor are up-regulated by pro-inflammatory signals. PPARalpha and beta are important for the rapid epithelialization of a skin wound and that each of them plays a specific role in this process. PPARalpha is mainly involved in the early inflammation phase of the healing, whereas PPARbeta is implicated in the control of keratinocyte proliferation. Particularly, the re-activation of PPARbeta influences three important properties of the activated keratinocytes that are vital for rapid wound closure (Tan *et al.*, 2003; Michalik *et al.*, 2001). On the basis of the previous data we are going to undertake a bioguided isolation using metalloproteinases, leukotrienes and nuclear factors as targets. Other properties of Haruan such as its antimicrobial, antifungal and platelet-aggregation properties might be actually contributing in synergy to the wound healing process as well as its intrinsic nutritional value. There were 14 amino acids being detected namely leucine, isoleucine, methione, tryptophane, lycine, histidine, alanine, oxyproline, tyrosine, theonine, glycine, serine, aspartic and glutamic acid that are the basis element for wound healing (Mat Jais, *et al*., 1994; 1998a). The amount in Vitamin A, an essential factor for wound healing, is relatively high (Mat Jais, *et al*., 1994; Westaby, 1985).

Antimicrobial and antifungal properties

Bacteria especially *Helicobacter pylori* that causes stomach ulcer and diarrhea, is one of the major health issues in Malaysia and other Asian countries, and plant materials have been the major source of natural therapeutic remedies or used to treat various infectious diseases including anti-microbial (Czygan, 1993; Ody 1993). Although plants are still the center of new screening for alternative, but due to problems of side-effect and limited efficacy (Nitta, *et al*., 2002; Souza, *et al*., 2003), we are looking into animal based resources and Haruan is one of the better candidates. In our preliminary works, Haruan's extracts had shown positive although mild results as anti-bacterial and anti-fungal agent. As part of the whole healing processes, anti-microbial activity, and anti-fungal in particular, is equally important and therefore our preliminary screening of the Haruan's extract against 13 filamentous fungus and 3 non-filamentous or yeast species obtained from the China General Microbiological Culture Center (CGMCC) has shown inhibition effects on the growth of three species labeled as 3.1601 *Neurospora crassa*, 3.3544 *Aleurisma keratinophilum* and 3.4655 *Cordyceps militaris* after 24, 46 and 64 hours. The same extract has also inhibited the growth f species 3.1930 *Botrytis pyramidalis* and 3.3727 *Paecilomyces fumosa-roseus* during the first 24 and 46 hours. This is very interesting for although the inhibition was not strong enough to kill the strain, but the partial inhibition by an animal based extract will be of a better use for human consumption to avoid unnecessary repercussion. We have not published the results for more works are now on-going.

Platelet-aggregation

Aggregation of platelet is one of the steps in blood clotting and wound healing, and Haruan's extracts not only induce aggregation in normal patients, but more

interestingly produced positive results in diabetic patients whom undergone drug treatments, when PAF and Collagen the placebo failed. This is promising for those suffering diabetic mellitus, and it is also will contribute as an alternative for dengue haemorrhagic patients (an on-going research project). Blood clotting is vital for both diabetic complication and dengue haemorrhagic to stop perfusion of blood that might be fatal.

Furthermore, anaesthesiologists routinely encounter problems with pre-operative evaluation of clot function and management of the pre-operative coagulopathies, especially among major surgical and anesthetic endeavors such as cardiac, thoracic and vascular procedures. In collaboration with Prof. Dr Lee Tat Leong, Department of Anaesthesiology, National University Hospital, Singapore and Associate Prof. Dr Kevin Croft at University Department of Medicine, Royal Perth Hospital, University of Western Australia, Perth, we are looking at the thromboelastrography (TEG) index as a useful adjunct to routine coagulation test for patients undergoing surgery. In this research, we evaluated the potential of Haruan's extracts as agent to improve TEG index, and discovered that the value of Slitting Point (SP), Reaction Time (R), Angle and the TEG Index between treatments (concentration of the extract) was highly significant. This is further supporting the potential of Haruan extracts to induce platelet aggregation and blood clotting (Mat Jais *et al.*, unpublished results).

Antinociceptive properties

The hydromethanolic fraction of Haruan fillets extracts produces a dose-dependent anti-nociceptive property, which is also essential in healing processes (Mat Jais, *et al*., 1998). This finding was recognized by the Society of Anaesthesiologists with the most original Paper Commendation Award at the Annual Scientific Meeting held in Singapore on 17 April 1997. The Haruan's extract is comparable to morphine in terms of anti-pain or anti-nociceptive properties and actually enhances its effects through a non-opioid mechanism without inducing any addictive behaviour in animal models. Heating the mucus extract to high temperatures led to minimal loss in antinociceptive activity; that both extracts maintained their activity within pH range 6.0–8.0 and naloxone pretreatment had no effect on the activity of either extract. That the mucus extract is relatively heat stable is similar to the finding for the fillet extract which is prepared at high temperature (and pressure). This suggests that the active ingredients in either are not denatured by heat, or minimally affected if at all. It would be consistent with a complex, stable macromolecule, most probably a glycoprotein or polypeptide, though we cannot rule out the possibility of a polysaccharide. Biochemical studies will be required to definitively characterise these extracts (Dambisya, *et al*., 1997a; 1997b; 1999; Mat Jais, *et al*., 1997; 1998a; Somchit, *et al*., 2004 and Zakaria, *et al*., 2004a; 2004b; 2004c; 2005a and 2005b).

HARUAN BASED MEDICINAL PRODUCTS

Rational of commercialization from the Malaysian point of view

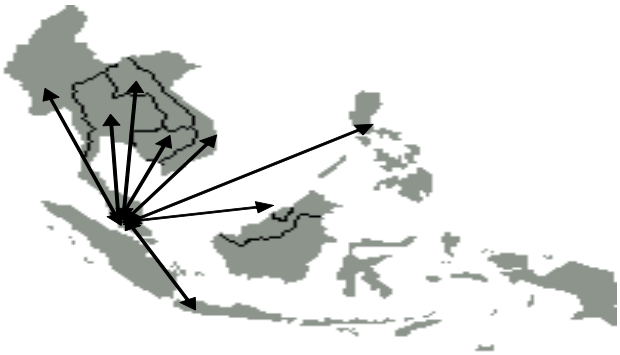
Government is spending at least RM600 millions or more on dermatological diseases, and the public on their owns are buying personal cares products and open selves medication totally to almost RM1 billions worth of imports, therefore is about time for us to come in with an alternative and by so doing reducing foreign exchange. Our almost preservative free non-steroidal products without side effects will be our main trade marks, and environmentally friendly with GAP certification label that will attract attention to consumers in US, Europe, Japan and other developed countries. There are more than 60 % of cosmetic and personal cares products containing either allergenic preservatives or/and steroidal compound(s). These chemical, when used in many skin treatment products had given serious medical issues, including miscarriage among pregnant ladies. This production will reduce the country annual of at least RM 600 - 700 millions dependent on import for skin related diseases or generally known as 'Exfoliation in Dermatitis' namely psoriasis, sclerosis, eczema and allergy; and at the same time create revenue and work opportunities to Malaysians. Natural products is expected to provide RM4 billions revenue in the next years, and Haruan fulfills all the requirements for commercialization either as a nutraceutical or medicine, namely

1. Haruan *C. striatus* is an indigenous species and all the information for farming activities to provide sustainable supplies of high quality fresh sample according to a Good Agriculture Practices (GAP) are well in placed
2. This is a truly Malaysian's product, and right from raw materials up to the finished products will be strictly in conformity to local and international standards. It will establishing Malaysian's contribution in health care industry later improvise to pharmaceutical productions, complete with

Intellectual Property Rights, Trade Marks and Global Brandings

3. This collaborative effort by an academia and local industry to produce the award winning Haruan Based Biomedical Products at MinDex/Innotex Kuala Lumpur 1996, is a project that has been recognized by Ministry of Science Technology and Innovation (MOSTI) as one of the six research to be commercialized in 1996) will create job opportunity, contribute to GDP, and more importantly provide alternative to reduce external dependency, which according to the Ministry of Health dermatological patients, the public on their owns on personal cares products and open selves medication is totaling to almost RM1 billion worth of annual imports
4. Our products are a non-steroidal with minimized chemical preservatives and additives, where it is estimated more than 60 % of those using cosmetic and personal cares products are allergy to preservative and steroid compound(s) in many skin treatment products that sometimes had given serious medical implication including miscarriage among pregnant women.

Figure 3. Geographical distribution of the selected stations in all the nine member nations in the South East Asia or ASEAN - Myanmar, Thailand, Laos, Vietnam, Cambodia, Philippines, Brunei, Indonesia and Singapore.



Haruan Based Biomedical Products (HBBP)

As part of a visionary project to develop and produce *Haruan Based Biomedical Products* (HBBP) for the domestic, regional and international market my team is now utilizing the existing facilities, experts and space within Taman Pertanian Jubli Perak Sultan Haji Ahmad Shah Kuantan (TPJPSHASK) or better know as Taman Pertanian Kuantan (TPK). This is being done jointly with the State Department of Agriculture (DOA) in Pahang.

The first challenge for such a goal was to establish optimal and sustainable source for Haruan, namely developing Good Agricultural Practices (GAP). The physical and chemical characteristics of the Haruan habitat was studied in detail (see biology of Haruan) and the farm stations set up their tanks of production with these parameters. The origin and genetic variability of the animals were also established. The raw material is therefore fully traceable and its composition constant.

CONCLUDING REMARKS

Animal drugs have been largely neglected as a source of medicines, and their potential is still to be realized. Haruan (*Channa striatus*) is a clear example as it is endowed with remarkable wound healing, anti-inflammatory, anti-nociceptive, platelet aggregation, as well as mild antimicrobial and antifungal properties. Its nutritional value is outstanding and actually contributes, at least in part, to the claimed wound healing properties. Haruan extracts are being developed from fully GAP certified stations and we are now focusing on identifying the bioactive compound(s), engaging clinical trial(s) and filing for patent(s). Latin-America, with its enormous natural heritage, must try to identify medicinal animal drugs and add value to its local economy following the example of Haruan.

REFERENCES

Czygan, FC. 1993. Kultugeschictae und mystic des Johanniskrautes. *Z. Phytothe*r. 5:276-282.

Dambisya YM, Lee TL, and Mat Jais AM. 1997a. Effects of *Channa striatus* Extracts on Morphine Analgesic. Proceedings of the 12th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology, Kuala Lumpur, pp 1.

Dambisya YM, Lee TL, Mat Jais AM. 1997b. Analgesic Properties of Extracts from Snakehead Fish, *Channa striatus*, Haruan. Presented at the 8th General Scientific Meeting of the Singapore Society of Anaesthesiology, Singapore, Indonesia, pp 41.

Dambisya YM, Lee TL, Sathivulu V, Mat Jais AM. 1999. Influence of Temperature, pH and naloxone on the Antinociceptive Activity of *Channa striatus* (Haruan) Extracts in Mice. *J. Ethnopharmacol.* 66: 181-186.

Eming SA, Krieg T, Davidson JM. 2007. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol.* 127(3): 514-525.

Heimann W. 1982. Fundamental of Food Chemistry. Avi. Publication Co., West Port Conneticut, USA, pp 27-233.

Inger RF, Kong CP. 1962. The Freshwater Fishes of North Borneo. Fieldiana Zoology, Chicago National History Museum Publi., Chicago, USA, pp 45, 154 - 156.

Jaafar, MI. 1985. *Makanan dan Kegunaannya.* Dewan Bahasa dan Pustaka, Kuala Lumpur, pp 274 - 275.

Kajima A, Patimah I, Siraj SS, Zalina MY, Sabri O. 1994. Laboratory Manual for mtDNA RFLPs Analysis in Fish Species. Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, Occassional Publication. No 12.

Kumar V. 1995. *Haplotypic Differences and Genetic Variability Revealed by Mitochondria DNA in Haruan, Channa striatus* Bloch, a Thesis Submitted to faculty of Fisheries and Marine Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia for the Degree of Bachelor of Science (Fishery), pp. 67, 89-93.

Lands WEM. 1986. *Fish and Human Health*. Academic Press Inc., Harcourt Brace Jovanovich, Publishers.

Ling, SW. 1977. *Aquaculture in Southeast Asia: a Historical Overview.* University of Washington Press, Seattle, USA, pp 21, 60 - 64.

Liu E; Shailaja S; Hoo BS, Mat Jais AM, Lee TL. 2001. Thromboelastographic Assessment of the Effect of Snakehead Fish Extracts on Whole Blood Coagulation. *Proc. Anaesthesia General Scientific Symp*., September.

Mat Jais AM. 1991. Haruan, *Channa striatus*, Farming in Backyard. *Proceedings of the Third Asian Conference of Technolology for Rural Development,* date, pp. 91, 230 - 232.

Mat Jais AM. 1997. Haruan *Channa striatus* as a Component of Health Food. *Proceedings of the Seminar of the Asian Food Technology*, pp 39 - 43.

Mat Jais AM. 2000. Malaysian's Environment in the New Millenium, *SETAC Globe*, May - June, 14 - 16.

Mat Jais AM, McCulloch R, Croft KD. 1994. Fatty and Amino Acid Composition of Haruan - Potential for Wound Healing. *Gen. Pharmacol*., 25, 947-950.

Mat Jais AM, Dambisya YM, Lee TL. 1997. Antinociceptive activity of *Channa striatus* Extracts in Mice. *J. Ethnopharmacology*, 57: 125- 130.

Mat Jais AM, Fung R, Bosi E, Platell C, McCauley R, Croft K. 1998a. Preliminary Evidence on the Potential of Haruan. *Malaysian Applied Biol.* 27: 50-51.

Mat Jais AM, Matori MF, Kittakoop P, Suwanborirux K. 1998b. Fatty Acid Composition in Mucus and Roe of Haruan, *Channa striatus*, for Wound Healing. *Gen. Pharmacol.* 30(4): 561-563.

Mat Jais AM, Prasat K, Croft KD. 2001. Distribution, Environmental Physiology and Genetic Variability of Haruan *Channa striatus*. *Malaysian Fisheries J.l*, 2(1): 192-197.

Michalik L, Desvergne B, Tan NS, Basu-Modak S, Escher P, Rieusset J, Peters JM, Kaya G, Gonzalez FJ, Zakany J, Metzger D, Chambon P, Duboule D, Wahli W. 2001. Impaired skin wound healing in peroxisome proliferator-activated receptor PPARalpha and PPARbeta mutant mice. *J. Cell Biol.* 154(4): 799-814.

Minear RA, Keith LH. 1982. Water Analysis. Academy Press Inc., Washington, USA, pp. 1, 85- 33.

Mohsin AK, Ambak MA. 1983. Freshwater Fishes of Peninsular Malaysia, Universiti of Putra Publications, Putra, Malaysia, pp 157-161.

Mori T, Bao DQ, Burke V, Puddey IB and Beilin LJ. 1999. Docosahexanoic Acid Lowers Ambulatory Blood Pressure and Heart Rate in Humans. *Hypertension*, 34(2):253-260.

Nitta T, Arai T, Takamatsu H, Inatomi Y, Murata H, Linuma M, Tanaka T, Ito T, Asai F, Ibrahim I, Nakanishi T and Watabe K, 2002. Antibacterial activity of extracts prepared from tropical and sub-tropical plants on methicillin-resistant *Staphylococus aureus*. *J. Health Sci.*, 48:273-276.

Ody P, 1993. The complex Medicinal Herbal. Dorling Kindersley Limited, New York, 132-171.

Page-McCaw A, Ewald AJ, Werb Z. 2007. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat. Rev. Mol. Cell Biol.* 8(3): 221-233.

Parks WC. 1999. Matrix metalloproteinases in repair. *Wound. Repair Regen.* 7(6): 423-432.

Rao TS, Yu SS, Djuric SW, Isakson PC. 1994. Phorbol ester-induced dermal inflammation in mice: evaluation of inhibitors of 5-lipoxygenase and antagonists of leukotriene B4 receptor. *J Lipid Mediat. Cell Signal*. 10(3): 213-228.

Root-Bernstein R, Root-Bernstein M. 1999. *Honey, Mud, Maggots and Other Medical Marvels: The sciences behind folks remedies and old wives' tales by*. Houghton Mifflin Company, Massachusetts, USA.

Rubin R. 2004. Maggots and leeches: Good medicine. *USA TODAY* http://www.usatoday.com/news/health/2004-07-07-leeches-maggots_x.htm [Posted 7/7/2004, Last accessed 23/07/2007]

Simopoulos AP. 2002. Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases. *J. Am. Coll. Nut*. 21(6):495-505.

Somchit MN, Solihah MH, Israf DA, Zuraini A, Arifah AK, Mat Jais AM. 2004. Effects of Three Local Malaysian *Channa* spp. Fish on Chronic Inflammation, Journal Oriental Pharmacy and Experimental Medicine. *J. Oriental Pharm. Exp. Med.* 5(1):91-94.

Souza IKH, deOliveira CMA, Ferri PH, de Oliviera JG Jr, de Souza AH Jr, de Fatima O, Fenandes L and Silva MRR, 2003. Antimicrobial activity of Hyptis ovalifolia towards dermatophytes. *Mem. Isnt. Oswaldo Cruz* 98:963-965.

Steffensen, B, Hakkinen L., Larjava H. 2001. Proteolytic events of wound-healing-coordinated interactions among matrix metalloproteinases (MMPs), integrins, and extracellular matrix molecules. *Crit. Rev. Oral Biol. Med.* 12(5):373-398.

Tan NS, Michalik L, Desvergne B, Wahli W. 2003, Peroxisome proliferator-activated receptor (PPAR)-

beta as a target for wound healing drugs: what is possible? *Am J Clin.Dermatol.* 4(8):523-530.

Wee KL. 1982. Snakeheads - Their Biology and Culture. In Muir JF, Robert RJ: *Recent Advances in Aquaculture*. West view Press, Boulder, Colorado, USA, pp. 181-213.

Westaby S, 1985. *Wound Healing*.2nd ed. The MacMillan Press Ltd., City Country, pp. 2 -7.

Zakaria ZA, Somchit MN, Sulaiman MR, Mat Jais AM. 2004a. Preliminary Investigation on the Antinociceptive Properties of Haruan *Channa striatus* Fillet Extracts with Various Solvent Systems. *Pakistan J. Biol. Sci*. 7(10):1706-1710.

Zakaria ZA, Somchit MN, Sulaiman MR, Mat Jais AM, Israf DA. 2004b. Non-Opioid Antinociceptive Activity of Fresh Haruan (*Channa striatus*) Fillet Extract. *J. Tech. Management* 2:6-11.

Zakaria ZA, Mat Jais AM, Sulaiman MR, Somchit MN. 2004c. Effects of α-Amylase, Protease and Lipase on Haruan *Channa striatus* Mucus Extracts Antinociceptive Activity in Mice. *Pakistan J. Biol. Sci*. 7(12): 2202-2207.

Zakaria ZA, Sulaiman MR, Somchit MN, Mat Jais AM. 2005a. Evaluation of the Onset and Offset Time of Aqueous Supernatant of Haruan *Channa striatus* Fillet Antinociceptive Administered Subcutaneously in Mice. *Pakistan J. Biol. Sci*. 8(3): 466-469.

Zakaria ZA, Sulaiman MR, Somchit MN, Justin EC, Daud I, Mat Jais AM. 2005b. The Effects of L-Arginine, D-Arginine, L-NAME and Methylene Blue on Haruan *Channa striatus* - Induced Peripheral Antinociception in Mice. *J. Pharm. Pharmac. Sci.* 8:199-206.

Zuraini A, Somchit MN, Solihah MH, Goh YM, Arifah AK, Zakaria MS, Somchit N., Rajion MA, Zakaria ZA, Mat Jais AM. 2006. Fatty Acid and Amino Acid Composition of Three Local Malaysian *Channa* spp Fish. *Food Chem.* 97: 674-678.

Derechos de Publicación © 2007 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 6 (3), 61 - 67.
BLACPMA ISSN 0717 7917

Artículo original/Original paper

First phytochemical studies on the genus *Baudouinia: B. fluggeiformis*, the main feeding source of *Propithecus verreauxi verreauxi*

[Primeros estudios fitoquímicos en el genero *Baudouinia: B. fluggeiformis*, la principal fuente de alimento de *Propithecus verreauxi verreauxi*]

Susanna BERTINI [1], Federico VANNELLI [1], Guido FLAMINI [1], Ivan NORSCIA [2], Silvio CHERICONI [1*]

[1] *Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy*
[2] *Dipartimento di Etologia, Ecologia, Evoluzione, Unità di Antropologia, Università di Pisa, via Volta 6, 56126 Pisa, Italy*

*Contact: chersil@hotmail.com

Received June 6 2007 Accepted July 23, 2007

Abstract

As part of an ethological-ecological project designed to explain on a chemical basis the feeding habits of the primate *Propithecus verreauxi verreauxi*, a lemur with reported self-medicating behaviour, we here study the phytochemical composition of the leaves of *Baudouinia fluggeiformis*, the preferred food source of these primates. More than 20 compounds were isolated and/or identified, including carotenoids, the flavanone hesperetin, several flavonols, catechins and gallotannins. This is the first phytochemical report on the genus Baudoinia, which is composed only by six species. The polyphenols were responsible for the high in vitro free radical scavenging activity of the methanol extract: the catechins -rich fractions were more active against peroxynitrite-induced tyrosine nitration while the fractions constituted mainly by flavonols showed a greater scavenger activity in the DPPH test. The hypothesis of pharmacophagy of *B. fluggeiformis* leaves by *P. verreauxi verreauxi* is discussed with emphasis on the protection against oxidative stress and prophylaxis of certain diseases endemic to the lemurs.

Keywords: Baudouinia fluggeiformis; Propithecus verreauxi verreauxi, pharmacophagy, polyphenols, carotenoids.

Resumen

El presente trabajo expone la fitoquímica de las hojas de *Baudouinia fluggeiformis* Baill. El interés en esta planta se debe a un proyecto eto-ecológico que pretende explicar las bases químicas de los hábitos alimentarios del primate *Propithecus verreauxi verreauxi,* un lemur con antecedentes de farmacofagia. Se aislaron o identificaron mas de 20 compuestos que incluyeron carotenoides, la flavanona hesperetina, diversos flavonoles, la flavona apigenina-6-C-neohesperidosido, así como catequinas y galotaninos. Esta es la primera vez que se hace un estudio fitoquímico del genero *Baudoinia,* que solo comprende seis especies en el mundo. Los polifenoles confieren un alto poder anti-radicalario al extracto total de las hojas: las fracciones ricas en catequinas fueron mas activas frente a la nitración de la tirosina inducida por peroxinitrito mientras que las fracciones ricas en flavonoles fueron mas activas frente al radical estable DPPH. La hipótesis de farmacofagia de hojas de *B. fluggeiformis* por *P. verreauxi verreauxi* es discutida en este trabajo con énfasis en la posible protección que el consumo de hojas de esta especie vegetal puede ofrecer a los Sifakas frente al stress oxidativo y la profilaxis de ciertas enfermedades endémicas entre lemures.

Palabras clave: Baudouinia fluggeiformis; Propithecus verreauxi verreauxi, farmacofagia, polifenoles, carotenoides.

INTRODUCTION

Verreaux Sifakas *(Propithecus verreauxi verreauxi)* is a primate species endemic to Western Madagascar. Studies at the Kirindy forest showed that these sifakas are highly selective in their plant-food choice, both in the sense of positive - feeding on rare plants - and negative - excluding abundant plants - selection (Carrai *et al.*, 1999).

Thirty-six plant species are known to compose the regular diet of these lemurs, but they spend the 97% of their feeding time only on nine of them (Carrai *et al.*, 2003). Among them, *Baudouinia fluggeiformis* Baill. is the preferred one (Norscia, 2002). This tree is an endemic plant of Madagascar belonging to the family

of Leguminosae, subfamily Caesalpinioideae, tribe Cassieae. The genus comprises only six species: *B. capuronii*, *B. fluggeiformis*, *B. louvelii*, *B. orientalis, B. rouxevillei,* and *B.sollyaeformis* (Du Puy *et al.*, 2002). No previous phytochemical studies for any of them has been yet reported and, hitherto, ours is the first phytochemical study on this genus. The popular Malagasy name of *B. fluggeiformis* is *Manjakabenitany*, meaning "Big King of the Earth", also reflects the importance attributed to this plant species by the natives. It is mainly due to its unusual wood, used by local people to make ornaments and objects for magical rituals: the trunk and branches are deeply folded, with dark to blackish-brown wood in the centre and whitish wood at the ends of the ridges (Du Puy *et al.*, 2002). These characteristics facilitate the identification of this rare tree.

There are antecedents of self-medication behaviour –or pharmacophagy- among sifaka females during the pregnancy and birth seasons (Carrai *et al.*, 2003). These authors hypothesized that the restricted selection of feeding sources exhibited by sifakas enhances both their health and chances of survival. Ingestion of tannins has been related with good health exhibited by periparturient females. Linking secondary metabolites present in the food with their contribution to health maintenance and physical performance of the consumers is a relatively new field of vertebrate behaviour, while lots of work had been made in arthropods for many years. In this study we aim first to establish the phytochemical composition of *B. fluggeiformis.* As illness, physical activity, and stress intensify oxidative processes (Frei and Higdon, 2003), we therefore studied the *in vitro* free radical scavenging and protective activity of different fractions of the methanol extract of *B. fluggeiformis* in an attempt to gain into the possible link between the nutraceutical value of this plant and its central role in the *Verreaux* Sifaka's diet.

MATERIALS AND METHODS

General Experimental Procedures

^{1}H and ^{13}C-NMR spectra of the isolated compounds were obtained with a Bruker AC200 spectrometer in CD_3OD, DMSO-*d6* and $CDCl_3$ using TMS as internal standard.

The following chromatographic media were used for purification: flash chromatography, Kieselgel© 60, 230 - 410 mesh (Merck, Germany); TLC, Kieselgel© 60 F_{254} precoated plates (Merck, Germany); chromatograms were visualized under UV light at 254 and 366 nm and sprayed with concentrated sulphuric acid or Naturstoffereagenz A-PEG reagents; Medium-pressure chromatography was done on a Büchi column (310 × 25 mm) packed with LiChroprep© diol, 40 - 63 μm (Merck, Germany) and solvents were flushed using a Waters 600E pump (Waters, USA); Solid Phase Extraction (SPE) was performed on Bond-Elut© RP-18 cartridges (Varian, USA); Gel-filtration chromatography was performed with Sephadex LH-20 (Pharmazia, Sweden).

The HPLC-UV analyses were performed with a Waters system (Waters Corporation, USA) consisting of a 600E pump, a 717 Plus auto-sampler and a 996 photodiode array detector, managed by Waters Millenium© v.3.2 software. The HPLC-MS-MS analyses were recorded with an ESI-MS LCQ Advantage© instrument with a Surveyor© LC pump system, supported by Xcalibur© software (Thermo Finnigan, USA). All HPLC analyses were performed using a Lichrosphere© 100 RP-18 column (5 μm, 250 × 4.6 mm) (Merck, Germany). The identification of the compounds was achieved by comparison of their retention times, UV-vis, and MS spectra of authentic samples.

The GC-FID analyses were obtained with a DANI GC 1000 (DANI, Italy) equipped with a 3% SE glass packed 80/100 Supelcoport© column (2.5 m × 3 mm) program: 160°C to 270°C, ΔT = 5.0°C/min, injector/detector temperature: 300°C; carrier gas: nitrogen (40 ml/min), volume of injection: 2 μl. The identification of the components was achieved by comparison of their retention times with those of pure authentic samples and the series of *n*-hydrocarbons.

Reference standards for α-carotene, cis-β-carotene, lutein and zeaxanthin were obtained from natural sources as described in literature (Vinkler and Ritcher 1972, Philip 1973, Daood *et al.*1987). β-carotene (Polichimica, Italy), *n*-alkanes (Sigma-Aldrich, Italy), hesperetin and quercetin-3-rhamnoside (Roth, Germany). Other flavonoids were isolated and characterized in our laboratory in previous studies.

Plant Material

Leaves were collected in June 2001 in the forest of Kirindy (Morondava, Madagascar, 44°39'E, 20°03'S) at an altitude of 30 m above sea level. The climate is characterized by a long dry season (April–November) and a short wet season (December– March). The mean annual rainfall is 800 mm, mostly in the rainy season. The temperature shows contrasting daily highs and lows (7 – 25°C) during the dry season, but is relatively

stable (25 – 30°C) during the wet season. Leaves were open air dried, then packed in sealed paper bags and stored in a fresh, dry atmosphere until used for chemical analyses. A voucher specimen (n3540/*Baudouinia fluggeiformis* /1) is deposited in the Herbarium Horti Pisani, Erbario generale - Nuove Acquisizioni section (University of Pisa, Italy).

Extraction

The ground dried leaves (380 g) of *B. fluggeiformis* were successively extracted at room temperature with increasing polarity solvents (2 l x three times, 5 days each, room temperature): petroleum ether, chloroform, chloroform/methanol 9:1, and methanol. After filtering and removal of the solvents under reduced pressure at up to 40°C, the respective petroleum ether (4.10 g), chloroform (6.90 g), chloroform / methanol (8.75 g), and methanol (33.65 g) extracts were obtained.

2,2-Diphenyl-1-picrylhydrazyl Radical (DPPH·) Test

Samples (10 μl) were added to a-solution of freshly prepared methanol solution of DPPH (200 μl, final concentration 100 μM) in 96-well microplates. After incubation at room temperature for 20 min. the absorbance at 490 nm was measured with a UV-spectrophotometer (Wallac Victor^2 1420 Multilabel Counter©, Perkin-Elmer, USA).

Peroxynitrite-Induced Tyrosine Nitration

This method is based on the determination, by HPLC-UV analyses, of the quantity of 3-Nitrotyrosine (3-NT) formed from the reaction between free tyrosine and peroxynitrite at a physiological pH (7.4). The estimated value of 3-NT is in inverse relation with the scavenger activity of the tested compound. The reaction was carried out by adding, under vortexing, the peroxynitrite (5 - 40 μl, 1 mM final concentration) to a solution containing the methanolic extract or pure compounds at the desired concentrations, tyrosine (2 mM) and HCO_3^- (50 mM) all dissolved in 50 mM phosphate buffer (pH 7.4). Test compounds were dissolved in methanol (final concentration 0.5%). Blanks, with or without methanol, were always performed to discard any interference of the solvent with the test. Quantitative determination of 3-NT was performed using an external standard calibration curve ($r^2 = 0.999$).

Peroxynitrite was synthesised from sodium nitrite/H_2O_2 acidified with HCl as previously described (Beckman *et al.*, 1994) and the residual H_2O_2 was removed by passing the solution through granular MnO_2. The yellowish solution was stored in aliquots at -80°C and the concentration of peroxynitrite evaluated immediately before use by measuring its absorbance at 302 nm ($\varepsilon = 1670\ M^{-1}cm^{-1}$). 3-NT was synthesized and purified according to established procedures (Keith and Powell, 1969). Standard solutions were prepared by successive dilutions with phosphate buffer (50 mM, pH 7.4).

Ascorbic acid (Riedel-de Haën, Germany) was used as reference compound. All other chemicals were of analytical grade and aqueous solution were prepared by using freshly deionised, ultra filtered water (Milli-Q© system, Waters, USA). Elution conditions for the HPLC detection of 3-NT were: 20 mM phosphate buffer, (pH 3.2)/methanol (92:8); flow 1 ml/min in isocratic mode, UV detection at 356 nm. All analyses were performed in triplicate and the IC_{50} calculated from dose-response regressions of an appropriate range of 4 – 5 concentrations.

RESULTS

Petroleum Ether Extract

A portion of the extract (500 mg) was re-dissolved in *n*-hexane and submitted to flash chromatography, eluting first with n-hexane (400 ml), then with n-hexane/chloroform 6:4 (350 ml), 1:1 (350 ml) and 2:8 (350 ml), chloroform (300 ml), and finally chloroform/methanol 1:1 (500 ml). After TLC analyses, the fractions of similar composition were combined thus obtaining 6 homogeneous fractions (F1-F6). Fractions F1, F4 and F6 contained only chlorophylls and were not further investigated. Fraction F2 (29 mg) consisted in pure β-carotene. Fraction F3 (61 mg) gave spontaneously a precipitate (8 mg) that, after filtration and GC analyses, resulted constituted by a mixture of n-triacontane and n-nonacosane. The filtrated supernatant of fraction F3 (53 mg) was submitted to preparative HPLC, (Solvent A: acetonitrile/water 9:1; Solvent B: ethyl acetate; Elution program: 0 min, A 100%, 16 min A 60%, 20 min A 0%; flow: 1 ml/min). The five isolated pure compounds were identified as the carotenoids zeaxanthin, lutein, α-carotene, β-carotene and cis-β-carotene by comparison of their UV-VIS spectra, HPLC retention times, ^{13}C NMR and MS-MS spectra with those of pure samples and data in literature (Hornero-Mendez and Mìnguez-Mosquera 1998;

Minguez-Mosquera and Hornero-Mendez 1993; de Pinho *et al.* 2001; Mercadante *et al.* 1999; Yen *et al.* 1996). Fraction F5 (34 mg) was submitted to SPE, eluting first with methanol/water 85:15 then with methanol/water 95:5 and finally with 100% methanol. Three fractions (H1-H3) were obtained. Fractions H1 and H3 contained only chlorophylls and were not further investigated. Fraction H2, was submitted to preparative HPLC chromatography under isocratic conditions (acetonitrile/0.1% aqueous formic acid 55:45, flow 1.5 ml/min), and it gave a flavonoid identified as hesperetin (16 mg) after comparison of its HPLC retention time and UV spectra with those of a commercial sample.

Chloroform Extract

The qualitative composition of this extract resulted identical to the above described petroleum ether extract.

Chloroform/Methanol 9:1 Extract

A portion of the extract (500 mg) was re-dissolved in methanol (1 ml) and submitted to SPE eluting with methanol/water 1:1 (25 ml), methanol/water 7:3 (25 ml), and finally with methanol/water 9:1 (25 ml) thus collecting three fractions (F1-F3). All the solvent mixtures were acidified adding 0.1% formic acid. Fraction F1 (226 mg) was submitted to HPLC/PDA chromatography under isocratic conditions (acetonitrile/0.2% aqueous formic acid water 17:83, flow 1.5 ml/min) to give two flavonols identified as quercetin-3-rhamnoside (62 mg) and 3-methoxyquercetin (29 mg) by comparison of their HPLC retention times and UV spectra with those of pure samples. Fraction F2 (67 mg) consisted in 3-methoxyquercetin according to HPLC analysis. Fraction F3 (116 mg) gave, after preparative TLC, 3-methoxykaempferol (70 mg). In order to check if the methyl ethers of quercetin and kaempferol were not artefacts, an extraction in chloroform/ethanol 9:1 was performed with 5 g of dried material defatted with petroleum ether. HPLC analyses in the above described conditions confirmed their presence in the original material.

Methanolic Extract

A portion of the extract (14 g) was re-dissolved in methanol (17 ml) and submitted to gel-filtration on a Sephadex LH-20 column, eluting with methanol 100%. Fractions were combined according to TLC analyses obtaining 8 homogeneous fractions (S1-S8). Fraction S1 (8.0 g) was not further investigated since, after TLC analyses, were constituted only by carbohydrates. Fraction S2 (270 mg) was submitted to preparative TLC (solvent system: ethyl acetate/acetic acid/formic acid/water 35:3:3:8; single run 10 cm) and gave two compounds identified by NMR analyses as apigenin-6-C-neohesperidoside (12.7 mg) (Wagner, 1979, Nikolov 1982) and 3-β-glucopyranosil gallic acid (9.7 mg) (Lu and Foo, 1999). Fraction S3 (320 mg) was submitted to medium-pressure chromatography eluting first with chloroform/methanol 8:2 (0.7 ml/min, 350 ml), then with chloroform/methanol 7:3 (1 ml/min, 500 ml) and contained, besides the same compounds present in S2, 1-*O*-galloyl-β-D-glucose (46 mg) that was identified by ^{1}H and ^{13}C NMR analyses (El-Mekkawy *et al.*, 1995). Fractions S4 (480 mg), S5 (580 mg) S6 (340 mg) were analysed by HPLC/PDA under isocratic conditions (acetonitrile/water 17:83 added with 0.2% formic acid, flow 1.5 ml/min) leading to the identification of rutin (Häkkinen and Auriola, 1998) and quercetin-3-glucoside (Lu and Foo, 1999) in fraction S4, quercetin-3-rhamnoside in fraction S5, and kaempferol 3-β-D-glucopyranoside (Lu and Foo, 1999) in fraction S6. Fraction S7 (550 mg) and S8 (240 mg) were analysed by HPLC/PDA and HPLC-MS-MS and resulted constituted by a mixture of (-)-epicatechin gallate and (-)-catechin gallate in the case of fraction S7 while fraction S8 contained (-)-epigallocatechin gallate, (-)-epigallocatechin and (-)-epicatechin (Zuo, 2002; Pelillo *et al.* 2002).

DPPH Test

The methanolic extract showed a remarkable scavenger activity with an IC_{50} of 13 μg/ml. Ascorbic acid exhibited an IC_{50} of 92 μg/ml. Among its more representative fractions, S4 (containing rutin and quercetin-3-glucoside) was the most active with an IC_{50} = 0.51 μg/ml followed by S3 (containing apigenin-6-C-neohesperidoside, 3-β-glucopiranosil gallic acid and 1-*O*-galloyl-β-D-glucose) with an IC50 = 1.46 μg/ml, and S7 (containing (-)-epicatechin gallate and (-)-catechin gallate) with an IC_{50} = 4.0 μg/ml.

Peroxynitrite-Induced Tyrosine Nitration

The methanol extract showed an IC_{50} value of 63.2 μg/ml. Ascorbic acid exhibit an IC_{50} of 70.2 μg/ml. Fraction S7, containing a mixture of (-)-epicatechin gallate and (-)-catechin gallate was the most active one in this test with an IC_{50} of 28.6 μg/ml, followed by

fractions S3 (containing apigenin-6-C-neohesperidoside, 3-β-glucopiranosyl gallic acid and 1-*O*-galloyl-β-D-glucose) and S5 (quercetin-3-rhamnoside) with IC_{50} = 85.16 and 43.3 μg/ml, respectively.

DISCUSSION

The phytochemistry of the species belonging to the genus *Baudouinia* remained unknown until now. Our study also aimed to gain into the feeding preference of *P. verreauxi verreauxi,* for *B. flugeiformis* and if it can be related to the secondary metabolites herein present.

Leaves from *B. fluggeiformis* contain mainly carotenoids, flavonoids, gallotannins and catechins. All these types of compounds have been linked with the maintenance of healthy conditions in mammals. Carotenoids have antioxidant properties and are considered of great importance for a good eye health (Newport and Lockwood, 2005). In particular lutein and zeaxanthin are unique compared to other dietary carotenoids because they selectively accumulate in the retina of mammals (Bone *et al.*, 2000) and make up a screening pigment, the macular pigment, which improve visual performance by providing a higher contrast sensitivity, less glare, and better contour perception (Mozaffariieh *et al.*, 2003). Lutein and zeaxanthin are better antioxidants that other carotenoids, such as beta-carotene, in preventing direct oxidation of both DNA and lipids in mammal's retina (Kruger *et al.*, 2002; Beatty *et al.*, 2002).

Classic theories about food selection in species of primates, based on the correlation of preference with the protein concentration of foods and the role of phenolic compounds as antinutritional substances, are being revisited, and there is mounting evidence suggesting that they may be a necessary component of a mammalian herbivore's diet (Palo, 1984; Mole and Waterman, 1987; Mowry *et al.*, 1996). Following consumption, the polyphenols remain predominantly in their conjugated forms and are primarily excreted intact in the urine but these forms keep their potent antioxidant properties (Rafi *et al.*, 2003).

Lemurs use to feed on tannins-rich plant species (Wood *et al.*, 2003). Catechins are of particular importance for sifakas as previous reports described the important presence of these compounds in the periparturient females' diet as a possible self-medication case (Carrai, 2003). In fact catechins are able to diminish the oxidative stress induced by infections (Frei and Higdon, 2003), a high risk for Sifaka females during the birth period. Furthermore, it is known that lemurs are susceptible to suffer from the iron storage disease (ISD), an often lethal syndrome characterized by elevated percentages of transferrin saturation. According to recent findings, a high level of iron-chelating catechins in the lemur's diet like (-)-catechin gallate, epicatechin gallate, (-)-epigallocatechin gallate, (-)-epicatechin, and (-)-epigallocatechin, all of them present in the leaves of *B. fluggeiformis*, decreases this parameter and contributes actively to the health of these animals when in captivity (Wood *et al.*, 2003). Many flavonoids are also endowed with iron-chelating properties and are reported to form inert complexes unable to initiate lipid peroxidation (Middleton, 2000), therefore, they may assist tannins in chelating dietary iron.

The antioxidant properties of flavonoids and catechins present *in B. fluggeiformis* has been determined in this work. These may give an indication of its nutraceutical value against oxidative stress caused by illness and physical activity (Frei and Higdon, 2003; Rafi *et al.*, 2003). The methanol extract of leaves of this plant species acts as a powerful radical scavenger in the DPPH test, being ten-fold more active than ascorbic acid, the fractions containing flavonols showing an even greater scavenger activity in this test. Furthermore the methanol extract was equivalent to ascorbic acid preventing *in vitro* peroxynitrite-induced formation of 3-nitrotyrosine, an important biomarker of the oxidative stress (Althaus *et al.*, 2000). In this model, the fractions containing catechins were more active.

Even if there are still many factors to be understood, i.e. the antioxidant potential of other plants available to these lemurs, the presence of such a wide range of antioxidants of known and proved beneficial activity in mammals (Singh and Bhat 2003, Jung *et al.*, 2003) may represent a promising clue to explain the particular preference of this primate for this plant species. Here we show that they are also able to scavenge nitrogen radicals in agreement with previous findings (Frei and Higdon, 2003). On the other hand catechins and some flavonoids with chelating properties are related to two nutrition deficiencies: iron leading to anaemia and protein indigestibility. A trade off must be achieved between antioxidant protection and these other problems in order to ascertain their actual dietary role in Sifakas.

CONCLUSIONS

In summary, the results of this work offers for first time the phytochemical profile of a species belonging to the genus *Baudouinia*. The more than twenty known secondary metabolites so far identified in our study are carotenoids, flavonoids, gallotannins and catechins. The lack of novelty of the chemical compounds so far isolated from *B. fluggeiformis* is however of ecological importance, as it may be linked with previous findings of a high dietary intake of polyphenols by *P. verreauxi verreauxi* and the importance of tannins-rich food to maintain the health of lemurs in captivity. Moreover, extracts from this species are able to inhibit *in vitro* formation of 3-NT, a biomarker of the oxidative stress. Further studies on the composition and nutraceutical properties of new yet chemically unknown plants eaten by *P. verreauxi verreauxi* are in progress.

Aknowledgements

The authors wish to thank the Malagasy Institutions that authorized the project and provided permits for plant exportation: the Tripartite Commission of the Madagascar Government, the Ministère des Eaux et Forêts, and the CFPF (Centre de Formation Professionnelle Forestière) at Morondava. Special thanks are due to Mr. C. Rakotondrasoa for the identification of the plant material, Peter Kappeler (DPZ, Göttingen–Germany) for logistic support at the Deutsches Primatenzentrum field in Kirindy, and to Valentina Carrai for guidance during sample collection.

REFERENCES

Althaus JS, Schmidt KR, Fountain ST, Tseng MT, Carroll TR, Galatsis P, Hall ED. 2000. LC-MS/MS detection of peroxynitrite-derived 3-nitrotyrosine in rat microvessel. *Free Rad. Biol. Med.* 29:1085-1095.

Beatty S, Koh H, Henson D, Boulton M. 2002. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey Ophtalmol.* 45: 115-134.

Beckman JS, Chen J, Ischiropoulos H, Crow JP. 1994. Oxidative chemistry of peroxynitrite. *Methods Enzymol.* 233:229-240.

Bone RA, Landrum JT, dixon Z, chen Y, Llerena CM. 2000. Lutein and zeaxhantin in the eyes, serum and diet of human subjects. *Exp. Eye Res.* 71:239-245.

Carrai V. 1999. Eco-etologia di *Propithecus verreauxi verreauxi*, lemure degla famiglia Indriidae. Dissertation, University of Pisa, Pisa, Italy, pp 45, 68-98.

Carrai V, Borgognini-Tarli SM, Huffman MA, Bardi M. 2003. Increase of tannin consumption by sifaka (*Propithecus verreauxi verreauxi*) females during the birth season: a case for self-medication in prosimians? *Primates*. 44:61-66.

Daood GH, Biacs P, Hoschke A, Vinkler M, Hajdù F. 1987. Separation and identification of tomato fruit pigments by TLC and HPLC. *Acta Aliment.* 16:339-350.

De Pinho PG, Ferreire ACS, Pinto MM, Benitez JG, Hogg TA. 2001. Determination of carotenoid profiles in grapes, musts and fortified wines from Douro varieties of *Vitis vinifera*. *J. Agric. Food Chem.* 49:5484-5488.

Du Puy DJ, Labat JN, Rabevohitra R, Villiers JF, Bosser J, Moat J. 2002. *The Leguminosae of Madagascar*. Royal Botanic Gardens, Kew, United Kingdom, pp 127.

El-Mekkawy S, Meselhy MR, Tomoco Kusumoto I, Kadota S, Hattori M, Namba T. 1995. Inhibitory effects of Egyptian folk medicines on human immunodeficiency virus (HIV) reverse transcriptase. *Chem. Pharm. Bull.* 43:641-648.

Frei B, Higdon JV. 2003. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *J. Nutr.* 133:3275S-3284S.

Häkkinen S, Auriola S. 1998. High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. *J. Chrom. A.* 829:91-100.

Hornero-Mendez D, Mìnguez-Mosquera MI. 1998. Isolation and identification of the carotenoid capsolutein form *Capsicum annuum* as cucurbitaxanthin A. *J. Agric. Food Chem.* 46:4087-4090.

Jung HA, Jung MJ, Kim JY, Chung HY, Choi JS. 2003. Inhibitory activity of flavonoids from *Prunus davidiana* and other flavonoids on total ROS and hydroxyl radical generation. *Arch. Pharm. Res.* 26:809-815.

Keith WG, Powell RE. 1969. Kinetics of decomposition of peroxynitrous acid. *J. Chem. Soc. A.* 1:90.

Kruger CL, Murphy M, De Freitas Z, Pfannuck F, Heinback J. 2002. An innovative approach to the determination of safety for a dietary ingredient derived from a new source: a case-study using a crystalline lutein product. *Food Chem. Toxicol.* 40:1535-1549.

Lu Y, Foo Y. 1999 . The polyphenol constituents of grape pomace. *Food Chem.* 65:1-8.

Mercadante AZ, Steck A, Pfander H. 1999. Carotenoids from guava (*Psidium guajava* L.): isolation and structure Isolation. *J. Agric. Food Chem.* 47:141-151.

Middleton E, Kandaswami C, Theoharides TC. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* 52:673–751.

Minguez-Mosquera MI, Hornero-Mendez D. 1993. Separation and quantification of the carotenoid

pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reverse phase HPLC. *J. Agric. Food Chem.* 41:1616-1620.

Mole S, Waterman PG. 1987. Tannins as antifeedants to mammalian herbivores- still an open question? pp. 572-587. In Waller GR: *Allelochemicals: role in agriculture and forestry*. American Chemical Society Symposium Series. American Chemical Society Press, Washington, DC, USA.

Mowry CB, Decker BS, Shure DJ. 1996 . The role of phytochemistry in dietary choices of Tana River red colobus monkeys *(Procolobus badius badius). Int. J. Primatol.* 17:63-84.

Mozaffarieh M, Sacu S, Wedrich A. 2003. The role of the carotenoids, lutein and zeaxanthin in protecting against age-related macular degeneration: a review based on controversial evidence. *Nutrition J.* 2:20.

Newport A, Lockwood B. 2005. Use of Nutraceuticals for eye health. *Pharm. J.* 275:261-264.

Nikolv N, Seligmann O, Wagner H, Horowitz RM, Gentili B. 1982 . New flavonoid glycosides from *Crataegus monogyna* and *Crataegus pentagyna*. *Planta Med.* 44:50-53.

Norscia I. 2002. PhD Dissertation, University of Pisa, Pisa (Italy), pp.85-86.

Palo RT. 1984. Distribution of birch *(Betula* spp.), willow *(Salix* spp.) and poplar *(Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. *J. Chem. Ecol.* 10:499-520.

Pelillo M, Biguzzi B, Bendini A, Gallina Toschi T, Vanzini M, Lercker G. 2002. Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins. *Food Chem.* 78:369-374.

Philip T. 1973. Nature of xanthophyll esterification in grapefruit. *J. Agric. Food. Chem.* 6:964-966.

Rafi MM, Yadav PN, Maeng IM. 2003. Targeting inflammation using nutraceuticals. *ACS Symposium Series* 859:48-63.

Singh B, Bhat TK. 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. *J. Agric. Food Chem.* 51, 5579-5597.

Vinkler M, Ritcher KA. 1972. Thin layer chromatography method to determine the pigment content (components) in the pericarp of paprika. *Acta Aliment.* 1:41-58.

Wagner H, Obermeier G, Seligmann O, Chari VM. 1979 . New flavone C-*O*-glycosides from *Melandrium album*. *Phytochemistry*. 18:907-910.

Yen GC, Wu SC, Duh PD. 1996. Extraction and identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). *J. Agric. Food Chem.* 44:1687-1690.

Wood C, Fang SG, Hunt A, Streich WJ. 2003. Increased iron absorption in lemurs: quantitative screening and assessment of dietary prevention. *Am. J. Primatol.* 61, 101-111.

Zuo Y, Chen H, Deng Y. 2002. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. *Talanta*. 57, 307–316.

Derechos de Publicación © 2007 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 6 (3), 68 - 72
BLACPMA ISSN 0717 7917

Artículo original/Original paper

Phytochemical and biological studies on *Nephelium longan*

[Estudios fitoquímicos y biológicos sobre *Nephelium longan*]

Khondaker M. RAHMAN[*], Kamrun NAHAR, Mohammad Gias Uddin KHAN, Choudhury M. HASAN[*]

Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Contact: KM Rahman, Lab G7, Cancer Research UK Gene Targeted Drug Design Research Group, School of Pharmacy, 29-39, Brunswick Square, London WC1N 1AX. khondaker.rahman@pharmacy.ac.uk

Received 20 April, 2007. Accepted 27 June, 2007.

Abstract

Extensive chromatographic separation and purification of the organic solvent extracts of *Nephelium longan* (Sapindaceae) stem bark afforded two compounds; scopoletin and stigmasterol. The structures of these compounds were determined by spectroscopic analyses, including ^{1}H and ^{13}C NMR. Different crude extracts (*n*-hexane, carbon tetrachloride, chloroform and methanol) were tested for antimicrobial activity by standard disc diffusion method known as the Kirby-Bauer method and cytotoxicity was measured by brine shrimp lethality bio-assay. In the brine shrimp lethality bio-assay, the plant extracts showed some promising results as compared to the standard vincristine sulphate, and the test results showed statistical validity. The chloroform and carbon tetrachloride extracts were subjected to antimicrobial and antifungal study and with some exceptions the results are insignificant compared to the standard antibiotic ampicillin.

Key words: Nephelium longan, Sapindaceae, scopoletin, stigmasterol, antimicrobial, cytotoxic activity, Artemia salina.

Resumen

Tras extensivo uso de técnicas cromatográficas, la separación y purificación de los extractos orgánicos de las cortezas de *Nephelium longan* (Sapindaceae) hemos aislado los compuestos escopoletina y estigmasterol. Las estructuras de estos compuestos se determinaron por métodos espectroscópicos usando ^{1}H y ^{13}C RMN. La actividad antimicrobiana de los extractos crudos (*n*-hexano, tetracloruro de carbono, cloroformo y metanol) fueron ensayados usando la difusión en disco estándar (método Kirby-Bauer) y la activadad citotóxica se midió con el ensayo de la *Artemia salina*. En la prueba de citotoxidad, los extractos mostraron efectos significativos comparados con vincristina. Para los estudios antimicrobianos solo se probaron los extractos clorofórmico y tetracloruro de carbono pero los resultados fueron insignificantes comparados con el antibiótico de referencia ampicilina.

Palabras clave: Nephelium longan, Sapindaceae, escopoletina, estigmasterol, actividad antimicrobiana, citotoxicidad, Artemia salina.

INTRODUCTION

Nephelium longan (Fam. - Sapindaceae; Bengali name – *Kathlichu*) is a tree of 30 or 40 ft in height and 45 ft in width, with rough-barked trunk to 2 1/2 ft thick and long, spreading, slightly drooping, heavily foliaged branches. The longan is native to China and India, and is cultivated in Bangladesh, Thailand, Cambodia, Laos, Vietnam and Taiwan (Hooker, 1897). Botanical synonyms for this species include *Dimocarpus longa*n Lour., *Euphoria longan* Steud., *Euphoria longana* Lam., and *Nephelium longana* Cambess. Closely allied to the glamorous lychee, in the family Sapindaceae, the longan, or lungan, also known as dragon's eye or eyeball, and as *mamoncillo chino* in Cuba, has been referred to as the "little brother of the lychee" (Morton, 1987).

The extract of the plant is anxiolytic (Okuyama *et al*.,1999) and anti-mutagenic (Minakata *et al*., 1985). No extensive work has been recorded previously on this plant. It has been reported to contain gallic acid, corilagin (an ellagitannin), ellagic acid (Rangkadilok *et al*., 2005), soyacerebrosides I and II, 1-O-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-(2'-lignoceroyl amino)-4,8-octadecadiene-1,3-diol (longan cerebroside I) and its 8*Z* isomer (longan cerebroside II), momor-cerebroside I, and phytolacca cerebroside (Ryu *et al*., 2003).

MATERIALS AND METHODS

General experimental procedures

^{1}H- and ^{13}C- NMR spectra were obtained from BCSIR (400 MHz Bruker NMR spectrometer with TMS as the internal reference). Silica gel (kieselgel G 60, mesh 70-230, particle size 0.043-0.063 mm) was used for column chromatography. PTLC was done on coated glass plates (kieselgel 60 PF_{254}, Merck). All solvents used in the study were purchased from Merck.

Plant material

The stems of *Nephelium longan* were collected in the surroundings of Comilla, Comilla district, Bangladesh in August 2004 and were taxonomically identified by Mrs. Mahbuba Begum (Chief Scientific Officer, Bangladesh National Herbarium) and a voucher specimen has been deposited there (DACB 21369).

Extraction and isolation

The air dried and pulverized plant material (200.0 g) was cold extracted with methanol and was successively partitioned with *n*-hexane, carbon tetrachloride and chloroform using modified Kupchan partitioning method. Evaporation under reduced pressure at 40ºC using a Buchii Rotary Evaporator provided 2.5, 1.1, 3.0, and 4.5 g of *n*-hexane, carbon tetrachloride, chloroform and methanol soluble materials, respectively. The *n*-hexane solubles were fractionated by column chromatography (CC) over silica gel (60-120 mesh) eluting with *n*-hexane, EtOAc and MeOH in order of increasing polarity to obtain a total of 30 fractions (each 50 ml). The eluates were combined together on the basis of TLC analysis. The fraction eluted with 10% EtOAc in *n*-hexane was subjected to PTLC (mobile phase, 20% EtOAc in toluene with few drops of acetic acid, multiple development) to obtain compound **1** and fraction eluted with 15% EtOAc in *n*-hexane in were subjected to PTLC (mobile phase, 25% EtOAc in toluene with few drops of acetic acid, multiple development) to obtain compound **2**.

Compound 1: ^{1}H- NMR (300 MHz, $CDCl_3$): δ 6.25 (1H, *d*, *J*=9.5 Hz, H-3), 7.57 (1H, *d*, *J*=9.5 Hz, H-4), 6.90 (1H, *s*, H-5), 3.94 (3H, *s*, OMe-6), 6.09 (1H, *s*, OH-7), 6.83 (1H, *s*, H-8); ^{13}C- NMR (125 MHz, $CDCl_3$): 161.5 (C-2), 103.3 (C-3), 114.0 (C-4), 113.5 (C-5), 143.3 (C-6), 144.1 (C-7), 107.6 (C-8), 149.8 (C-9), 111.6 (C-10), 56.5 (OMe-7).

Compound 2: ^{1}H- NMR (400 MHz, $CDCl_3$): δ 3.55 (1H, *m*, H-3), 5.37 (1H, *m*, H-6), 0.90 (1H, H-20), 5.16 (1H, *dd*, *J*=15.0, 6.5 Hz, H-22), 5.03 (1H, *dd*, *J*=15.0, 9.0 Hz, H-23), 0.70 (3H, *s*, Me-18), 1.03 (3H, *s*, Me-19), 0.94 (3H, *d*, Me-21), 0.84 (3H, *d*, Me-26), 0.86 (3H, *d*, Me-27), 0.82 (3H, *t*, Me-29).

Antimicrobial Screening

The microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. The antibacterial activity of the test samples was measured by standard disc diffusion method following the protocols described by Bauer *et al*. (1966). Standard ampicillin disc and blank sterile filter paper disc (BBL, Cocksville USA, 6 mm in diameter) were used as positive and negative controls, respectively. A total of 16 microorganisms were used for the experiment. They are listed in tables 1 and 2.

Cytotoxicity Activities

All the tested extractives were dissolved in DMSO; the final concentrations were achieved by serial dilution from 50 to 0.39 μg/ml and cytotoxicity was evaluated by the Brine shrimp lethality bioassay. The assay was performed using three replicates and the results were compared with the standard, vincristine sulfate. DMSO was used as a negative control. For hatching, eggs were kept in brine with a constant oxygen supply for 48 h; the mature nauplii were then used in the experiment (Meyer *et al.*, 1982; Persoone, 1988).

For the statistical validity of the results in the cytotoxicty analysis, the LC_{50}'s obtained from triplicate experiments and corresponding 95% confidence limits were calculated for the acute tests utilizing the computer program CT-TOX that uses the Binomial, Moving Average Angle, Probit, Spearman-Karber analyses (CTDEP, 1990; Vanhaecke *et al*., 1981).The statistical analysis used was dependent on the dose response of the test organisms. When multiple methods produced valid LC_{50} values, the method that produced the narrowest 95% confidence limits was chosen. The Chi-square statistic for heterogeneity of variance was calculated for every set of data and compared with the tabular (critical) value to indicate how well the data fit the model.

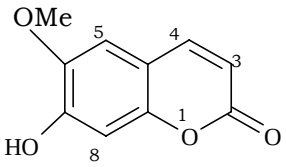
RESULTS AND DISCUSSION

Repetitive chromatography of the *n*-hexane soluble of a methanol extract of *N. longan* stems afforded two

compounds **1** and **2**. Compound **1** was obtained as a white gum, which appeared as a blue spot on TLC plate under UV light at 254 nm. It also exhibited a blue fluorescence under UV light at 366 nm. The compound was identified as scopoletin by comparing the ^{1}H NMR data with those published for this compound (Aldrich, 1992).

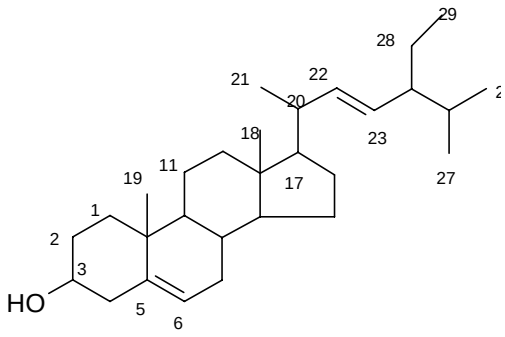
The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** displayed signals characteristic of a 6,7-dioxygenated coumarin. The spectrum revealed two doublets at δ 6.28 and δ 7.60 characteristic of H-3 and H-4 protons respectively of the pyrone ring of a coumarin. The presence of two aromatic proton singlets at δ 6.92 and δ 6.85 were attributable to H-5 and H-8 respectively. In this spectrum a three-proton singlet at δ 3.95 was assigned for a methoxy group. Besides, a singlet at δ 6.09 could be attributable for a hydroxyl group. On this basis it was identified as scopoletin.

Figure 1. Scopoletin (**1**)



Compound **2** was obtained as white needle shaped crystal, which gave purple colour with vanillin-sulfuric acid spray reagent on TLC plate. The compound was identified as stigmasterol by comparing the ^{1}H NMR data with those published for the compound (Aldrich, 1992). The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) revealed a one-proton multiplet at δ 3.55, the position and multiplicity of which is indicative of H-3 of the steroidal nucleus. The typical signal for H-6 of the steroidal skeleton was evident from a multiplet at δ 5.37 integrating one proton. The olefinic protons H-22 and H-23 appeared as characteristic downfield signals at δ 5.16 and 5.03 respectively in the ^{1}H NMR spectrum. Each of the signals were observed as double doublets (*J*=15.0 Hz, 8.3 Hz) which is indicative of *trans* coupling with the olefinic proton and vicinal coupling with neighbouring methine proton. The spectrum further revealed signals at δ 0.70 and δ 1.03 (3H each) assignable to the protons of two tertiary methyl groups at C- 18 and C - 19 respectively. In addition, two doublets (each 3H) centered at δ 0.82 (*J* = 6.0 Hz) and δ 0.84 (*J* = 6.0 Hz) could be ascribed to the methyl groups at C - 29 and C-26 respectively. The doublet at δ 0.94 integrating three-proton was demonstrative of a methyl group at C-21. These ^{1}H-NMR spectral features are characteristics of a steroidal carbon skeleton of stigmasterol and these are in close agreement to those data published for stigmasterol. Therefore, it was identified as stigmasterol.

Figure 2. Stigmasterol (**2**)



In our preliminary antimicrobial screening, the chloroform and carbon tetrachloride showed moderate activity against *Vibrio mimicus* and carbon tetrachloride extract showed mild to moderate activity against *Staphylococcus aureus* and *Vibrio parahaemolyticus*, as compared to the standard ampicillin (Table 1). Both chloroform and carbon tetrachloride extracts showed moderate antifungal activity against *Candida albicans* and *Aspergillus* niger (Table 2). The toxicological study showed some promising results for carbon tetrachloride extract which yielded LC_{50} of 3.13 μg/ml. Chloroform and methanol extracts showed moderate cytotoxic activity, LC_{50} 17.17 μg/ml and 13.63 μg/ml respectively, whereas the positive control vincristine sulphate demonstrated an LC_{50} of 0.44 μg/ml.

CONCLUSIONS

The phytochemical study on the *n*-hexane soluble fraction yielded two pure compounds, scopoletin and stigmasterol, whose structures were established through comparison with published results. In the brine shrimp lethality bio-assay, the plant extracts showed some promising results as compared to the standard vincristine sulphate, and the test results showed statistical validity. The chloroform and carbon tetrachloride extracts were subjected to antimicrobial and antifungal study and with some exceptions the results are insignificant compared to the standard antibiotic ampicillin.

Table 1. Antibacterial activity of extracts of *Nephelium longan*

Species	Diameter of Zone of Inhibition (mm)		
	$CHCl_3$ extract (100 µg/disc)	CCl_4 extract (100 µg/disc)	Ampicillin (30 µg/disc)
Gram Positive			
Bacillus megaterium	7	7	14
Bacillus subtilis	-	-	8
Sarcina lutea	-	-	17
Staphylococcus aureus	-	7	10
Bacillus cereus	7	-	10
Gram Negative			
Pseudomonas aeruginosa	7	-	15
Escherichia coli	-	-	9
Salmonella typhi	-	7	15
Shigella boydii	-	-	11
Shigella dysenteriae	-	-	6
Vibrio mimicus	7	7	8
Salmonella paratyphi	-	-	9
Vibrio parahaemolyticus	-	7	12

'-' indicates no sensitivity

Table 2: Antifungal activity of extracts of *Nephelium longan*

Species	Diameter of Zone of Inhibition (mm)		
	$CHCl_3$ extract (100 µg/disc)	CCl_4 extract (100 µg/disc)	Ampicillin (30 µg/disc)
Saccharromyces cerevaceae	-	-	7
Candida albicans	7	7	10
Asperigillus niger	7	7	9

'-' indicates no sensitivity

Table 3. Cytotoxicity of extracts of *Nephelium longan* on brine shrimps

Sample	LC_{50} (µg/ml)	95% Confidence Limit	Regression equation	χ^2	
				Calculated	Tabular
Vincristine	0.44	0.20-0.98	y=3.1817+0.407x	1.125	15.507
Hexane extract	29.93	17.78-49.71	y=0.238+0.7131x	1.766	15.507
CCl_4 extract	3.13	1.08-9.04	y=4.048+0.3411x	1.604	15.507
$CHCl_3$ extract	17.17	8.59-34.34	y=3.1507+0.3828x	1.943	15.507
MeOH extract	13.63	8.04-23.12	y=1.6987+0.5892x	2.703	15.507

Acknowledgments

The authors are thankful to Institute of Nutrition and Food Sciences (INFS) for supplying the test bacteria, BCSIR, Dhaka for running the NMR spectra and Bangladesh National Herbarium for identifying the plant.

REFERENCES

Aldrich Chemical Co. 1992. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra,* Vol. 3. Aldrich Chemical Company, p 569B.

Bauer AW, Kirby WWM, Sherris JC, Truck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J . Clin. Pathol*. 45:493-496.

CTDEP. 1989. CT-TOX Multimethod Computer Program. IBM PC Version 1.0, Statistical Software package developed by the Bureau of Water Management, Connecticut Department of Environmental Protection, Water Toxics Laboratory, Hartford, CT,USA.

Hooker, JD. 1897. *The flora of British India,* Vol I. L Reeve & Company, UK, p 688.

Meyer BN, Ferringni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for bioactive plant constituents. *Planta Med.* 45:31-32.

Minakata H, Komura H, Tamura SY, Ohfune Y, Nakanishi K, Kada T. 1985. Antimutagenic unusual amino acids from plants. *Experientia.* 41(12):1622-1623.

Morton J. 1987. Longan. In: Morton JF. Fruits of warm climates, Florida Flair Books, Miami, FL, USA. pp. 259–262

Okuyama E, Ebihara H, Takeuchi H, Yamazaki M. 1999. Adenosine, the anxiolytic-like principle of the Arillus of *Euphoria longana*. *Planta Med.* 65(2):115-119.

Persoone G. 1988. Proceedings of the international symposium on brine shrimp, *Artemia salina.* University Press, Wittern, Belgium, pp 1-3.

Rangkadilok N, Worasuttayangkurn L, Bennett RN, Satayavivad J. 2005. Identification and quantification of polyphenolic compounds in Longan (*Euphoria longana* Lam.) fruit. *J Agric Food Chem.* 53(5):1387-1392.

Ryu J, Kim JS, Kang SS. 2003. Cerebrosides from *Longan Arillus. Arch Pharm Res.* 26(2):138-142.

Vanhaecke P, Persoone G, Claus C, Sorgeloos P. 1981. Proposal for a short-term toxicity test with *Artemia* nauplii. *Ecotoxicol. Env. Safety* 5:382-387

BLACPMA ISSN 0717 7917

Artículo original/Original paper

Styrylpyrone glucosides with antimicrobial activity from *Senecio mannii* Hook. (Asteraceae)

[Glucósidos de estirilpironas con actividad antimicrobiana aislados de *Senecio mannii* Hook. (Asteraceae)]

Jean Claude NDOM [1*] Joseph Tanyi MBAFOR [2*] Zanetsie KAKAM [2] Ngeufa HAPPI [1] Juliette Catherine VARDAMIDES [1] Luc Mbaze MEVA'A [1], Theophile Mpondo NGANDO [1], Zacharias Tanee FOMUM [2]

[1]*Department of Chemistry, University of Douala, Faculty of Science P.O. Box 2415, Douala, Cameroon.*
[2]*Department of Chemistry, University of Younde I, Faculty of Science, P.O. Box 812, Yaounde, Cameroon.*

*Contact: Tel: +237-694-71-51; E-mail 1: ndomjefr@yahoo.fr (JC Ndom); E-mail 2: Jtmbafor@yahoo.fr (JT Mbafor).

Received June 6 2007 Accepted July 23, 2007

Abstract

Phytochemical investigation of the methanol extract of the dried ground aerial plants of *Senecio manii* gave two new compounds 4-methoxy-6-(11-O-ß-D-glucopyranosylstyryl)-α-pyrone (1) and 4-methoxy-6-(11-O-α-L-rhamnopyranosylstyryl)-α-pyrone (2) as well as the four known compounds, namely 4-methoxy-6-(11-hydroxystyryl)-α-pyrone (3), 4-methoxy-6-(12-hydroxystyryl)-α-pyrone (4), 4-methoxy-6-(12-O-ß-D-glucopyranosylstyryl)-α-pyrone (5) and α-amyrin. Their structures were established based on spectroscometric analysis. The styrylpyrone derivatives showed a significant antimicrobial activity.

Keywords: Senecio mannii; Asteraceae; styrylpyrones; antimicrobial activity; antifungal activity.

Resumen

Estudios fitoquímicos del extracto metanólico de las partes aéreas secas y pulverizadas de *Senecio manii* dieron lugar al aislamiento y determinación de dos nuevos compuestos, 4-metoxi-6-(11-O-ß-D-glucopiranosilestiril)-α-pirona (1) y 4-metoxi-6-(11-O-α-L-ramnopiranosilestiril)-α-pirona (2) asi como cuatro compuestos conocidos: 4-metoxi-6-(11-hidroxiestiril)-α-pirona (3), 4-metoxi-6-(12-hidroxiestiril)-α-pirona (4), 4-metoxi-6-(12-O-ß-D-glucopiranosilestiril)-α-pirona (5) y α-amyrina. Sus estructuras fueron establecidas en base a análisis espectroscópicos. Las estirilpironas demostraron estar dotadas de una significativa actividad antimicrobiana.

Palabras clave: Senecio mannii; Asteraceae; estirilpironas; actividad antimicrobiana; actividad antifungica

INTRODUCTION

Senecio mannii (Asteraceae) is a perennial medicinal herb native to African mount. It can be found in the South West, West and Nord West provinces of Cameroon where it is used locally to treat microbial and fungal diseases (Dalziel, 1937; Hutchinson and Dalziel, 1958; Bouquet and Debray, 1974). Previous pharmacological studies presented antimicrobial and antifungal activity of the total extract of *S. inaequidens* DC, *S. vulgaris* and *S. boissieri* (Steenkamp *et al.*, 2001; Louzzo *et al.*, 2004). Several species of *Senecio* permit to isolate a large variety of secondary metabolites include flavonoids (Ragaa and Nabiel., 1981), chalcones (D'Agostino *et al.*, 1991), acetophenones (Urones *et al.*, 1987), xanthones (Catalano *et al.*, 1996), triterpenoids (Rücker *et al.*, 1999). Recently we reported the isolation of cacalolide and shikimic acid from this genus (Ndom *et al.*, 2006). As part of our continuing search of bioactive compounds from the plant genus *Senecio*, we have now investigated the constituents of *S. mannii*. We report here the isolation and structural elucidation of two new compounds as well as the antimicrobial activity of compounds **3 - 4** evaluation.

MATERIAL AND METHODS

Plant material

Air-dried and ground aerial plants of *S. mannii* Hook. were collected in November 2006 at Limbe

locality to mount Cameroon (2200 m), South - West of Cameroon. The sample was identified by Mr Ndive Elias from Botanical gardens, Limbe, Cameroon, where a voucher specimen (IC12394) is deposited.

Extraction and Isolation

The air-dried and ground aerial plants of *S. mannii* (1.5 kg) were immersed in MeOH at room temperature during 72 hours. After removing the solvents by evaporation under reduced pressure, the obtained crude extract (76.7 g) was chromatographed over silica gel 60 (230-400 mesh ASTM, Merck), using *n*-hexane (Hex) and ethylacetate (EtOAc) in increasing polarity order. A total of 75 subfractions (ca. 200 mL each) were collected and combined in ten fractions (A-J) based on TLC analysis. Fraction D (7.8 g) is the combined subfractions 30-46 eluted with mixture Hex-EtOAc (8.5:1.5). Fraction F (5.2 g) was set up of subfractions 62-77 eluted with mixture Hex-EtOAc (4:1). Fraction G (4.0 g) was set up of subfractions 78-82 eluted with mixture Hex-EtOAc (7.5:2.5). Main fraction D was chromatographed over silica gel 60C (20-40 μm) column with mixture hex-EtOAc gradient. 25 fractions (*ca*. 100 mL each) were collected and combined based on TLC. Fractions 5-24 were further chromatographed over preparative TLC (Silica gel 60C, 20-40 μm) using mixture Hex-EtOAc gradient (8.5:1.5) to give 4-methoxy-6-(11-hydroxystyryl)-α-pyrone (**3)** (15.6 mg) and 4-methoxy-6-(12-hydroxystyryl)-α-pyrone (**4)** (31.2 mg). Fraction F was chromatographed over silica gel 60C (20-40 μm) column with mixture Hex-EtOAc gradient. 28 fractions of (*ca*. 100 mL each) were collected and combined based on TLC. Fractions 1-12 and 16-26 were successively further chromatographed over silica gel 60C (20-40 μm) with mixture Hex-EtOAc (4:1) to give α-amyrin (40 mg) and 4-methoxy-6-(12-O-ß-D-glucopyranosylstyryl)-α-pyrone **(5)** (20.3 mg).

Main fraction G was chromatographed over silica gel 60C (20-40 μm) column with a gradient. 45 fractions of (*ca*. 100 mL each) were collected and combined based on TLC. Fractions 7-29 were chromatographed using preparative TLC on silica gel 60C (20-40 μm) with a mixture Hex-EtOAc as eluent to give 4-methoxy-6-(11-O-ß-D-glucopyranosylstyryl) -α-pyrone **(1)** (26.8 mg) and 4-methoxy-6-(11-O-α-L-rhamnopyranosylstyryl)-α-pyrone **(2)** (29.4 mg).

Identification of Compounds

Melting points were determined on a Buchi apparatus and were uncorrected. UV spectra were obtained on a Shimadzu- 265 Spectrophotometer and recorded in methanol. IR spectra were recorded on a Perkin-Elmer 727B spectrometer in KBr discs. The HR-ESI-TOF-MS were obtained in the positive ion mode on pulsar mass spectrometer. ^{1}H and ^{13}C-NMR spectra were obtained with a Brucker model equipped with a 5 mm ^{1}H and ^{13}C (ATP) probe operating at 300 and 75 MHz, respectively, with TMS as internal standard. Homonuclear ^{1}H connectivities were determined by using the COSY and NOESY (mixing time 500 ms experiments) experiments. One-bond ^{1}H-^{13}C connectivities were determined with HMQC (Heteronuclear Multiple Quantum Connectivity by 2D-multiple) gradient pulse factor selection. Two - and three-bond ^{1}H-^{13}C connectivities were determined by HMBC (Heteronuclear Multiple Bond Connectivity by 2D-multiple Quantum) experiment. Chemical shifts were reported in δ (ppm) and coupling constants (J) were measured in Hz. Precoated aluminium sheets silica gel 60 F_{254} TLC (Thin Layer Chromatographic) plates was used to check the purity of compounds and preparative chromatography. Spots were visualised by UV lamp (254 nm and 365 nm) or by 50% H_2SO_4 reagent. All reagents used were of analytical grades.

4-methoxy-6-(11-O-ß-D-glucopyranosylstyryl)-α-pyrone (1)

White crystals; (CH_2Cl_2); mp 262-264° C; UV λ^{MeOH}_{max} (logε) nm: 224 (4.03), 264 (3.85), 309 (3.33), 328 (3.28), 360 (3.23); IR (KBr) υ_{max} 3450, 1750, 1631, 1517 cm^{-1}; ^{1}H NMR and ^{13}C NMR see table 1; HR-ESI-TOF-MS $[M+H]^+$ m/z. 407.1263 (calc. for $C_{20}H_{22}O_9$ 407.1264); EIMS *m/z* (rel. int.): 406 (100) $[M]^+$.

Acid hydrolysis of 4-methoxy-6-(11-O-ß-D-glucopyranosylstyryl)-α-pyrone:

The sample (25 mg) was dissolved in 7% H_2SO_4 and refluxed on a water bath for 4 hours. The reaction mixture was diluted with 20 mL of H_20 and extracted with CH_2Cl_2. Evaporation of solvent followed by purification of the residue by prep. TLC over silica gel with toluene-Me_2CO (10:3) as eluente gave a white compound identified as 4-methoxy-6-(11-hydroxystyryl)-α-pyrone by comparison with its physical and spectral data (Keith *et al.*, 1967).

Identification of the sugar moiety: the aqueous phase after extraction with CH_2Cl_2 was neutralised with 1M NaOH and evaporated in vacuum. H_2O was added to the residue and the mixture was again evaporated in vacuum to remove all the impurities. The residue obtained was compared to standard sugars by TLC using n-BuOH-toluene-pyridine-H_2O (5:1:3:3)

(BTPW).The sugar was detected with aniline hydrogen phalate, and shown to consist to D-glucose. For GLC analysis, the residue was dissolved in TRISIL (0.05 ml: N-(trimethylsilyl)-imidazole in pyridine), left at room temp. for 15 min, and analysed by GLC on a SHIMADZU GC-GA gas chromatograph, glass column 2.6 mm x 2mn packed with 1.5 % SE-30 on chromosorb W, detector FID injection temp. 150° carrier gas N_2 (40 mL min^{-1}). The GLC peaks of the silylated derivative of the residue and glucose had the same retention time (R_t 4.9 min).

4-methoxy-6-(11-O-α-L-rhamnopyranosylstyryl)-α-pyrone (2)

White crystals, (CH_2Cl_2); mp 252-254°C; UV λ^{MeOH}_{max} (logε) nm 228 (4.01), 247 (3.81), 252 (2.64), 266 (3.87), 300 (3.36), 307 (3.50), 328 (3.30), 360 (3.25), IR (KBr) υ_{max} 3450, 1750, 1631,1517 cm^{-1}; ^1H NMR and ^{13}C-NMR see table 3; HR-ESI-TOF-MS $[M+H]^+$ at m/z 390.1314 (calc.$C_{20}H_{22}O_8$ 390.1315). EIMS m/z (rel. int.): 390 (100) $[M]^+$.

Identification of the sugar moiety: the sample (18.7 mg) was hydrolysed as described above and the aglycone was identified as 4-methoxy-6-(11-hydroxystyryl)-α-pyrone by comparison with its physical and spectral data (Keith *et al.*, 1967). The sugar was identified as L-rhamnose by comparison of its trimethylsilylated derivative to that of standard sugars using GLC.

Antibacterial activity

An aliquot of the crude extract of *S. mannii* was serially diluted to stand a range of 1.0 - 0.01 mg/mL in 2% acetone final concentrations. Compounds **3** and **4** were diluted to final concentrations of 100, 10.0, 5.0 and 0.5 µg/mL in 2% acetone. The plant extract and isolated pure compounds (sterilised by filtering through a 0.22 µm filter) were added to 5 mL of sterilised nutrient agar in Petri dishes and swirled carefully before solidifying. The organisms were streaked in radial patterns on the agar plates (Mitsher *et al.*, 1976). Plates were hatched at 37°C in the dark and examined after 24h and 48h. Complete inhibition of growth of bioactive compounds was required to be considered active.

The controls consisted of Petri dishes containing only nutrient agar and others containing nutrient agar in 2% acetone. Each treatment was analysed in triplicate. The extract and purified active principles from *S. mannii* were tested against five randomly - selected bacteria by agar dilution method (Turnbull and Kramer., 1991). A gram-positive bacteria tested showed significant activity in Compounds **3** and **4** (Table 1). But, the minimum inhibitory concentration (MIC) was very significant with compound **3** than **4**. Compounds **3** and **4** were not active on gram-negative bacteria except for *Pseudomonas aeruginosa* which was significantly inhibited at an MIC of 0.1µg/mL. These results are agreeing with previously reported of similar antimicrobial activity to *Senecio* genus (Louzzo *et al.*, 2004).

Antifungal activity.

The plant extract as well as compounds **3** and **4** were subjected to the same treatment as noted above except that instead of streaking bacteria into the agar, cultured fungal inocula discs were carefully deposited at the centre of each Petri dish. Plates were incubated at 25 °C in the dark and examined after 24 and 48 hours. Complete inhibition of growth was similarly required for compounds to be declared active. Controls were also prepared containing only nutrient agar on nutrient agar in 2% acetone. The growth of three fungal species *Fusarium solani*, *Aspergillus flavus* and *Candida glutamate* were significantly inhibited at a low MIC's by compound **4** than compound **3**.

In this study, we observed that compounds **3** and **4** have the ability to inhibit the growth of all fungal species tested. These results confirm the use of these compounds as broad spectrum antimicrobial agent. This probably explains the use of extracts from this plant by traditional healers against a certain number of infections because antibacterial activity seems to be related to the presence of phenolic compounds.

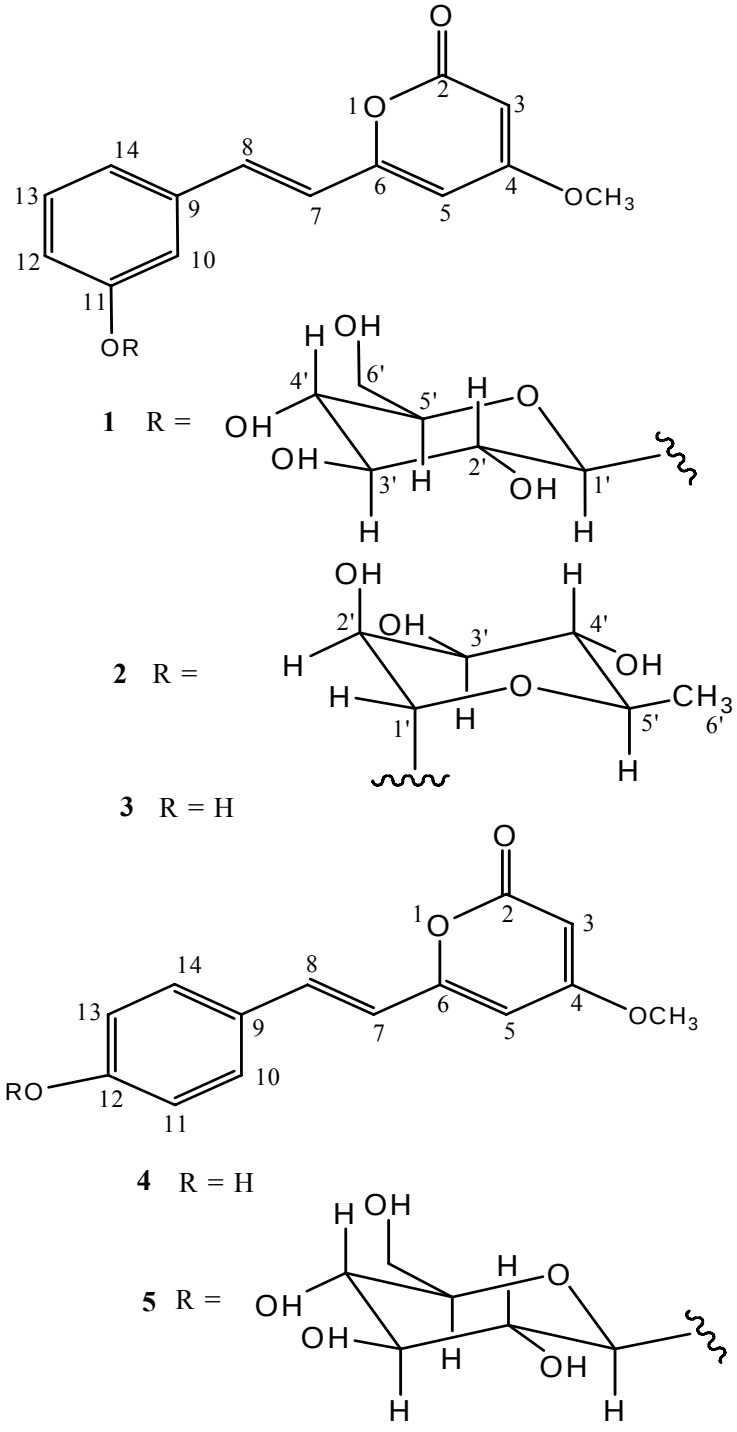
RESULTS AND DISCUSSION

The perennial plant of *S. manii* was extracted with methanol. The extract was subjected to silica gel column chromatography, eluting with increased parts of ethylacetate in hexane to give six compounds numbered **1-6**.

Compound **3-6** were identical with 4-methoxy-6-(11-hydroxystyryl)-α-pyrone (Keith *et al.*, 1967), 4-methoxy-6-(12-hydroxystyryl)-α-pyrone (Dharmaratne *et al.*, 2002; Whitton, *et al.*, 2003), α-amyrin (Loucam *et al.*, 1973) and 4-methoxy-6-(12-O-ß-D-glucopyranosylstyryl)-α-pyrone (Wang *et al.*, 2004).

Compound **1** obtained as white crystals from a solution in CH_2Cl_2, was found to be a glucoside by the Molish test. The positive high resolution electrospray-TOF mass spectrum running on an API QSTAR pulsar mass spectrometer (HR-ESI-MS) showed a

Figure 1. Styrylpirones isolated from *S. manii*



1 R =

2 R =

5 R =

Table 1. Antibacterial activity of the crude acetone extract from the ground aerial plants of S. mannii and isolated compounds 3 and 4.

Bacteria species	**Gram (+/-)**	**Minimum inhibitory concentration**		
		Crude extract (mg/ml)	**3 (µg/mL)**	**4 (µg/mL)**
Straphylococcus aureus ATCC13709	+	1.0	0.1	0.5
Escherichia coli. ATCC 25922	-	-	-	-
Escherichia coli. ATCC 35218	-	1.0	-	-
Klebsiella pneumonia. ATCC 10031	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	-	1.0	0.1	0.1

(-) Not active

Table 2. Antifungal activity of the crude acetone extract from the ground aerial plants of *S. mannii* and isolated compounds **3** and **4.**

Fungal species	**Minimum inhibitory concentration**		
	Crude extract (mg/ml)	**3** (µg /mL)	**4** (µg /mL)
Fusarium solani	1.0	0.1	0.5
Aspergillus flavus	0.01	1.0	0.5
Candida glutamate	0.05	1.0	1.0

Table 3. ^{1}H (500 MHz) and ^{13}C (125 MHz) assignments for 4-methoxy-6-(11-O-α-L-rhamnopyranosylstyryl) -α-pyrone **(1)** and 4-methoxy-6-(11-O-α-L-rhamnopyranosylstyryl)-α-pyrone **(2)** in DMSO-d_6. Assignments were based on HMQC, HMBC and NOESY experiments.

Attribution	**(1)**			**(2)**		
	^{1}H	**^{13}C**	**HMBC**	**^{1}H**	**^{13}C**	**HMBC**
2		162.6	H-3		162.7	H-3
3	5.60 (d, J = 2.2)	88.6	H-5	5.61(d, J = 2.2)	88.4	H-5
4		170.6	OCH_3-15		170.8	OCH_3-15
5	6.15 (d, J = 2.2)	101.8	H-3, H-7	6.25(d, J = 2.2)	100.7	H-3, H-7
6		157.9	H-8		158.6	H-8
7	6.75 (d, J = 13.0)	117.4	H-5	6.83 (d, J = 16.0)	117.7	H-5
8	6.50 (d, 13.0)	131.1	H-10, H-14	6.76 (d, J = 16.0)	133.8	H-10, H-14
9		134.3	H-13		127.7	H-13
10	6.85 (dd, 2.0, 1.9)	114.9	H-12, H-14	6.83 (dd, 8.7, 1.8)	128.7	H-12, H-14
11		158.8	H-13		116.5	H-13
12	6.66 (ddd, 8.7, 1.9, 1.8)	115.6	H-10, H-14	6.70(ddd, 8.5, 1.9, 1.7)	158.3	H-10, H-14
13	7.30 (dd, 8.7, 8.6))	131.1		7.25 (dd, J = 8.4, 8.5)	117.7	
14	6.80 (ddd, J = 8.6, 2.0, 1.9)	116.7	H-10, H-12	7.20 (ddd, J = 8.4, 1.9, 1.7)	128.9	H-10, H-12
15-OCH_3	3.5 (s)	56.3		3.5 (s)	56.1	
1’	4.9 (d, 7.4)	100.2		4.25 (d, 1.4)	102.1	
2’	3.27(m)	73.1		3.85 (t, J = 3.7)	71.9	
3’	3.30 (m)	76.5		3.65 (t, J = 10)	69.8	
4’	3.10 (m)	69.6		3.40 (t, 9.7)	66.6	
5’	3.37 (m)	77.0		3.3 (t, 9.7)	75.1	
6’	3.45 (dd, J = 11.7. 1.8)	60.6		3.5 (m)	66.6	
	3.60 (dd, J = 11.7, 1.8)			0.95 (d, J = 6)	16.1	

pseudomolecular ion peak $(M+H)^+$ at *m/z.* 407.1263 (calc.$C_{20}H_{22}O_9$ 407.1264). In the EIMS, the mass fragment arise at *m/z.* 244 (M^+- glucosyl) indicated the presence of glucosyl moiety.

The UV spectrum exhibited absorption maxima of a typical styrylpyrone at λ_{max} (MeOH) 224, 264, 309, 328, 360 nm (Dharmaratne *et al.*, 2002) suggesting the presence of a conjugated chromophore.

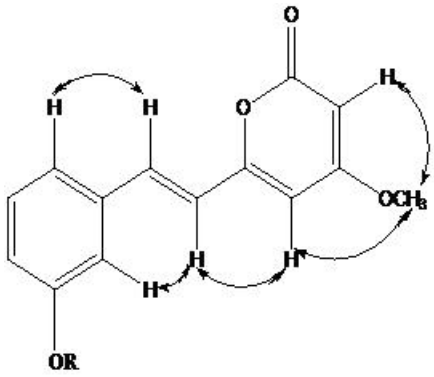
The IR spectrum of **1** showed characteristic bands for olefin groups (1631, 1517, 820 cm^{-1}) and carbonyl functionalities (1709, 1630 cm^{-1}).

The ^1H NMR spectra revealed characteristic signals at δ5.60 (d, J = 2.2 Hz, H-3) and at δ6.15 (d, J = 2.2 Hz, H-5) fixed in meta position and one methoxy group at δ3.5 (s) (Table 3). The ^{13}C NMR spectrum (Table 3) presented signals with a carbonyl group at δ162.6 (C-2), a quaternary oxygenated aromatic carbon at δ157.9 (C-6), a methoxy group at δ56.3 (C-15), a quaternary oxygenated aromatic carbon bearing an methoxy group at δ170.6 (C-4) and two aromatic methine at δ101.8 (C-5) and δ88.6 (C-3). All these data confirmed the presence of a α- pyrone ring.

On the other hand the ^1H NMR spectra of **1** revealed a typical AB system of two protons, one at δ6.75 (d, J= 13.0 Hz, H-7) and another at δ6.50 (d, J = 13.0 Hz, H-8). The value of the coupling constant indicated the trans configuration; four aromatic protons in which the ^1H NMR signals for H-10 and H-14 appeared close together (δ6.85 and 6.80 respectively) and a part of H-14 (ddd, J= 8.6, 2.0, 1.9 Hz) overlapped with the H-10 (dd, J = 2.0, 1.9 Hz) signal to give a broad band and two others at δ6.66 (ddd, J = 8.7, 1.8, 1.9 Hz, H-12) and δ7.30 (dd, J = 8.7, 8.6 Hz, H-13). This hypothesis was confirmed by the ^{13}C NMR Jmod spectrum of **1** which showed two olefin carbons at δ117.4 (C-7) and δ131.1 (C-8) four aromatic methyne carbons at δ114.9 (C-10), δ115.6 (C-12), δ131.1 (C-13), δ116.7 (C-14) and two quaternary aromatic carbons one bearing an glucosyl group at δ158.8 (C-11) and another at δ134.3 (C-9) (Table 3). All these data showed that compound **1** possess a styryl moiety. From this evidence, the structure of **1** resembled that of styrylpyrone derivatives (Rezende *et al.*, 1971; Dutta *et al.*, 1972; Veit *et al.*, 1993, 1995; Dharmaratne *et al.*, 2002).

HMBC correlations between C-2 (δ162.6) / H-3, C-4 (δ170.6) / OCH_3, C-3 (δ88.6) / H-5, C-5 (δ 101.8) / H-3, C-5 (δ101.8) / H-7, C-6 (δ157.9) / H-8, C-7 (δ117.4) / H-5 allow us to fixe the methoxy group at position 4. On the other hand, HMBC correlations between, C-8 (δ131.1) / H-14, C-8 (δ131.1) / H-10, C-9 (δ134.3) / H-13, C-9 (δ134.3) / H-7, C-10 (δ114.9) / H-14, C-11 (δ158.8) / H-13 aid to locate the glucosyl group in position 11.The coupling (COSY) relationship established completely different patterns in compound **1.** NOESY correlations between H-3 (δ 5.60) / OCH_3-4), H-5 (δ6.15) / OCH_3-4, H-5 (δ6.15) / H-7(δ6.75), H-8 (δ6.50) / H-14 (δ6.80), H-7(δ6.75) / H-10 (δ6.85) (Fig 1) and the various observed coupling constants (Table 3) indicated the close spatial proximity of particular protons and the site of O-methylation in position 4. The presence of a *E*-styrylpyrone moiety was evident by the characteristic shifts and couplings in these spectra (Table 3) and by comparison with data on compound **3** (Franca *et al.*, 1973) and styrylpyrones published earlier (Benerji *et al.*, 1980; Ganzeran and Khan., 1999).

Fig.2: Selected NOESY Correlations



The compound was subjected to acid hydrolysis with 7% H_2SO_4 to yield aglycone that was identified as 4-methoxy-6-(11-hydroxystyryl)-α-pyrone **3** from its physical and spectral data ^1H, ^{13}C NMR (Keith *et al.*, 1967)

The sugar moiety was identified by TLC and GLC of its TMSi derivative as glucose. This was confirmed ^{13}C NMR signals at δ100.2, 77.0, 76.5, 73.1, 69.6, and 60.6 agreeing with published data for D-glucose (Veit *et al.*, 1995). A β-D-glucopyranosyl configuration was deduced from the coupling constant (J = 7.9 Hz) of the anomeric proton signal at δ4.9 (H-1') in the ^1H NMR (Veit *et al.*, 1995).

The site of attachment of the glucose moiety was confirmed by correlation between H-1' and C-11, the absence of NOESY correlations between pyrone protons and glucosyl protons in the NOESY spectra and 2D HMBC, ^1H-^1H COSY, ^{13}C-^1H, optimized for long range couplings. Thus, the structure of **1** was clearly defined as 4-methoxy-6-(11-O-ß-D-glucopyranosylstyryl)-α-pyrone.

Compound **2** obtained as white crystals from a solution in CH_2Cl_2 was found to be a glucoside similar to compound **1** from its positive response to the Molish test and its ^1H NMR and ^{13}C NMR data. The positive high resolution electrospray-TOF mass spectrum running on an API QSTAR pulsar mass spectrometer (HR-ESI-MS) showed a pseudomolecular ion peak $(M+H)^+$ at (*m/z*. 390.1314 calc.$C_{20}H_{22}O_8$ 390.1315).

Acid hydrolysis under the same conditions as mentioned above for compound **1** gave for compound **2** a glycone identified as L-rhamnose by TLC and GLC of its TMSi derivative, confirmed by ^{13}C NMR signals at δ102.1, 75.1, 71.9, 69.8, 66.6 and 16.7 matching with those published for L-rhamnopyranose (Muzitano *et al.* 2006)

Mass fragment peak at (*m/z.* 244, M^+- rhamnosyl) indicated the presence of rhamnose moiety. In the ^1H NMR spectrum, a doublet at δ4.25 (J = 1.4 Hz) was assigned to the anomeric proton and confirmed the α-rhamnose configuration (Mamdouh *et al.*, 1999). An additional secondary methyl group at δ16.1 replaced the signal for the carbon of the hydroxymethylene group of the glucose in compound **1** (Table 3) further suggesting that the glycone in compound **2** is α-L-rhamnose. The rhamnose moiety was placed at C-11 from close similarity observed between the carbon signals in compounds **1** and **2** and the 2D COSY experiment. The structure of compound **2** was therefore elucidated as 4-methoxy-6-(11-O-α-L-rhamnopyranosylstyryl)-α-pyrone.

Acknowledgments.

This investigation was supported by J.C.Ndom research grants (RGA) N°.05-305 RG/CHE/AF/AC from the Third World Academy of Science (TWAS). We are also grateful to the Museum National d'Histoire Naturelle de Paris for recording the NMR and Mass spectra of the isolated compounds and Dr Tchoumbougnang Francois in the laboratory of biochemistry at the University of Douala for evaluating the antimicrobial activity.

REFERENCES

Banerji A, Saddhanta AK, Acharyya AK, Dutta CP. 1980. Carbon-13 NMR spectroscopy of kavapyrones. *Org. Magn. Res.* 13:345-348.

Bouquet A, Debray L. 1974. Plantes Médicinales de la Côte-d'Ivoire. *Travaux et documents de l'ORSTOM* 32:83-87.

Catalano S, Luschi S, Flamini GP, Cioni PL, Nieri M, Morelli I. 1996. Xanthone from *Senecio mikanioides* leaves. *Phytochemistry* 42:1605-1607.

D'Agostino M, De Feo V, De Simone F, Pizza C. 1991. Three chalcones from *Senecio pseudotites. Phytochemistry* 30: 2440-2441.

Dharmaratne HR, Nanayakkara NP, Khan IA. 2002. Kavalactones from *Piper methysticum* and their ^{13}C NMR spectroscopic analyses. *Phytochemistry* 59:429-433.

Dalziel JM. 1937. The useful Plants of West Tropical Africa. The crown agents for the colonies, London, p. 140-141.

Dutta CP, Roy LPK, Chatterjee A. 1972. Constituents of *Piper methysticum. Phytochemistry* 11: 2891-2892.

Franca NC, Gottlieb OR, Suarez AM. 1973. 6-Phenylethyl-5,6-dihydro-2-pyrones from *Aniba gigantifolia. Phytochemistry* 12:1182-1184.

Ganzeran M, Khan IA. 1999. Analytic technique for the determination of lactones in p*iper methysticum* Forst. *Chromatographia* 50: 649-653.

Hutchinson J, Dalziel JM. 1958. Flora of West Tropical Africa. The white flies Press, Ltd, London and Tom Bridge, UK, pp. 377-383.

Keith BD, Leslie ER, Wynn JD, Mir I. 1967. 4-methoxy-pyran-2-one *J. Chem. Soc.* VOL:413-419.

Lou-cam CA. 1973. Triterpenoids of *Isertia hypoleuca* leaves. *Phytochemistry* 12: 475-476.

Louzzo MR, Statti GA, Tundis R, Conforti F, Bonesi M, Autelitano G, Houghton PJ, Miljkovic BA, Menichini F. 2004. Antibacterial and antifungal activity of *Senecio inaequidens* DC and *Senecio vulgaris* L. *Phytother. Res.* 18:777-779.

Mitsher LA, Leu R, Bathala MS, Wu W, Beal JL. 1976. Antimicrobial agents from higher plants. I. Introduction, rational and methodology. *Lloydia* 35: 157-166.

Mamdouh M, Abou-Zaid, Constance N. 1999. 1-O-galloyl-α-rhamnose from *Acer rubrum Phytochemistry* 52:1629-1631.

Muzitano MF, Tinoco LW, Guette C, Kaiser CR, Rossi-Bergmann B, Costa SS. 2006. The antileishmanial activity assessment of unusual flavonoids from *Kalanchoe pinnata. Phytochemistry* 18:2071-2077.

Ndom JC, Mbafor JT, Azebaze AG, Vardamides JC, Kakam Z, Kamdem AF, Deville A, Ngando TM, Fomum ZT. 2006. Secondary Metabolites from *Senecio burtonii*. *Phytochemistry* 67: 838-842.

Ragaa MA, Nabiel AM. 1981. Flavonoids of three local *Senecio species. Phytochemistry* 20: 1180-1181.

Rezende CM, Bülow MV, Gottlieb OR, Pincho SL, Da Rocha AI 1971 .The 2-pyrones of *Aniba* species. *Phytochemistry* 10:3167-3172.

Rücker G, Manns D, Schenkel EP, Hartmann R, Heinzmann BM. 1999. Triterpenes with a new 9-epi-cucurbitan skeleton from *Senecio selloi. Phytochemistry* 52:1587-1591.

Steenkamp V, Stewart JM, Van der Merwe S, Zukerman M, Crowter NJ. 2001 .The effect of *Senecio boissieri* a new jacaranone derivative. *Fitoterapia* 71:91-93.

Turnbull PC, Kramer JM. 1991. Bacillus. In Burlows A, Hausler Jr WJ, Hermann KI, Isenberg HD, Shadomy HJ. *Manuals of Clinical Microbiology* 5th Ed . American Society for Microbiology, Washington, DC, USA, pp. 296-303.

Urones JG, De Pascual Teresa J, Marcos IS, Fernandez Moro R, Basabe Barcala P, Sexmero Cuadrado MJ. 1987. Acetophenones and terpenoids from *Senecio gallicus. Phytochemistry* 26:1113-1111.

Veit M, Geiger H, Kast B, Beckert C, Horn C, Markham KR, Wong H, Czygan FC 1995. Styrylpyrone glucosides from *Equisetum. Phytochemistry* 39:915-917.

Veit M, Geiger H, Wray V, Abou-Mandour AA, Rodzinsky W, Witte L, Strack D, Czygan FC. 1993. Equisetumpyrone, a styrylpyrone glucoside in gametophytes from *Equisetum arvense. Phytochemistry* 32:1029-1032.

Wang AX, Qi Z, Zhong-Jian J. 2004. A new furobenzopyranone and other constituents from *Anaphalis lactea. Pharmazie* 59:807-811.

Whitton PA, Lau A, Salisbury A, Whitehouse J, Evans CS. 2003. Kava lactones and the kava-kava controversy. *Phytochemistry* 64:673-679.

BLACPMA ISSN 0717 7917

Artículo original/Original paper

In vitro and *in vivo* activity of berberine on the blood trypomastigote from *Trypanosoma cruzi*

[Actividad de la berberina en tripomastigotes sanguíneos de *Trypanosoma cruzi*]

Guillermo SCHINELLA [1,2,*], Horacio TOURNIER [1,2], Anibal ZAIDENBERG [3]

[1]*Cátedra de Farmacología Básica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calles 60 y 120, 1900 - La Plata, Argentina.*
[2] *Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina.*
[3] *IDIP – Instituto de Desarrollo e Investigaciones Pediátricas (Hospital de Niños 'Sor María Ludovica'), La Plata, Argentina.*

*Contact: schinell@uv.es

Received 20 May 2007. Accepted 30 July 2007

Abstract

Blood transfusion has been found to be an important mechanism in the transmission of the Chagas disease and there is a need for new agents able to inhibit the infectiveness of *Trypanosoma cruzi* in stored blood. The methanolic extract of the root of *Coptis chinensis* and the alkaloid berberine has been previously identified as effective trypanicidal agents both *in vitro* and *in vivo* and could be promising prophylactic drugs to address this issue. The extract of *C. chinensis* and its main bioactive, berberine, resulted more active against Bra C15C2 clone epimastigotes in axenic cultures than allopurinol, the antitrypanosomal drug of reference, with IC50 values of 1.7 µ/ml and 0.3 µg/ml (0.81 µM), respectively. The *in vitro* anti-trypanosomal activities of the methanolic extract of roots of *C. chinensis* and its main bioactive, berberine, were tested using epimastigotes of the clon Bra C15C2. Berberine (250 µg/ml) and crystal violet were equally effective in preventing transfusion-mediated infection of CF1 mice with the clon H510C8C3. However berberine (30 mg/kg/day, p.o.) for 30 days was unable to enhance the survival of already infected animals. The mechanism of protection may encompass both diminished parasitaemia in stored blood as well as inactivation of the infectiveness of the parasites, opening a new perspective in the fight against Trypanosomiasis.

Keywords: Trypanosoma cruzi; Coptis chinensis, berberine, blood transfusion, prophylaxis

Resumen

Las transfusiones sanguíneas son un importante factor de transmisión de la enfermedad de Chagas y se necesitan agentes profilácticos para inhibir la infección por *Tripanosoma cruzi* presente en los bancos de sangre procedentes de donantes infectados. *Coptis chinensis* y el alcaloide berberina han sido previamente identificados como efectivos tripanocidas tanto *in vitro* como *in vivo* y por tanto son potencialmente interesantes como agentes profilácticos. El extracto metanólico de raíces de *C. chinensis* y su principal compuesto activo, berberine, resultaron activos frente a epimastigotes del clon Bra C15C2 en cultivos axénicos, con IC_{50s} de 1.7 µ/ml y 0.3 µg/ml (0.81 µM), respectivamente. Berberina (250 µg/ml) y violeta de genciana fueron igualmente efectivos en la prevención de la infección por trypomastigotes del clon H510C8C3 mediada por transfusión sanguínea en un modelo con ratones CF1. Sin embargo, berberina (30 mg/kg/día, p.o.) durante 30 días no pudo mejorar la supervivencia de ratones CF1 previamente infectados por transfusión. El mecanismo de protección podría implicar tanto una cierta citotoxicidad contra el parásito sin llegar a eliminarlo completamente en sangre almacenada; así como una inactivación del potencial infectivo del parasito, lo cual abre una nueva perspectiva en la lucha contra la Tripanosomiasis

Palabras clave: Trypanosoma cruzi; Coptis chinensis, berberina, transfusión sanguínea, profilaxis

INTRODUCTION

American trypanosomiasis or Chagas'disease is after malaria, the most prevalent vector-borne illness in Latin America and is caused by a protozoan parasite, *Trypanosoma cruzi*, which is transmitted to humans by triatomine bugs. The disease is associated with poverty in rural areas in Central and South America. In 1996 it was estimated that between 16 and 18 million people were infected, of whom over 6 million would develop clinically overt disease and 45000 would die per year. (WHO, 2007). This microorganism has a complex life cycle with different developmental stages in different hosts. It multiplies inside mammalians cells as amastigotes and after that are released into the bloodstream as trypomastigotes; which can infect other cells or be ingested by the insect vector. In the gut of Triatomine, trypomastigotes differentiate to the other reproductive forms, epimastigotes; which at the rectal ampoule transform into the infective metacyclic trypomastigotes; which are unloaded with the bug's excreta and to reach the bloodstream of the vertebrate hosts (Tyler and Engman, 2001). Blood transfusion is the second most important mechanism of transmission of Chagas' disease (Docampo *et al.*, 1988). This fact is of epidemiological importance and it has become a major health problem in South and Central America because of the migration of infected individuals into and out of the Americas (Schmunis *et al.*, 2001).

Current chemotherapy recommended for the treatment and the prevention of Chagas disease has serious limitations because of their limited effectiveness and important drug-related side effects (Stoppani, 1999; Paulino *et al.*, 2005). The exposed reasons make the search of new chemopreventive or chemotherapeutic agents an urgent priority.

One of the current approaches in the quest for new trypanocidal drugs relies on screening the biological activity of natural products. Different products with a very broad range of structural types have been assessed against *T. cruzi* in cultures or in infected animals (Sepúlveda-Boza and Cassels, 1996) and, among them, plant-derived products containing alkaloids have shown very promising anti-trypanosomal activity (Cavin *et al.*, 1987; Rojas de Arias *et al.*, 1994). We recently showed how the methanolic extract of roots of *Coptis chinensis* was able to inhibit the growth of epimastigotes of *T. cruzi* with a 25-fold higher potency than reference compounds normally used in these kind of screenings (Schinella *et al.*, 2002). Berberine is one of the major protoberberine alkaloids present in extracts of *C. chinensis* and it has a good demonstrated anti-protozoal activity in different models (Phillipson and Wright, 1991; Cavin *et al.*, 1987; Abe *et al.*, 2002, 2004).

This research deals with the evaluation of the trypanocidal properties of berberine against epimastigotes in axenic cultures and blood trypomastigotes of different clones of *T. cruzi* as well as its potential as prophylactic agent in transfusion mediated trypanosomiasis.

MATERIALS AND METHODS

Chemicals

Culture mediums were from Gibco BRL (Life Technologies, NY, USA). Berberine and other chemicals for the assays were of analytical grade (Sigma Co., St. Louis). The plant material was purchased from Asia Natural Products (Amposta, Spain) and certified by The School of Medicine of the University of Beijing as fitting the pharmaceutical standards for its use in Traditional Chinese Medicine (macroscopic characters, microscopic characters and berberine content of 5-7% according the Chinese pharmacopoeia). The methanolic extract of the root of *Coptis chinensis* was prepared as previously described (Schinella *et al.*, 2002).

Animals

Female CF1 mice (c.a. 30 g each) from the Biological Institute (La Plata, Argentina) were used. They were kept in standard environmental conditions and fed with rodent diet with tap water *ad libitum*.

Analytical high - performance liquid chromatography (HPLC) - diode array detector (DAD) analyses.

HPLC-DAD analysis was performed on a Merck-Hitachi system equipped with a Pump L-6200, L-7455 Diode Array Detector and Auto Sampler L-7200, injection valve (Reodyne), loop of 100 µl, precolumn Lichrospher© C18 (4 × 4 mm, 5 µm, Merck), and column Lichrospher© C18 (250 × 4 mm, 5 µm, Merck). The data were collected and processed with the software DAD-Manager (Merck-Hitachi).

The analysis of the extract was carried out with the following mobile phase: A (H2O + trifluoroacetic acid 0.01%) and B (methanol + trifluoroacetic acid 0.01%); elution profile: isocratic 40% B.

In vitro antiprotozoal assay

T. cruzi epimastigotes (clone Bra C15C2) were cultured in F29 media supplemented with 10% (v/v) heat-inactivated fetal calf serum at 27 °C, with an *inoculum* of 5×10^5 cells per ml. Compounds were added at different concentrations and all assays were carried out in triplicate. Final dimethyl sulphoxide (DMSO) concentration was always less than 0.5%. After 72 h of contact with the samples, parasites were stained with Wrigth-Giemsa and counted in a Neubauer chamber. The activity of the compounds was assessed by comparison with the negative control (DMSO) and allopurinol was used as positive control (Zaidenberg *et al.*, 1999).

Transfusion assays with pre- treatment of the infected blood

Blood from CF1 mice infected with the clon H510C8C3 of *T. cruzi* was collected on the fourteenth day, when parasitaemia peaks (Zaidenberg *et al.*, 1999). The final concentration of 1 x 10^7 trypomastigotes/ml of blood was obtained by diluting the samples with blood from healthy CF1 mice. The assays were performed in Eppendorf© tubes with 400 µl of blood in the presence of berberine (250 µg/ml), gentian violet (250 µg/ml) or vehicle (DMSO) (Mafezoli *et al.*, 2000). The microcentrifugue tubes were incubated 24 h at 4° C and the presence of parasites monitored by microscopy. The blood was then inoculated in healthy CF1 mice (n = 3 per group). Survival was monitored daily during a period of 60 days.

Transfusion assay with post-treatment of the recipients

Male mice CF-1 (20-25 g, three groups/10 animal each) were infected with $1x10^5$ trypomastigotes of *T. cruzi* (clone H510C8C3). After 24 h the groups were treated with berberine (30 mg/kg/day, p.o.), benznidazol (100 mg/kg/day), or vehicle (0.2 ml de sterile, bidistilled water/day) for 30 days. Parasitaemia and survival were monitored during 40 days after the infection (Zaidenberg e*t al.*, 1999).

Statistical analysis

Data were expressed as mean ± S.D. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. Differences were considered significant at $P\leq0.05$. The inhibitory concentration 50% (IC_{50}) was calculated from the concentration/effect regression line. In each case, an appropriate range of 3 – 4 concentrations was used. Kaplan Meir method was used for the analysis of the survival curve.

RESULTS AND DISCUSSION

The inhibitory activities of berberine and *C. chinensis* extract on the *in vitro* growth of the *T. cruzi* epimastigote form are shown in Figure 2. The concentration of the alkaloid producing half-maximal inhibition (IC_{50}) was 0.3 µg/ml (0.81 µM), one order of magnitude lower than that observed with the extract of *C. chinensis* (IC_{50} = 1.7 µg/ml).

Figure 1. Chemical structure of berberine.

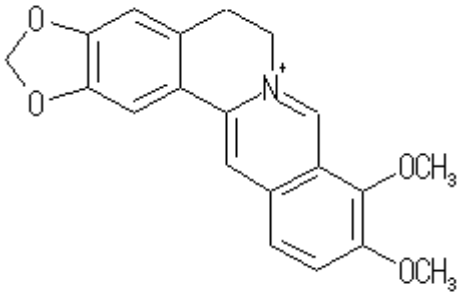


Figure 2. (A) HPLC Chromatogram of the methanolic extract of *C. chinensis*. (B) HPLC Chromatogram of berberina in the same elution conditions (C) Spectra of the pure berberine and of the main peak in the extract.

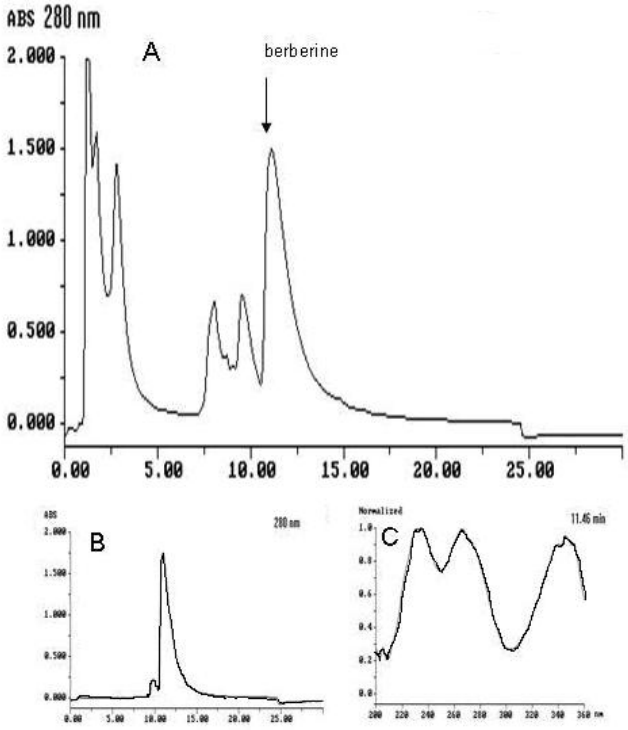


Table 1. Mortality of the mice inoculated with infected blood pre-treated with berberine or crystal violet during a period of 60 days.

Treatments	Mortality
Control (DMSO)	3/3
Berberine	0/3
Crystal violet	0/3

According the Chinese standards the content in berberine of *Rhizoma coptidis* should be close to 70 mg/g of drug (Ji *et al.*,, 1999) and correspondingly the WHO monographs also states that the content in berberine of this drug must be between 5-7% (WHO, 1999). The University of Beijing certified that our material was abiding these standards and we confirmed by HPLC analysis that berberine was present in the extract as one of the major peaks (see Figure 2).

It was previously reported that berberine is active against epimastigote of *T. cruzi* (Cavin *et al.*, 1987; Abe *et al.*, 2002). However, in the conditions of our assay, berberine shows higher potency than that observed by Cavin *et al*. (1987). Moreover it was reported that berberine is able to immobilize all the epimastigote forms of *T. cruzi* after 48 h incubation at 26 °C at a concentration 300 µg/ml (Abe *et al.*, 2002) whilst in our conditions we observe the complete absence of viable forms of the parasite in the culture medium at only 100 µg/ml. These discrepancies are probably attributable to the different sensitivities of each strain of the parasite and/or the influence of the different culture mediums used in the assays, highlighting how carefully the data reported in literature have to be compared.

Although axenic cultures of *T. cruzi* epimastigotes are a useful model to identify active compounds against the parasite it does not take into account the different sensitivity of the other stages of *T. cruzi* present in the vertebrate hosts. Blood transfusion has been found to be an important mechanism in the transmission of the parasite. Thus, compounds able to prevent transfusion mediated infections could constitute an invaluable clinical tool in the fight against trypanosomiasis.

Crystal violet –also called gentian violet- is a recognised trypanocidal compound currently used in clinics to prevent blood borne infections during transfusion. No acute toxic side effects are reported even after administration of large amounts of gentian violet-treated blood (Docampo and Moreno, 1990). However, the long-term toxicity of this agent for blood recipients is still an open issue (Moraes-Souza and Bordin, 1996) as well as having the unpleasant effect of colouring the patient's skin. In our experimental conditions crystal violet produced the total lysis of the parasites after 24 hours of incubation but some parasites were still observed in blood treated with the alkaloid. Nevertheless, no parasitaemia was observed in the animals inoculated with the treated blood samples and there was no difference in the survival between both groups of animals. Control animals showed a high level parasitaemia and started to die at the 29th day (see Table 1). This suggests that the parasites observed in the blood treated with the alkaloid were rendered unable to infect the transfusion recipients.

Figure 3. Effects on the growth of *T. cruzi* epimastigotes in axenic cultures incubated in the presence of *C. chinensis* extract and berberine.

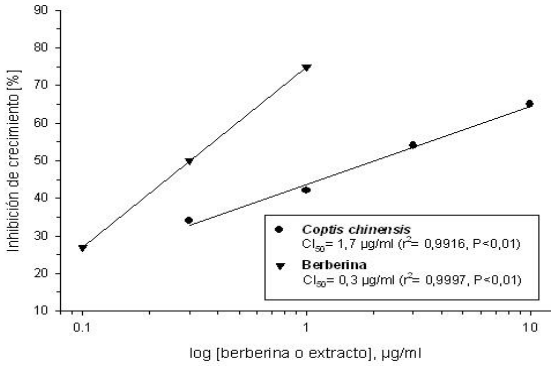
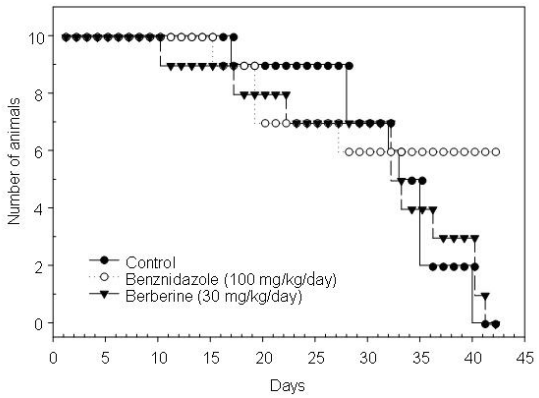


Figure 4. Survival of CF1 mice transfused with infected blood. Mice were treated daily with benznidazole and berberine.



After establishing that berberine had antiparasitic activity against epimastigotes and trypomastigotes *in vitro*, we investigated the effects of the alkaloid on already infected mice. In our model, berberine was unable to delay the death of previously infected animals (see Figure 3). The lack of efficacy in the treatment could be attributable to different factors

including an insufficient dose of the alkaloid, but benznidazol, at higher doses, did not protect the mice either. It has been reported that the bioavailability of berberine is quite poor in experimental models in dogs and rats and P-glycoprotein has been implicated (Pan *et al.*, 2002) This alkalois also ha delay the emptying of the stomach in human volunteers (Xin *et al.*, 2006). New experiments with higher doses are necessary in order to establish if berberine can provide an alternative to current drugs against the infection with *T. cruzi*.

CONCLUSIONS

To our knowledge this is the first communication of the activity of berberine as a prophylactic agent in transfusion experiments using the blood trypomastigote form of *T. cruzi*. Berberine failed to cure mice already infected by transfusions, but this alkaloid effectively protected transfusion recipients when used as a prophylactic drug in stored blood. Berberine somehow renders the parasites unable to proliferate *in vivo* by a not yet determined mechanism, thus opening new perspectives in the fight against Trypanosomasis.

REFERENCES

Abe F, Nagafugi S, Yamauchi T, Okabe H, Maki J, Higo H, Akahane H, Aguilar A, Jiménez-Estrada M, Reyes-Chilpa R. 2002. Trypanocidal constituents in plants 1. Evaluation of some Mexican plants for their trypanocidal activity and active constituents in Guaco, roots of *Aristolochia taliscana*. *Biol. Pharm. Bull.* 25:1188-1191.

Abe F, Nagafuji S, Okabe H, Akahane H, Estrada-Muniz E, Huerta-Reyes M, Reyes-Chilpa R. 2004. Trypanocidal constituents in plants 3. Leaves of *Garcinia intermedia* and heartwood of *Calophyllum brasiliense*. *Biol Pharm Bull.* 27(1):141-143.

Cavin JC, Krassner SM, Rodríguez E. 1987. Plant-derived alkaloids active against *Tripanosoma cruzi*. *J. Ethnopharmacol.* 19:89-94.

Docampo R, Moreno SN, Gadelha FR, de Souza W, Cruz FS. 1988.Prevention of Chagas' disease resulting from blood transfusion by treatment of blood: toxicity and mode of action of gentian violet. *Biomed Environ Sci.* 1(4):406-413.

Docampo R, Moreno SN. 1990. The metabolism and mode of action of gentian violet. *Drug Metab Rev.* 22(2-3):161-178.

Ji S, Chai Y, Zhang G, Wu Y, Liang D, Xu Z. 1999. Determination of berberine in *Rhizoma coptidis* and its preparations by non-aqueous capillary electrophoresis *Biomed. Chromatogr*. 13: 439–441.

Mafezoli J, Vieira PC, Fernandes JB, da Silva MFGF, de Alburquerque S. 2000. In Vitro activity of Rutaceae species against the trypomastigote form of *Trypanosoma cruzi*. *J. Ethnopharmacol.* 73:335-340.

Moraes-Souza H, Bordin JO. 1996. Strategies for prevention of transfusion-associated Chagas' disease. *Transfus Med Rev.* 10(3):161-170.

Pan GY, Wang GJ, Liu XD, Fawcett JP, Xie YY. 2002 . The involvement of P-glycoprotein in berberine absorption. *Pharmacol Toxicol.*91(4):193-197.

Paulino M, Iribarne F, Dubin M, Aguilera-Morales S, Tapia O, Stoppani AO. 2005. The chemotherapy of Chagas' disease: an overview. *Mini Rev. Med. Chem.* 5:499-519.

Phillipson JD, Wright CW. 1991. Medicinal plants against protozoal diseases. *Trans. R. Soc. Trop. Med. Hyg.* 85:18-21.

Rojas de Arias A, Inchausti A, Ascurrat M, Fleitas N, Rodríguez E. 1994. In vitro activity and mutagenicity of bisbenzylisoquinolines and quinones against *Trypanosoma cruzi* trypomastigotes. *Phytother. Res.* 8:141-144.

Schinella GR, Tournier HA, Prieto JM, Rios JL, Buschiazzo H, Zaidenberg A. 2002. Inhibition of *Trypanosoma cruzi* growth by medical plant extracts. *Fitoterapia* 73:569-575.

Schmunis GA, Zicker F, Cruz JR, Cuchi P. 2001. Safety of blood supply for infectious diseases in Latin American countries, 1994-1997. *Am. J. Trop. Med.* 65:924-930

Sepúlveda-Boza S, Cassels BK. 1996. Plants metabolites active against *Trypanosoma cruzi*. *Planta Med.* 62:98-105

Stoppani AOM. 1999. The chemotherapy of Chagas disease. *Medicina* (Buenos Aires) 59:147-165

Tyler KM, Engman DM. 2001. The life cycle of *Trypanosoma cruzi* revisited. *International Journal of Parasitology* 31: 472-481

WHO. 1999. *Rhizoma Coptidis*. WHO monographs on selected medical plants Vol. 1. WHO, Geneva, pp 105-114.

WHO. 2007. *Triatomine bugs*. www.who.int/entity/water_sanitation_health/resources/vector210to222.pdf [Consulted 04/12/07], p.213.

Xin HW, Wu XC, Li Q, Yu AR, Zhong MY, Liu YY. 2006. The effects of berberine on the pharmacokinetics of cyclosporin A in healthy volunteers. *Methods Find Exp Clin Pharmacol.* 28(1):25-9.

Zaidenberg A, Tournier H, Schinella G, Marin G, Buschiazzo H. 1999. Effects of Trifluralin on *Trypanosoma cruzi in vitro* and *in vivo*. *Pharmacol. Toxicol.* 84:98-100.

V JORNADAS DE ECONOMÍA Y SOCIEDAD DEL NOA
23 y 24 de Agosto de 2007; San Miguel de Tucumán – Argentina
Email : aresnoa@webmail.unt.edu.ar

PRIMER CONGRESO INTERNACIONAL DEL AGUA Y EL AMBIENTE
29 al 31 de Agosto de 2007; Caldas – Colombia
http://www.udistrital.edu.co/comunidad/eventos/1ciaya

CURSO DE POSTGRADO
"ETNOBOTANICA APLICADA: DEFINICION Y ALCANCES"
29 de Agosto al 1 de Septiembre de 2007; La Plata – Argentina
e-mail: aladio@crub.uncoma.edu.ar

55th INTERNATIONAL CONGRESS AND ANNUAL MEETING OF THE SOCIETY FOR
MEDICINAL PLANT RESEARCH
2 - 6 de Septiembre de 2007; Graz, Austria
http://www.ga2007.org

XVI CONGRESO ITALO-LATINOAMERICANO DE ETNOMEDICINA
"Carlo L. Spegazzini"
4 al 8 de Septiembre de 2007; La Plata – Argentina
http://www.xvicongresosilae.com.ar

Conjuntamente se realizará la
SEGUNDA REUNION ANUAL DE BLACPMA
5 y 6 de Septiembre de 2007

SEMINARIO SOBRE DESARROLLO ECONÓMICO TERRITORIAL EN AMÉRICA LATINA
4 al 7 de Septiembre de 2007; Santiago – Chile
E.mail: Alicia.williner@cepal.org

1º FORO DE ECONOMÍA SOCIAL DE LA MACRO REGIÓN AtaCaLaR.
"LA UNIVERSIDAD Y LAS ORGANIZACIONES EN LA ECONOMÍA SOCIAL Y SOLIDARIA"
6 al 8 de Septiembre de 2007; Catamarca – Argentina
foroecosocatacalar@yahoo.com.ar
secretario@coordreg-catamarca.gov.ar

VII ENCUENTRO DE POSTGRADOS IBEROAMERICANOS SOBRE DESARROLLO Y
POLÍTICAS TERRITORIALES
6 y 7 de Septiembre de 2007; Río Cuarto – Argentina
www.eco.unrc.edu.ar/postgrado/viiseminario.htm

XXXI JORNADAS ARGENTINAS DE BOTÁNICA
20 al 25 septiembre, 2007; Corrientes, Argentina
http://www.botanicargentina.com.ar/jornadas.htm

PRIMER CONGRESO INTERNACIONAL DE EDUCACIÓN PARA EL DESARROLLO
SOSTENIBLE: UNA VISIÓN DE LA DECADA
25 al 29 de Septiembre de 2007; Bogotá – Colombia
www.unicolmayor.edu.co
mavilanr@unicolmayor.edu.co
congresoeducacionsostenible@yahoo.es

CURSO AVANZADO DE FITOTERAPIA
APLICACIÓN CLINICA DE LOS MEDICAMENTOS HERBOLARIOS
26 al 28 de Septiembre de 2007; México DF – México
informes@phytomedicamenta.com

III REUNIÓN BINACIONAL DE ECOLOGÍA CHILE-ARGENTINA
30 septiembre al 4 octubre, 2007; La Serena, Chile
http://www.socecol.cl

SEGUNDO CONGRESO LATINOAMERICANO DE PARQUES NACIONALES
Y OTRAS ÁREAS PROTEGIDAS: CONSERVACIÓN, INTEGRACIÓN Y BIENESTAR
PARA LOS PUEBLOS DE AMÉRICA LATINA
30 de Septiembre al 06 de Octubre de 2007; Bariloche – Argentina
http://www.congresolatinoparques2007.org:80/

XVIII CONGRESO FARMACÉUTICO ARGENTINO
4 al 6 de Octubre de 2007; Mendoza – Argentina
http://www.congresomedicamento.com.ar/

XLII CONGRESO NACIONAL DE CIENCIAS BIOLÓGICAS
8 al 12 de Octubre de 2007; Barranquilla – Colombia
http://www.asociacioncolombianadecienciasbiologicas.org/

SEGUNDO SIMPOSIO INTERNACIONAL
"LA INVESTIGACIÓN EN LA UNIVERSIDAD: EXPERIENCIAS INNOVADORAS DE
INVESTIGACIÓN ARTICULADAS A LA DOCENCIA Y A LA EXTENSIÓN"
17 al 20 de Octubre de 2007; San Salvador de Jujuy – Argentina
E-mail: areainvestigación@ucse.edu.ar

VI INTERNATIONAL SYMPOSIUM ON NATURAL PRODUCTS AND ITS APPLICATIONS
24 al 26 de Octubre de 2007; Chillán – Chile
Informes Dr. Carlos Céspedes
cespedes_leonardo@yahoo.com
BLACPMA Boletín oficial de este evento

IV CONGRESO INTERNACIONAL DE ORDENAMIENTO TERRITORIAL
13 al 17 de Noviembre de 2007; Potosí – Bolivia
Web: http://www.naya.org.ar/eventos/

VII REUNIÓN ARGENTINA DE CLADÍSTICA Y BIOGEOGRAFÍA
14 al 16 noviembre, 2007; San Isidro – Buenos Aires, Argentina
Informes: VII_reunion_cladistica@darwin.edu.ar

SEGUNDA CONFERENCIA CIENTÍFICA DE ORQUÍDEAS DE LOS ANDES
14 al 17 noviembre, 2007; Loja, Ecuador
jpsuarez@utpl.edu.ec , dpvelez@utpl.edu.ec ,
http://www.andeanorchids2007.org

VI CONGRESO CONTINENTAL
III CONGRESO IBEROAMERICANO Y DEL CARIBE DE PRODUCTOS Y MEDICINAS NATURALES
26 al 30 de Noviembre de 2007; Ciudad de La Habana – Cuba
www.loseventos.cu/productosymedicinanatural2007

III INTERNATIONAL CONGRESS OF PHARMACOLOGY AND THERAPEUTICS, VII NATIONAL CONGRESS OF CUBAN SOCIETY OF PHARMACOLOGY, V NATIONAL WORKSHOP OF PHARMACOEPIDEMIOLOGY, I NATIONAL SYMPOSIUM OF PHARMACOLOGY TEACHING, I SYMPOSIA OF THERAPEUTIC UPDATING IN CANCER, OPHTHALMOLOGY AND ENDOCRINOLOGY,
I SYMPOSIUM CUBA-CANADA OF HOMEOPATHY
11 al 14 de Diciembre de 2007; La Habana – Cuba
http://www.scf.sld.cu/html/congreso/english/congress2007.htm

BLACPMA adheres the 'Salvador Declaration'
BLACPMA se adhiere a la "Declaración de Salvador"

The Developing World Perspective

Open access means unrestricted access to and use of scientific information. It has growing support worldwide and it is received with enthusiasm and high expectations in the developing world. Open Access promotes equity. For the developing world, Open Access will increase scientists and academics capacity to both access and contribute to world science.
Historically the circulation of scientific information in developing countries has been impeded by a number of barriers including economic models, infrastructure, policies, language and culture.
Consequently, WE, the participants of the International Seminar on Open Access - parallel meeting of the 9th World Congress on Health Information and Libraries and the 7th Regional Congress of Information in Health Sciences agree that

1. Scientific and technological research is essential for social and economic development.
2. Scientific communication is a crucial and inherent part of the activities of research and development. Science advances more effectively when there is unrestricted access to scientific information.
3. More broadly, open access enables education and use of scientific information by the public.
4. In a world that is increasingly globalized, with science claiming to be universal, exclusion from access to information is not acceptable. It is important that access be considered as a universal right, independent of any region.
5. Open Access must facilitate developing countries' active participation in the worldwide exchange of scientific information, including free access to the heritage of scientific knowledge, effective participation in the process of generation and dissemination of knowledge, and strengthening the coverage of topics of direct relevance to developing countries.
6. Developing countries already have pioneering initiatives that promote Open Access and therefore they should play an important role in shaping Open Access worldwide.

Therefore,

1. We urge governments to make Open Access a high priority in science policies including:
2. requiring that publicly funded research is made available through Open Access;
3. considering the cost of publication as part of the cost of research;
4. strengthening the local OA journals, repositories and other relevant initiatives;
5. promoting integration of developing countries scientific information in the worldwide body of knowledge.

We call on all stakeholders in the international community to work together to ensure that scientific information is openly accessible and freely available to all, forever.

La Perspectiva del Mundo en Desarrollo

Acceso Libre significa acceso a y uso no restringido de la información científica. Este concepto tiene un apoyo internacional cada vez mayor y ha generado grandes expectativas en el mundo en desarrollo. El concepto es equitativo y para los Países en Vías de Desarrollo (PVD) el Acceso Libre (AL) incrementara la capacidad de científicos y académicos para poder contribuir a la ciencia mundial.
Históricamente la circulación de información científica en PVD ha sido impedida por ciertas barreras incluyendo modelos económicos, infraestructuras, política, lenguaje y cultura.
Consecuentemente, NOSOTROS, los participantes del Seminario Internacional sobre el Acceso Libre, reunión paralela del 9 Congreso Mundial de información y Librerías sobre la Salud y el 7 Congreso Regional en Información en Ciencias de la Salud acuerdan que

1. El desarrollo científico y tecnológico es fundamental para el desarrollo económico y social
2. La información científica es una parte inherente y crucial de las actividades de investigación y desarrollo. Los avances científicos son mas efectivos cuado existe un acceso no restringido a la información científica.
3. Más generalmente, Acceso Libre permite la educación y uso de información científica por parte del publico.
4. En un mundo cada vez más globalizado, donde la ciencia clama ser universal, la exclusión del acceso a la información es inaceptable. Es muy importante que este acceso sea considerado como un derecho universal, independiente de cada región.
5. Acceso Libre debe facilitar a los PVD su participación activa en el intercambio mundial de información científica, incluyendo libre acceso a la herencia científica, participación efectiva en el proceso de generación y diseminación del conocimiento y fortalecimiento de la cobertura de tópicos de directa relevancia a los PVD.
6. Los PVD ya han desarrollado experiencias pioneras que promueven el Acceso Libre y por tanto deben tener un papel importante en darle forma al proyecto de Acceso Libre a nivel Mundial.

Por tanto,

1. Urgimos a los gobiernos a hacer del Acceso Libre una prioridad en las políticas científicas incluyendo:
2. la exigencia de que toda investigación subvencionada públicamente sea accesible libremente
3. la consideración de que los costes de publicación son parte integrante del coste de investigación;
4. fortalecimiento de las publicaciones locales de Libre Acceso, repositorios y otras iniciativas relevantes;
5. la promoción de la integración de la información científica generada por los PVD en el cuerpo mundial de conocimientos.

Hacemos un llamamiento a todas las partes de la comunidad internacional para trabajar juntos con el fin de asegurar que la informaron cientifica sea accesible libre y gratuitamente a todos y por siempre.

BLACPMA

ISSN 0717 7917
www.blacpma.cl

-Así es -respondió don Quijote- y no hay que hacer caso destas cosas de encantamentos, ni hay para qué tomar cólera ni enojo con ellas; que, como son invisibles y fantásticas, no hallaremos de quién vengarnos, aunque más lo procuremos. Levántate, Sancho, si puedes, y llama al alcaide desta fortaleza, y procura que se me dé un poco de aceite, vino, sal y romero para hacer el salutífero bálsamo; que en verdad que creo que lo he bien menester ahora, porque se me va mucha sangre de la herida que esta fantasma me ha dado.

Levantóse Sancho con harto dolor de sus huesos, y fue a escuras donde estaba el ventero; y, encontrándose con el cuadrillero, que estaba escuchando en qué paraba su enemigo, le dijo:

-Señor, quien quiera que seáis, hacednos merced y beneficio de darnos un poco de romero, aceite, sal y vino, que es menester para curar uno de los mejores caballeros andantes que hay en la tierra, el cual yace en aquella cama, malferido por las manos del encantado moro que está en esta venta.(…)

En resolución, él tomó sus simples, de los cuales hizo un compuesto mezclándolos todos y cociéndolos un buen espacio, hasta que le pareció que estaban en su punto. Pidió luego alguna redoma para echallo, y como no la hubo en la venta, se resolvió de ponello en una alcuza o aceitera de hoja de lata, de quien el ventero le hizo grata donación, y luego dijo sobre la alcuza más de ochenta paternostres y otras tantas avemarías, salves y credos, y a cada palabra acompañaba una cruz, a modo de bendición; a todo lo cual se hallaron presentes Sancho, el ventero y cuadrillero; que ya el harriero sosegadamente andaba entendiendo en el beneficio de sus machos.

Miguel de Cervantes y Saavedra, "Don Quijote de la Mancha"** , **Parte I, Cap XVII

Plantas Medicinales en la Literatura

Con el auspicio de

SOCIEDAD LATINOAMERICANA DE FITOQUÍMICA

Registrada en INDEX COPERNICUS®, LATINDEX®, IMBIOMED®