Therapeutic potential of natural products: research and development

Carla Hollandino, DSc

College of Pharmacy, Federal University of Rio de Janeiro, Brazil

Session 3, 19th ICSB

April 8th, 2019
Brazil emerges as one of the leading nations in the world scenario to afford new molecules with biodynamic activity.

• The Amazon rainforest is the largest in the world;
• About 60% is located in Brazil;
• It accounts for about 10% of the known species found in the world.

NASA satellite image.
It is estimated that only about 1-2% of the Brazilian native plant species have been successfully studied!!!
Brazilian Academic Consortium for Integrative Health

Since June 2018
<table>
<thead>
<tr>
<th>HEART HEALTH</th>
<th>MIND &amp; MOOD</th>
<th>PAIN</th>
<th>STAYING HEALTHY</th>
<th>CANCER</th>
</tr>
</thead>
</table>

Mindfulness meditation may ease anxiety, mental stress

Posted January 08, 2019, 12:00 PM, Updated December 22, 2017, 12:00 PM

Julie Cariss
Executive Editor, Harvard Health Letter

My mom began meditating decades ago, long before the mind-calming practice had entered the wider public consciousness. Today, at age 81, she still goes to a weekly meditation group and quotes Thich Nhat Hanh, a Zen Buddhist monk known for his practice of mindful meditation, or "present-focused awareness."

Welcome to IKIM

The Institute of Complementary and Integrative Medicine (IKIM) is part of the University of Bern and integrates conventional and complementary medicine in research, teaching, and patient care.

It consists of four divisions: Anthroposophically extended Medicine, Classical Homeopathy, Neural Therapy, and Traditional Chinese Medicine/Acupuncture.

Carla Holandino
April 8th, 2019
29 Integrative and Complementary Health Practices (ICHP): Brazil, January, 2018
Number and distribution of Brazilian universities who perform the Integrative and Complementary Health Practices (ICHP) research: 37 Universities, 71 Researchers (February, 2018).

Northeast 24.3%
Midwest 5.4%
Southeast 48.6%
South 21.6%
Orbignya speciosa (Mart.) Barb. Rodr.: Babassu

Carla Hollandino
April 8th, 2019
Antinociceptive effect of the Orbignya speciosa Mart. (Babassu) leaves: Evidence for the involvement of apigenin

Mariana Martins Gomes Pinheiro a, Fábio Boylan b,1, Patrícia Dias Fernandes a,4

a Universidade Federal do Rio de Janeiro, Instituto de Ciências Farmacêuticas, Laboratório de Farmacologia de Informação e de Saúde mental, Complexo Universitário, Rio de Janeiro, Brazil
b School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland. Twenty Twenty Three Westland Row, Dublin 2, Ireland

ABSTRACT

Aims: Babassu is the common Brazilian name of Orbignya speciosa Mart. (Arecaceae). The fruits are used for several disorders. In the present study, the antinociceptive effects of the ethanol extract (EE) and dichloromethane fraction (DF) obtained from leaves were investigated, as well as apigenin using nociceptive models (acetic acid-induced abdominal writhing, formalin, and hot plate).

Methods: Mice were treated with EE, DF (100, 50 and 100 mg/kg, p.o.), apigenin (1 mg/kg, p.o.), morphine (5 mg/kg, s.c.), acetylsalicylic acid (1000 mg/kg, p.o.) or vehicle (0.1 ml, p.o.). The EE and DF reduced the contractions induced by acetic acid. Both also reduced the licking response in the formalin model. In the hot plate model, the antinociceptive effects were at least equal to that shown by morphine. To elucidate the antinociceptive mechanism of action of EE, DF and apigenin in the animals we re-created with atropine (neuromuscular blocking agent; 1 mg/kg, s.c.), naloxone (opioid receptor antagonist; 1 mg/kg, s.c.), 1-hydroxy arginine methyl ester (L-NAME; nitric oxide synthase inhibitor; 3 mg/kg, s.c.) or mecamylamine (nicotinic receptor antagonist; 2 mg/kg, s.c.) and evaluated in the hot plate model.

Key findings: The antinociception produced by EE was abolished by atropine, naloxone or mecamylamine. The effect of apigenin was significantly blocked by atropine or naloxone.

Significance: The results obtained indicated that EE and DF have antinociceptive activity that is mediated, at least in part, by opioid and cholinergic systems. This effect can be attributed to the presence of apigenin, a flavonoid in the dichloromethane fraction.

© 2012 Elsevier Inc. Open access under the Elsevier CC License.
Cell viability ($ID_{50}$).

<table>
<thead>
<tr>
<th></th>
<th>($ID_{50}$) ($\mu$g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.3 ± 0.8$^a$</td>
</tr>
<tr>
<td></td>
<td>33.9 ± 4.3$^b$</td>
</tr>
<tr>
<td></td>
<td>55.0 ± 6.1$^b$</td>
</tr>
<tr>
<td></td>
<td>48.8 ± 5.7$^b$</td>
</tr>
<tr>
<td></td>
<td>127.0 ± 14.3$^c$</td>
</tr>
<tr>
<td></td>
<td>141.2 ± 15.4$^c$</td>
</tr>
</tbody>
</table>

Fig. 3 – Giemsa-staining of mammalian cells in the absence and in the presence of ethanol 1.20% 1200$\mu$g/mL of OSEME. Cells were cultured and stained as described under Materials and Meth. Panels A, B and C: HL-60 cells control, 1.20% ethanol and 1200$\mu$g/mL of OSEME, respectively; panels D, E and F: K562 cells control, 1.20% ethanol and 1200$\mu$g/mL of OSEME, respectively; panels G and H: K562-Lucena 1 cells control, 1.20% ethanol and 1200$\mu$g/mL of OSEME, respectively; panels I, J, and L: MCF-7 cells control, 1.20% ethanol and 1200$\mu$g/mL of OSEME, respectively; panels M, N and O: 3T3-L1 cells control, 1.20% ethanol and 1200$\mu$g/mL of OSEME, respectively. Scale bars = 50μm.

(a, b or c) were statistically indicated under Material and Meth. as shown in Figure 1.
Effects of a nanocomposite containing *Orbignya speciosa* lipophilic extract on Benign Prostatic Hyperplasia


*Departamento de Fisiologia e Anatomia, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil.*

*School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland.*

**Abstract**

Ephrotharmacological relevance: Lower urinary tract symptoms (LUTS) are a common complaint among aging men and are usually caused by Benign Prostatic Hyperplasia (BPH). A number of medical treatments for LUTS/BPH exist, such as α-blockers, 5α-reductase inhibitors, phytotherapeutic drugs and combination therapies. *Babassu* is the common name of a Brazilian native palm tree called *Orbignya* species, whose kernels are commonly used (often entirely or as a grounded powder), in parts of Brazil for the treatment of urinary disorders. This study investigates the effects of *Orbignya* species nanoparticle extract, a newly developed phytotherapeutic formulation derived from the kernels of babassu, in the treatment of BPH.

**Materials and methods:** *Orbignya* species extract was obtained from the kernels, a nanoparticles particle system was developed and acute toxicity test was performed. BPH primary stromal cells and tissue cultures were established and treated with 300 μg/mL *Orbignya* species nanoparticles (NanoOSE) extract in order to evaluate its effects on apoptosis induction, cytotoxicity, cell morphology and proliferation.

**Results:** Our results indicate that NanoOSE shows no toxicity in animals and acts specifically by promoting morphological cell changes, reducing cell proliferation as well as inducing necrosis/apoptosis on BPH cells and tissues.

**Conclusion:** This study provided the first report of the successful use of NanoOSE on BPH treatment which corroborates with the popular use of the kernels of this plant. The results also suggest the potential of NanoOSE as a candidate new phytotherapeutic agent on the management of BPH.
Piper cabralanum: antitumoral nanotechnology strategy
Passiflora mucronata: antioxidant and apoptotic potential

Isabel C.V. da Silva1,2, Goran N. Kaluderovic2, Pollyana F. de Oliveira3, Dépice O. Guimarães3, Carla H. Quaresma4, Andrea Porzel5, Michelle P. Montano5, Ludger A. Wessjohann2 and Ivan C.R. Leal1

1Laboratório de Produtos Naturais e Bioensaios, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Departamento de Produtos Naturais e Alimentos, CCS, Rio de Janeiro, RJ, Brazil; 2Department of Biocatalysis, Leibniz Institute of Plant Biochemistry, Weinberg 7, D-06120 Halle (Saale), Germany; 3Laboratório de Produção Industrial, Curso de Farmácia, Universidade Federal do Rio de Janeiro, Campus Maracanã, Polo Norte Câncer (MCT), Macau, RJ, Brazil; 4Laboratório Multidisciplinar de Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Departamento de Farmácia, CCS, Rio de Janeiro, RJ, Brazil

Abstract: Background: P. mucronata (Pan) covers from South America, Brazil and is characterized as “Manacá da Rainha.” It is used in folk medicine for its soothing properties and in treating insomnia.

Objective: The present study for the first time analyzed the antioxidant and cytotoxicity of the hydroalcoholic leaves extract and fractions from Pan.

Methods: The cytotoxicity test will be evaluated by different assays (MTT and CV) against human prostate cancer (PC2) and mouse malignant melanoma (B16F10) cell lines, and the antioxidant test by DPPH method.

Results: B-Amyrin, oleanolic acid, β-sitosterol and stigmasterol were isolated of the most active, human fractions. These substances were tested against the human cell line B-16F10 and stigmasterol showed the most relevant activity to PC2 in CV assay and, oleanolic acid to B16F10 by the MTT assay. In addition, it was possible to indicate that the mode of cell death for stigmasterol, presumably is apoptosis. In terms of antioxidant activity, the hydroalcoholic leaves extract presented higher activity (EC50 133.3 μg/mL) compared to the flower (EC50 1272 μg/mL) and fruit (EC50 207 μg/mL) extracts. By the HPLC-HRMS, it was possible to identify the presence of flavones in the leaves extract (iso-kaempferol, schaftoside, iso-sclerostirol, visnagin, isoviscnagin, orientin).

Conclusion: P. mucronata leaves fraction showed prominent cytoprotective effect against cancer cell lines, and stigmasterol contributes to this activity, inducing apoptosis of these cells. Furthermore, as other Passiflora species, Pan extracts have antioxidant activity and flavones are its major phenolic compounds.
Passiflora mucronata: antioxidant and apoptotic potential

Apoptosis cell death
Euphorbia tirucalli Lineu: antitumoral potential

Table 3: Chemical compounds in Euphorbia tirucalli extracts determined from ESI(-) FT-ICR MS data

<table>
<thead>
<tr>
<th>Molecular formula or M (DBE)</th>
<th>MG</th>
<th>PA</th>
<th>MT</th>
<th>SC</th>
<th>Arraiolos do Cabo/RJ</th>
<th>UFRJ/RJ</th>
<th>Proposed structure (Class of Natural Product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{10}H_{16}O_{18} (3)</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>Malic acid glycoside</td>
</tr>
<tr>
<td>C_{14}H_{20}O_{12} (12)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>Ellagic acid (phenolic)</td>
</tr>
<tr>
<td>C_{16}H_{18}O_{9} (9)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>Mono-caffeylquinic acid (phenylpropanoid)</td>
</tr>
<tr>
<td>C_{20}H_{20}O_{11} (12)</td>
<td>+++</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Quercetin (flavonoid)</td>
</tr>
<tr>
<td>C_{20}H_{18}O_{10} (7)</td>
<td>nd</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>+</td>
<td>nd</td>
<td>Acid triterpene (triterpene)</td>
</tr>
<tr>
<td>C_{18}H_{16}O_{13} (4)</td>
<td>nd</td>
<td>++</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>++</td>
<td>Malic acid glycoside</td>
</tr>
<tr>
<td>C_{22}H_{20}O_{14} (12)</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>2,3-(S)-Hexahydroxydiphenoyl-D-glucose</td>
</tr>
<tr>
<td>C_{20}H_{20}O_{14} (11)</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(ellagitannin)</td>
</tr>
<tr>
<td>C_{27}H_{22}O_{18} (17)</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>2,3-Di-O-galloyl-D-glucose (galloyltannin)</td>
</tr>
</tbody>
</table>

nd = not detected; +++high intensity of [M-H] ions; ++medium intensity of [M-H] ions; + low intensity of [M-H] ions
Chronic toxicological effects of high diluted solutions of Aveloz (Euphorbia tirucalli L.) on healthy mice: a preliminary study

Marina OBN Varrochio1 (UFS), Cristiane Pereira1, Fernanda Salles2, Telisa Gonçalves3, Elaine Duarte3, Carolina Leoco Aguiar3, Gleyse Morena Barbosa3, Nelson Ortes3, Alexandre dos Santos Freitas1, Paulo Eduardo Mancarreto2, Maysa Goulart Branco1, Ricardo Kusser1, Carla Holandino2,4

Federal University of Rio de Janeiro,1University of Rio de Janeiro

ABSTRACT

The samples extracted from Euphorbia tirucalli, a plant popularly known as Aveloz, is used in complementary medicine to treat various dermatological conditions. The current study aims to investigate the chronic toxicological effects of high diluted (100X) solutions of Euphorbia tirucalli L. on healthy mice. The diluted solutions were prepared using 2% alcohol and administered through subcutaneous injection at concentrations of 0.001% and 0.0001% for 14 days. No changes were observed in the body weight, food and water intake, behavior, or hematological and biochemical parameters. However, a significant increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was observed in the group receiving 0.0001% solution, suggestive of liver damage. In conclusion, the high diluted solutions of Euphorbia tirucalli L. (100X) can be used safely in the treatment of various dermatological conditions.

Keywords: Euphorbia tirucalli; Toxicology; High Dilution; Mouse

High dilutions of Euphorbia tirucalli L. (AVELOZ) modify the viability and glycolytic metabolism of cell lines

Carolina Leoco Aguiar1, Gleyse Morena Barbosa1, Gleyse Morena Barbosa1, Marina Cristina Braga Antunes Varrochio1, Veaslo Loz Viegas1, Ricardo Kusser1, Fernanda Sumae2, Mauro Solem3, Carla Holandino2,4

Laboratory of Pharmaceutical Sciences, Department of Medicine, Faculty of Pharmacy, Federal University of Rio de Janeiro, Brazil

ABSTRACT

The effects of Euphorbia tirucalli L. (AVELOZ) on cell viability and glycolytic metabolism were evaluated in various cell lines, including human prostate and breast cancer cells. Aveloz has been shown to have antitumoral properties, and its effect on cell viability and metabolism was studied using high diluted (100X) solutions. The results showed a significant decrease in viability and an increase in lactate production, indicating a shift from aerobic to anaerobic metabolism, which is characteristic of cancer cells. These findings suggest that Aveloz may have potential as an anti-cancer agent through its ability to alter the metabolic profile of cancer cells.

Keywords: Euphorbia tirucalli; Aveloz; PFT-1; High Dilution; Cell metabolism
U.S.-Brazil Collaborative Biomedical Research Program (R01 Clinical Trial Optional)

Therapeutic potential and immune mechanisms involved with *Euphorbia tirucalli* in cancer
Viscum album

Urech & Baumgartner, 2015

Fig. 2. Seasonal variations of total viscotoxin and mistletoe lectin concentrations in *V. album* during the vegetation cycle of leaf development (adapted from [52]).
1) Metabolomic profile of *Viscum album* from different host trees;
2) Metabolomic profile of *Viscum album* mother tinctures;
3) Antitumoral activity of *Viscum album* preparations;
4) Development of new *Viscum album* formulations.
Metabolomic profile of *Viscum album* from different host trees

- 2 young leaves (1 year)
- 1 old leave (2 years)
- 1 young stem (1 year)
- 1 old stem (2 years)
- 3 berries (green and ripe)

*Viscum album in Switzerland*
Metabolomic profile of *Viscum album* from different host trees

*Viscum album ssp album*

*Malus*  
*Quercus*  
*Ulmus*

*Viscum album ssp abietis*  
*(Abies alba)*

*Viscum album ssp austriacum*  
*(Pinus sylvestris)*

*Viscum album in Switzerland*
Collections to Metabolomic: Mirio Grazi and *Malus domestica* Movie
Phytochemical profile and \textit{in vitro} antitumoral effects of \textit{Viscum album} mother tinctures

Mother Tincture: first day
Dry residue Biological TLC, UHPLC

(ANSM, 2010; ANVISA, 2011)
Viscum album ethanolic tinctures and antitumoral potential

Original article

Phenolic compounds from Viscum album tinctures enhanced antitumor activity in melanoma murine cancer cells


ARTICLE INFO

Article history:
Received 20 August 2017
Accepted 26 January 2018
Available online 31 January 2018

Keywords:
Viscum album
Mistletoe
Lignans
Phenolic compounds
Antitumoral

ABSTRACT

Cancer is one of the biggest problems in public health worldwide. Plants have been shown important role in anticancer research. Viscum album L. (Santalaceae), commonly known as mistletoe, is a semi-parasitic plant that grows on different host trees. In complementary medicine, extracts from European mistletoe (Viscum album L.) have been used in the treatment of cancer. The study was conducted to identify chemical composition and antitumoral potential of Viscum album tinctures. Chemical analysis performed by high resolution chromatography equipped with high resolution mass spectrometer identified caffeic acid, chlorogenic acid, sakuranetin, sossakuranetin, syringin 4-O-glucoside, syringin 4-O-apioyl-glucoside, salvilignoside C and ligulolignoside A compounds. Some of these compounds are probably responsible for the reduction of tumoral cellular growth in a dose-dependent manner. It was observed that melanoma murine cells (B16F10) were more sensitive to V. album tinctures than human leukemic cells (K562), besides non-tumoral cells (MA-104) had a much lower cytotoxicity to them. Apoptotic-like cells were observed under light microscopy and were confirmed by a typical DNA fragmentation pattern. Additionally, flow cytometry results using Annexin V/FITC permitted to quantitatively increased expression of early and late apoptotic markers on tumoral cells, confirming augmented Sub G0 population, which was probably associated with a consistent decrease in G1, and an increase in S or G2/M populations. Results indicate the chemical composition of V. album tinctures influences the mechanisms of in vivo tumoral cell death, suggesting a potential use in cancer pharmacotherapy research.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Conclusions

1) It is very important to elucidate the phytochemical profile of the plants’ extracts in order to increase the pharmacologic uses of natural substances.

2) The use of sensitive methodology as well as adequate statistical approach are fundamental to guarantee the quality control of raw herbal sources.
THANK YOU VERY MUCH FOR YOUR ATTENTION!

cholandino@gmail.com
cholandino@pharma.ufrj.br