Evaluation of the inhibition of skin matrix metalloproteinases by *Pothomorphe umbellata* root extract

Cristina Dislich Ropke
Essential aspects of UV radiation (UVR) induced carcinogenesis

Direct and oxidative DNA modification
Neoplastic transformation

Immunosuppression
Inhibition of the immunologic recognition of the tumor

Induction of signaling cascades
Upregulation of MMP
Down-regulation of procollagen type I
Invasion

CANCER
Metalloproteinases

- The MMPs are endopeptidases that can cleave virtually any component of the ECM.

- The MMPs are synthesized as inactive ZYMOGENS (pro-MMPs). They are kept inactive by an interaction between a cysteine-sulphhydryl group in the propeptide domain and the zinc ion bound to the catalytic domain: activation requires proteolytic removal of the propeptide prodomain.

- MMPs can promote cancer progression by increasing cancer-cell growth, migration, invasion, metastasis and angiogenesis.

(Egeblad & Werb, 2002)
Regulation of MMPs

The activity of MMPs is regulated at three levels: synthesis (primarily transcription), proteolytic activation of the zymogen and inhibition of proteolitic activity by specific endogenous inhibitors.

(Rittié & Fisher, 2002)
UV-induced signalig cascades

- Activation of cell surface growth factor and cytokine receptors
- Inhibition of transforming growth factor (TGF)-β signaling
- Activation is enhanced by concomitant production of ROS

(Rittié & Fisher, 2002)
Metalloproteinases and photoaging

- UV irradiation of human skin causes extracellular matrix degradation via induction of transcription factor AP-1, and subsequent increases MMP production.
Inhibition of the enzymatic activity of MMPs

- TIMPS - tissue inhibitors of metalloproteinases
- Direct inhibition of the catalytic domain
- Chelation of Zn $^{2+}$
Antioxidant activity and MMP inhibition

- Oral administration of GTP resulted in inhibition of UVB-induced expression of matrix degrading MMP (MMP-2, MMP-3, MMP-7 and MMP-9) in hairless mouse skin (Vayalil et al., 2004)
- Metabolites of Maritime Pine Bark Extract (Grimm et al., 2004)
*Pothomorphe umbellata* L. Miq

- *Pothomorphe umbellata*, a plant of Piperaceae family, is widely used in Brazilian folk medicine for treatment of liver diseases and healing of skin wounds.

- **Pariparoba**

- The roots of *P. umbellata* were included in the first edition of the Brazilian Pharmacopea
Pothomorphe umbellata L. Miq
**Pothomorphe umbellata** L. Miq

*in vitro* results

- Crude root ethanolic extracts of *P. umbellata* demonstrated a significant activity in the prevention of *in vitro* spontaneous brain lipid peroxidation evaluated by TBARS and chemiluminescence (CL) emission (Barros *et al.*, 1996)

This activity was attributed to 4-nerolidylcathecol, a compound isolated from the hexane extracts of roots and leaves of *P. umbellata*

- the total reactive potential of the *P. umbellata* extract was higher than that obtained for the isolated 4-NC, suggesting the presence in the extracts of additional compounds with antioxidant activity (Desmarchelier *et al.*, 1997)
**Pothomorphe umbellata** L. Miq

*in vivo* results

- Topical application of *P. umbellata* root extract reduced the lipid peroxidation of skin homogenates (TBARS and CL) in 97%\(^1\)
- Antioxidant activity 2.5 higher than that of \(\alpha\)-tocopherol\(^1\)
- Preserved endogenous \(\alpha\)-tocopherol concentration in the skin, after acute irradiation with UVB\(^2\)

\(^1\)Ropke, *Dissertação de Mestrado* 1998;
\(^2\)Ropke *et al.*, *Photochem. Photobiol.* 2003
**Pothomorphe umbellata** L. Miq

*in vivo* results

- *P. umbellata* extract was able to reduce the incidence of visible and histological skin alterations in chronically UV-irradiated mice

Photoprotective effect of *Pothomorphe umbellata* root extract against ultraviolet radiation induced chronic skin damage in the hairless mouse

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In Vitro and In Vivo Inhibition of Skin Matrix Metalloproteinases by Pothomorphe umbeliata Root Extract

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Figure 2. A: Zymograms of skin homogenates incubated in the presence of increasing concentrations of 4-NC and increasing concentrations of P. umbeliata extract. B: The bar graphs represent the intensities of the band obtained from gelatin zymography by densitometry. The data shown are mean values ± SD of three independent experiments.
Inhibition of MMP-9 induction after acute UVB exposure

- UVB dose: 0.23 J/cm²
- Sacrifice time: 2h after irradiation

Figure 3. A: Gelatinase activities in the nonirradiated group (C), the irradiated control group (I), the gel-treated group (G), the *P. umbellata*-treated group and the irradiated group (Pu). A: Typical gelatin zymographic pattern. MMP-9 activities were lower in the *P. umbellata* group than in the nonirradiated or irradiated control groups. B: Skin section photomicrograph showing the absence of neutrophil infiltration 2 h after the last irradiation (original magnification, ×400; hematoxylin-eosin stain). C: Each MMP band was densitometrically quantified by computer imaging analysis. Bars represent mean values ± SD (*n* = 6). *P* < .05.
Effect of topical application of *P. umbellata* extract on MMP-2 and 9 on the skin chronically exposed to UVB radiation

- **Groups:** control, UVB, UVB+vehicle, UVB+*P. umbellata* (treated 2 h prior irradiation for 4 weeks)
- **Lamp:** UVB Philips TL 12RS 40W
- **Dose:** 13.17 KJ/m² (4 times weekly)
- **Sacrifice:** 2 h after irradiation
- **Zimography** – Acrilamid SDS-page gel, containing 0,5% of gelatin
- **Densitometer** GS-700 BIO-RAD
Results

P- MW Standard
C- Control
I- Irradiated group
G- Irradiated group treated with vehicle
Pu- Irradiated group treated with the *P. umbellata* gel
Additional compounds with MMP inhibitory activity

- Fractioning of *P. umbellata* root extract
- *In vitro* gelatin zymography with fractions without 4-NC
Results

MMP9
MMP2

4-NC

4-NC

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Chemical stability and SPF determination of *Pothomorphe umbellata* extract gel and photostability of 4-nerolidylcatechol


<table>
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<tr>
<th>Sample</th>
<th>SPF</th>
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<tr>
<td>Homosalate 8%</td>
<td>7.86 ± 0.12</td>
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<tr>
<td><em>P.umbellataro</em> extract gel 1.41%</td>
<td>3.35 ± 0.02</td>
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<tr>
<td>Isolated 4-nerolidylcatechol</td>
<td>4.00 ± 0.59</td>
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<tr>
<td>Crude<em>P.umbellataro</em> extract</td>
<td>21.53 ± 0.04</td>
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Conclusions

• Either by its previously demonstrated antioxidant properties, as by the inhibitory effects on MMPs hereby shown, our combined data may provide a rational basis for the use of standardized *P. umbellata* extract in prophylaxis and therapy of photodamage

• There are other compounds in the *P. umbellata* extract with MMP inhibition activity
Patent USP/Fapesp PCT/BR03/00134 "Use of *Pothomorphe umbellata* extract, composition on basis of *Pothomorphe umbellata* extract and method of application of the *Pothomorphe umbellata* extract", 2003

Patent USP/Fapesp PI 0504720-0 “Process of obtainment of cathecol and derivatives as from plants of the gender *Pothomorphe*, formulations and use of them”, 2005
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Thank you for your attention!