



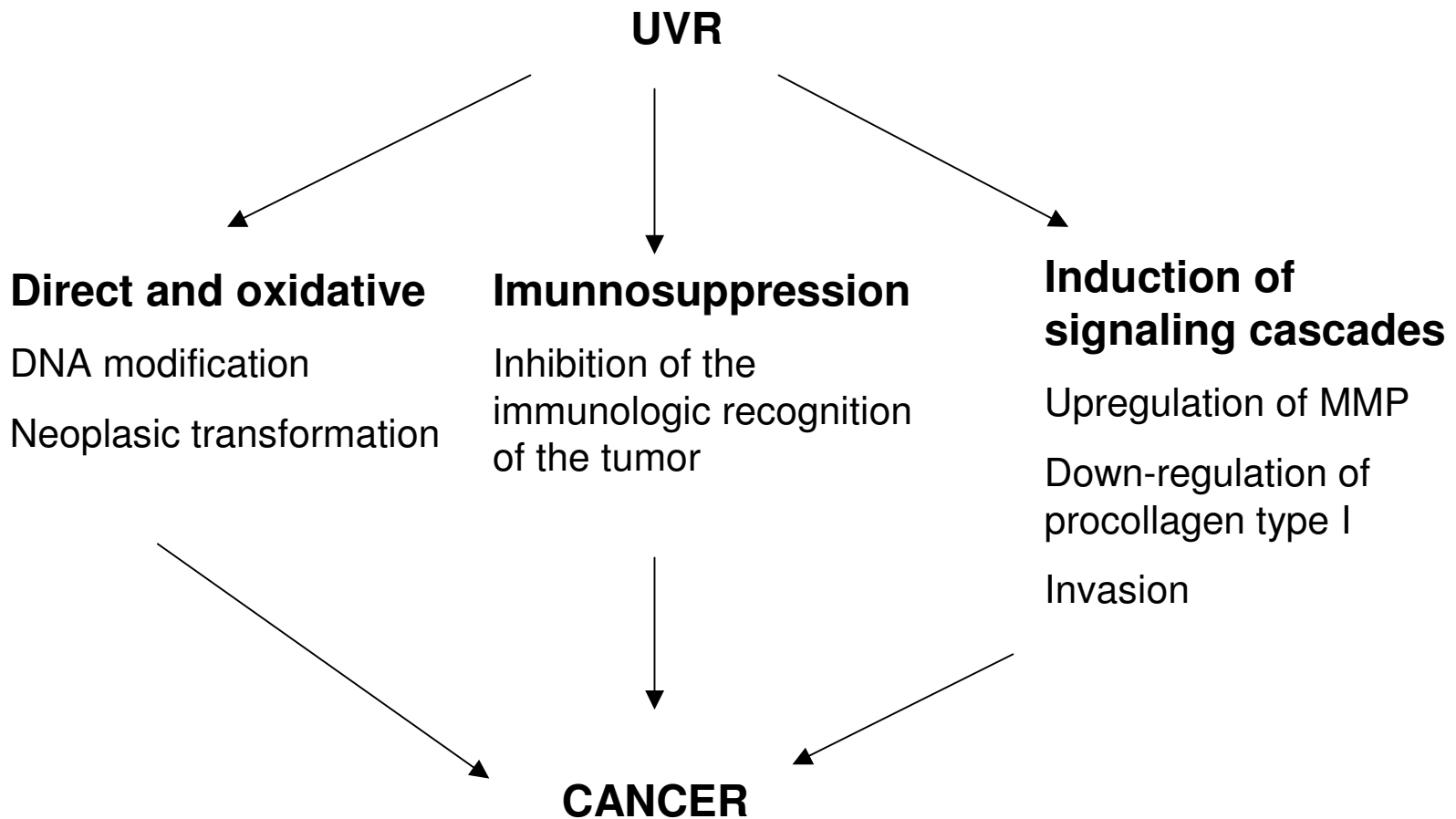
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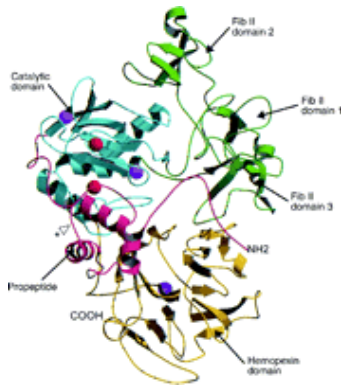


Evaluation of the inhibition of skin
matrix metalloproteinases by
Pothomorphe umbellata root extract

Cristina Dislich Ropke

Essential aspects of UV radiation (UVR) induced carcinogenesis





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-sulphydryl 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

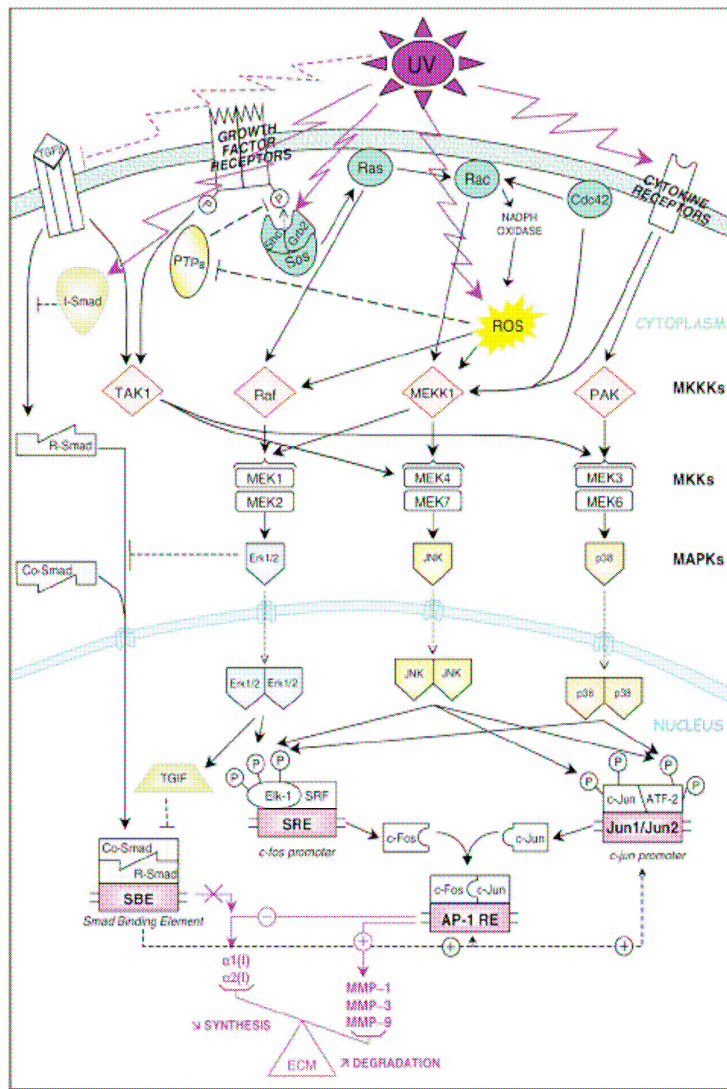
(Egeblad & Werb, 2002)

Regulation of MMPs

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

(Rittié & Fisher, 2002)

UV-induced signaling 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)

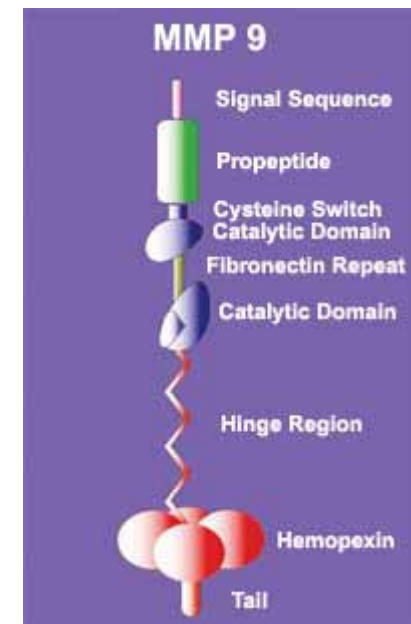
Metalloproteinases and photoaging



- UV irradiation of human skin causes extracellular matrix degradation via induction of transcription factor AP-1, and subsequent increases MMP production

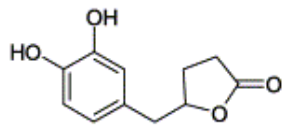
Inhibition of the enzymatic activity of MMPs

- TIMPS - tissue inhibitors of metalloproteinases
- Direct inhibition of the catalytic domain
- Chelation of Zn^{2+}



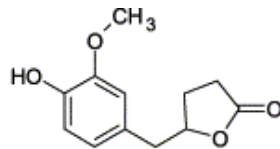
Antioxidant activity and MMP inhibition

- 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)



M1

δ -(3,4-dihydroxyphenyl)- γ -valerolactone



M2

δ -(3-methoxy-4-hydroxyphenyl)- γ -valerolactone



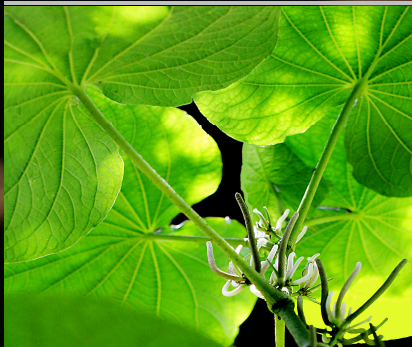
Pothomorphe umbellata L. Miq



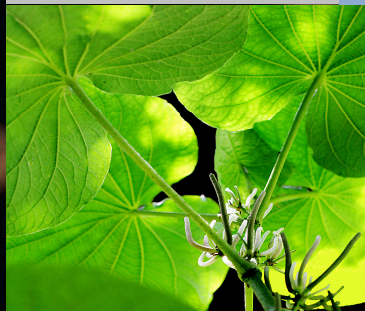
- *Pothomorphe umbellata*, a plant of Piperaceae family, is widely used in Brazilian folk medicine for treatment of liver diseases and healing of skin wounds.

- **Pariparoba**

- The roots of *P. umbellata* were included in the first edition of the Brazilian Pharmacopeia



Pothomorphe umbellata L. Miq



Pothomorphe umbellata L. Miq

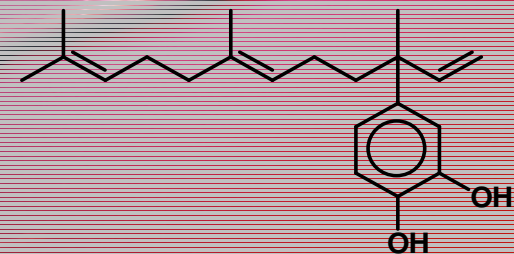
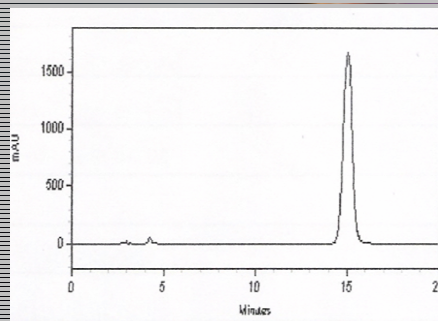
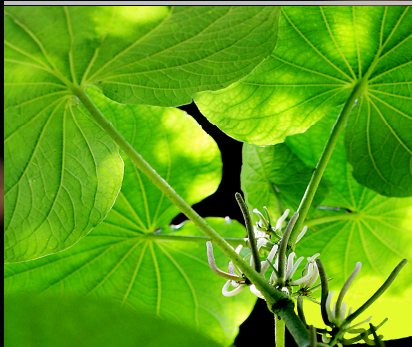
in vitro results



- 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-nerolidylcatechol**, 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)



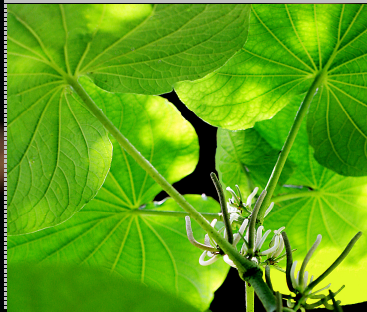
Kijjoa *et al.*, 1980

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 α -tocopherol¹
- Preserved endogenous α -tocopherol concentration in the skin, after acute irradiation with UVB²



¹Ropke, **Dissertação de Mestrado** 1998;

²Ropke *et al.*, **Photochem. Photobiol.** 2003

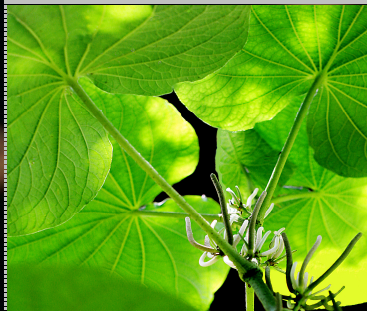
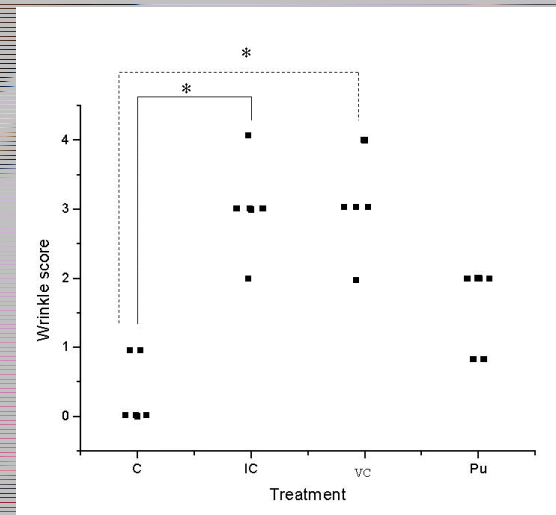
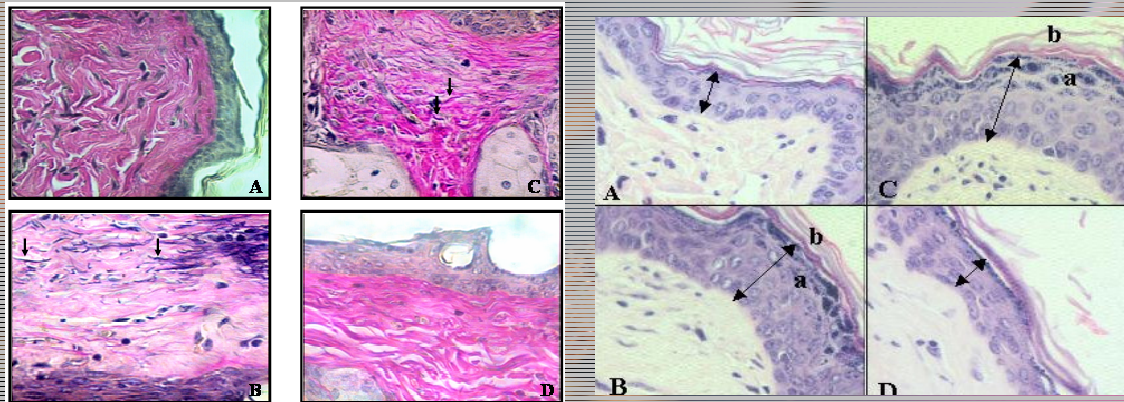
³Ropke *et al.*, **Clin. Exp. Dermatol.**, 2005, ⁴Ropke *et al.*, **Photochem. Photobiol.** 2006

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 umbellata* Root Extract

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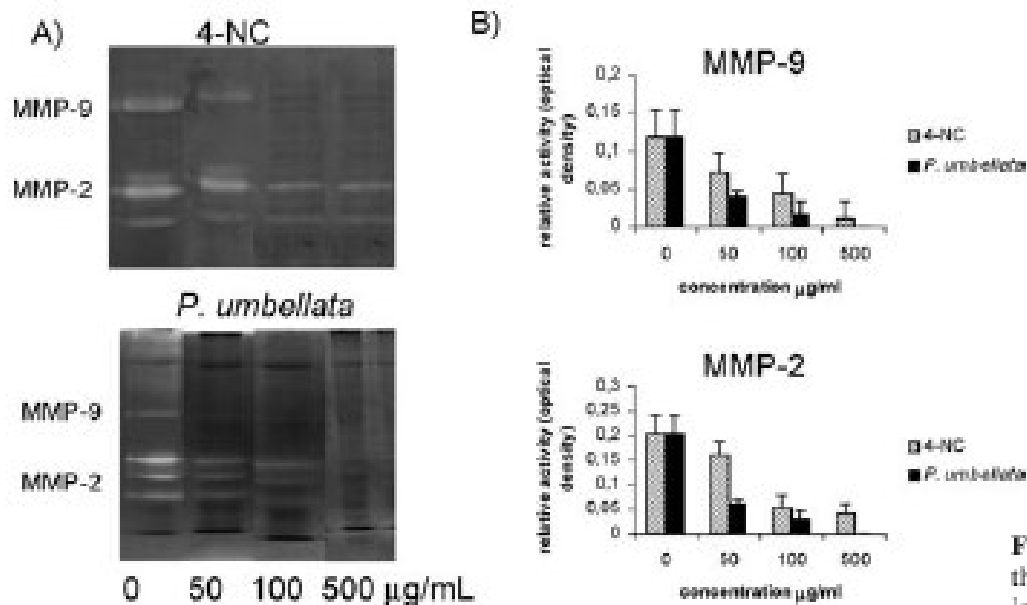
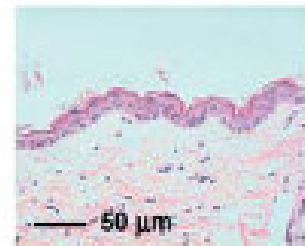


Figure 2. A: Zymograms of skin homogenates incubated in the presence of increasing concentrations of 4-NC and increasing concentrations of *P. umbellata* extract. B: The bar graphs represent the intensities of the band obtained from gelatin zymography by densitometry. The data shown are mean values \pm SD of three independent experiments.

Inhibition of MMP-9 induction after acute UVB exposure



B)



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

C)

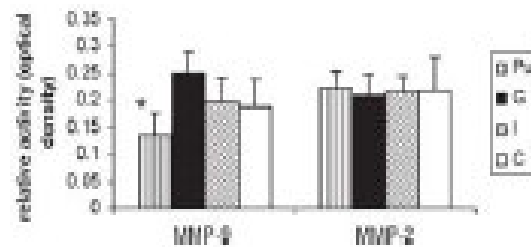
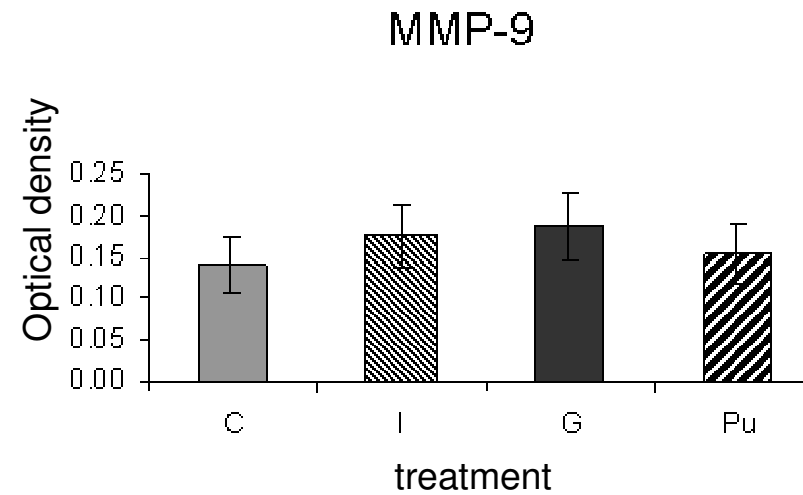
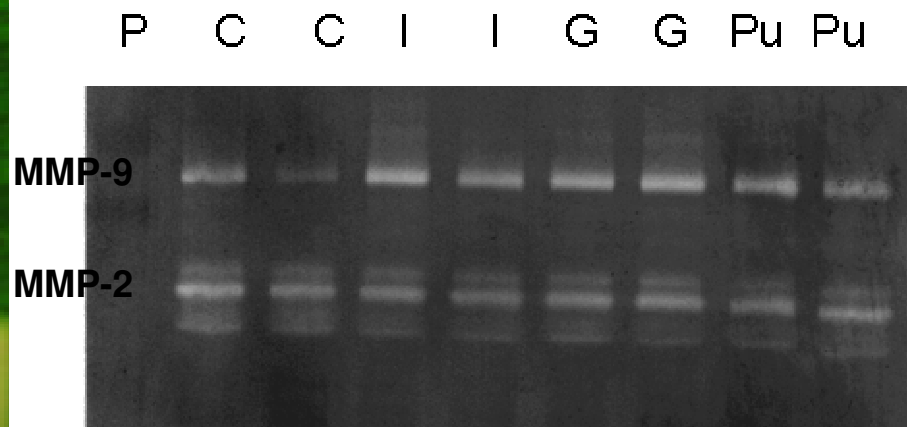


Figure 3. A: Gelatinase activities in the nonirradiated group (C), the irradiated control group (I), the gel-treated group (G), the *P. umbellata*-treated group and the irradiated group (Pu). A: Typical gelatin zymographic pattern. MMP-9 activities were lower in the *P. umbellata* group than in the nonirradiated or irradiated control groups. B: Skin section photomicrograph showing the absence of neutrophil infiltration 2 h after the last irradiation (original magnification, $\times 400$; hematoxylin-eosin stain). C: Each MMP band was densitometrically quantified by computer imaging analysis. Bars represent mean values \pm 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

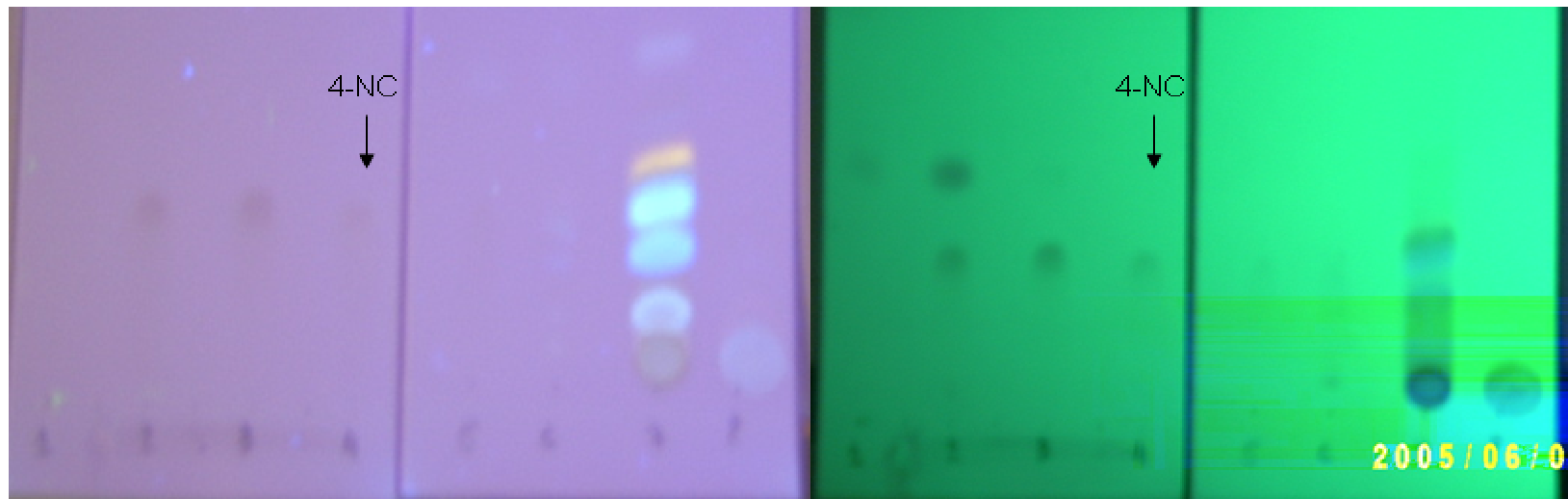
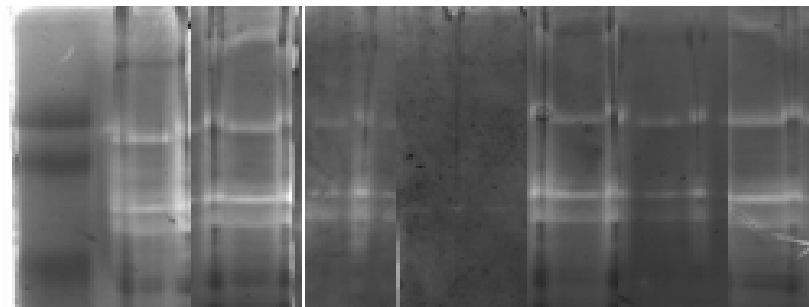
- Fractioning of *P. umbellata* root extract
- *In vitro* gelatin zymography with fractions without 4-NC



Results

P F1 F2 F3 F4 F5 F7 F8

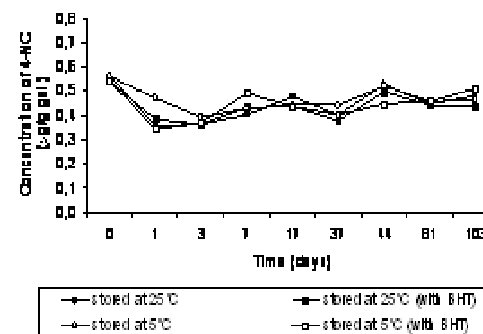
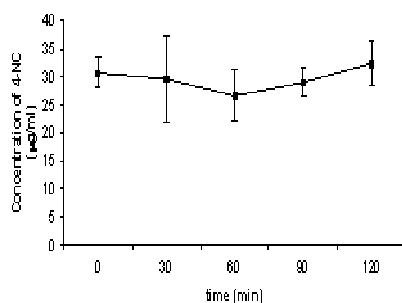
MMP9
MMP2



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

Vanessa V. da Silva*, Cristina D. Ropke, Rebeca L. de Almeida,
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Tânia C.H. Sawada, Silvia B.M. Barros

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Sample	SPF
Homosalate 8%	7.86 ± 0.12
<i>P.umbellata</i> root extract gel 1.41%	3.35 ± 0.02
Isolated 4-nerolidylcathecol	4.00 ± 0.59
Crude <i>P.umbellata</i> root extract	21.53 ± 0.04

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 catechol and derivatives as from plants of the gender *Pothomorphe*, formulations and use of them", 2005

Team

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Thank you for your attention!