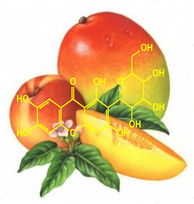
Cytotoxicity Screen of Mangiferin and its Major Metabolite Norathyriol in Human Tumor Cell Lines

Souza, J. R. R. [1,2], Feitosa, J. P. A. [2], Ricardo, N. M. P. S [2], Trevisan, M. T. S. [2], Frei, E. [1], Ulrich C. M.[1], Owen, R. W. [1]

[1]Division of Preventive Oncology, National Center For Tumor Diseases, Im Neuenheimer Feld 460/German Cancer Research Center, Im Neuenheimer Feld 581, Heidelberg, Germany

[2]Department of Organic and Inorganic Chemistry, Federal University of Ceará – P. O. Box: 6.021, ZIP-Code: 60455-760, Fortaleza, Ceará, Brazil



INTRODUCTION

⇒Many natural products are available worldwide as potential chemoprotective agents against commonly occurring cancers[1].

⇒Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-beta-D-glucoside) is a xanthone glycoside present mainly in *Mangifera indica, L.*[2] and has been reported to have anticancer, antioxidant and other activities[3]

⇒ Mangiferin is reported to have a very low bioavailability: Tmax (time point of maximum plasma concentration) of mangiferin was found to be 38.64 ng.mL^{-1} after administration of 0.9 g in a human trial[4]. That result shows that mangiferin could be mainly available in the colon.

⇒ Many studies have shown that human fecal bacteria (HFB) can have a great impact on polyphenol metabolism and that bioconverted forms of polyphenols (metabolites) probably have more biological importance than the native form present in diets[5].

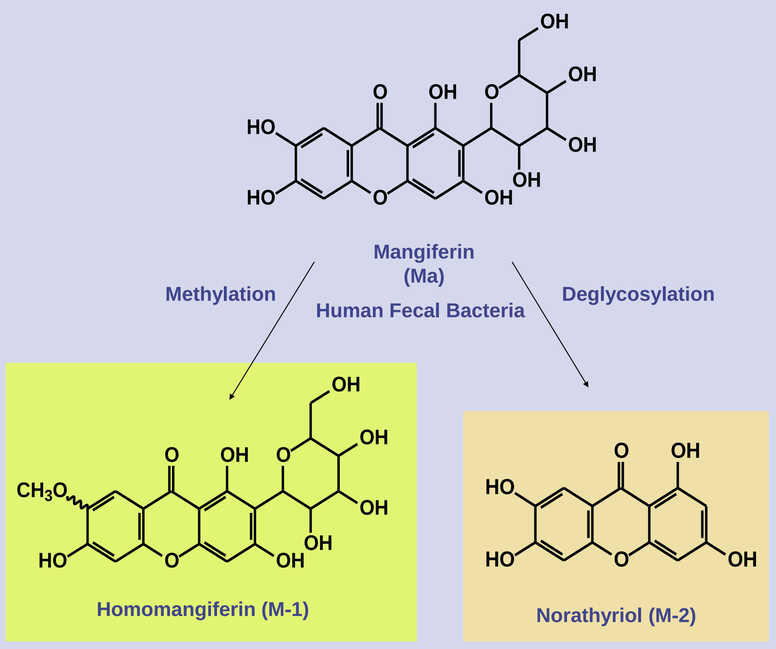


Figure 1 – Mangiferin metabolism by HFB *in vitro.*

METHODOLOGY

⇒ Intestinal simulation reactions (ISR) were performed to produce mangiferin metabolites by strict anaerobic fermentation *in vitro* using mixed fecal bacteria from volunteers samples (n = 3);

⇒ Mangiferin (50 mg) was mixed with 100 mg of a fecal sample in 100 mL of sterilized BHI broth inside 100 mL sterilized and uncovered Duran bottles. The bottles were placed along with two AnaeroGen sachets and an indicator of anaerobiosis in an anaerobic container that was hermetically closed;

⇒The container was incubated for 5 days (120h) at 37 °C and the solution was occasionally stirred. Samples (app. 1 mL) were taken from Duran bottles inside the container using a syringe connected to a tubular system coupled to the anaerobic container, centrifuged in eppendorf tubes at 14,000 r.p.m. for 5 minutes and afterwards, an aliquot of the samples (10 or 20 µL) were analysed by HPLC-ESI-MS.;

⇒Hewlett-Packard (HP) 1090 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a reverse-phase C18 column (250 mm × 4 mm i.d., 5 µm; Phenomenex, Aschaffenburg, Germany).

RESULTS

ISR of Mangiferin – Volunteer 1

⇒ Metabolites were screened every 12 h by HPLC-ESI-MS following solid-phase extraction. Norathyriol was purified by semipreparative reverse-phase HPLC (not shown).

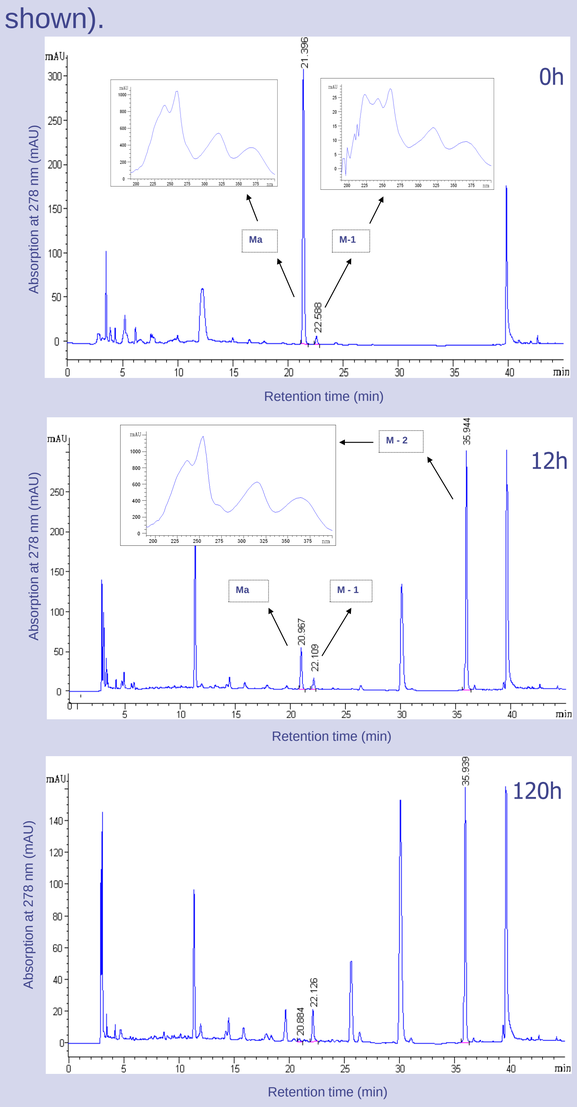


Figure 2 – HPLC-ESI-MS Screen at 0, 12 and 120h time points.

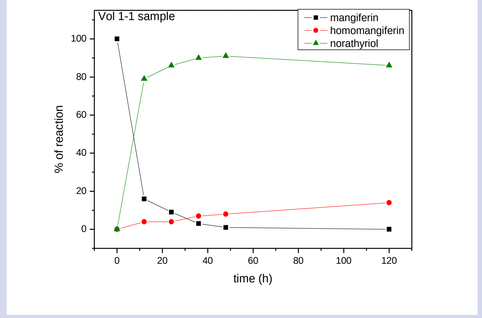
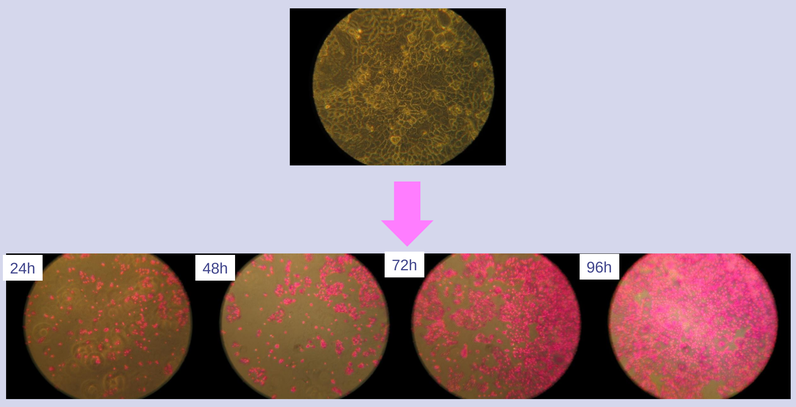


Figure 3 – Mangiferin metabolic kinetics

Cytotoxicity Assays of Mangiferin and Metabolites

⇒ Cytotoxicity of mangiferin (not shown) and norathyriol were tested in two human tumor cell lines, a lung adenocarcinoma (A240286S) and a colon tumor (Caco-2) cell line.

Figure 4 – Caco-2 cells growth curve pictures in Sulforhodamine B assay.



⇒ Norathyriol was much more cytotoxic than mangiferin with an IC_{50} of 51 µM in both the Caco-2 and lung cancer cells.

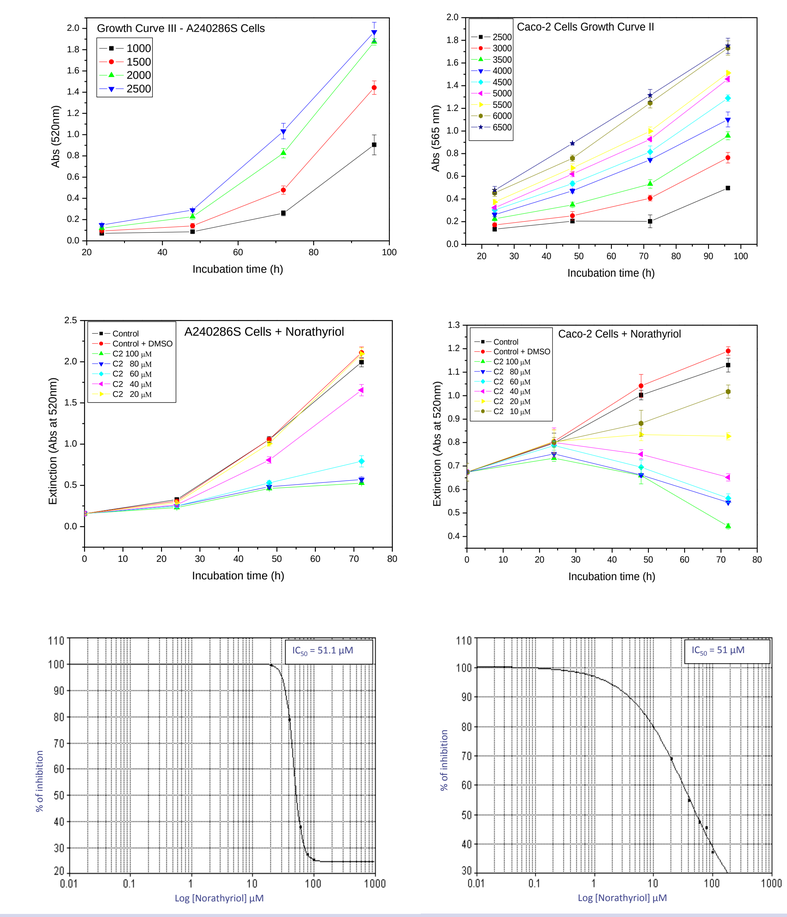


Figure 5 – Growth, Inhibition and IC_{50} curves.

CONCLUSION

⇒ The major metabolite was the aglycone of mangiferin namely norathyriol (confirmed by MS spectra and ^{1}H, ^{13}C NMR);

⇒ Norathyriol was much more cytotoxic than mangiferin with an IC_{50} of 51 µM in both the Caco-2 and lung cancer cells whereas mangiferin was ineffective. Showing potential as a chemopreventive agent against both colon and lung cancer;

⇒García-Rivera *et al.,*[3] have identified various target molecules in different transcription factor NFkB cancer pathways in metastatic breast cancer cells, which are sensitive to treatment with mangiferin. The mechanism of action of Norathyriol is currently under study in our laboratories.

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Acknowledgements

C A P E S

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