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Mangifera indica L. extract (Vimang®) inhibits 2-deoxyribose damage induced by Fe (III) plus ascorbate

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Vimang® is an aqueous extract of selected species of *Mangifera indica* L, used in Cuba as a nutritional antioxidant supplement. Many *in vitro* and *in vivo* models of oxidative stress have been used to elucidate the antioxidant mechanisms of this extract. To further characterize the mechanism of Vimang® action, its effect on the degradation of 2-deoxyribose induced by Fe (III)-EDTA plus ascorbate or plus hypoxanthine/xanthine oxidase was studied. Vimang® was shown to be a potent inhibitor of 2-deoxyribose degradation mediated by Fe (III)-EDTA plus ascorbate or superoxide (O_2^-). The results revealed that Vimang®, at concentrations higher than 50 μM mangiferin equivalent, was equally effective in preventing degradation of both 15 mM and 1.5 mM 2-deoxyribose. At a fixed Fe (III) concentration, increasing the concentration of ligands (either EDTA or citrate) caused a significant reduction in the protective effects of Vimang®. When ascorbate was replaced by O_2^- (formed by hypoxanthine and xanthine oxidase) the protective efficiency of Vimang® was also inversely related to EDTA concentration. The results strongly indicate that Vimang® does not block 2-deoxyribose degradation by simply trapping $^{\bullet}OH$ radicals. Rather, Vimang® seems to act as an antioxidant by complexing iron ions, rendering them inactive or poorly active in the Fenton reaction. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: Mangifera indica; Vimang; iron chelator; antioxidant.

INTRODUCTION

Interest in natural antioxidants has risen dramatically in recent times due to: (i) the antioxidative efficacy of a variety of phytochemicals; (ii) the consensus that foods rich in certain phytochemicals can affect the aetiology and pathology of chronic diseases and the aging process; (iii) the public's preconceived belief that natural compounds are innately safer than synthetic compounds and are thus more commercially acceptable (Seeram *et al.*, 2005; Liu, 2003; Meydani, 2002).

Polyphenolic compounds, including a large group of flavonoids, are abundant in vegetables, fruits, wine and tea. These compounds are generally known to possess potent antioxidant properties (Rice-Evans *et al.*, 1995), which depend on their free radical scavenging capacity and on their iron chelating activity (Morel *et al.*, 1994).

Recently, a standard aqueous stem bark extract from selected species of *Mangifera indica* L. (Anacardiaceae), which has been used as a food supplement in Cuba under the brand name of Vimang®, was reported to have potent *in vivo* and *in vitro* antioxidant activity (Martínez *et al.*, 2000; Sánchez *et al.*, 2000). Chemical studies performed with this extract permitted the isolation and identification of phenolic acids, phenolic esters, flavan-3-ols, mangiferin, which is the predominant component of this extract (Núñez-Sellés *et al.*, 2002), and micronutrients such as selenium (Center of Pharmaceutical Chemistry, 1998; Capote *et al.*, 1998).

A recent report demonstrated that an aqueous extract of *Mangifera indica* L stem bark (Vimang®) inhibited bleomycin-iron dependent and copper-phenantroline dependent DNA-damage. It also showed that the natural extract inhibited phospholipids peroxidation, with a powerful effect on nonenzymic peroxidation (with Fe (III)/ascorbate) (Martínez *et al.*, 2000). Although this work suggests some iron-complexing ability as an antilipoperoxidative mechanism of the natural extract, the exact mechanisms of iron interaction with Vimang® have not been established yet. To further characterize these mechanisms, the effect of the *Mangifera indica* L. extract on the iron-mediated degradation of 2-deoxyribose was studied.

MATERIALS AND METHODS

Plant extract. *Mangifera indica* L was collected from a cultivated field located in the region of Pinar del Río, Cuba. Voucher specimens (code 41722) were deposited at the Herbarium of the Academy of Science, guarded by the Institute of Ecology and Systematic from

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Ministry of Science, Technology and Environment, La Habana, Cuba. *Mangifera indica* L. extract (Vimang®) was kindly donated by the Centre of Pharmaceutical Chemistry (Havana, Cuba). It melts at 210–215 °C with decomposition. The chemical composition of this extract has been characterized by chromatographic (planar, liquid and gas) methods, mass spectrometry and UV/Vis spectrophotometry (Núñez-Sellés *et al.*, 2002). The total polyphenol content of Vimang® determined by the Folin-Ciocalteu method and expressed as gallic acid equivalents, was 34 g/100 g dry weight. The mangiferin content was determined by HPLC methods (Centre of Pharmaceutical Chemistry, 1998) and represents 16% of the powdered extract.

Reagents and solutions. Ascorbic acid, phosphate sodium salts, citrate, EDTA, dimethyl sulfoxide (DMSO), 2-deoxyribose, salicylate, thiobarbituric acid (TBA), hypoxanthine and xanthine oxidase were purchased from Sigma Chemical Co. (St Louis, MO). All other reagents were of analytical purity.

Stock solutions of Vimang® were prepared by dissolving the solid extract in DMSO, in a concentration range 5–100 mM, equivalent to mangiferin concentration (1.3–26.0 μg/mL powdered extract). Experiments were carried out with 1/1000 dilution (5–100 μM mangiferin concentration equivalents) to prevent solvent influence on the results.

Stock solutions of EDTA were neutralized with HCl/NaOH. Ferric chloride stock solutions (1 mM) were prepared daily in 10 mM HCl. Solutions of Fe (III) – EDTA (1:1 and 1:5 ratio) and Fe (III) – citrate (1:2 ratio) were also freshly prepared in HCl 10 mM. Stock solutions of 1% TBA were freshly prepared in 50 mM NaOH. All solutions were made with milli-Q deionized water.

Deoxyribose assay. The formation of $^{\bullet}OH$ radicals was measured using 2-deoxyribose oxidative degradation. The principle of the assay is the quantification of the main 2-deoxyribose degradation product, malonaldehyde (MDA), by its condensation with TBA (Hermes-Lima *et al.*, 2000). Typical reactions were started by addition of ascorbate (2 mM final concentration) to 1 mL of a solution containing 10 mM phosphate buffer (pH 7.2), 15 or 1.5 mM 2-deoxyribose, 50, 250 mM EDTA or 100 μM citrate, 10 or 50 μM Fe (III) and 5, 10, 25, 50 or 100 μM Vimang® (or no Vimang®). Ascorbate was added approximately 10 min after the addition of Vimang®. Reactions were carried out for 30 min at 37 °C in a shaking bath to ensure continuous flow of O_2 into the tubes and terminated by the addition of 1 mL of 4% phosphoric acid (v/v) followed by 1 mL 1% TBA solution. After boiling for 15 min, the absorbance of solutions at 532 nm was recorded. 'Zero time' absorbance values (where ascorbate is added to solutions after the phosphoric acid and TBA) were subtracted from the values obtained for each experimental condition.

When the hypoxanthine/xanthine oxidase system was substituted for ascorbate in reducing Fe (III) to Fe (II), reactions were started by adding 0.025 U xanthine oxidase (Hermes-Lima *et al*., 2000).

Vimang® (100 μM mangiferin equivalent) did not interfere with the reaction of MDA and TBA (data not shown).

The results shown as 'Damage to 2-deoxyribose (% of control)' were calculated as: [(sample A_{532} – 'zero time' A_{532})/(Control A_{532} – 'zero time')] × 100.

Statistical analysis. The results are presented as the mean ± SD and statistical significance between the groups was determined by means of one-way analysis of variance (ANOVA) followed by unpaired Student's t-test to determine statistical significance. Values of p lower than 0.05 ($p < 0.05$) were considered as indicative of significance.

RESULTS AND DISCUSSION

The inhibitory action of Vimang® on 2-deoxyribose degradation mediated by Fe (III)-EDTA plus ascorbate was investigated.

Competition studies were performed in order to evaluate the effectiveness of Vimang® and two $^{\bullet}OH$ scavengers (DMSO and salicylate) in protecting 1.5 or 15 mM 2-deoxyribose from iron-mediated oxidative damage (Fig. 1). The $^{\bullet}OH$ scavengers at 20 mM protected 15 mM 2-deoxyribose significantly less than 1.5 mM 2-deoxyribose ($p < 0.05$).

Vimang® protection at 5, 10 and 25 μM showed a similar pattern to those of DMSO and salicylate ($p < 0.05$), but was equally effective in preventing oxidative degradation of both 1.5 and 15 mM 2-deoxyribose at higher concentrations (50–100 μM).

These results suggest that Vimang® at elevated concentrations, in contrast to typical $^{\bullet}OH$ scavengers, does not interfere with the reaction between 2-deoxyribose and $^{\bullet}OH$ radicals and supports the proposal

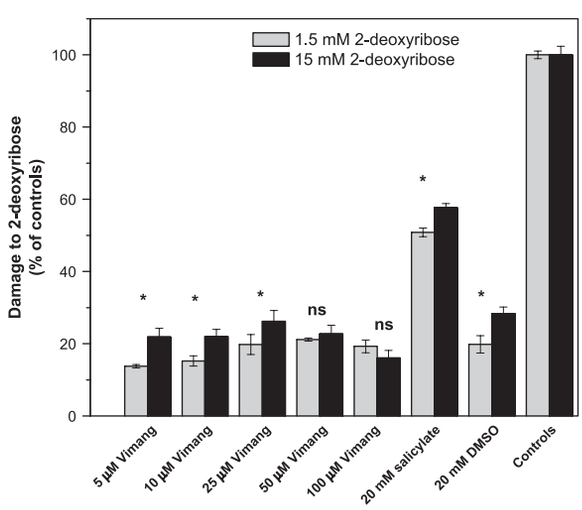

Figure 1. Titration graphics for the effect of Vimang and $^{\bullet}OH$ scavengers DMSO and salicylate on oxidative damage to 1.5 or 15 mM 2-deoxyribose induced by Fe (III)–EDTA plus ascorbate. Solutions were incubated for 30 min at 37 °C and contained 10 mM phosphate buffer (pH 7.2), 2-deoxyribose (1.5 or 15 mM), 50 μM EDTA and 10 μM Fe (III). The concentrations of Vimang, DMSO and salicylate are as indicated on the *x*-axis. Reactions were started by addition of ascorbate to a final concentration of 2 mM. The bars show mean ± SD ($n = 3$). Controls contain only DMSO (14.3 μM), which is the solvent concentration in the Vimang samples. A_{532} values for controls were 0.35 ± 0.01 and 1.06 ± 0.11 for 1.5 and 15 mM 2-deoxyribose, respectively. One tailed *t*-test was used for statistical analyses; * $p < 0.05$; ns, not significant.

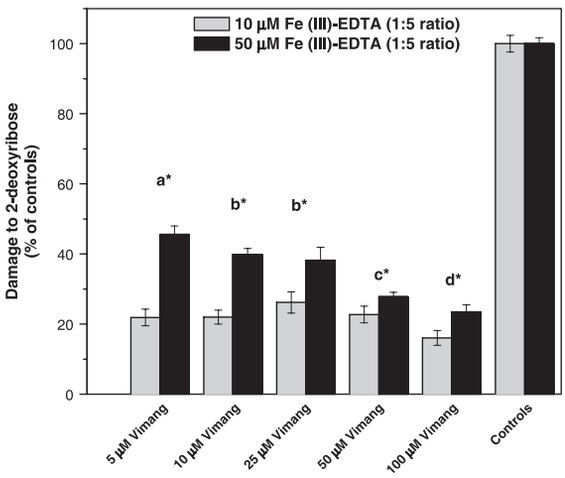

Figure 2. Effect of Vimang concentration on 2-deoxyribose degradation caused by Fe (III)–EDTA plus ascorbate. Experimental conditions are as described in the legend to Fig. 1, except that two concentrations of Fe (III) were used; 10 and 50 μM. Fe (III)–EDTA concentration ratios were kept at 1:5. The bars show mean ± SD ($n = 3$). Controls are also as described in the legend of Fig. 1, except that the A_{532} were 1.06 ± 0.11 and 2.45 ± 0.12 for 10 and 50 μM Fe (III)–EDTA, respectively. One tailed *t*-test was used for statistical analyses; different letters indicate significant difference ($p < 0.05$) between Vimang concentrations at 50 μM Fe (III). * Significance ($p < 0.05$) versus respective reactions with 50 μM Fe (III).

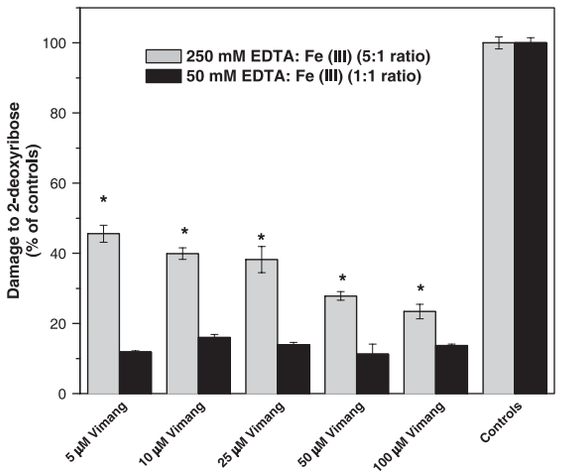

Figure 3. Effect of Vimang concentration on 2-deoxyribose degradation caused by Fe (III)–EDTA plus ascorbate. Solutions were incubated for 30 min at 37 °C and contained 10 mM phosphate buffer (pH 7.2), 15 mM 2-deoxyribose, 50 μM Fe (III)–EDTA (with 50 or 250 μM EDTA), Vimang (5–100 μM) and 2 mM ascorbate. The bars show mean ± SD ($n = 3$). Controls are as described in the legend of Fig. 1, except that the A_{532} were 2.03 ± 0.26 and 2.45 ± 0.12 for 50 and 250 μM EDTA, respectively. Significance versus respective reactions with 50 μM EDTA (one tailed *t*-test): * $p < 0.05$.

that it acts by preventing •OH formation from Fe (III) – EDTA plus ascorbate rather than by trapping •OH radicals.

Vimang® at 5 μM allowed only 14%–20% of iron-induced damage to 1.5/15 mM 2-deoxyribose. DMSO and salicylate at mM concentrations afforded less protection than Vimang® at μM levels. These results are consistent with the fact that classical antioxidants are effective only in mM concentrations in aqueous solutions and suggest that Vimang® works as an antioxidant by a different mechanism than the •OH scavengers.

The non-existence of a dose-dependent profile in Vimang® protection from 2-deoxyribose degradation at 10 μM Fe (III) could be due to a complete iron complexation by 5 μM Vimang® concentration; therefore a comparative study was performed to evaluate the effectiveness of Vimang® in preventing 10 μM or 50 μM iron-induced damage to 15 mM 2-deoxyribose. Figure 2 shows that Vimang® was more effective in preventing 2-deoxyribose degradation at 10 than at 50 μM Fe (III) concentration ($p < 0.05$). The figure also shows that Vimang® reduced 50 μM iron-induced damage to 2-deoxyribose in a dose-dependent fashion (a, b, c, d, $p < 0.05$).

Titration of Vimang® in the 2-deoxyribose assay was performed with two different concentrations of EDTA at a fixed Fe (III) concentration of 50 μM (Fig. 3). Vimang® was less effective in preventing 2-deoxyribose degradation at higher concentrations of EDTA ($p < 0.05$). For example, the degradation of 2-deoxyribose at 5 μM Vimang® concentration increased from 11.8% to 45.6%, which represents 33.8% protection reduction at higher EDTA concentrations. These data suggest that Vimang® inhibits 2-deoxyribose degradation by removing Fe (III) from EDTA, forming a complex with iron that cannot participate in the Fenton reaction. With high levels of EDTA, less iron

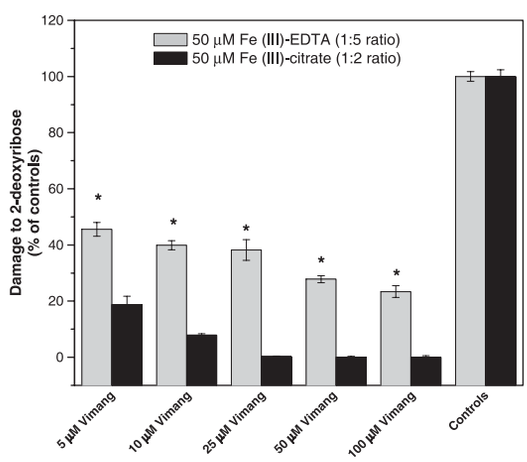

Figure 4. Effect of Vimang concentration on 2-deoxyribose degradation caused by Fe (III)–citrate (1:2) and Fe (III)–EDTA (1:5) plus ascorbate. Solutions were incubated for 30 min at 37 °C and contained 10 mM phosphate buffer (pH 7.2),15 mM 2- deoxyribose, 50 μM Fe (III), 100 μM citrate and 250 μM EDTA. The concentrations of Vimang are as indicated on the *x*-axis. The reactions were started by the addition of ascorbate to a final concentration of 2 mM. The bars show mean ± SD ($n = 3$). The controls are as described in the legend of Fig. 1. A_{532} values for controls were 0.64 ± 0.02 and 2.45 ± 0.12 for citrate and EDTA, respectively. One tailed *t*-test was used for statistical analyses; significance versus respective reactions: * $p < 0.05$.

is available for complexation with Vimang®, resulting in less protection against 2-deoxyribose degradation. A similar experiment was performed with complexes of citrate and Fe (III) (Fig. 4). These results show that citrate allows for a greater protection of 2-deoxyribose by Vimang® ($p < 0.05$) than EDTA. Since citrate forms a weaker complex with Fe (III) compared with EDTA (Roche *et al.*, 1990), Vimang® removes the metal from citrate more easily, resulting in more effective protection against iron-mediated damage to 2-deoxyribose.

Most of the experiments show high efficiency of Vimang® in preventing iron-induced 2-deoxyribose damage that could also be explained by the formation of a redox active mangiferin–Fe (III) complex that mimics superoxide dismutase activity, which maintains the Fe-chelate within a continuous Fe (III)–Fe (II)–Fe (III) conversion with no significant accumulation of Fe (II) or superoxide. A recent report demonstrated that the 2:1 flavonoids–Fe (III) complexes were more effective than the parent compounds in scavenging superoxide radicals generated by xanthine oxidase/hypoxanthine (Moridani *et al.*, 2003).

It was recently shown (Pardo Andreu *et al.*, 2005) that Vimang® prevented Fe (II) citrate induced lipoperoxidation in isolated rat liver mitochondria, mainly through its ability to stimulate the rate of oxygen consumption (insensitive to superoxide dismutase) due to Fe (II) autoxidation, forming complexes with Fe (III) that do not participate in Fenton-type reactions and lipid peroxidation.

Vimang® also protects 2-deoxyribose from oxidative damage induced by hypoxanthine, xanthine oxidase and Fe (III) – EDTA. Superoxide radicals (O_2^-) formed in the hypoxanthine/xanthine oxidase system substitute for ascorbate in reducing Fe (III) to Fe (II). Three different concentrations of EDTA were tested (50, 100 and 250 μM) with 50 μM Fe (III) (Fig. 5). The protective efficiency of Vimang® (at 25 or 50 μM) was inversely related to the EDTA concentration tested ($p < 0.05$), which suggested that the antioxidant action of Vimang® could be based on its ability to remove Fe (III) from EDTA.

Martínez *et al.* (2001) recently reported that lipoperoxidation induced by addition of ascorbic acid and Fe (III) to rat liver microsomes was markedly inhibited by Vimang® (IC_{50} = 0.0126% w/v). They also showed a potent antioxidant action of Vimang® in the NADPH-dependent peroxidation system, in addition to the previously invoked mechanism, which might be due to its ability to scavenge H_2O_2. Similar results were obtained by Sato *et al.* (1992) who evaluated mangiferin in the same model.

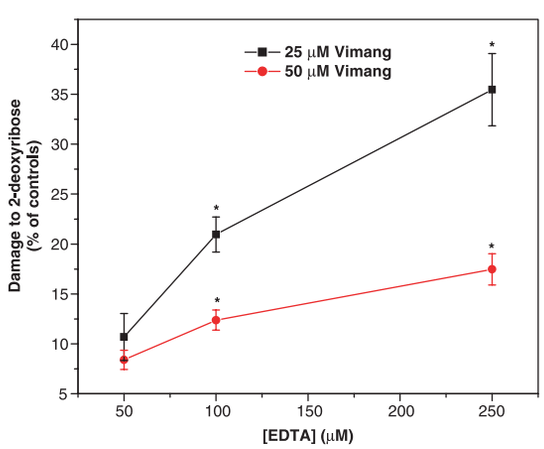

Figure 5. Effect of Vimang (25 and 50 μM) on the oxidative degradation of 2-deoxyribose induced by 200 μM hypoxanthine, 0.025 U xanthine oxidase and 50 μM Fe (III)–EDTA. Solutions were incubated for 30 min at 37 °C and contained 10 mM phosphate buffer (pH 7.2), 15 mM 2- deoxyribose, 50 μM Fe (III). The concentrations of EDTA are as indicated on the *x*-axis. Reactions were started by adding xanthine oxidase. A_{532} values for controls were 0.64 ± 0.02, 0.59 ± 0.12 and 0.63 ± 0.07 for 50, 100 and 250 μM EDTA, respectively. The figures show mean ± SD (n = 3). One tailed *t*-test was used for statistical analyses; significance versus respective reactions with different EDTA concentrations: * $p < 0.05$.

In the Vimang® extract, the major polyphenolic component is mangiferin, a glucosylxanthone, which is probably the main substance responsible for the extract antioxidant and antiinflammatory properties (Martinez *et al.*, 2000; Martinez *et al.*, 2001; Sanchez *et al.*, 2000; Garrido *et al.*, 2001; Garrido *et al.*, 2004) and could also be strongly implicated in its iron complexing properties, although further research must be done to clarify mangiferin–iron interactions.

Fe (III)–EDTA/ascorbate slowly generates $^{\bullet}OH$ radicals, causing 2-deoxyribose degradation (Hermes-Lima *et al.*, 1994). The process of $^{\bullet}OH$ formation and 2-deoxyribose damage may occur *via* the following sequence of reactions:

$$\text{Fe(III)} - \text{EDTA} + \text{ascorbate} \rightarrow \text{Fe(II)} - \text{EDTA} + \text{ascorbyl} \quad (1)$$

$$\text{Fe(II)} - \text{EDTA} + O_2 \rightarrow \text{Fe(III)} - \text{EDTA} + O_2^- \quad (2)$$

$$2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2 \quad (3)$$

$$H_2O_2 + \text{Fe(II)} - \text{EDTA} \rightarrow \text{Fe(III)} - \text{EDTA} + OH^- + {}^{\bullet}OH \quad (4)$$

$$^{\bullet}OH + \text{2-deoxyribose} \rightarrow \text{degradation products} \quad (5)$$

According to these reactions, Vimang® could prevent iron-induced 2-deoxyribose damage: (i) by removing Fe (III) from EDTA (Eqn 1), which slows the rate of Fe (III) reduction by ascorbate or O_2^-; (ii) by removing Fe (II) from EDTA (Eqn 2), which prevents iron participation in Fenton reactions; (iii) by reducing the H_2O_2 concentration, a fundamental component in Fenton-type reaction (Eqn 3); (iv) by scavenging superoxide and/or $^{\bullet}OH$ radicals (Eqns 3 and 5).

The mechanism of antioxidant polyphenol action has usually been attributed to $^{\bullet}OH$ scavenging activity (Sah *et al.*, 1995) and this could be true for Vimang®, which contains a polyphenol mixture of 34% w/w. However, the results strongly indicate that Vimang® does not block 2-deoxyribose degradation by simply trapping $^{\bullet}OH$ radicals. Rather, Vimang® seems to act as an antioxidant by complexing iron, forming complexes that block iron participation in Fenton reactions. Grinberg *et al.* (1997) also concluded that the protective activity of tea polyphenols against $^{\bullet}OH$-dependent salicylate hydroxylation was due to iron chelation.

It is of particular interest that a 5 μM mangiferin-equivalent concentration of Vimang was able to produce significant protection against 2-deoxyribose damage mediated by 10 or 50 μM iron. These concentrations are lower than those of classical iron chelators such as deferoxamine and pyridoxal isonicotinoyl hydrazone (Mauricio *et al.*, 2003). Since *in vivo* levels of free or loosely bound iron are hardly higher than 1 μM, even in iron overload (Halliwell and Gutteridge, 1999), very low concentrations of the extract components would afford protection against iron-mediated damage. These results suggest that the observed *in vitro* effects of this compound could be relevant to *in vivo* conditions of iron overload. Indeed, the efficacy in the ethnomedical use of Vimang® as an antiinflammatory and cancer preventive agent (Guevara *et al.*, 1998) could

be partially attributed to its iron complexing ability, because these human pathologies are related to increased intracellular iron levels (Sheth and Brittenham, 2000).

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REFERENCES

Capote R, Guardado I, Novoa H *et al.* 1998. Chemical-analytical characterization of aqueous extract of *Mangifera indica* L. *Rev Cub Quim* **10**: 111–112.

Centre of Pharmaceutical Chemistry. 1998. Pharmaceutical compositions including a mixture of polyphenols, terpenoids, steroids, fatty acids, and microelements with antioxidant, analgesic, anti-inflammatory, and anti-spasmodic properties. *Patent 203/98*; OCPI-Havana, Cuba.

Dorman HJD, Hiltunen R. 2004. Fe (III) reductive and free radical-scavenging properties of summer savory (*Satureja hortensis* L.) extract and subfractions. *Food Chem* **88**: 193–199.

Garrido G, Gonzalez D, Delporte C *et al.* 2001. Analgesic and anti-inflammatory effects of *Mangifera indica* L. extract (Vimang). *Phytother Res* **15**: 18–21.

Garrido G, Gonzalez D, Lemus Y *et al.* 2004. *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract (VIMANG). *Pharmacol Res* **50**: 143–149.

Grinberg LN, Newmark H, Kitrossky N, Rahamin E, Chevion M, Rachmilewitz EA. 1997. Protective effects of tea polyphenols against oxidative damage to red blood cells. *Biochem Pharmacol* **54**: 973–978.

Guevara GM, González LS, Alvarez LA, Riaño MA, Garrido GG, Núñez-Selles AJ. 2004. Uso etnomédico de la corteza de *Mangifera indica* L. en Cuba. *Rev Cubana Plant Med*. **9**: 40–46.

Halliwell B, Gutteridge JMC. 1999. Antioxidant defences. In *Free Radical in Biology and Medicine*, Halliwell B, Gutteridge JMC (eds). Oxford University Press: Oxford; 105–245.

Hermes-Lima M, Ponka P, Schulman MH. 2000. The iron chelator pyridoxal isonicotinoyl hydrazone (PIH) and its analogues prevent damage to 2-deoxyribose mediated by ferric iron plus ascorbate. *Biochim Biophys Acta* **1523**: 154–160.

Hermes-Lima M, Wang EM, Schulman HM, Storey KB, Ponka P. 1994. Deoxyribose degradation catalyzed by Fe (III) EDTA: kinetic aspects and potential usefulness for submicromolar iron measurements. *Mol Cell Biochem* **137**: 65–73.

Liu RH. 2003. Health benefits of fruit and vegetable are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* **78**: 517S–520S.

Martinez G, Delgado R, Perez G, Garrido G, Núñez-Sellés AJ, Leon OS. 2000. Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L extract (VIMANG). *Phytother Res* **14**: 424–427.

Martinez G, Giuliani A, Leon OS, Perez G, Nunez-Selles AJ. 2001. Effect of *Mangifera indica* L. extract (QF808) on protein and hepatic microsome peroxidation. *Phytother Res* **15**: 581–585.

Mauricio AQ, Lopez KBG, Gomes CS, Oliveira RG, Alonso A, Hermes-Lima M. 2003. Pyridoxal isonicotinoyl hydrazone inhibits iron-induced ascorbate oxidation and ascorbyl radical formation. *Biochim Biophys Acta* **1620**: 15–24.

Meydani M. 2002. The Boyd Orr lecture. Nutrition interventions in aging and age associated disease. *Proc Nutr Soc* **61**: 165–171.

Morel I, Lescoast G, Cillard P, Cillard J. 1994. Role of flavonoids and iron chelation in antioxidant action. *Methods Enzymol* **234**: 437–443.

Moridani, MY, Pourahmad J, Bui H, Siraki A, O'Brien PJ. 2003. Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. *Free Radic Biol Med* **34**: 243–253.

Núñez-Sellés A, Vélez-Castro H, Agüero-Agüero J *et al.* 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars, fatty acids and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as nutritional supplement. *J Agric Food Chem* **50**: 762–766.

Pardo-Andreu G, Delgado R, Velho J, Inada MN, Curti C, Vercesi AE. 2005. *Mangifera indica* L. extract (Vimang) inhibits Fe^{2+}-citrate-induced lipoperoxidation in isolated rat liver mitochondria. *Pharmacol Res* **51**: 427–435.

Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res* **22**: 375–383.

Roche M, Desbarres J, Colin C, Hardy A, Bauer D. 1990. *Chimie des Solutions*. Collection Info Chimie, Technique et Documentation, Lavoisier: Paris.

Sah NK, Kumar S, Subramanian M, Devasagayam TP. 1995. Variation in the modulation of superoxide-induced single strand breaks in plasmid pBR322 DNA by biological antioxidants. *Biochem Mol Biol Int* **35**: 291–296.

Sánchez GM, Re L, Giuliani A, Núñez-Sellés A, Davison GP, León- Hernández OS. 2000. Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. *Pharmacol Res* **42**: 565–573.

Sato T, Kawamoto A, Tamura A, Tatsum Y, Fuiii T. 1992. Mechanism of antioxidant action of Pueraria glycoside (PG)-1 (an isoflavonoid) and mangiferin (a xanthonoid). *Chem Pharm Bull* **40**: 721–724.

Seeram NP, Adams LS, Henning SM *et al.* 2005. *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalgin, ellagic acid and total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J Nutr Biochem* **16**: 360–367.

Sheth S, Brittenham GM. 2000. Genetic disorders affecting proteins of iron metabolism: clinical implications. *Annu Rev Med* **51**: 443–464.

