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Evaluation of the *in vitro* Antioxidant Activity of *Mangifera indica* L. Extract (Vimang)

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An extract of *Mangifera indica* L. (Vimang) was tested *in vitro* for its antioxidant activity using commonly accepted assays. It showed a powerful scavenger activity of hydroxyl radicals and hypochlorous acid and acted as an iron chelator. The extract also showed a significant inhibitory effect on the peroxidation of rat-brain phospholipid and inhibited DNA damage by bleomycin or copper-phenanthroline systems. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Mangifera indica L.; antioxidant activity; DNA damage; lipid peroxidation.

INTRODUCTION

There are increasing suggestions that reactive oxygen species may play a role in the pathogenesis of cancer (Dreher and Junod, 1996; Oberley and Oberley, 1997) and in other diseases including inflammation, bacterial infections, AIDS, etc (Maxwell, 1997).

Several authors have reported pharmacological activities of *Mangifera indica* (Anacardiaceae) extracts including antispasmodic analgesic, antipyretic and other effects (Coe and Anderson, 1996; Awe *et al.*, 1998; Das *et al.*, 1989), but very little on the antioxidant activity. There are, however, reports that flavonoids possess antioxidant activity (Catapano, 1997). Since these are also components of Vimang, we tested the extract of *Mangifera indica* for antioxidant effects *in vitro* (i.e. protection against oxygen-derived species) and the possible pro-oxidant action, using commonly accepted assays.

MATERIAL AND METHODS

Plant extract. Stem bark extract of *M. indica* was prepared as previously described of (OCPI Patent 203/1998) and dissolved in distilled water for pharmacological studies.

Phospholipid peroxidation. The ability of Vimang to inhibit peroxidation of membrane lipids at pH 7.4 was tested using rat-brain phospholipid as described by Aruoma *et al.* (1992).

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The interference of the extract with colour development during the TBARS assay was determined by adding a previously determined concentration of Vimang to the TBA reagents and used to determine the extent of peroxidation of rat-brain phospholipids (Aruoma *et al.*, 1996).

Bleomycin-iron dependent and copper-phenanthroline dependent DNA damage. The bleomycin and the copper-phenanthroline assays were conducted to test the pro-oxidant activity of Vimang. Salmon testes DNA was used as a target in these assays (Auroma, 1993).

Deoxyribose assay. The deoxyribose assay (Auroma, 1994) was used to detect hydroxyl radical (OH·) scavenger activity and the iron-binding ability of Vimang.

Reaction with hypochlorous acid (HOCl). This was studied using the elastase assay (Wasil *et al.*, 1987). Control experiments were also carried out to test any direct effect of Vimang on α_1-antiproteinase (α_1-AP) or elastase (Aruoma *et al.*, 1992).

Thiobarbituric acid-reactive substance (TBARS) assay. TBARS were determined as an indicator of phospholipid peroxidation and as a measure of the extent of DNA and deoxyribose damage. The assay was conducted as described by Buege and Aust (1978).

All chemicals and reagents used were purchased from Sigma (St Louis MO, USA) Aldrich, or BDH (Poole).

RESULTS AND DISCUSSION

Rat brain phospholipids undergo spontaneous autoxidation or rapid non-enzymatic peroxidation when incubated in the presence of ferric chloride and ascorbic acid. The effects of Vimang on spontaneous and non-enzymatic peroxidation are shown in Table 1. Vimang inhibited

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Table 1. Effect of Vimang (*Mangifera indica* extract) on rat brain phospholipid peroxidation

Spontaneous autoxidation			Non-enzymic peroxidation		
Sample	Extent of peroxidation (A 532 nm)	Inhibition (%)	Sample	Extent of peroxidation (A 532 nm)	Inhibition (%)
None	0.341	–	None	1.317	–
Trolox C	0.187	45	–	–	–
Vimang conc			Vimang conc		
0.2	0.163	52	0.024	0.351	73
0.1	0.205	40	0.012	0.428	67
0.05	0.208	39	0.659	0.659	50
0.025	0.255	25	0.003	1.138	14
0.0125	0.290	15	0.0015	1.290	2

Trolox C, established antioxidant, positive control.
Values are mean of triplicate experiments, varying by not more than 10%.
Vimang concentrations expressed as %w/v.

Table 2. Effects of Vimang on bleomycin-iron and copper phenanthroline-mediated DNA damage[a]

	Extent of DNA damage (A_{532} nm)			
Sample	Bleom-Fe^{3+}	Bleom + Fe^{3+} + Asc	Cu^{2+}-Phenant	Cu^{2+}-Phenant + Asc
None	0.019	0.163	0.169	0.233
Asc	0.163	–	0.233	–
Vimang conc				
0.024	0.021	0.008	0.042	0.083
0.012	0.018	0.022	0.065	0.102
0.006	0.026	0.029	0.173	0.115
0.003	0.014	0.041	0.114	0.154
0.0015	0.020	0.105	0.138	0.231

Values are the means from triplicate experiments with values agreeing to within 5%.
Bleom, bleomycin; Asc, ascorbate 0.2 mM; Phenant, phenanthroline.
Vimang concentration are indicated as % w/v.

phospholipid peroxidation showing a powerful effect on non-enzymic peroxidation (IC_{50} = 0.008% w/v,) compared to with spontaneous autoxidation (IC_{50} = 0.209%). This difference may have been caused by an iron-binding or free radical iron scavenging ability of the extract. In addition, the inhibition of phospholipid peroxidation by Vimang in the spontaneous autoxidation system was higher than those determined for other plant extracts in similar assays (Aruoma *et al.*, 1996).

Bleomycin and iron III (Fe^{3+}) and cupric (Cu^{2+}) ions with phenanthroline mixtures, in the presence of a reducing agent, mediate damage to DNA which could be detected using a TBA assay (Auroma, 1993). Data in Table 2 show that the extract did not promote DNA damage in either of these systems. In both cases, the extent of DNA damage was similar to or lower than the basal value which demonstrated that it did not show pro-oxidant activity.

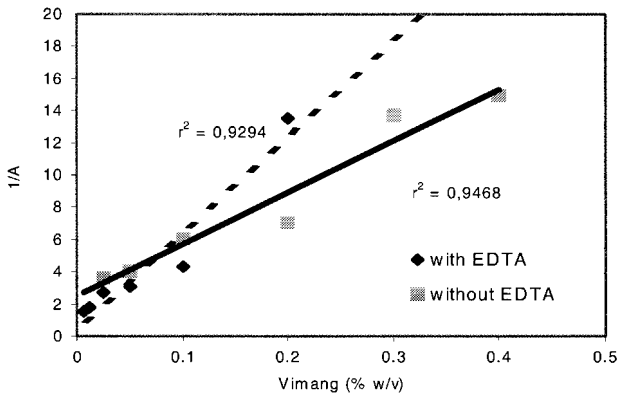

Figure 1. Action of Vimang extract on hydroxyl radical-dependent degradation of deoxyribose.

In the assay where ascorbic acid was added, the extract showed a concentration-dependent protection to DNA damage. The square correlation coefficient between the logarithm of concentration and the percentage of DNA protection was 0.80 and 0.88 for bleomycin-iron III/ascorbic acid and phenanthroline-cupric II ions/ascorbic acid, respectively. The half protective concentration was estimated to be 0.016% and 0.0084% w/v in each case.

These differences are probably related to the way in which each method caused damage to DNA. In the bleomycin-iron III system, the sugar moiety of DNA is predominantly damaged, while in the copper phenanthroline system the damage seems to be predominantly directed at the bases (Aruoma, 1993).

Hydroxyl radicals (OH·) are generated in a reaction mixture containing ascorbate, hydrogen peroxide and iron III-EDTA at pH 7.4 and measured by their ability to degrade the sugar deoxyribose (Aruoma, 1994). The extent of inhibition is dependent on the concentration of the scavenger and its rate constant for reaction with OH·. Vimang was found to be a powerful scavenger of the hydroxyl radicals (Fig. 1, with EDTA). The EC_{50} for the deoxyribose degradation was 0.011% w/v.

When ferric ions were added to the reaction mixture as iron III chloride ($FeCl_3$) instead of EDTA complex, some of the ions formed a complex with deoxyribose. This

Table 3. Scavenging of hypochlorous acid (HClO) by Vimang extract

	Elastase activity ($\Delta A_{410\ nm}\ s^{-1} \times 10^{-3}$)	Activity of α_1-AP in inhibiting elastase (%)
A. Addition to reaction mixtures		
Elastase (0.03 mg/mL)	13.2	–
+ HOCl (75 μM)	13.0	1
+ α_1AP (0.77 mg/mL)	0.0	100
+ (HOCl + α_1AP)	12.9	2
+ QF−808 0.10	13.0	1
+ QF−808 0.10 + α_1AP	0.0	100
B. Addition of Vimang to elastase assay mixture HOCl + α_1-antiproteinase + elastase		
0.10	5.5	58
0.06	6.2	53
0.04	5.6	57
0.02	8.3	37
0.01	9.6	27

Values are the average of triplicate measurements that agree to within 10%. Activity of α_1-AP in inhibiting elastase was calculated as 100−% of elastase activity detected.
Vimang concentrations are indicated as % w/v.

complex could be reduced by ascorbate to iron II, which could remain attached to deoxyribose and subsequently react with hydrogen peroxide. This reaction produced the necessary OH· radicals, which immediately started the deoxyribose degradation in a site-specific manner. The resulting deoxyribose degradation is not inhibited by OH· scavengers at moderate concentration. Molecules that can inhibit deoxyribose degradation are those that have iron-binding capacity and can withdraw the iron ions from the deoxyribose and render them inactive or poorly active in the Fenton reaction (Aruoma, 1994). Figure 1 (without EDTA) shows that Vimang had an inhibitory effect on deoxyribose degradation when $FeCl_3$-EDTA was replaced by $FeCl_3$. EC_{50} here was 0.117% w/v. It indicated that Vimang is a good scavenger of OH· and had a significant effect on the reduction of ion-dependent OH· generation from H_2O_2, probably by chelating iron.

Hypochlorous acid (HOCl) is produced by the neutrophyl-derived enzyme myeloperoxidase at the site of inflammation and when activated neutrophyls infiltrate reoxygenated tissue (Wasil *et al.*, 1987). HOCl can attack many biological molecules, but one of the most important is α_1-antiproteinase, the major inhibitor in human plasma of proteolytic enzymes, such as elastase. Thus, the ability of a compound to protect α_1-antiproteinase against inactivation by HOCl in a physiologically meaningful way, is a good test of whether that compound might be capable of scavenging HOCl at a biologically significant rate (Wasil *et al.*, 1987). Table 3 shows that Vimang did not affect the elastase activity or the inhibitory action of α_1-antiproteinase, while preincubation of HOCl with Vimang at different concentrations prevented the inhibition of α_1-antiproteinase added subsequently. Hence, it could scavenge HOCl in a concentration-dependent manner. There was a linear correlation between log conc Vimang and the activity of α_1-antiproteinase for the inhibition of elastase ($r = 0.93$), giving an EC_{50} of 0.04% w/v.

Since this extract has been used in traditional medicine as an antiinflammatory and cancer preventive agent, it may be that its efficacy could be partially attributed to its free radical scavenging activity. This behaviour has been also reported in *Uncaria tomentosa* bark extracts (Desmarchelier *et al.*, 1997), and results observed in *Mangifera indica* are slightly higher than those obtained with *U. tormentosa* extracts.

On the other hand, in the Vimang extract, the major component is mangiferin, a flavonoid (glucosylxanthone) which has been reported to have various pharmacological activities, such as antitumour, immunomodulatory and anti-HIV effects (Guha *et al.*, 1996), inhibition of respiratory burst in rat neutrophils (Hsu *et al.*, 1997), and antioxidant activity (Born *et al.*, 1996). In our investigations, the antioxidant activity detected is probably due to the mangiferin present in the extract.

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