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Evaluation of the antioxidant properties of a methanolic extract from 'Juice Plus fruit' and 'Juice Plus vegetable' (dietary supplements)

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The antioxidant properties of methanolic extracts from 'Juice Plus fruit' and 'Juice Plus vegetable', which are sold in capsule form as dietary supplements, were evaluated using a range of established *in vitro* antioxidant assays. The values are compared to representative extracts from equivalent amounts (by dry weight) of selected fruits and vegetables prepared by the same method. Although there are some differences between the results of each assay, in general Juice Plus performed about equally to the fresh fruit and vegetables on a dry weight basis. This implies that, based on *in vitro* assays to measure antioxidant potential, one capsule of 'Juice Plus fruit' or 'Juice Plus vegetable' (weighing 1 g) is equivalent to about 10 g (fresh weight) of fruit or vegetable. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Based on data from a large number of nutritional, biochemical, epidemiological and food studies, the World Health Organization recommends the consumption of at least five portions of fruit and/or vegetables per day. The health benefits from increased consumption of fruit and vegetables arise from an increased intake of antioxidant vitamins and other nutrients (Krinsky, 1993; Manson *et al.*, 1993; Block, 1993; Packer, 1992), and extra benefit may be obtained from an adequate consumption of 'extranutrients'[1], such as sulphur-containing compounds, flavonoids and other phenolic compounds (Johnson *et al.*, 1994). As an alternative to consuming five portions per day, some manufacturers are now selling dietary supplements which are aimed at topping up or increasing the dietary intake of antioxidants. The benefits of taking supplements containing mixtures of pure known antioxidants (e.g. vitamins E and C) can generally be assessed from the many published studies on these compounds (e.g. Gershoff, 1993; Packer, 1991; Machlin, 1991). However, there are now formulations available which are based on whole or partial extracts of foods. These are more difficult to assess since, in addition to vitamins, they may contain a wide range of compounds which may be classed as 'extranutrients'. Juice Plus capsules are an example of this new type of supplement, and they are sold as direct replacements for fruit and vegetables in the diet of individuals who are too busy to shop and prepare five portions of produce every day.

To assess the antioxidant properties of these materials, we have used a range of established assays to analyse the antioxidant nature of Juice Plus fruit (JPF) and Juice Plus vegetable (JPV) capsules. JPF capsules contain dried fruit juice (33%), dried fruit pulp (19%) (from apples, oranges, pineapples, cranberries and peaches), citrus fibre (30%), together with gelatin, vitamin C, papaya powder, pineapple powder and beta-carotene. JPV capsules contain dried juice (32%) and pulp (21%) (from carrot, parsley, beet, broccoli, kale, cabbage and spinach), sugarbeet fibre (33%), gelatin and beta-carotene (0.21%). The results are assessed relative to comparable amounts of freeze-dried fruit and vegetables.

MATERIALS AND METHODS

Materials

Juice Plus fruit blend and Juice Plus vegetable blend were a gift from Premier Incomes, UK. Aliquots of non-diseased human liver were supplied by the Transplant Unit of Addenbrookes Hospital, Cambridge, UK, and

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[1]'Extranutrients' are bioactive non-vitamin components of the diet, and are also called non-nutrient bioactive components, dietary phytoprotectants or protective factors.

were stored at −40°C. NADPH, ADP, ferrozine, deoxyribose and other biochemicals were obtained from Sigma Chemical Co., Poole, UK.

Preparation of extracts

Extracts were made essentially as described by Tawfiq *et al.* (1994), using a standard method to extract components, as described below. Aliquots (0.5 g) of the material inside the capsule were ground to a fine powder using a mortar and pestle, before suspension in 30 ml of 70% methanol by vortex mixing. The samples were boiled for 15 min, cooled to room temperature and centrifuged for 5 min at 5000***g***. The supernatant was retained and the pellet resuspended in 30 ml of 70% methanol. This suspension was boiled for a further 15 min before cooling and centrifugation. The collected supernatants were combined and the methanol removed by rotary evaporation to a volume of less than 10 ml, before adjusting to a final volume of 15 ml with water.

Preparation of human liver microsomes

Liver microsomes were prepared according to the method of Lambert (1989). The buffer used throughout was 20 mM Bis/Tris propane pH 7.0/250 mM sucrose/150 mM KCl. Microsomes were washed with 150 mM Tris/HCl pH 8.0 before being aliquoted and stored at −70°C in 250 mM sucrose at a protein concentration of 10–20 mg ml^{-1}. Microsomes were used within 1 week of preparation.

Inhibition of ascorbate/iron-induced peroxidation

Liver microsomes (0–0.4 mg protein) were suspended in 150 mM KCl containing 0.2 mM $FeCl_3$ and various amounts of the extracts from the supplements as indicated. Peroxidation was initiated with 0.05 mM ascorbate in a final volume of 0.4 ml. After 40 min at 37°C, the reaction was terminated by addition of 0.8 ml of 20% (w/v) trichloroacetic acid/0.4% thiobarbituric acid/0.25 M HCl and 0.01 ml of butylated hydoxytoluene (5%) in ethanol. The production of Thiobarbituric acid reactive substances was measured after incubation for 15 min at 80°C (Buege and Aust, 1978).

Inhibition of NADPH/iron-induced peroxidation

Liver microsomes (0–0.4 mg protein) were suspended in 20 mM Bis-Tris propane buffer pH 7.0/150 mM KCl/0.8 mM ADP/0.2 mM $FeCl_3$ (freshly prepared in water) and various amounts of extract added. Peroxidation was initiated with 0.4 mM NADPH and the samples treated as described above.

Iron reduction

Ascorbate (0.125 mM), $FeCl_3$ (0.175 mM) and KCl (146 mM) were mixed in the presence or absence of extracts from the supplements in a final volume of 0.4 ml. After incubation at 37°C for 40 min, ferrozine was added to 0.5 mM. The amount of Fe(II) was determined spectrophotometrically at 562 nm (Stookey, 1970).

Deoxyribose assay

The deoxyribose assay was performed as described by Halliwell *et al.* (1987).

Protein determination

Microsomal protein concentrations were determined using the bicinchoninic acid method using bovine serum albumin as standard (Smith *et al.*, 1985).

RESULTS

Deoxyribose damage

Addition of ferric–EDTA to hydrogen peroxide and ascorbate at pH 7.4 leads to the production of hydroxyl radicals which can degrade deoxyribose into thiobarbituric acid reactive substances. Other compounds added to the reaction mixture may compete with the deoxyribose for hydroxyl radicals and inhibit sugar degradation. When iron is added to the mixture as ferric chloride instead of iron–EDTA, some of the Fe(III) ions bind to deoxyribose. Thus the damage to the sugar

Table 1. Deoxyribose and iron-reduction assays

Extract	Deoxyribose assay (A_{535})		Iron reduction	
	+EDTA	−EDTA	A_{562}	% of control
Water	1.80 ± 0.12	0.35 ± 0.03	0.90	100
Hypotaurine	0.35 ± 0.03	0.12 ± 0.01	nd	nd
JPF	0.38 ± 0.03	0.32 ± 0.02	1.97	219
JPV	0.22 ± 0.01	0.17 ± 0.01	0.85	94
Grapefruit (R)	0.31 ± 0.01	0.14 ± 0.01	0.76	84
Apple (R)	0.63 ± 0.05	0.25 ± 0.01	0.87	97
Broccoli (R)	0.83 ± 0.06	0.28 ± 0.02	1.06	118
White cabbage (A)	0.39 ± 0.04	0.16 ± 0.01	1.06	118

Extracts (330 μg dry weight) were added to assays as described in Materials and methods. Hypotaurine (50 mM) was added as a standard free radical scavenger (O. I. Aruorna, personal communication) and positive control. nd, not determined.
JPV, Juice Plus vegetable blend; JPF, Juice Plus fruit blend. R, raw; A, after autolysis.

becomes site specific and the hydroxyl radicals formed by the bound iron immediately attack the deoxyribose. The ability of a compound to inhibit deoxyribose degradation under these conditions reflects its iron chelating ability and the ability of its iron complex to participate in Fenton chemistry (Halliwell *et al.*, 1987).

The results on extracts from the Juice Plus capsules are shown in Table 1. For comparison, results obtained on addition of extracts from whole fruits and vegetables, made by the same procedure (Plumb *et al.*, 1995), are also shown. Both JPV and JPF are effective at scavenging hydroxyl radicals, as are grapefruit and white cabbage. JPF is the poorest at chelating iron, whereas JPV is equivalent to extracts from grapefruit or white cabbage.

Ascorbate/iron-induced lipid peroxidation

The ability of extracts from JPV and JPF to inhibit free radical mediated damage of lipids was also tested. Figure 1 shows the effect on ascorbate/iron-mediated peroxidation of human liver microsomes, and the results are again compared to extracts from fruits and vegetables. Poor inhibition is observed on adding extracts from vegetables or from JPV. However, extracts from fruits are good inhibitors in this system, in the order: plum > JPF > grapefruit.

The ability of the samples to influence the initial step in iron/ascorbate-induced peroxidation (iron(III) reduction by ascorbate) was assessed (Table 1). JPF markedly increased the amount of iron(II) produced, and this was greater than any of the other extracts.

NADPH/iron-induced lipid peroxidation

The ability of the extracts to inhibit NADPH/iron-induced lipid peroxidation was examined. Peroxidation is induced via the NADPH cytochrome P450 reductase–cytochrome P450 electron transport chain present in liver microsomes. The results, presented in Fig. 2, show that JPV and JPF both have only weak antioxidant properties even at 660 μg dry weight per assay. This is compared to extracts from fruit and vegetables, which are much better inhibitors of peroxidation when the radicals are generated enzymically.

DISCUSSION

We have tested the ability of extracts from Juice Plus capsules to scavenge free radicals, chelate iron, affect the reduction of iron(III) by ascorbate, and to inhibit two types of lipid peroxidation. All of these methods were carried out *in vitro*, and it is appreciated by the authors that these results cannot be directly extrapolated to the efficacy of the antioxidants *in vivo*. However, the aim of this paper is simply to compare the antioxidant potential of the supplements with representative extracts from whole fruits and vegetables.

In this context, the results show that extracts from the Juice Plus capsules do not differ considerably from extracts made from fresh fruit and vegetables in their ability to inhibit a range of established *in vitro* antioxidant assays, when the results are expressed on a dry weight basis. There are obviously differences between assays, but these reflect differences in the radical generating system and the target molecule(s). From these data, therefore, it is clear that 1 g of the contents of a Juice Plus capsule is approximately equivalent to 1 g of a freeze-dried preparation from representative fruits or vegetables as assessed by these antioxidant assays. Since most fruits and vegetables are about 90% water, then one capsule (which weighs 1 g) is equivalent to about 10 g of constituent fruit or vegetable. Since a portion of fruit or vegetable is about 100 g, then it follows that one portion of fresh fruit or vegetable is equivalent to ten capsules on the basis of antioxidant

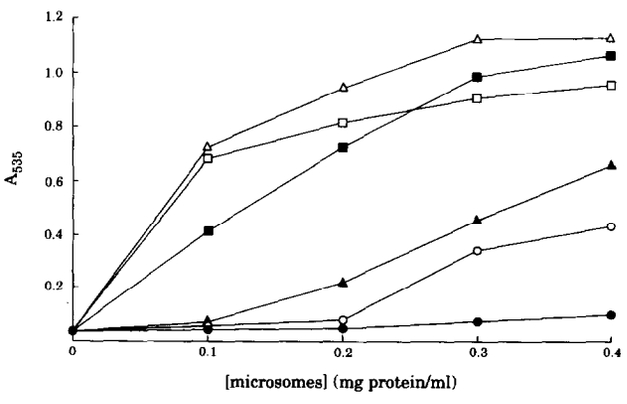

Fig. 1. Inhibition of iron/ascorbate-induced peroxidation of human liver microsomes. Microsomes from human liver in 150 mM KCl were incubated with ascorbate (0.05 mM), $FeCl_3$ (0.2 mM) and extracts (0.02 ml, containing 660 μg dry weight of the material to be tested) from white cabbage (after autolysis; ■), grapefruit (495 μg, after autolysis; ▲), plum (after autolysis; ●), JPV (□), JPF (○) and control (△). Extract from broccoli (raw) was the same as the control.

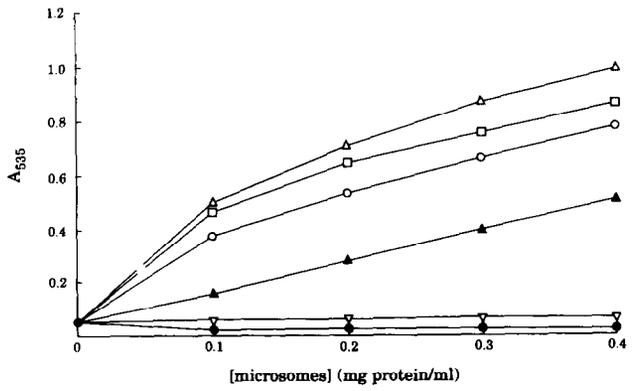

Fig. 2. Inhibition of NADPH/iron-induced lipid peroxidation of human liver microsomes. Microsomes from human liver in 20 mM Bis-Tris propane buffer pH 7.0/150 mM KCl were incubated with NADPH (0.4 mM), $FeCl_3$ (0.2 mM), ADP (0.8 mM) and extracts (0.02 ml, containing 660 μg dry weight of the material to be tested) from white cabbage (after autolysis; ■), grapefruit (495 μg, after autolysis; ▲), plum (after autolysis; ●), broccoli (330 μg, raw; (▽), JPV (□), JPF (○) and control (△).

activity. It is also recognized by the authors that there are other means of determining the health benefits of a food or supplement and that, in this paper, we have focused only on the water-soluble antioxidants. Further work is necessary to determine these other putative effects.

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