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Antioxidant Activity of Five Vegetables Traditionally Consumed by South-Asian Migrants in Bradford, Yorkshire, UK

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Five vegetables traditionally consumed among South-Asian migrants in Bradford (Yorkshire, UK) were tested for their free radical scavenging activity (FRSA) in the DPPH (1,1-diphenyl-2-picrylhydrazil radical) screening assay (using extracts prepared both by cold maceration and also by boiling the plant in the solvent under reflux) and for their *in vitro* non-enzymatic inhibition of bovine brain lipid peroxidation.

In both antioxidant assays a strong activity was shown by extracts derived from okra (*Abelmoschus esculentus*, Malvaceae) fruits and charungli (*Caralluma edulis*, Asclepiadaceae) aerial parts.

Extracts from bitter melon (*Momordica charantia*, Cucurbitaceae) and angular loofah (*Luffa acutangula*) showed a significant difference in the FRSA between the extract obtained by using cold maceration and that prepared by boiling the plant in the solvent under reflux, suggesting the chemical composition of the plant changed during the heating process, leading to an increase in the amount of antioxidant components.

These findings confirm the great interest of the nutraceutical sciences in extracts of *Caralluma edulis*, whose phytochemistry and phytopharmacology should be investigated further in order to detect possible phytotherapeutic uses in the prevention of ageing related diseases (ARDs) and Alzheimer disease (AD).

Keywords: antioxidant; lipid peroxidation; ethnopharmacology; *Caralluma edulis*; migrants; Asia.

INTRODUCTION

Very few studies have analysed the management, culinary use and health benefiting properties of food plants traditionally consumed among migrant communities; ethnobotanical surveys on these phenomena have mostly interested the USA (Kuebel and Tucker, 1988; Ikeda *et al.*, 1991; Corlett *et al.*, 2003; Nguyen, 2004) while research on the food habits of migrants in Central and Northern Europe have been developed mainly under the perspective of biodiversity studies (Gladis, 2002, 2003), food anthropology (Jonsson *et al.*, 2002a, 2002b) or sociology (Mayer-Renschhausen *et al.*, 2002 and a few chapters therein).

In traditional societies, nutrition and health care are interconnected and many plants are consumed as food in order to benefit health (Etkin, 1996; Pieroni, 2000; Pieroni *et al.*, 2002a, 2002b; Pieroni and Price, 2005). The nutraceutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables is regarded worldwide as an important area of the nutritional and phytotherapic research (Lionis *et al.*, 1998; Grivetti and Ogle, 2000; Karakaya *et al.*, 2001; Ogle *et al.*, 2001a, 2001b, 2001c; Johnson and Grivetti, 2002; Owen and Johns, 2002; Coulaidis *et al.*, 2003; Ogle *et al.*, 2003; Zeghichi *et al.*, 2003a, 2003b; El and Karakaya, 2004).

On the other hand, oxidative stress is intricately linked with ageing related diseases (ARDs) and longevity: antioxidant activity was recently studied in relation to CNS disorders (Perry *et al.*, 2001). The metabolism of phospholipids is in fact associated with neuronal death in Alzheimer's disease (AD) and antioxidants such as vitamin E play an important role in ß-amyloid (Aß) aggregation (Murray and Lynch, 1998; Varadarjan *et al.*, 2001), while very recently the Cache County Study demonstrated that the use of vitamin E and vitamin C supplements in combination is associated with a reduced prevalence and incidence of AD (Zandi *et al.*, 2004). Ayasolla *et al.* (2004) have suggested a role for ceramide in inducible nitric oxide synthase induction and NO production in AD pathobiology and provided a possible explanation for the beneficial effects of vitamin E therapy in AD patients.

The aim of the present study was to evaluate the *in vitro* antioxidant activity of uncommon vegetables, traditionally consumed during the winter months in south-Asian (Pakistani and Northern Indian) migrant households of the Bradford area (West Yorkshire, North England, UK).

MATERIALS AND METHODS

Plant material and preparation of crude plant extracts. Vegetables were bought in small Asian greengrocers in the Bradford area.

Botanical identification was carried out by A. Pieroni and by taxonomists of the Department of Botany,

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Punjab University, Lahore, Pakistan. Voucher specimens are deposited at the Herbarium of the School of Pharmacy of the Bradford University.

Plant parts of the five vegetables were collected and extracted at room temperature. 20 g of plant material was extracted in 200 mL of methanol:water (4:1) for 7 days in the dark at room temperature; the same procedure was repeated with fresh plant parts and the same solvent, extracting under reflux for 30 min. The whole extracts were filtered, and used for the two following assays.

Evaluation of the free radical scavenging activity (FRSA) in the 1,1-diphenyl-2-picrylhydrazil radical ($DPPH^{\cdot}$) assay. The antioxidant activity using the DPPH assay was assessed by the method of Pieroni *et al.* (2002a). Plant parts were peeled as is usually done in the migrant cuisine. 20 g of mixed plant was extracted in 200 mL of methanol: water (4:1) at room temperature and protected from light for 7 days. A second batch of extracts was prepared extracting with 200 mL of the same solvent under reflux for 30 min.

1.5 mL of each extract was added to 1.5 mL of DPPH 0.1 mM dissolved in methanol. Absorbance was read at 517 nm after 5 min. The FRSA was calculated as $[1\text{-}(A\text{-}B)/C] \times 100$, where A is the absorbance of 1.5 mL of the crude extract solution mixed with an equal volume of the DPPH solution; B the absorbance of 1.5 mL of the crude extract solution mixed with an equal volume of methanol and C the absorbance of a blank prepared by mixing 1.5 mL of the DPPH with an equal volume of methanol: water (4:1). An extract of arctic root (*Rhodiola rosea*, Crassulaceae) was used as a control.

Evaluation of the inhibition of lipid peroxidation in liposomes. An assay for non-enzymatic lipid peroxidation was carried out by slightly modifying the procedures of Houghton *et al.* (1995), Burits and Bucar (2000) and Pieroni *et al.* (2002b). Phospholipid liposomes were prepared from Type VII Folch bovine brain extract (Sigma), suspended in phosphate buffer (5 mg/mL). Before the test the suspension was sonicated with ice until a milky solution was obtained. The reaction mixture contained 1 mL of the liposomal suspension, 100 µL phosphate buffer, 500 µL of plant extract (obtained by using cold maceration), 200 µL $FeCl_3$ (1 mM in H_2O) and 200 µL ascorbic acid (1 mM in H_2O).

Samples were incubated at 37 °C for 1 h, then 2 mL thiobarbituric acid (1% in 50 mM NaOH), 2 mL trichloracetic acid (2.8% in H_2O) and 200 µL 2,6-ditert-butyl-p-kresol (2% in EtOH 98%) were added. The samples were incubated at 80 °C for 20 min. After cooling, 2.5 mL of *n*-butanol was added and the reaction mixtures were centrifuged. Since the UV-absorption was too high the organic layer was diluted 1:1 with *n*-butanol. The absorbance of the organic layer was measured at 532 nm. Inhibition (I) of lipid peroxidation (%) was calculated using the following equation: $I(\%) = 100 \times [(X - Y)/X)]$, where X was the absorbance of the negative control reaction (containing no inhibitor) and Y was the absorbance in the presence of the inhibitor.

An extract of tassel hyacinth (*Leopoldia comosa*, Liliaceae s.l.) fresh bulbs, which have been found to have remarkable inhibiting properties on lipid peroxidation (Pieroni *et al.*, 2002b) was used as a control.

RESULTS

During approximately 20 interviews with customers of south-Asian greengrocers (mostly elderly) in Bradford, conducted in January–February 2004, five vegetables, which are traditionally bought, cooked and consumed in Pakistani and Northern Indian households during this time of year, were identified. They were the fruits of bitter melon (*Momordica charantia*), drumstick (*Moringa oleifera*), okra (*Abelmoschus esculentus*), angular loofah (*Luffa acutangula*) and the succulent aerial parts of charungli (*Caralluma edulis*, Fig. 1).

Specific recent data on the antioxidative properties of the edible parts of these species do not occur in the scientific literature to date.

They are mostly imported from Pakistan and Northern India, where they are locally cultivated or even (*Caralluma*) harvested from the wild. Consumption of these vegetables is very important among the older generations, and unfortunately it is less important among the youngest members of the communities.

Traditional culinary uses of the selected plants are reported in Table 1.

Detailed ethnobotanical data on these and other taxa used by South-Asian migrants in Bradford will be discussed elsewhere.

Free radical scavenging activity (FRSA)

Figure 2 reports the FRSAs in the DPPH assay of both extracts obtained using cold maceration (7 days) or an extraction under reflux (30 min).

Charungli and okra extracts showed a FRSA activity which was comparable to that recorded for the reference (arctic root). Moreover, the aim of the comparison between both extracts was to assess the eventual change of the antioxidant activity, due to the heating process. The aim of the experiment was to mimic the heating process that occurs during cooking, as all these

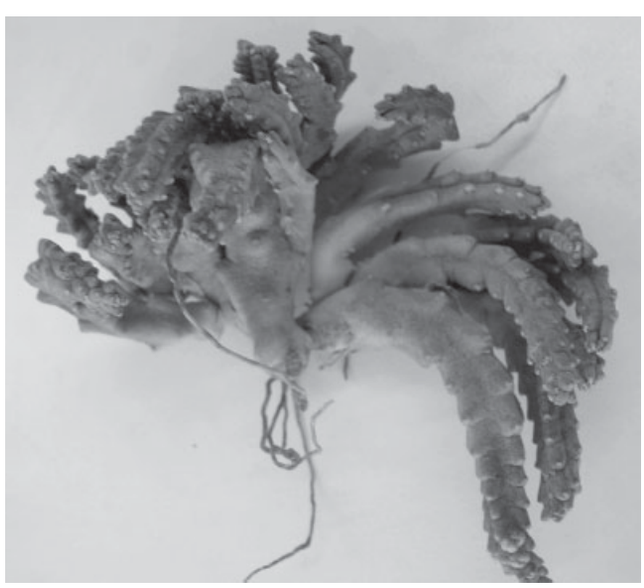

Figure 1. Charungli (*Caralluma edulis*, Asclepiadaceae).

Table 1. Traditional culinary uses of the five chosen vegetables, bought, processed and consumed in South-Asian (Pakistani/Northern Indian) migrant households in Bradford

Scientific name and botanical family	English name	Pakistani/Indian vernacular name	Part used as food	Traditional culinary uses	Period of use among migrants in Bradford
Moringa oleifera LAM. (Moringaceae)	Drumstick tree/ Ben oil tree	Dodi	Fruits	Fried and stuffed with potatoes	All year around
Luffa acutangula (L.) ROXB. (Cucurbitaceae)	Angular loofah	Turyah	Fruits	Cooked with lentils and fried onions	All year around
Abelmoschus esculentus (L.) Moench. (Malvaceae)	Okra	Bhindi	Fruits	Sautéed with onions and made into a curry	All year round
Momordica charantia L. (Cucurbitaceae)	Bitter melon	Karela	Fruits	Fried and stuffed with potatoes	All year round
Caralluma edulis (EDGEW.) BENTH. EX HOOK. F. (Asclepiadaceae)	Charungli	Charungli	Aerial parts	Cooked with meat as a curry	Winter time only

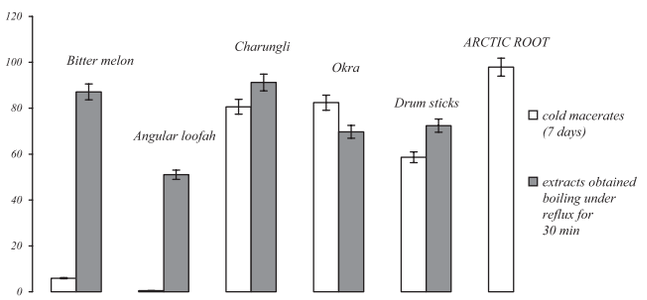

Figure 2. FRSA in the DPPH assay of the south-Asian vegetable extracts, obtained by using cold maceration (7 days) and extraction under reflux (30 min) (n = 3 experiments).

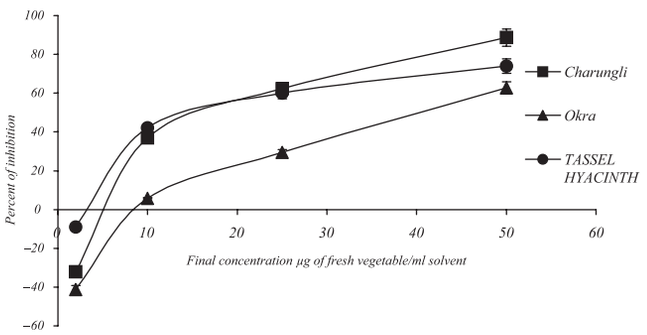

Figure 3. *In vitro* inhibitory activity of brain lipid peroxidation by the two most active south-Asian vegetables (n = 3 experiments).

vegetables are consumed cooked. The FRSA activity of the extracts obtained by heating under reflux was higher for all extracts in comparison with the results obtained by cold maceration, the only exception being okra. However, the most relevant differences were shown by bitter melon and angular loofah.

In vitro non-enzymatic inhibition of the lipid peroxidation in liposomes

For the two most interesting extracts (charungli and okra) the *in vitro* non-enzymatic inhibition of lipid peroxidation in liposomes was assessed (Fig. 3), chosen for determining antioxidative activity related to CNS disorders. In this assay, both extracts showed significant antioxidant activity similar to the control (tassel hyacinth, *Leopoldia comosa*). These two extracts showed a dose-dependent activity.

DISCUSSION

The interest in the search for new natural antioxidants has grown over the past years because reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to ageing related diseases (Finkel and Holbrook, 2000). Synthetic antioxidants can generally cause problems of toxicity.

Research on the antioxidant activity of less used and neglected vegetables then becomes very important, in order to provide new scientific evidence and insight into the growing modern nutraceutical market.

While the antioxidant activity of bitter melon (*Momordica charantia*) is relatively well known, our results showed a significant difference in the FRSA between the extracts of the fresh edible fruits obtained by using cold maceration and those prepared by boiling the plant in the solvent under reflux, suggesting that the heating process led to a relevant increase of antioxidant components; the same phenomenon was observed for angular loofah (*Luffa acutangula*).

Abelmoschus esculentus fruits have been well studied, especially in food technology, but rarely for their pharmacology: one recent study has pointed to the effects of glycosylated compounds from okra fruits in inhibiting the adhesion of *Helibacter pylori* to human gastric mucosa (Lengsfeld *et al.*, 2004).

Caralluma species have been used for centuries in semi-arid areas of Pakistan as emergency foods (Atal *et al.*, 1980); while *Caralluma edulis* is known for its antidiabetic properties (Wadood *et al.*, 1989) and other

Caralluma species for their antihyperglycemic activity (Venktash *et al.*, 2003). Extracts from *Caralluma* species are nowadays the object of an increasing interest for small nutraceutical enterprises (see for example: Gencor Pacific, 2004).

Caralluma species have shown antiinflammatory (Ramesh *et al.*, 1998, 1999a; Zakaria *et al.*, 2001) and gastric mucosa protecting and antiulcer (Al-Harbi *et al.*, 1994; Zakaria *et al.*, 2002) properties.

The phytochemistry of the same genus *Caralluma* (whose complex botanical taxonomy has been the object of a very extensive proposal for revision, Plowes, 1995) is characterized by many pregnane glycosides, while recently megastigmane glycosides also have been isolated from *Caralluma negevensis* (Bader *et al.*, 2003), as well as a few flavons (Ramesh *et al.*, 1999b; Bader *et al.*, 2003).

Phytochemical investigations on the middle polar and polar polyphenolic fractions of *Caralluma* species could be worthwhile, in order to find a correlation between the antioxidant activity shown and the phytochemistry of this plant.

On the other hand, the health benefits of minor plant-derived diet products of migrants can have implications in the social medical field and in health policies as well.

For example, epidemiological studies on the South-Asian migrants in Northern England have shown that South-Asian children living in UK have a similar incidence of diabetes to the indigenous population, while in Asia children have a low incidence of Type 1 diabetes, suggesting that these rapid changes are due to environmental factors (Feltbower *et al.*, 2002). The same patterns have been observed for the incidence of cancer, and Winter *et al.* (1999) underlined a transition from a lower cancer risk in the country of origin to a high incidence in the new country of residence (UK), suggesting that detrimental changes in the lifestyle and other exposures have occurred in the migrant south-Asian population.

In-depth studies concerning the potential epidemiological impact of the (decreasing) consumption of these traditional vegetables to the health status of South-Asian migrants in UK, as well as their ethnopharmacology, could yield interesting findings.

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