POLYPHENOLS OF SOME INDIAN VEGETABLES

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VEGETABLES have assumed greater importance in human nutrition because of the recognition of the beneficial effects of dietary fibre and polyphenols, two constituents that were considered insignificant in the past. Dietary fibre includes all the unassimilable structural plant materials like cellulose, hemicelluloses and pectins. These materials have important effects on gut function because of their bulk, ability to absorb and retain water, and their being substrates for the bacteria of the gut[1]. The interest in food phenolics owes its origin to 'vitamin P', a group of polyphenols better known as 'permeability factors'. These compounds increase capillary resistance and thus prevent subcutaneous capillary bleeding. Rutin, the 3-rutinoside of quercetin, and flavonones of the *Citrus* fruits formed the principal components of vitamin P. In addition to their role in decreasing capillary bleeding, these compounds are reported to prolong life in scorbutic guinea pigs and help in overcoming vascular purpurea. But none of these substances has been shown to have a true vitamin effect and the designation was dropped in 1950 on the recommendation of the American Society of Biological Chemists and the American Institute of Nutrition[2]. At present the term 'bioflavonoids' is used to denote all the flavonoids exhibiting some pharmacological activity.

Though minerals, vitamins and even dietary fibre of vegetables are known in great detail, no conscientious effort seemed to be made to understand the phenolic chemistry of vegetables. A thorough knowledge of the phenols and their possible role in digestive processes would help to ascertain the beneficial/toxic effects of these compounds. In the present work, 11 leafy vegetables and 5 fruit vegetables commonly used in India were analysed qualitatively for their flavonoids and related compounds. Since glycosidic flavonoids partly lose their sugar residues in the human body to yield the aglycones[3], an attempt was made to identify the aglycones from the acid-hydrolysed extracts of the plants.

All the vegetables were collected fresh from Baroda. The fresh plant materials were analysed using standard procedures[4–6]. Authentic samples were used to confirm the identity of the flavonoids.

The distribution of the various phenolics in 16 vegetables is presented in table 1. All the leafy vegetables contained various flavonoids in the leaves. Flavonols were widely distributed, being found in all of them except *Colocasia*. Most of the vegetables contained quercetin and/or kaempferol and their various methoxylated derivatives. The 6/8-hydroxylated flavonols gossypetin and quercetagetin were seen in *Moringa* leaves. In cabbage and *Hibiscus sabdariffa* flavonols were in traces. The leaves of *Colocasia* contained flavones (apigenin and luteolin) instead of flavonols. None of the plants screened contained glycoflavones. Proanthocyanidins were seen in the leaves of *Moringa* while anthocyanins were located in *H. sabdariffa* and *Ipomoea aquatica*. Coumarins were present in both the umbelliferous plants screened, viz. *Anethum* and *Coriandrum*.

Proanthocyanidins were widespread among the fruit vegetables. Only *Cyamopsis tetragonoloba* was free of them. *C. tetragonoloba* was also peculiar in containing quercetin and kaempferol. Flavonols were in traces in both *Hibiscus esculentus* and *Vigna anguiculata*.

The presence of flavonols in most of the vegetables that are regularly consumed is noteworthy. It is also significant that most of the flavonoids possess 3′,4′-dihydroxy/dimethoxy substituents. Though their pharmacological effects have not been proved conclusively, the flavonoids are found to exert beneficial effects in more than fifty diseases. According to De Eds[7] the flavonoids with free hydroxyl groups at the 3′,4′-positions (quercetin, gossypetin, quercetagetin and luteolin) exert beneficial effects on the capillaries by (i) chelating metals and thus sparing ascorbate from oxidation, (ii) prolonging epinephrine action by inhibiting *O*-methyl transferase, and (iii) stimulating the pituitary–adrenal axis. The flavonoids with multiple methoxy groups (7,4′-diOMe kaempferol, 3′,4′-diOMe quercetin, etc.) play an important role in the circulatory system by reducing aggregation of erythrocytes (which occurs during illness or injury) by site-specific membrane surface effects, and improve the microcirculation within the body[8].

Since the coumarins are also known to act as diuretics, vasodilators and oestrogens[9], the vegetables containing them, viz. *Coriandrum* and *Anethum*, may exhibit these medicinal properties.

Since the flavonoids and/or coumarins are present in most of the vegetables, a critical appraisal of their

Table 1 *Polyphenols in some vegetables of India*

	Polyphenol*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Leafy vegetables														
Brassica oleracea var. *capitata* L.						+								
Raphanus sativus L.			+	+	+									
Hibiscus subdariffa L.						+							+	
Moringa oleifera Lam.							+			+	+	+		
Trigonella foenum-graecum L.			+				+							
Sesbania sesban (L.) Merr.			+			+							+	
Anethum graveolens L.				+					+					+
Coriandrum sativum L.						+	+		+					+
Ipomoea aquatica Forsk.							+	+					+	
Argyreia nervosa Boj.						+								
Colocasia antiquorum Schott.	+	+											+	
Fruit vegetables														
Hibiscus esculentus L.									+			+		
Moringa oleifera Lam.												+		
Vigna unguiculata (L.) Walp.									+			+		
Cyamopsis tetragonoloba Taub.			+			+								
Dolichos lablab L.												+		

*1, Apigenin; 2, 3′,4′-diOMe luteolin; 3, kaempferol; 4, 4′-OMe kaempferol; 5, 7,4′-diOMe kaempferol; 6, quercetin; 7, 3′-OMe quercetin; 8, 4′-OMe quercetin; 9, 3′,4′-diOMe quercetin; 10, gossypetin; 11, quercetagetin; 12, proanthocyanidins; 13, anthocyanins; 14, coumarins.

metabolic fate and toxic properties, if any, is necessary. Simple phenols undergo various hydroxylation/dehydroxylation reactions or get oxidized to phenolic acids. Ingested quercetin has been found to get excreted as glucuronides or sulphates or to get metabolized to phenylacetic acids[10]. Phenylacetic acid is converted to phenylalanine or conjugated with glutamine to form phenylacetylglutamine and excreted in urine. Phenylacetic acid possesses antispasmodic properties[11].

The presence of proanthocyanidins in most of the fruit vegetables is interesting. These compounds undergo hydrolytic and polymerization reactions in the highly acidic alimentary tract and produce condensed tannins. The tannins are known astringents and are antimicrobial in nature. But they also interfere with the absorption of iron in the human body and may bind to enzymes, rendering them inactive.

The present work emphasizes the necessity of evaluating the various effects of phenolics in the human systam. It also brings to focus the importance of minor components of foods, which are often overlooked. A proper understanding of their role in the human body will facilitate the judicious use of vegetables effectively.

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INHIBITION OF PHOTOSYNTHESIS BY OXYFLUORFEN

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OXYFLUORFEN, a diphenyl ether (DPE) group herbicide, has been found to be very effective against broad- and micro-leaved weeds[1,2]. The mechanism of herbicidal injury induced by oxyfluorfen is still unclear. Most of the DPE herbicides have a light-dependent mode of action but some do not[3,4]. Oxyfluorfen requires light for herbicidal action[4,5] and the evidence suggests that the photo-oxidative damage caused by oxyfluorfen involves photosynthetic electron transport[6-8]. Data in support of oxyfluorfen action independent of photosynthetic electron transport are also available[9-11]. Recently, Haworth and Hess[11] have shown that oxyfluorfen-induced herbicidal injury by generation of singlet oxygen is independent of photosystem I (PS I) electron transport, in agreement with the previous findings on the requirement of oxygen for DPE herbicidal activity[12,13].

In the present communication, we report that photo-oxidative damage to chloroplast membranes is due to the inhibition of photosystem II (PS II) electron transport by oxyfluorfen, which may involve binding of oxyfluorfen to pigment complex(es) or any other component of the membrane.

Seeds of *Oryza sativa* (var. CSR-4) obtained from the College of Agriculture, Indore, were soaked in water for 24 h and grown under laboratory conditions in plastic containers. Oxyfluorfen (1, 3, 5 and 7 ppm) was sprayed on 15-day-old rice plants, and studies were carried out every alternate day.

Chloroplasts were isolated from freshly cut leaves of rice plants according to Karabourniotis *et al.*[14] in ice-cold isolation medium containing 400 mM sucrose, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$, 10 mM KCl, 150 mM NH_4Cl, 2 mg/ml BSA (fraction V) and 4 g/l polyvinylpyrrolidone (PVP-10). The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged for one min at 3000 *g*. The supernatant was discarded and the pellet was suspended in minimal volume of isolation medium, but without PVP.

Chlorophylls were extracted in cold 80% acetone under dim green light and the amount of chlorophylls in the extract was estimated according to Mackenny[16].

Heat treatment of spinach chloroplasts (to destroy water splitting complex) was carried out by incubating chloroplasts at 48°C for 4 min and thereby stopping electron flow to PS II. Diphenylcarbazide (DPC) was used (final concentration 0.5 mM) as donor of electrons on oxidizing side of PS II.

Type C spinach chloroplasts were used in experiments to study the mode of action of oxyfluorfen and to calculate I_{50} values at different chlorophyll concentrations. For other experiments type C rice chloroplasts were used.

The photoreduction of 2,6-dichlorophenol indophenol (DCIP) (PS II activity, $H_2O \rightarrow$ DCIP) was measured spectrophotometrically (Shimadzu model UV–VIS 160) according to Mohanty *et al.*[17] The chloroplasts were illuminated for 30 sec with saturating white light (50 W/m^2). The incident beam was passed through a water filter to cut off the infrared radiation. The reaction mixture in a final volume of 3 ml contained chloroplasts equivalent to 10 μg chlorophyll/ml, 20 μM DCIP, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$ and 5 mM NH_4Cl. The photoreduction of DCIP was measured spectrophotometrically at 605 nm and the rate of DCIP reduction was calculated using an extinction coefficient of 21 mM^{-1}.

Spraying of oxyfluorfen resulted in visible chlorosis, curling of leaf and appearance of irregular burnt patches. The visible injury was apparently more at higher concentrations (5 to 7 ppm) of oxyfluorfen. The effect of different concentrations of oxyfluorfen on total chlorophyll content of rice leaves is shown in figure 1. At low concentration of oxyfluorfen (1 ppm), there was a slight decrease in chlorophyll content up to 24 h after spray, followed by recovery. However, at higher concentrations of oxyfluorfen, there was no recovery but a further decrease. There was about 80% decrease in total chlorophyll content of leaves of rice plants sprayed