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Bioactive Phytochemicals In Indian Foods

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Many plant foods have been described to have curative value in the ancient Indian Ayurvedic medical system and in folk medicine[1]. In recent years, there has been a surge of interest in plant foods as sources of phytochemicals which may have a useful role in the prevention of diseases such as cancer, diabetes, cardiovascular diseases, cataract, gallstones, etc.

In a recent popular article[2], the latest findings on the role of phytochemicals, present in fruits and vegetables, in the prevention of cancer and heart disease and the underlying mechanisms, have been lucidly discussed.

The National Institutes of Health (NIH), USA, have recently announced a new plan for more detailed study of food chemistry and of the interactions of compounds of foods with the body and genome. This new branch of research has been named 'Bionutrition'[3].

A wide range of 'non-nutrient' chemicals is present in foods with different biological activity and potency. The class of chemicals which has been identified in plant foods with biological activity is given in Table1.

Although these chemicals have been reported to be present in a wide range of plant foods, their efficacy in disease prevention has been clearly established only in the case of some chemicals or class of chemicals. Phytochemicals in foods with proven health benefits are considered here.

DIETARY FIBRE

Most plant foods in their native state contain indigestible residue which used to be designated as crude fibre. During the 1960s, however, it was recognised that indigestible residue in foods consists of polysaccharides which have the potential of preventing, in humans, certain diseases such as atherosclerosis, diabetes and cancer[4]. These are designated as 'dietary fibre'.

The role of dietary fibre in disease prevention was discussed in an earlier article[5] in this *Bulletin*. This subject is therefore not being discussed in detail here.

Currently, dietary fibre has been redesignated as Non-Starch Polysaccharides (NSP). NSP is not a single entity but consists of a wide range of complex polysaccharides such as cellulose, gums, mucins and hemi-cellulose and lignins with different chemical, physico-chemical and physiological properties.

Although many plant foods contain several of the NSP components, all of them may not exert the desired health benefit to the same degree. Only some foods with specific type of NSP at a reasonably high level of intake can exert the expected beneficial effect. For example, *fenugreek seeds* contain nearly 40 per cent gum which can have beneficial effect on controlling blood glucose levels only at the intake of 80-100 g/day[6].

ANTIOXIDANTS

Oxidants and free radicals such as singlet molecular oxygen ($O_2^{\cdot}$), superoxide, ($\dot{O}_2$), hydroxyl ($\dot{O}H$), peroxide (O-O-H) and lipid peroxide ($L\dot{O}\dot{O}$) radicals are known to cause tissue damage[7]. Such tissue injury, when it becomes cumulative, is considered to play an important role in the pathology of several diseases such as cancer, cataract, atherosclerosis, ischaemia, pulmonary dysfunction and radiation damage. Lipid peroxidation of membrane lipids, circulating lipoprotein lipids including cholesterol, oxidative damage of cellular proteins and DNA, and lens proteins in retina are all considered to be the mechanism by which the oxygen-free radicals and peroxide cause these diseases. These oxidants and free radical species are generated in cells during utilisation of O_2 which is so essential for life sustenance.

Other sources of these free radical species are environmental pollutants such as smoke, carcinogenic chemicals and radiation, etc. There are, however, protective mechanisms in the body against these dangerous

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oxygen radicals. Superoxide dismutase (SOD) can convert the $\dot{O}_2$ radical to hydrogen peroxide which can be further destroyed by catalase. Glutathione reductase is also a part of the *in vivo* defence system against oxidation damage of cells. This *in vivo* system of detoxifying oxygen free radicals may not be capable of neutralising all the free radicals produced in the body as well as those derived from the environment. Several chemicals in foods with antioxidant properties can, however, quench the oxidants and free radicals and help to counteract their damaging effects. Such protective action by the antioxidants is mediated through several mechanisms such as chain termination, radical scavenging, oxygen quenching, etc.

A large number of antioxidants, both nutritive and non-nutritive, occur in foods and these are listed in Table 2. Besides β-carotene, vitamin C and vitamin E which are nutrients, a number of carotenoids[8,9], phenols[10], and flavonoids[11] which occur in plant foods possess the antioxidant property. Most plant foods contain phenols and flavonoids. *Green leafy vegetables* (GLV), fruits and yellow vegetables are particularly rich in carotenoids, flavonoids and vitamin C. GLV and fruits such as *papaya* can be an inexpensive source of carotenoids. More than 50 per cent of the carotenoids present in these foods do not have provitamin A activity but possess antioxidant properties. *Turmeric*, which is widely used in Indian cooking, contains the yellow colouring principle — curcumin, which is a powerful antioxidant. Vitamin E is present in vegetable oils and the germ portion of cereals. *Palm oil*, which is rich in both tocopherols and tocotrienols and carotenoids, can be an important source of antioxidants in our diet. Foods popularly advocated as rich sources of vitamin C such as *amla* and *guava* can also be rich and inexpensive sources of antioxidants. Besides being rich in vitamin C, these two fruits also contain phenolic components including flavonoids. Habitual Indian diets contain antioxidants derived from different food sources.

The total antioxidant potential of a food or a diet can be determined by its effect on preventing lipid peroxidation in an *in vitro* system[7]. However, the potency of antioxidants present in food *in vivo* will depend not only on their levels but also on their bioavailability, that is, the extent to which the active form of the antioxidants is released from the food in the gut and absorbed. Some of the flavonoids and phenolic antioxidants are rather poorly absorbed. The antioxidant potency *in vivo* can be determined in man by measuring the effect of their consumption on the lipid peroxide levels in plasma and accessible cells.

Earlier, the lipid peroxide levels were measured in terms of TBA reactive substances which are considered rather non-specific. Currently, however, sophisticated methods have been developed for separating the lipid peroxides and their breakdown products before estimating their levels. Methods employing HPLC, GCMS, chemiluminesence, ^{13}C-NMR and enzymatic methods have been developed for assessing the lipid peroxidation levels in body fluids and tissues[7].

The beneficial influence of antioxidants and antioxidant-rich foods on disease prevention, particularly cancer, has been established through extensive studies in animals and *in vitro* systems. In humans, however, the association between the intake of dietary antioxidants and protection against diseases such as cancer, cardiovascular diseases, cataract, etc, have been largely based on epidemiological studies.

There have been several recent epidemiological studies to implicate dietary antioxidant phytochemicals such as carotenoids[8,9], flavonoids[11] and phenolic compounds[10] as protecting agents against cancer and cardiovascular diseases. Ziegler *et al*[12] have shown that intake of carotenoids such as lutein, zeaxanthin, β-cryptoxanthin, lycopene, besides β-carotenes, through fruits and vegetables, resulting in elevated levels of blood carotenoids, is associated with reduced risk of lung cancer. Hertog *et al*[13] have shown in an epidemiological study in the Netherlands that regular intake (26 mg/day) of flavonoids (quercetin, kaemferol) through vegetables and fruits among the elderly reduced the risk of death from coronary heart disease. Flavonoids from all the fruits and vegetables consumed were estimated by HPLC. These flavonoids act by scavenging superoxide anion, singlet oxygen, lipid peroxide radicals and through sequestering metal ions. Quercetin, the major flavonol, inhibits oxidation and cytotoxicity of LDL. Flavonoids also inhibit cyclo-oxygenase leading to lower platelet aggregation and reduced thrombotic tendencies.

Another study by Frankel *et al*[14] reports that phenolic substances in red wine inhibit oxidation of human low density lipoproteins providing an

Table 1
Bioactive Phytochemicals In Foods

Classification	Main groups of compounds	Biological function
Non-Starch Polysaccharides (NSP)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	(a) Water holding capacity (b) Delay in nutrient absorption (c) Binding toxins and bile acids
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid, anthocyanine, phenolic indoles	(a) Oxygen free radical quenching (b) Inhibition of lipid peroxidation
Detoxifying agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, diterpenes, carotenoids, retinoids, cyanates, phytosterols, methyl xanthines, protease inhibitors	(a) Inhibition of activation of procarcinogens (b) Inducers of drug metabolising enzymes (c) Binding of carcinogens (d) Inhibitors of tumourogenesis
Others	Alkaloids, volatile flavour compounds, biogenic amines, terpenoids and other isoprenoid compounds	(a) Neuropharmacological (b) Antioxidant (c) Cancer chemo-prevention

explanation for the low prevalence of atherosclerosis and heart diseases (CHD) in some regions of France in spite of high intake of saturated fat. This anomaly, called the 'French paradox', is attributed to the regular consumption of red wine rich in phenolic substances.

Such epidemiological studies to correlate the intake of phytochemicals with the low prevalence of cancer or heart diseases have their limitations, since several other environmental, dietary and genetic factors can influence these diseases. However, the effect of consuming antioxidant containing foods on lipid peroxide levels, which are responsible for the cell damage leading to these diseases, can be directly determined as described above.

ANTIOXIDANTS IN INDIAN FOODS

Although we have data on vitamin antioxidant (ascorbic acid, tocopherol and carotene) content of Indian foods reported in the food composition tables, we have very limited data on other antioxidant chemicals in foods, such as individual flavonoid and other phenolic compounds. The total phenolic and polyphenolic content of the major food groups which form part of Indian dietaries have, however, been reported. Although the data on carotene (active provitamin A) content of foods are available, the contents of other carotenoids have been reported only for a few fruits such as the mango and papaya. Carotene, in many foods, constitutes only 20-50 per cent of total carotenoid pigments.

On the basis of available data on antioxidant nutrient content and dietary intake, it would be possible to estimate the dietary antioxidant intake of different population groups. The intake of nutritive antioxidants, β-carotene and vitamin C is quite low in the low income groups, inadequate even to meet their minimal recommended intake. The intake of these nutrients in the upper income groups is, however, adequate to meet their nutritional needs. Whether they are adequate enough to discharge their antioxidant function effectively is still not known.

Similarly, the intake of tocopherols, 50 per cent of which is derived from cereals and pulses, is somewhat lower in the low income groups but the deficit is not as large as in the case of other vitamins. Such intakes of vitamin antioxidants are much lower among preschool children of low income groups who suffer from various vitamin deficiencies more frequently than the adults. It can be postulated that in poor income populations, antioxidant intakes may not be adequate to offer protection against diseases caused by oxygen free radicals and lipid peroxides, due to the low levels of consumption of fruits and vegetables.

Indian diets, which are largely based on plant foods, contain several antioxidant chemicals such as phenols, flavonoids and carotenoids. *Spices* which are an invariable component of Indian diets, contain phenolic compounds which can function as antioxidants. *Turmeric,* and its active principle curcumin, has been shown to be an effective antioxidant which inhibits lipid peroxide induced DNA damage[15]. Besides curcumin, other spice principles such as eugenol from *cloves* and capsaicin from *red chillies* have been shown to inhibit lipid peroxidation in a dose dependent manner[16]. It would be useful if the content of these antioxidant chemicals and the total antioxidant potential of habitual diets of different groups of population is determined by both *in vitro* and *in vivo* methods.

DETOXIFICATION BY PHYTOCHEMICALS

An important role of phytochemicals in foods in health promotion and disease prevention is the detoxification of drugs, toxicants, carcinogens and mutagens. These toxic chemicals, which are foreign to the body (xenobiotics), are detoxified mainly in two phases[17,18].

Phase I metabolism or biotransformation involves reactions such as oxygenation, oxidation, reduction, dehalogenation and desulfuration, all of which introduce new functional groups into the lyophilic toxic molecules, rendering them more hydrophilic and more rapidly excretable. The microsomal mixed function oxidase (MFO) system is responsible for many aspects of phase I metabolism of xenobiotics. Paradoxically, phase I drug metabolising enzymes, whose main function is detoxification, can also activate some of the biologically inactive compounds (procarcinogens) into toxic compounds (namely, Cl_4).

Phase II metabolism, which has a more important role in the detoxification of xenobiotics, involves synthetic reactions in which glucuronic acid, glutathione, glycine or amino acids are conjugated to the functional groups of the foreign chemical compound or its phase I metabolic product, making them even more polar and readily excretable. The enzymes involved in phase II metabolism are glutathione transferase, NADPH quinone reductase, UDP glucuronosyl transferase and epoxide reductase[19]. These enzymes are induced by a wide variety of chemicals including several phytochemicals present in foods and help in detoxifying the toxins.

In this respect the phytochemicals themselves behave like foreign compounds and vary in their potencies in inducing the drug metabolising enzymes. These protective inducers are identified and their potencies measured by employing a simple cell culture system in which the effect of phytochemicals on the specific activ-

Table 2
Dietary Antioxidants

Nutrient	Non-nutrient
β-carotene — Provitamin A Ascorbic acid — Vitamin C	Carotenoids (Lycopene, xanthophyls) Lutein, α- and γ- carotenes (cryptoxanthine, zeaxanthine)
Tocopherols Tocotrienols Riboflavin	Flavonoids (quercetin, myricetin, quercetagatin, gossypetin)
Sulphur amino acids	Anthocyanins
Cysteine and methionine	Isoflavones
Selenium	Phenolic compounds (catechin) Indoles

ity of quinone reductase is measured[20].

There have been extensive studies during the past few decades on the effect of phytochemicals present in foods on drug metabolising enzymes (DME)[18,19,21,22]. A wide range of phytochemicals has been shown to induce drug metabolising enzymes and protect against carcinogens and toxins. Some chemicals induce both phase I and phase II enzymes and others such as phenolic compounds are monofunctional. The balance between phase I and phase II enzymes is an important determinant of whether exposure to a carcinogen will result in toxicity or not.

Phytochemicals which are strong inducers of MFO are safrole, xanthenes, flavones and indoles. Rats maintained on a diet containing a higher content of indoles, such as *sprouts* and *cabbage,* have a higher level of induced intestinal MFO activity and show an altered response to chemical carcinogens. Humans on similar diets show an enhanced hepatic metabolism of antipyrine. Coumarine derivatives present in fruits and vegetables, namely p-corumeric acid and chlorogenic acids present in *tomatoes, strawberries, pineapples* and *green peppers* can induce glutathione S-transferase and inhibit chemical carcinogenesis.

Vegetables of the crucifera family such as *cabbage, broccoli, Brussel sprouts* and *cauliflower* contain a number of indole derivatives such as indole-3-carbinol and isothiocyanates, which can act as anticarcinogens by inducing phase II enzymes of drug metabolism. Recently, Talalay and coworkers from NIH, USA, have isolated an isothiocyanate, named 'sulphorafane' from *broccoli* which is a powerful inducer of phase II drug metabolising enzymes[23,24]. A screening of a wide range of vegetables[20] indicated that *broccoli* and *green onions* had the highest activity with regard to induction of quinone reductase. Phenolic compounds in *green* and *black tea* (catechin, flavonols, theaflavin) have been reported to inhibit cytochrome P-450 mixed function oxygenase and enhance phase II enzymes glutathione S-transferase, quinone reductase[25].

Table 3
Anticarcinogens In Plant Foods

Category	Compounds
Compounds preventing formation of carcinogens from precursor	Ascorbic acid, $\alpha+\gamma$ tocopherols, caffeic acid, ferulic acid
Blocking agents	*Phenols:* Ellagic acid, caffeic acid, ferulic acid, p-hydroxy cinnamic acid *Indoles:* Indole-3-acetonitrile, indole-3-carbinol, 3-3' indolymethane *Aromatic isothiocyanates:* Benzyl isothiocyanates, phenyl isothiocyanates *Coumarins:* Coumarin, limethin *Flavones:* Quercetin pentamethyl ether *Diterpenes:* Kahweal palmitate *Retinoids & carotenoids:* Retinyl palmitate, retinyl acetate, β-carotene
Suppressing agents	Soyabean protease inhibitor, benzyl-isothiocyanate, β-sitosterol, caffein, fumaric acid, selenium

BLOCKING AND SUPPRESSING AGENTS

According to Wattenberg[21,22], several minor chemical constituents of foods inhibit carcinogenesis by blocking and suppressing action. The blocking agents, besides enhancing the metabolic disposal of carcinogens as discussed above, prevent the active carcinogen reaching the target tissue by sequestering them (for example, glutathione and plant phenolic compounds). Suppressing agents are those which inhibit carcinogenesis, presumably acting at the cellular level, when administered subsequent or just prior to exposure to a carcinogen from which cancer would result in the absence of a suppressor. Compounds in foods which act through the above mechanisms as anti-cancer agents are given in Table 3.

Isoprenoid compounds in fruits, vegetables, cereal grains and essential oils, namely limonene, besides inducing phase II drug metabolising enzymes also act as suppressing agents. They suppress tumour growth by suppressing HMG CoA reductase activity, a rate limiting step in cholesterol biosynthesis[26]. Such an inhibition depletes cells of intermediate products required for cell proliferation. By inhibiting cholesterol synthesis, these compounds can also protect against cardiovascular diseases. *Garlic*, widely used as a spice, contains sulphur compounds, allilic sulphides, which can induce phase II drug metabolising enzymes and inactivate carcinogens. *Garlic*, after mild processing, has been shown to yield a compound — agoene which can prevent platelet aggregation. This action of *garlic* may be related to the reported beneficial effect of garlic in heart diseases[27].

It can be seen that several compounds which act as antioxidants (ascorbic acid, tocopherols, phenols) also possess inhibitory properties. Indoles and aromatic isothiocyanates are important inducers of DME. Phenols can also bind carcinogens to prevent their action at the cellular level. Protease inhibitors, plant sterols and caffein can act as suppressing agents. Although these compounds (Table 3) occur widely, the precise content of several of these compounds in foods is yet to be determined.

It must, however, be remembered that phenolic compounds and thiocyanates also have antinutritional properties. Polyphenols can bind dietary iron and make it unavailable. Thiocyanate compounds as well as phenols are also goitrogenic. Thiocyanates, isothiocyanates and indoles are powerful inducers of DME. The former two occur widely in vegetables belonging to the crucifera family, namely *cabbage*, *mustard*, *rape greens* and seeds.

These chemicals can be separated employing HPLC and GC. The inhibitory potential of these compounds can be tested both *in vitro* and *in vivo* on the enzyme systems that actively convert precarcinogens to carcinogens and on drug metabolising enzymes, and their effect on sequestering carcinogens and preventing their binding to cell membrane and DNA.

OTHER PHYTOCHEMICALS IN PLANT FOODS

Besides the compounds described under the above three classes of major biologically active phytochemicals, which have been extensively investigated, there are a number of other chemicals whose possible biological activity is yet to be established firmly. Among those are amines and non-protein amino acids; alkaloids and other nitrogen compounds and plant sterols.

It has been reported recently that *Vicia fabia* beans are rich in L-dopa and these beans are being used in the treatment of Parkinson's disease caused by the cerebral deficiency of dopamine[28].

Caffein is an alkaloid which can act as a stimulant. A fair quantity of caffein is ingested through *coffee* and *tea*. As mentioned above, caffein can act as a blocking agent in carcinogenesis.

Certain plant sterols have been shown to have hypocholesterolemic potential. The hypocholesterolemic effect of *rice bran oil* has been attributed to plant sterols present in the unsaponifiable fraction of the oils[29].

FUTURE RESEARCH

It will thus be seen that foods contain a large number of chemical compounds, other than nutrients, which have a wide range of biological activity and the potential of offering protection against different diseases such as diabetes, cancer, cardiovascular diseases, cataract, etc. Some vitamins also share these properties.

As in the case of nutrients, there is a need to determine the optimal dietary intake of these chemicals to provide maximal protection against these diseases. As a prelude to this type of research, there is a need for precise quantitative data on the content of these chemicals in commonly consumed foods and on their bioavailability and biological protency.

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NUTRITION NEWS

- **The XXVII Annual Meeting of the Nutrition Society of India**: At this meeting held on November 24 and 25, 1994, at the National Institute of Nutrition, Hyderabad, Dr B.N. Tandon was elected President of the Society succeeding Dr K.T. Achaya. The Executive Committee for the term 1994-96 also includes Dr Subadra Seshadri (Vice President), Dr Kamala Krishnaswamy (Vice President), Dr Usha Chandrashekhar (Secretary), Dr J.C. Raghuram (Joint Secretary) and Dr Hanumantha Rao (Treasurer).

The scientific programme for the meeting included:

- The *18th Gopalan Oration* on 'Assessing Energy Needs — Recent Advances' by *Prof W.P.T. James*

- The *Srikantia Memorial Lecture* on 'Problems and Prospects in Upscaling Nutrition Projects to Programmes', by *Dr Tara Gopaldas*

- Symposia on 'Food Safety and Food Quality' and on 'Diet Related Chronic Diseases'; and,

- Panel discussion on the 'National Nutrition Policy and Plan of Action'.

- **The 21st Kamla Puri Sabharwal Memorial Lecture** on 'Chemical Contaminants in Drinking Water — Public Health Issues and Challenges' was given by Dr A.K. Susheela at the Lady Irwin College, New Delhi on December 15, 1994. Dr C. Gopalan presided.