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# POLYPHENOLS: ANTIOXIDANT MAVERICK TO TACKLE NON-COMMUNICABLE DISEASES

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## ABSTRACT

In recent years, healthy diet is gaining increased importance in the face of rampant Non-communicable diseases (NCDs) scenario India is reverberating with. Polyphenols, forming largest category of phytochemicals, are gaining unprecedented interest as 'life span essentials' in scientific settings. They are ubiquitous plant secondary metabolites, structurally diversified group ranging from simple phenols i.e. phenolic acids to complex phenols i.e. condensed tannins. Their antioxidant mechanisms: both direct (free radical scavenging, metal chelation) and indirect (cell signal modulation, phytochemical hormetic pathways) have been well-corroborated to attenuate oxidative stress and eventually culminates in health promotion and decreased incidence of NCDs. In this line, recommended dietary allowance has not been set precisely yet and in general, calls for increased frequency of their consumption from varied plant sources (fruits, vegetables, whole cereals, pulses, nuts) on a long term basis to reap its benefits.

**KEYWORDS:** polyphenols, antioxidants, non-communicable diseases

## INTRODUCTION

### BURDEN OF NON COMMUNICABLE DISEASES (NCDS) IN INDIA

NCDs in India have become a big scourge by accounting for more disability-adjusted life years (DALY) (Bloom et al, 2014), and 53% deaths in India originating from cardiovascular diseases (24%), chronic respiratory diseases (11%), cancer (6%), diabetes (2%) (Sharma K, 2013). A report 'Global Burden of Cancer-2013' covering 28 cancer groups of 188 countries from 1990-2013 cited that global mortality rate from NCDs (57% in 1990 to 70% in 2013) has outstripped chorus of communicable, maternal, neonatal, and nutritional diseases (34% in 1990 to 22% in 2013); and those originating from cancer increased from 12% in 1990 to 15% in 2013, with abrupt hike to 60% in Indian scenario and new cancer cases almost doubled during the period (Fitzmaurice et al, 2015). In 2005, India witnessed the "highest loss in potentially productive years of life" worldwide, leading killer being reported as cardiovascular disease (Chakma and Gupta, 2014). This scenario indicates multi-pronged etiology i.e. increased sedentary lifestyle, increased shift towards processed foods, high-calorie diet, and decreased consumption of coarse cereals, fruits and vegetables which thereof calls for the urgent action needed to be taken on therapeutic modifications viz. increased consumption of coarse grains, inclusion of dietary fibre, decreased and judicious selection of processed foods, as a prudent adjunct with structured physical activity.

### OXIDATIVE STRESS - A COMMON DENOMINATOR IN PATHOGENESIS OF NCDS

Oxidative stress is defined as 'prooxidant-antioxidant imbalance in favour of the former, leading to potential biomolecular damage afflicted by free radicals i.e. Reactive Oxygen and Nitrogen Species {RONS- ROS ($O_2^{\cdot-}$, $H_20_2$, $OH^{\cdot}$, $RO^{\cdot}$, $ROO^{\cdot}$); RNS ($NO^{\cdot}$, $NO_2$, $ONOO^{-}$}, and disruption of redox signalling and control' (Sies H, 1991; Halliwell B, 2007; Jones DP, 2006). Operational concepts in the definition entail *prooxidant-antioxidant imbalance* which decodes that RONS, at low and steady concentrations exquisitely controlled by body antioxidants, are cardinal for normal physiological functioning i.e. immunity, muscular contraction, nerve transmission, gene expression, reproduction, apoptosis and so forth, and that oxidative stress results due to high, toxic levels of RONS supplied either by environmental stressors or emasculated antioxidant systems (Rahal et al, 2014); *biomolecular damage* involving a) Lipid peroxidation of cell membranes through formation of peroxyl

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radicals, hydroperoxides, endoperoxides, carbonyl products [HNE (4-Hydroxynonenal), MDA (Malondialdehyde)], which if not curtailed by antioxidants, facilitates formation of advanced-lipoxidation end products (ALE) due to carbonyl products-induced DNA and protein damage b) Oxidative modification of proteins (i.e. metabolic enzymes with Fe-S clusters (GADPH), detoxification enzymes [Cu-Zn SOD (superoxide dismutase with copper and zinc at its active site)], oxidized-proteins removal systems (proteasomes), kinases, transcription factors, receptors particularly those with cysteine, methionine, tyrosine, phenylalanine c) DNA damage by inflicting oxidative injury to purine, pyrimidine bases and deoxy ribose sugar leading to single-strand, double-strand DNA breaks d) increased intracellular levels of free calcium and iron leading to metal-catalyzed radical production and mitochondrial dysfuction (Avery SV, 2011); *hyperactivation of signal transduction mechanisms* [Hypoxia Inducible Factors (HIFs), Mitogen Activated Protein Kinases (MAPKs), Nuclear factor kappa-light chain-enhancer of activated B cells (NF-Kb), Phosphoinositide 3-kinase (PI3K)] associated with defiant cell growth and inflammatory responses; the term 'signal' itself addresses pro-oxidant shift in cell's redox state and carries information from outside of the cell to inside via oxidative modification {oxidising thiol moiety (-SH) of cysteine in proteins to disulphide bond (-SS-)} of the proteins i.e. receptors, protein kinases [viz. non-receptor tyrosine kinases (Src family kinases), c-Jun N-terminal kinases (JNK) and p38 MAPK signaling, insulin receptor kinase], protein phosphatases (viz. tyrosine phosphatases), transcription factors [viz. activated protein-1 (AP-1) and NF-$_{K}$B, bacterial OxyR] (Valko et al, 2007; Schieber and Chandel, 2014).

Oxidative stress has been implicated to play a prudent role in the pathology of 120 diseases, with robust *in vivo* (rats and humans) evidences for cancer, neurodegenerative, cardiovascular, autoimmune, inflammatory diseases (Halliwell B; 2010; Perl et al, 2013; Rahman et al, 2012). Oxidative assaults purported to cause cancer comprise DNA damage, lipid peroxidation, aberrant signalling mechanisms [hyperactivation of cancer causing proteins viz. MAPK/AP-1, NF-Kb, epidermal growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF); mutations in tumour suppressor protein p53], apoptotic inhibition due to Bcl-2 overexpression and elevated thioredoxin; cardiovascular disease entail peroxidation of cell membrane and circulating LDL, hyperactivation of ROS generating enzymes {xanthine oxido-reducatse (XOR) and NAD(P)H oxidase} (Valko et al, 2007); neurodegeneration involve mitochondrial dysfunction, accumulation of toxic protein aggregates, defiant inflammatory state, and defects in aberrant protein clearance (Reynolds et al, 2007); diabetes encompass insulin resistance due to deranged insulin signalling and pancreatic β-cell failure (Chang and Chuang, 2010); autoimmune diseases encompass NF-kB-mediated release of pro-inflammatory cytokines [(interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNFα), interferon beta (IFNβ)] and proliferation of immune cells (Schieber and Chandel, 2014)

## ANTIOXIDANT DEFINITIONS

Antioxidant definition has various versions. Halliwell and Gutteridge, 1995; Halliwell B, 2007 defined antioxidants as 'any substance that, when present at low concentrations compared to that of an oxidizable substrate (lipids, proteins, DNA, carbohydrates), significantly delays or inhibits oxidation of that substrate and remove oxidative damage inflicted to it'. Khlebnikov et al, 2007 cited it as 'any substance that directly scavenges ROS or indirectly acts to upregulate antioxidant defences or inhibit ROS production'. Pamplona and costantini, 2011 broadly defined it as any mechanism, structure and/or substance that prevent, delays, removes or protects against oxidative non-enzymatic chemical modification (damage) to a target molecule. Blomhoff, 2005 defined it as 'A redox active compound that limits oxidative stress on non-enzymatic reaction with a reactive oxidant'.

## SIGNIFICANCE OF DIETARY ANTIOXIDANTS

In the 21$^{st}$ century, people need to get more antioxidants , popularly extolled as *'life span essentials'* in the diet to offset the oxidative assaults laid on too thick by deplorably polluted environment, refined and convenient approaches to modern lifestyle, and also the rampant burden of NCDs. It has been estimated that a healthy diet can prevent approximately 30% of all cancers (dong et al, 2007). Various epidemiological investigations and intervention trials have purported dietary/native antioxidants (present in nutritional doses), instead of supplemental antioxidants, to be effective candidates against chronic health complications (Bouayed and Bohn, 2010). Supplemental administration of antioxidant vitamins and minerals (Vitamins A, C, E, selenium) either singly or in combination in healthy subjects and smokers has shown no effect in reducing mortality from cancer and cardiovascular disease, and in many cases has exacerbated it (Lindsay and Astley, 2002).

Phytochemicals are gaining unprecedented recognition, apparent from their citation as *'foods for the 21$^{st}$ century'*. They are non-nutritive, bio-active plant chemicals in fruits, vegetables, grains with health benefits; and broadly categorised as carotenoids, phenolics, alkaloids, nitrogen-containing and organosulfur compounds (Irina and

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Mohamed, 2012). Polyphenols, forming the largest class of phytochemicals and popularly cited as *life span essentials*, are gaining robust interest attributed to their potent antioxidant activity, ubiquitous presence in plant foods and documented literature corroborating their health-promoting and disease-preventing potential.

## NOMENCLATURE OF POLYPHENOLS

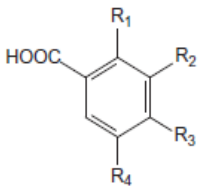
Dawn on the concept 'Polyphenols' seeded from the 'French paradox' report, a france situation of decreased coronary heart disease (CHD) risk, despite high saturated fat intake, attributed to red wine consumption as a carrier of protective phenolic compounds (Renauds and De Lorgeril, 1992). Chemically, polyphenols are the products of secondary metabolism in plants (literally, they are produced in plants as defensive mechanisms against ultraviolet radiation or aggression by pathogens, pesticides); characterized by hydroxylated phenyl moieties; more than 8000 species have been identified in plant species (Soto-Vaca et al, 2012)

Polyphenols comprise two main classes - the flavonoids and nonflavonoids, based on the number of phenol rings and the way in which these rings interact, and range from simple i.e. phenolic acids to complex compunds i.e. tannins. Flavonoids are polyphenolic compounds comprising 15 carbons, with two aromatic rings (A and B) connected by a three-carbon bridge (C6–C3–C6), and difference in its sub-classes arise from the hydroxylation pattern of the ring-structure, the degree of saturation of the C-ring, and the substitution of the 3-position.The nonflavonoid group may be separated into two different classes: (1) the phenolic acids, including the hydroxybenzoic acids (HBAs; C1–C3 skeleton) and hydroxycinnamic acids (HCAs; C3–C6 skeleton), and (2) the stilbenes (C6–C2–C6 skeleton) (Vauzour D, 2012). Tannins are high molecular-weight phenolic compounds having two sub-classes - condensed tannins (proanthocyanidins) and hydrolysable tannins. Proanthocyanidins are polyhydroxyflavan oligomers or polymers, with most common sub-cateogories as procyanidins (made of catechin/epicatechin monomers) , prodelphinidins (made of gallocatechin/epigallocatechin monomers), propelargonidins (made of afzelechin/epiafzelechin monomers) linked through B-type linkage (4→6 or 4→8 carbon-carbon linkage) or A-type linkage (C2→C7 ether-linkage) ccur either as hydrolysable or condensed tannins. Hydrolysable tannins are condensation products of phenolic acids (gallic, ellagic acids) and sugars, mostly glucose (Serrano et al, 2009).About benzoic acid derivatives, common ones are salicyclic acid, gallic acid, vanillic acid, syringic acid, p-hydroxybenzoic acid, protocatechuic acid, may be present in soluble or conjugated forms, their content in edible plants is low exception being blackberries, raspberries and tea. Common cinnamic acid derivatives are caffeic acid, chlorogenic acid, ferulic acid, coumaric acid.

## STRUCTURES OF POLYPHENOLS

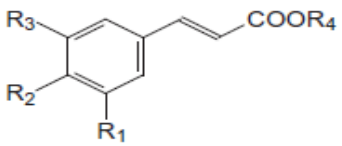
***Phenolic acids***

- Hydroxybenzoic acids (C6-C1)




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Gallic acid | H | OH | OH | OH |
| Protocatechuic acid | H | OH | OH | H |
| Genistic acid | OH | H | H | OH |
| *p*-Hydroxybenzoic acid | H | H | OH | H |
| Vanillic acid | H | $OCH_3$ | OH | H |
| Syringic acid | H | $OCH_3$ | OH | $OCH_3$ |
| Salicylic acid | OH | H | H | H |

- Hydroxycinnamic acids (C6-C3)




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Caffeic acid | OH | OH | H | H |
| Chlorogenic acid | OH | OH | H | Quinic acid |
| Ferulic acid | $OCH_3$ | OH | H | H |
| *p*-Coumaric acid | H | OH | H | H |
| *m*-Coumaric acid | OH | H | H | H |

*Flavonoids (C6-C3-C6)*

- Flavonols

| | 5 | 7 | 3' | 4' | 5' |
|---|---|---|---|---|---|

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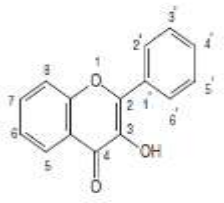
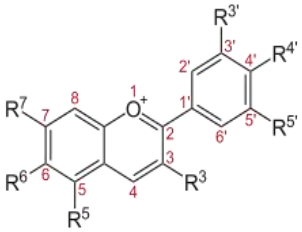


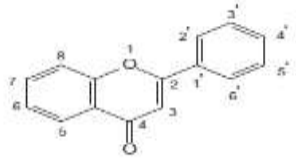
| | | | | | |
|---|---|---|---|---|---|
| Quercetin | OH | OH | OH | OH | H |
| Kaempferol | OH | OH | H | OH | H |
| Myricetin | OH | OH | OH | OH | OH |
| Isorhamnetin | OH | OH | $OCH_3$ | OH | H |
| Rutin | Quercetin + Rutinoside at C-3 position | | | | |

- Anthocyanins



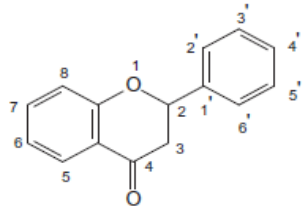
| | $R^{3'}$ | $R^{4'}$ | $R^{5'}$ | $R^3$ | $R^5$ | $R^6$ | $R^7$ |
|---|---|---|---|---|---|---|---|
| Pelargonidin | H | OH | H | OH | OH | H | OH |
| Cyanidin | OH | OH | H | OH | OH | H | OH |
| Delphinidin | OH | OH | OH | OH | OH | H | OH |
| Peonidin | $OCH_3$ | OH | H | OH | OH | H | OH |
| Petunidin | $OCH_3$ | OH | OH | OH | OH | H | OH |
| Malvidin | $OCH_3$ | OH | $OCH_3$ | OH | OH | H | OH |

- Flavones



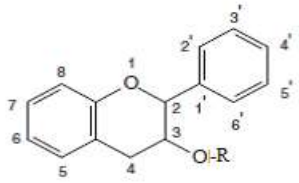
| | 3’ | 4’ | 5’ | 5 | 7 | 8 |
|---|---|---|---|---|---|---|
| Apigenin | H | OH | H | OH | OH | H |
| Luteolin | OH | OH | H | OH | OH | H |
| Orientin | OH | OH | H | OH | OH | Glc |
| Vitexin | H | OH | H | OH | OH | Glc |

- Flavanones



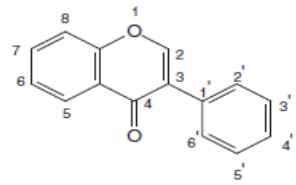
| | 3’ | 4’ | 5 | 7 |
|---|---|---|---|---|
| Hesperetin | OH | $OCH_3$ | OH | OH |
| Hesperidin | OH | $OCH_3$ | OH | rutinose |
| Naringenin | H | OH | OH | OH |
| Naringin | H | OH | OH | 2-*O*-α-L-Rhamnosyl-D-glucoside |
| Eriodictyol | OH | OH | OH | OH |

- Flavanols



| | 3’ | 4’ | 5’ | R | 5 | 7 |
|---|---|---|---|---|---|---|
| Epi(-catechin) | OH | OH | H | H | OH | OH |
| Epigallocatechin | OH | OH | OH | H | OH | OH |
| Epicatechin gallate | OH | OH | H | gallate | OH | OH |
| Epigallocatechin gallate | OH | OH | OH | gallate | OH | OH |

- Isoflavones



| | 4’ | 5 | 6 | 7 |
|---|---|---|---|---|
| Daidzein | OH | H | H | OH |
| Genistein | OH | OH | H | OH |
| Glycetein | OH | H | $OCH_3$ | OH |
| Formononetin | $OCH_3$ | H | H | OH |
| Biochanin A | $OCH_3$ | OH | H | OH |

caffeic, ferulic, sinapic, p-coumaric, chlorogenic acids, rarely found in free form and the bound forms are glycosylated derivatives or esters of quinic, shikimic or tartaric acid i.e. chlorogenic acid (5-caffeoylquinic acid). Cinnamic acid derivatives are more abundant in fruits compared to benzoic acid counterparts with higher concentration in outer parts (3). Flavonoids sub-classes include flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols. 1) Flavonols- double bond between C2 and C3 with hydroxyl group in C3 position,

represent most ubiquitous flavonoid with quercetin and kaempferol as the most representative compound, main sources include onions, curly kale, leeks, broccoli, blueberries, tea and red wine; present in glycosylated form with sugar moieties as glucose, rhamnose, galactose, arabinose, xylose, glucuronic acid 2) Flavones- double bond between C2 and C3; chiefly consist of glycosides of luteolin and apigenin; less common flavonoid with parsley and celery the only important edible sources 3) Flavanones- saturated three carbon chain and an oxygen atom in C4, generally glycosylated by disaccharides (neohesperidose or rutinose) at C7, main sources are citrus fruits with naringenin, hesperetin and eriodictyol being the main aglycones in grapefruit, oranges and lemons respectively; whole fruit contain 5 times as much as a glass of orange juice due to high flavonone content in albedo and membranes; also present in tomatoes and certain aromatic plants such as mint 4) Isoflavones- structutally similar to flavonols with b ring in c3 position, chiefly consists of genistein, daidzein and glycitein in four forms i.e. aglycone, 7-*O*-glucoside, 6''-*O*-acetyl-7-*O*-glucoside, and 6''-*O*-malonyl-7-*O*-glucoside; exclusively present in leguminous plants in aglycone or glucosylated form 5) Anthocyanins- water soluble pigments responsible for red, blue, purple colors of fruits and vegetables, most common are malvidin, petunidin, cyanidin, peonidin, delphinidin, and pelargonidin, generally found as glycosides with glucose, galactose and arabinose, widely distributed in human diet (red wine, certain cereals and vegetables) with fruits being most abundant sources; found mainly in skin except for some red fruits (cherries and strawberries) 6) Flavanols- saturated three carbon bridge with hydroxyl group in c3 position; present in monomeric and polymeric form (catechins and proanthocyanidins respectively); not glycosylated; present in fruits as catechin and epicatechin and in leguminous plants, grapes, and more importantly in tea as gallocatechin, epigallocatechin, and epigallocatechin gallate; proanthocyanidins (condensed tannins) are dimers, oligomers, and polymers of catechins that are bound together by links between C4 and C8; very hard to estimate their content in foods due to wide range of structures and molecular weights; responsible for astringency of fruits and beverages and bitterness of chocolate (26). The concentrations of phenolic and polyphenolic in plants are influenced by growing conditons, moisture, attack by plant pathogens, germination, extent of ripeness, processing and storage conditions (Soto-Vaca et al, 2012).

Major sources of polyphenols in human diet include fruits, vegetables, cereals, legumes, beverages, cocoa products, herbs and spices, oilseeds. There is sea of evidence on fruits and vegetables polyphenols, and however, the importance of cereals, legumes, oilseeds as a source of antioxidants is often undervalued.

In human studies, Flavonoid intake was found to be inversely associated with renal cell carcinoma, colorectal cancer, breast cancer, prostate cancer, improved cognition, reduced incidence of neurodegeneration, reduced blood pressure, improved insulin sensitivity and cardioprotection (Stevenson and Hurst, 2007). A 12 years follow-up study on 807 subjects aged 65 years or older reported that high concentrations of a urinary biomarker of polyphenol intake caused 30% reduction in all-cause mortality (Zamora-Ros et al, 2013). In a 25-day human intervention trial on healthy non-smokers, 600 g/ day of fruit and vegetables induced increased glutathione peroxidase activity by ~15% and decreased plasma lipid oxidation rates, without significantly changing any of the many other measured markers of redox status. A report by Soto-vaca et al, 2012 cited soya isoflavones to activate endothelial nitric oxide synthase which is associated with Nrf2-mediated antioxidant gene expression and stimulation of prostacyclin (inhibits platelet activation) in human endothelial cells.

## ANTIOXIDANT MECHANISMS OF POLYPHENOLS

***Direct antioxidant effects-*** Direct antioxidant effects of polyphenols include free radical scavenging, metal chelation, and inhibition of radical-producing enzymes (Xanthine oxidase, lipoxygenase, cyclo-oxygenase, nitric oxide synthase, myeloperoxidase, angiotensin converting enzyme, tyrosine kinase) and cancer-agonisting enzyme (telomerase) (Stevenson and Hurst, 2007). These antioxidant mechanisms emanate from structure-activity relationship (SAR) of polyphenols, particularly flavonoids and deciphered as number of hydroxyl groups as well as their positions {3'-, 4'- OH groups on B rings (also called as catechol group), 4'-OH group on B ring and 3-OH group on C ring}; unsaturation between 2 and 3 carbon atoms on C ring; and 4-oxo function of C ring (Fraga CG, 2007; Irina and Mohamed, 2012).

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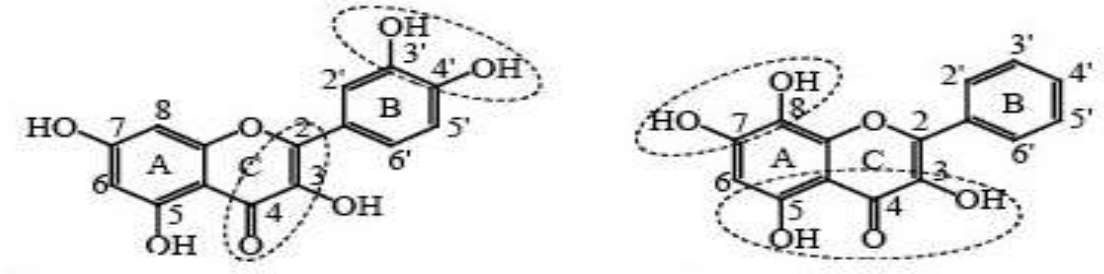


Radical (hydroxyl, peroxyl, superoxide, peroxynitrite) scavenging ability of polyphenols attributes to their hydrogen atom donating property leading to radical stabilization with concomitant formation of less reactive and more stable phenoxyl radical which is stabilized by resonance, or may react with another radical to form stable quinone structure (Irina and Mohamed, 2012).

Inhibition of metal-catalysed hydroxyl radical formation is mediated through auto-oxidation of $Fe^{2+}$ (or $Cu^{+}$) or formation of inactive complex with $Fe^{2+}$ (or $Cu^{+}$) and structures implied to this advantage are hydroxyl-keto group (3-OH or 5-OH with 4-C=O) , as well as catechol moiety (Dai and Mumper, 2010).

***Bioavailability***- Direct antioxidant effects of polyphenols are the function of their bioavailability (bioaccessibility + bioavailability + utilization coefficient). However, their bioavailability in humans have been consistently reported to be low, and even their increased consumption correspond to low plasma antioxidant concentrations (<1 µM/L) in the body against the ones (high µM to low mM) required to elicit free radical scavenging and metal chelation actions (Halliwell et al, 2005; Halliwell and Gutteridge, 2006). The factors purported to affect their bioavailability are low absorbability (attributed to their significant (90-95%) presence as esterified, polymerized, glycosylated forms in foods, post-absorptive metabolism-derived loss of antioxidant function and antioxidant non-selectivity against ROS-heterogeneity (Shen et al, 2007). Metabolism-derived loss of antioxidant function explains the conjugation processes (glucuronidation, sulphation, methylation) that happens after absorption in liver and small intestine and diminish antioxidant potential due to addition of glucuronyl, sulphate, methyl groups to phenolic structure in exchange of antioxidative-hydroxyl groups, and more evidence to this line advocates that this constraint can be relented by increasing the consumption and frequency of polyphenol-rich foods, as a function of increased polyphenol aglycones outstripping aforesaid conjugation processes.

However, non-bioaccessible polyphenolic fractions (oligomeric and polymeric proanthocyanidins) have been reported to provide local antioxidant activity in GIT, attributed to their higher concentrations in these areas, pre-absorptively {i.e. efficient complex-forming capacity of oligomeric and polymeric proanthocyanidins with digestive enzymes during the course of digestion eventually culminating in enzyme inhibition and glycaemic control, and polymerization-dependent (upto seven units) radical (hydroxyl, superoxide, peroxyl) scavenging and metal chelation} and post-absorptively (non-absorbable proanthocyanidin fractions remain in colonic lumen and counteract the effects of dietary pro-oxidants produced during colonic microbial metabolism (Halliwell et al, 2005).

Improved gut microbial health as another dimension of polyphenol bioactivity is gaining unprecedented recognition. This is elucidated as prebiotic effects of polyphenols i.e. provision of fermentable substrates to colonic microflora which transcends to upregulation of beneficial microbial community and cumulative effect on systemic antioxidant activity emanating from absorbable phenolic metabolites (Visioli et al, 2011; Cardona et al, 2013). A study by Yamakoshi et al, 2001 reported that proanthocyanidin rich extract from grape seeds given to healthy adults for 2 weeks was able to significantly increase the number of bifidobacteria. Dolara et al, Dietary supplementation of dealcoholized, proanthocyanidin-rich red wine extract for 16 weeks documented a shift from the predominance of bacteroides, clostridium, and propionibacterium species to a predominance of bacteroides, lactobacillus and bifidobacterium species in rats. Jackson et al 2011 extolled Equol, a gut microbial-facilitated precursor of daidzein isoflavone, to be a more potent antioxidant than daidzein itself and prudent against cardiovascular disease via halting metal-induced oxidation of LDL, increasing the transcription of endothelial nitric oxide synthase, downregulating the expression of NADPH oxidase and abating serum levels of C-reactive protein. Recent evidence on polyphenolic bioavailability states that the most abundant polyphenols in diet are not necessarily those leading to highest concentrations in the human body in a way that gallic acid and isoflavones are the most absorbed fractions followed by catechins, flavanones and quercetin glucosides while

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proanthocyanidins, galloylated tea catechins and anthocyanins are least absorbed polyphenols (Manach et al, 2005).

***Indirect antioxidant effects-*** Irrespective of their plasma concentrations, polyphenols have been documented to directly interact with cell membranes components i.e. proteins due to their structural propensity of establishing intramolecular hydrogen bonding (IHB) with proteins. And since polyphenols are plant secondary metabolites, their interaction develops low level oxidative stress in cells as a consequence of their evolutionary mechanism of defence against physical and biological damage, which in turn sets the ground for signal transduction cascade associated with endogenous antioxidant upregulation, reduced inflammation, and reduced tumour proliferation. These pathways are:

***Modulation of signalling pathways and health promotion***

Several polyphenols including resveratrol, catechins, curcumin, genistein, caffeic acid, ellagic acid, theaflavins, coumarins are documented to prevent and treat cancer by inhibiting transcriptional factors viz. NF-Kb and AP-1, protein kinases viz. Protein kinase B (AKT) and MAPK, growth factor receptors, cyclooxygenase (COX-2) enzyme associated with tumour survival, proliferation, invasion, angiogenesis and metastasis (Shanmugam et al, 2011). Robust evidence for polyphenols (chlorogenic acid, resveratrol, genistein, quercetin, catechins, curcumin, flavonols, flavones, anthocyanins), carotenoids and organosulfur compounds to act as anti-obesity agents exist and the molecular mechanisms explaining to the benefit are transcriptional activation of peroxisome proliferator-activated receptors (PPARs) {they modulate lipolysis, glucose transport, improved insulin sensitivity}, inhibition of liver X receptor (LXR) {which favours free fatty acid, triglyceride, cholesterol synthesis)}, and inhibition of NF-kB {master of inflammatory responses} (Ali et al, 2014; Meydeni and Hasan, 2010; Williams et al, 2013). A study by Shanmugam et al, 2011 provided an exhaustive review on dietary phytochemicals to act as promising chemo-preventive and treatment strategies in *in vivo* and *in vitro* models. They include curcumin (turmeric), resveratrol (red grapes, peanuts and berries), genistein (soyabean), diallyl sulphide (allium), S-allyl cysteine (allium), allicin (garlic), lycopene (tomato), capsaicin (red chilli), diosgenin (fenugreek), 6-gingerol (ginger), ellagic acid (pomegranate), ursolic acid (apple, pears, prunes), silymarin (milk thistle), anethol (anise, camphor, and fennel), catechins (green tea), eugenol (cloves), indole-3-carbinol (cruciferous vegetables), limonene (citrus fruits), beta carotene (carrots) and dietary fiber and are well-documented to downregulate various signal transduction, transcription and apoptotic pathways {NF-$_K$B, AP-1, AKT, signal transducer and activator of transcription (STATS), endothelial growth factor receptor (EGFR), COX-2, histone deacetylases (HDAC), Bcl2 (B-cell lymphoma 2) family, C-X-C-motif chemokine-receptor 4 (CXCR4)} associated with cell hyperproliferation, angiogenesis and metastasis. A study by Pasten et al 2007 conducted in cultured human coronary artery endothelial cells has also demonstrated a beneficial effect of polyphenols (ie, catechin and quercetin) on the expression of the plasminogen activator inhibitor-1 gene, potentially providing a further biological explanation to the cardiovascular protective role of these molecules. Quercetin and naringenin have been reported to inhibit certain cytochrome P450 enzymes (CYP1A1 and CYP3A4) involved in the bioactivation of chemical carcinogens, constituting another proposed chemopreventive mechanism of polyphenols against cancer development including lung cancer (Bouayed and Bohn, 2010). Citrus flavonoids i.e. hesperidin, neohesperidin, hesperetin confer neuroprotection via signal modulation of Protein Kinase A (PKA), serine threonine kinase/Protein Kinase B (Akt/PKB), Protein Kinase C (PKC), extracellular signal-regulated kinases (ERK1/2), p38 MAPKs and JNK pathways related to antioxidant protection viz. inhibition of ROS formation and caspase 3 activity, decrease in membrane and DNA damage, enhance of antioxidant enzyme activity, and maintainence of calcium homeostasis and mitochondrial potential (Hwang et al, 2012)

***Activation of cellular antioxidant response (Keap1/Nrf2/ARE pathway)***

Nuclear factor erythroid 2-related factor 2 (Nrf2), cited as master regulator of endogenous antioxidant response, is a transcriptional factor activated as an cellular adaptation to mild oxidative stress. Biochemical mechanism of Nrf2 activation starts from oxidation of cysteine residues in Kelch-like ECH-associated protein-1 (Keap1) leading to Keap-Nrf2 dissociation in cytosol followed by Nrf2 activation and its translocation to nucleus, binding with antioxidant response element (ARE), eventually resulting in upregulation of a) glutamate cysteine ligase and glutathione synthetase (enzymes required for glutathione biosynthesis), b) superoxide dismutase, peroxiredoxins, thioredoxins, thioredoxin reductase (enzymes catalysing radical scavenging and reduction processes), c) heme-oxygenase 1 (enzymes trigerring catabolism of pro-oxidant heme), d) metallothionein (free iron, copper, zing sequestering proteins), e) NAD(P)H:quinine oxidoreductases (enzyme detoxifying highly reactive quinones), f) glutathione-s-transferase (enzyme involved in glutathione conjugation with xenobiotic substrates for latter's detoxification), g) Heat shock proteins (prevent aberrant protein accumulation in cells) (Stefanson and Bakovic,

2014). Furthermore, Nrf2 activation is coupled with NF-kB deactivation. NF-kB is a transcriptional factor which is known to regulate high-grade oxidative stress and control tumour cell survival, proliferation, metastasis, invasion and angiogenesis via NF-kB/ TNF-α sinaling cascade. Polyphenol-induced Nrf2 activation is popularly known as *Xenohormesis*, a metaphor for beneficial actions of low-dose polyphenols, obtained from natural food sources, in human body (Poljsak, 2011). Polyphenols documented to induce Nrf2/ARE response are curcumin, flavonols (quercetin, fisetin), flavan-3-ol (epigallocatechin-3-gallate), isoflavone (daidzein), stilbene (resveratrol), cinnamic acid derivatives (caffeic and ferulic acid esters), gallic acid (Magesh et al, 2012).

***Activation of Sirtuin 1 (SIRT1)***

Polyphenols postulated to activate SIRT1 are curcumin, catechins, resveratrol, quercetin. Sirtuins are $NAD^+$-lysine deacetylases, which by causing deacetylation of cell proteins, are cited to have two-pronged cellular antioxidant mechanisms- activation of transcriptional factor Forkhead box 3 (FOXO3) and inhibition of transcriptional factor NF-kB. FOXO3 activation confers upregulation of antioxidant enzymes {catalse, SOD2 (having manganese at its active site)}, reduction in neuronal toxicity associated with aberrant protein aggregation; and NF-kB inhibition entails anti-proliferative activity in tumor cells and reduction in oxidative stress (Chung et al, 2010).

## RECOMMENDED AND ACTUAL DIETARY INTAKE OF POLYPHENOLS

The current dietary advice for polyphenols, in healthy adults, is to consume 5 portions of fruit and vegetables every day, each portion being depicted as 100 g (Williamson and Holst, 2008). World Health Organisation (WHO) recommended 400 g of fruit and vegetable intake, that excludes potato, cassava and starchy tubers.

A survey conducted on 1,001 subjects from five cities namely, NCR, Mumbai, Kolkata, Hyderabad, Chennai and belonging to middle and upper income groups have pin-pointed Indians to have sub-optimal intake of fruits and vegetables (120 g of fruits and 160 g of vegetables, totalling 280 g) in Indians (Mukherjee et al, 2016) and worse, increased consumption of low-fibre and refined carbohydrates (Bhattacharya, 2015) which is well-reported to be devoid of antioxidative polyphenols. Therefore, an holistic approach comprising of varied dietary sources viz. fruits, vegetables, whole cereal grains and legumes, nuts) calls for critical attention to peak antioxidant load within the body.

## CONCLUSIONS

Dietary Antioxidants are cardinal as complementary systems in body antioxidant network, especially in the face of evolutionary pro-oxidant shift in cell's redox balance (accumulated oxidative damage, progressively inefficient antioxidant system and more efficient iron storage) as one ages. Polyphenols as prudent components of dietary antioxidants are gaining burgeoning interest in scientific and nutrition research attributed to their ubiquitous presence in plant foods, effective direct and indirect antioxidant mechanisms to attenuate lifestyle- and diet-related chronic diseases and inconsistent evidence of nutrient-antioxidant supplements (β-carotene, ascorbic acid, vitamin E and selenium); which thereof culminates in a nutritional guideline that increased frequency of polyphenols as a long term nutritional approach is vital to manifest their antioxidant benefits associated with health promotion and disease prevention. And the best way to obtain myriad of antioxidants is to consume variety of plant foods grouped in fruits, vegetables, whole cereals and pulses, and nuts. WHO recommended minimum 400 g of fruit and vegetable intake, excluding potato, cassava and other starchy tubers.

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