1. Polyphenols

Polyphenols are naturally occurring chemicals that are characterized by the presence of multiples of phenol structural units. The term ‘polyphenol’ have been in use since 1894 (http://www.merriamwebster.com/dictionary/polyphenol). The word 'poly' is derived from the ancient Greek word 'polus', means 'many, much' and the word phenol refers to the chemical structure which is an aromatic benzenoid (phenyl) ring with an hydroxyl (-OH) group and hence the suffix '-ol'.

Phenolic compounds or the polyphenols arise biogenetically from two main synthetic pathways: the shikimate pathway and the acetate pathway (Harborne, 1989). The quantity and quality of the polyphenols present in plant foods can vary significantly due to different factors, such as plant genetics and cultivar, soil composition and growing conditions, maturity state, and post-harvest conditions, and so on (Jaffery et al., 2003). However, since the presence of polyphenols constitute part of the plants innate defense mechanisms, polyphenol synthesis has been shown to be stimulated under conditions of stress, such as temperature alterations, UV exposure, and pathogenic attacks (Dixon & Paiva, 1995). Hence, polyphenols contribute significantly to plant physiology by playing key roles in growth, reproduction, pigmentation, protect from pathogens and predators which is due to their potent astringency and hence are known as phytoalexins (Bravo, 1998). These compounds may be present in free or esterified/etherified, soluble form as well as in insoluble form, found bound to cell wall constituents such as polysaccharides, protein, lignin, cutin or suberin (Clifford, 1999; Adom & Liu, 2002; Naczk & Shahidi, 2004).

1.1. Classification of polyphenols

Classification of polyphenols is based on their source of origin, biological function, and chemical structure. The unique physical, chemical, and biological properties of particular members of the polyphenol class are defined by the number and characteristic of phenol structures. Majority of polyphenols in plants exist as glycosides with different sugar units and acylated sugars at different positions of the polyphenol skeletons. Polyphenols are divided into many classes and subclasses depending on their chemical structure. Main classes of polyphenols are phenolic acids, flavonoids, lignans and tannins and each class is addressed below individually.
**Phenolic acids**

Phenolic acids are non-flavonoid polyphenolic, aromatic secondary plant metabolites that are widely distributed in plant kingdom and also account for one third of our diet. Though called polyphenols, phenolic acids comprise molecules that are with one phenol ring. In vascular plants phenolic acids are produced as breakdown products of lignin and cell wall polymers, as by-products of monolignol pathway, by means of shikimic acid through the phenyl propanoid pathway. In addition, some phenolic acids are produced by microbes. There are two unique structures of phenolic acids *i.e.*, phenyl propanoid type with C₆-C₃ back bone (hydroxyl cinnamic acids) and phenyl methyl type with C₆-C₁ (hydroxyl benzoic acids), the former type found in plant cell walls and lignin whereas later type in microbes (Croteau *et al.*, 2000). Gallic acid is extensively found in tea, tea leaves may contain upto 4.5 g/kg fresh weight of gallic acid (Tomas-Barberan & Clifford, 2000). Among hydroxycinnamic acids, caffeic, ferulic, *p*-coumaric and sinapic acids are most common in grains (Gallardo *et al.*, 2006).

**Flavonoids**

Flavonoids are the largest and best studied group of polyphenols, which include several thousands of low molecular weight phenolic compounds, belonging to 7 major subgroups ─ flavonols, flavones, flavanols, flavanones, flavanonols, anthocyanidins, and isoflavonoids. Among these, flavones, flavonols, and flavanones are the most abundant naturally occurring flavonoids. These molecules are characterized by the presence of flavan nucleus and are known as C₆-C₃-C₆ phenolics. Flavonoids occur as non-conjugated/glycosylated (aglycones), and mostly as glycosylated derivatives. More than 8000 polyphenolic compounds have been identified in various plant species, among them over 4000 are flavonoids (Harborne & Williams, 2000; Cheynier, 2005). Flavonoids are biologically active molecules which have been studied as potential components of functional foods, owing to their benefits for human health (Middleton *et al.*, 2000; Graf *et al.*, 2005). Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and herbs. An increase in the research on flavonoids is mainly because of the French paradox, according to which, a low cardiovascular mortality rate is observed in Mediterranean populations in association with red wine consumption and especially a high saturated fat intake. The
daily intake of the flavonoids constitutes about 2/3 of the total intake of dietary polyphenols (Manach et al., 2004). Flavonoids act as a light screen against damaging UV radiation in young plants, are good antioxidants, enzyme inhibitors, and precursors of several toxic substances, and hence provide resistance against pathogens. In addition, flavonoids may function as photosensitizing and energy-transferring compounds, and take part in the control of plant growth and development along with plant hormones (Harborne, 1988). Furthermore, these compounds have been implicated in defense against other plants, fungi, insects, and bacteria as regulators of interactions between beneficial fungi, herbivores, and insects, or as important constituents of animal diets, both nutritionally and medically (Berhow, 1998, Nijveldt et al., 2001).

Green and black tea contains about 25% flavonoids. Most consumed flavonoid is quercetin and the richest sources are apple, onion and tea (Hertog et al., 1993). Other important sources of flavonoids are citrus fruits, pomegranates, berries, grapes, olive oil, cocoa, coffee, walnuts, peanuts, and other fruits and vegetables. Most dietary flavonoids occur in food as O-glycosides (Hammerstone et al., 2000). The most common glycosidic unit is glucose, but other examples include glucorhamnose, galactose, arabinose, and rhamnose (Cook & Samman, 1996).

Isoflavones are found mostly in legumes, but soybeans are the principle human dietary source. Isoflavones are present in plant foods either as the aglycone (genistein or daidzein) or as different glycosides, including acetyl and malonyl glycosides and the β-glucosides of daidzein and genistein (Kudou et al., 1991). Soy isoflavones can reduce blood cholesterol and can help to prevent osteoporosis. Soy flavonoids are also used to ease menopausal symptoms.

The most widely occurring and structurally diverse flavonoids are flavones and flavonols (Harborne et al., 1999). Flavones are frequently found in grains and herbs rather than in fruits and participate in taste. The main flavanols are catechins which are very abundant in tea, other sources are red wine and chocolate. The best studied flavanol also include quercetin and kaempferol.

Flavanones occur in chick peas, cumin, hawthorn berry, licorice, peppermint, rowanberry but citrus fruits are the richest source wherein the characteristic flavor is contributed by the flavanones. Hesperidin is being reported in cumin and peppermint.
Anthocyanins are pigments found in red fruits such as cherries, plums, strawberries, raspberries, blackberries, grapes, red currants and black currants. Anthocyanins often occur in a complex mixture. Grape extracts can have glucosides, acetyl glucosides and coumaryl glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin.

**Lignans**

Lignans are polyphenols associated with dietary fiber and found to produce important physiological effects. Even though widely distributed, lignans are relatively less studied owing in part to difficulties in their quantification, isolation and analysis. Flax seeds are identified as the richest source of lignan Seco-isolariciresinol, followed by tea. Generally plant lignans occur in the form of glycosides and are considered to be one of the several classes of phytoestrogen.

**Stilbenes**

The stilbenes are often in plants that are not routinely consumed for food, or in the non edible tissue. The major dietary sources of stilbenes are grapes, grape juices and wine, and peanuts and peanut butter with lesser amounts found in berries, red cabbage, spinach and certain herbs. Resveratrol is one of the most best studied stilbenes from grapes and red wine.

1.2. **Sources of polyphenols**

The most important dietary sources of polyphenols are fruits, tea and seed coat of grains. Cloves (15188 mg/100 g) stand first among 100 top ranked foods containing polyphenols while rose wine (10 mg/100 g) occupies last position in the ranking (Perez-Jimenez, 2010). High levels of polyphenols mainly found in the fruit skins and seeds may reveal only the measured extractable polyphenol content of a fruit or seed, though the non-extractable polyphenols are not considered.
Table-1. Major classes of polyphenols

<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>Non-Flavonoids</th>
<th>Cereal grains, Blackberry, Strawberry, Raspberry, Black currant</th>
<th>Cereal grains, Apple cider, Blueberry, Kiwi</th>
<th>Wine, Peanuts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td>p-hydroxy benzoic acid&lt;br&gt;Protocatechuic acid&lt;br&gt;Gallic acid&lt;br&gt;Vanillic acid&lt;br&gt;Syringic acid</td>
<td>R&lt;sub&gt;2&lt;/sub&gt; R&lt;sub&gt;3&lt;/sub&gt; R&lt;sub&gt;4&lt;/sub&gt; R&lt;sub&gt;5&lt;/sub&gt;</td>
<td>H H OH H</td>
<td>H OH OH OH OH</td>
</tr>
<tr>
<td>a) Hydroxy-benzoic acids</td>
<td>p-coumaric acid&lt;br&gt;Caffeic acid&lt;br&gt;Ferulic acid&lt;br&gt;Sinapic acid&lt;br&gt;Chlorogenic acid</td>
<td>R&lt;sub&gt;2&lt;/sub&gt; R&lt;sub&gt;3&lt;/sub&gt; R&lt;sub&gt;4&lt;/sub&gt; R&lt;sub&gt;5&lt;/sub&gt;</td>
<td>H H OH H</td>
<td>H OH OH H</td>
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<tr>
<td>b) Hydroxy-cinnamic acids</td>
<td></td>
<td></td>
<td>H OCH&lt;sub&gt;3&lt;/sub&gt; OH H</td>
<td></td>
</tr>
<tr>
<td>Stilbenes</td>
<td>Pterostilbene&lt;br&gt;Resveratrol&lt;br&gt;Piceid</td>
<td>R&lt;sub&gt;2&lt;/sub&gt; R&lt;sub&gt;3&lt;/sub&gt;</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>OH OH</td>
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<td></td>
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<td></td>
<td>OGlc</td>
<td>OH</td>
</tr>
<tr>
<td>Lignans</td>
<td>Structure</td>
<td>Description</td>
<td>Sources</td>
<td></td>
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<tr>
<td>---------</td>
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</tr>
<tr>
<td><a href="#">Lignan 1</a></td>
<td>Secoisolariciresinol, sesamol, enterodiol</td>
<td>(1). 7,7'-epoxylignane (2). 2,7'-cycloalkane (3). 8,4'-oxyneolignane</td>
<td>Flax seeds, Sesame seeds, Cereals</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tannins</th>
<th>Structure</th>
<th>Description</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="#">Tannin</a></td>
<td>Tannic acid, Ellagitannins</td>
<td>Hydrolysable Tannins, Proanthocyanidins, Phlorotannins</td>
<td>Legumes, Nuts, Berries</td>
</tr>
</tbody>
</table>

*Note: The images of the structures are placeholders and should be replaced with actual chemical structures.*
<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Structure</th>
<th>Chemical Formula</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavanols</td>
<td><img src="image1" alt="Flavanols Structure" /></td>
<td>R₁OH R₄OH gallate</td>
<td>Green tea, Red wine, Chocolate, Black tea, Grape seed</td>
</tr>
<tr>
<td>Flavonols</td>
<td><img src="image2" alt="Flavonols Structure" /></td>
<td>R₂H R₃H OH</td>
<td>Apple, Onion, Buckwheat, Curly kale, Leek, Cherry tomato, Broccoli, Blueberry Apricot, Beans, Black grape, Tomato, Black tea, Green tea, Red wine</td>
</tr>
<tr>
<td>Isoflavones</td>
<td><img src="image3" alt="Isoflavones Structure" /></td>
<td>R₂OH R₃OH</td>
<td>Soy milk, Soy extract, Soy nuts</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td>Cyanidin</td>
<td>Delphinidin</td>
<td>Malvidin</td>
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<tr>
<td><img src="image1.png" alt="Anthocyanidins" /></td>
<td>R₁</td>
<td>OH</td>
<td>R₂</td>
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<tr>
<td></td>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td>OCH₃</td>
<td>OC</td>
<td>H₃</td>
</tr>
<tr>
<td></td>
<td>Blackberry, Black currant, Blueberry, Black grape, Cherry, Rhubarb, Strawberry Red wine Plum, Red cabbage, Orange juice, Grapefruit juice</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavanones</th>
<th>Taxifolin</th>
<th>Naringenin</th>
<th>Hesperetin</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image2.png" alt="Flavanones" /></td>
<td>R₁</td>
<td>R₂</td>
<td>R₃</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>OH</td>
<td>OH</td>
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<td></td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Citrus, Orange peel</td>
<td></td>
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</tbody>
</table>
1.3. Factors affecting polyphenol content of plant foods

Thermal processing

According to several earlier reports, thermal processing methods generally reduce the total phenolic content and their antioxidant activities. Effects of various heating methods (frying, microwave heating, and boiling with water in a pressure cooker) on virgin olive oils showed severe losses of polyphenols and degradation of oil in all the methods tested (Brenes et al., 2002). Another study showed a significant reduction radical-scavenging activity and total polyphenol content and ascorbic acid content in cooked peppers (Chuah et al., 2008). This may be due to the leaching of antioxidant compounds from the pepper into the cooking water. On the contrary, roasting significantly increased total polyphenols in little millet which was mainly due to an increase in tannin content (Pradeep & Guha, 2011). Cooking (at 121 °C) without soaking and retaining the cooked water increased antioxidant capacity in beans (Phaseolus vulgaris L.) (Rocha-Guzman et al., 2007), in contrast, reduced the tannin content in beans and chickpeas (Wang et al., 2010). Tryptophan and phenolic acid contents of eight commercial potato varieties were affected differently by domestic cooking (boiling, microwaving, and baking). Further, a decline in the total phenolic contents was observed in all varieties after cooking in contrast, the antioxidant activity was not affected by these cooking conditions (Xu et al., 2009).

Effect of common cooking practices (boiling, steaming, and frying) on phytochemicals (polyphenols, carotenoids and glucosinolates) and total antioxidant capacities of three vegetables - carrots, courgettes (Zucchini), and broccoli, revealed that processed vegetables offer lower nutritional quality and for each vegetable a cooking method would be preferred to preserve the nutritional and physicochemical qualities (Miglio et al., 2008). In the case of boiling treatment, limited discoloration and better preservation of antioxidants (carotenoids) were observed in all the vegetables. Pre-treatment of cherry tomatoes with a solution containing citric acid–NaCl–CaCl₂ following drying at one of the three different temperatures (40, 60, 80 °C) affected the antioxidant activity and the phenolic acid content (Rizzo et al., 2015). An increases in the content of flavonoids, phenolic acids and main glucosinolates as well as the carotene, lutein and vitamin E was observed on steam-cooking of broccoli in comparison with the fresh ones (Gliszczyńska-Świgło et al.,...
Effect of soaking and roasting on bioactive components and antioxidant potential in dry beans was investigated and this study revealed that these processing methods influenced the total phenolic, flavonoid and antioxidant contents in selected samples (Boateng et al., 2008). High temperature and short time roasting (in a forced air convection oven at 130 °C for 33 min) of cashew nuts and testa effectively enhanced the antioxidant activity (Chandrasekara & Shahidi, 2011).

Drying of fruits

Freeze-drying, which is a shelf life enhancing and microbiological contamination reducing method, has been reported to cause polyphenol losses. This is possibly via the disruption of cell compartments and degradation of polyphenols by enzyme activities e.g., polyphenol oxidase (Bohn, 2014). Freeze-drying of watermelon, papaya, star fruit, muskmelon, and mango resulted in significant loss (30-50%) of phenolic contents (Shofian et al., 2011). Among microwave vacuum drying, freeze-drying and hot-air drying, the dehydration methods used for raspberries revealed that about 80% loss of phenols were recorded with freeze-drying and hot-air drying while it was lowest with microwave vacuum drying (Mejia-Meza et al., 2010). A study revealed that sun drying lowered the total polyphenols, anthocyanins and antioxidant activities in figs (Kamiloglu & Capanoglu, 2015).

Germination of grains

Germination increased total polyphenol, total flavonoid and tannin content in little millet (Pradeep & Guha, 2011). As per another study on the effect of different processing on the nutritional composition of Dolichos lablab seeds, germination showed similar effect on the tannin content (Osman, 2007). This was also supported by the report that germination increased the total polyphenols in five mung bean cultivars and hydrolysable tannins, condensed tannins increased with an increase in the period of germination (Tajoddin et al., 2014).

Dehulling

Dehulling and thermal treatment affected the phenolic content and antioxidants of millet grains (Chandrasekara et al., 2012). Significant reduction in protein, polyphenols and phytic acid contents and considerable increase in in vitro protein digestibility was observed in dehulled cultivars (Standard & Ugandi) of pearl millet
(Hag et al., 2002). Significant reduction of the total extractable phenolics (total phenolics, resorcinols, catechols and galloyls) was observed in dehulled legumes (cowpea, mung bean and brown kidney bean) compared to other processes such as water soaking at different pHs, germination and cooking (Towo et al., 2003). Dehulling decreased the total phenol content of whole grains of several millets such as kodo, finger, proso, foxtail, little and pearl millet (Chandrasekara et al., 2012). Similar kind of result was reported by Hag et al. (2002) in pearl millet. This may due to the fact that in cereal grains the phenolic compounds are mainly concentrated in the outer layers of the grain (Zielinski & Kozlowska, 2000; Awika et al., 2005).

**Storage**

Phenolic profile is affected by storage through the oxidative stress followed by the formation of oxidized phenols (Armaforte et al., 2007). Storage duration decreased polyphenols, flavonoids in food mix containing chickpea and amaranth stored at different temperatures (Oghbaei & Prakash, 2015). Influence of long-term storage (five years) on the total phenolic and antioxidant capacity of almond hull and shell from different genotypes was assessed by Dolatabadi et al. (2015). The study showed no decrease in the amount of total phenolics on storage for five years, however the same influenced antioxidant and antiradical potential of almond hull and shell. Kivi et al. (2014) observed a significant enhancement of antioxidant capacity, total phenolics, and anthocyanins in raspberry fruit stored at 10 °C. Storage-life and the antioxidants level in grapes were well retained at low temperature storage (2–4 °C) with restricted ventilation packaging (1–2%), as observed by Doshi and Adsule (2008).

Effect of storage period (one year) and different packaging materials viz., cans, high density polyethylene (HDPE) bottles, polypropylene (PP) bottles and oriented polypropylene/aluminium/linear low density polyethylene (OPP/Al/LLDPE) standing pouches on total phenolic content and antioxidant activity of *Centella asiatica* drinks was studied (Siah et al., 2011). Total phenolic content and antioxidant activity remained stable for the first month of storage in all types of packaging materials. Except for the standing pouches which recorded the highest reading and other packaging materials recorded the significant decrease all through the storage period. At 0 °C storage temperature, a significant decrease in both the total antioxidant
activity and the total phenol content of the pinotage, cabernet sauvignon, chardonnay and chenin blanc wines was observed during bottle ageing over 12 months of storage (de Beer et al., 2005).

2. Health effects of polyphenols

Polyphenols are well studied for their immense beneficial physiological roles. Polyphenols recorded their role as protective agents against cardiovascular disease and certain forms of cancer, including breast, esophageal, gastrointestinal, lung and skin cancer, type 2 diabetes, cardiovascular diseases, and hypertension, antioxidant, anti-hyperglycemic, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity.

![Fig. 1. Health effects or biological functions of polyphenols](image)

**Antioxidant activity**

Several epidemiologic studies revealed consumption of polyphenol-rich foods and beverages are useful in lowering the risk of oxidative stress-related diseases. Fruits such as rosehip (*Rosa canina*), chokeberry (*Aronia melanocarpa*), hawthorn (*Crataegus monogyna*), blackcurrant (*Ribes nigrum*), blueberry (*Vaccinium myrtillus*) and
rowanberry (Sorbus aucuparia) have highest antioxidant activity (Denev et al., 2014). Studies have revealed that hydroxy cinnamic acids are more potent antioxidants as compared to corresponding hydroxy benzoic acids (Andreasen et al., 2001). Polyphenols may improve cell survival rate as antioxidants or may induce apoptosis and prevent tumor growth as pro-oxidants (Lambert et al., 2004). Antioxidant and antimicrobial activity of polyphenols obtained from milled fractions of whole flour, seed coat of finger millet have been reported (Viswanath et al., 2009). Antioxidant activity was carried out by β-carotene-linoleic acid assay and antimicrobial activity was determined against Bacillus cereus and Aspergillus flavus. Highest antioxidant activity of 86% and higher antimicrobial activity was recorded with seed coat extract than whole flour extract with 27% antioxidant activity.

Radovanović et al. (2013) studied the total phenolics, antimicrobial and antioxidant activities polyphenolic extracts obtained from three wild red wild berry fruit species, viz, European cornel (Cornus mas), blackthorn (Prunus spinosa L.) and wild blackberry (Rubus fruticosus). Highest amount of total phenols, 8625 mg/kg was found in European cornel. In addition, gallic acid, caffeic acid, p-coumaric acid, (+)-catechin, procyanidin B2, (-)-epicatechin, ferulic acid, quercetin, rutin and quercetin-3-glicoside were the main polyphenols in the fruit extracts. Antioxidant activity was estimated by 2,2’-diphenyl-1-picrylhydrazyl (DPPH) test. All three plant extracts exhibited the high scavenging effect on DPPH radical. A positive correlation between the antioxidant activity of plant extracts and their phenolic content has been documented (Qusti et al., 2010). This study also revealed higher concentration of phenolic acids in dried fruits than in fresh ones.

Using bran, flour and whole grain of different wheat varieties, Vaher et al. (2010) investigated the phenolic content, and antioxidant assay by DPPH radical scavenging capability. This study revealed highest phenolic content in bran (1258 to 3157 μg/g) followed by grains (168 to 459 μg/g) and flour (44 to 140 μg/g) and major phenolic acid found in wheat varieties was ferulic acid. Variation in the antioxidant activity was observed among different wheat varieties with varied phenolic compounds and a higher antioxidant activity was observed in extracts with a higher phenolic content. Fibre-rich fractions (coarse and fine fractions) from wheat, finger millet, pearl millet and sorghum were investigated for their antioxidant activity after treating with digestive enzymes (Siddiq & Prakash, 2015). Finger millet and pearl millet found to contain more
polyphenols and flavonoids respectively. In addition to a higher rate of antioxidant components, enzyme-treated samples showed higher total antioxidant activity and were higher in millet flours in comparison to wheat.

**Anti-carcinogenic activity**

Several studies suggested that anti-carcinogenic effects of a number of polyphenols including quercetin, catechins, isoflavones, lignans, flavanones, ellagic acid resveratrol, or curcumin were proved to be effective against oral, abdomen, duodenum, colon, liver, lung, breast and skin cancers in some models. Dietary polyphenols alter pleiotropic effects on cancer cells by acting as chemopreventive blocker agents or chemopreventive suppressor agents, or both (de Kok et al., 2008). In the case of human cancer cell lines administered polyphenols found to act as protective agents either by reducing the number of tumors or their growth (Jhonson et al., 1994). α-Mangostin, phenolic xanthone of Mangosteen (*Garcinia mangostana*), has found to be effective against prostate, breast, lung, and colorectal cancer (Shan et al., 2011). Similar results were reported by several researchers regarding the anticarcinogenic effect of α-Mangostin against breast cancer (Shibata et al., 2011), prostate cancer (Han et al., 2009; Johnson et al., 2012).

The resistance of lung cancer cells against apoptotic effect of various antitumor drugs has led to challenges in its curability. Polyphenols extracted from dried leaves of green tea (*Camellia sinensis*) and ginger (*Zingiber officinale*) recorded their antineoplastic effect on non small cell lung cancer cells (NSCLC-NCI-H460) *in vitro* (Hessien et al., 2012). Intake of green tea is protective against skin cancer in humans (Katiyar et al., 2000) and also prevents oral cancer in smokers by reducing smoking-induced DNA damage and cell growth inhibition (Schwartz et al., 2005). Similarly, consumption of polyphenols rich fruits, vegetables (Hamer et al., 2008), red wine (Scalbert et al., 2005), and green tea (Yang et al., 2000; Scalbert et al., 2005; Yang et al., 2002) reduce the incidence of colorectal cancer. Tea polyphenols are protective against human oral cancer (Li et al., 1999).

**Antidiabetic activity**

Dietary polyphenols are successively used for various aspects of diabetes mellitus. Polyphenols reduce intestinal absorption of carbohydrate, modulate the
enzymes involved in glucose metabolism, improve β-cell function and insulin action, stimulate insulin secretion, and the antioxidative and anti-inflammatory properties of these components. These properties of dietary polyphenols together with their antioxidant and anti-inflammatory properties are known to contribute to its hypoglycemic effect (Iwai et al., 2006; Iwai, 2008; Cabrera et al., 2006). Quercetin, a flavonoid of onion has been found to be strong anti-diabetic compound. Quercetin has ability to protect against the alterations in diabetic patients during oxidative stress, lipid peroxidation and inhibition of antioxidant system during diabetes (Rizvi & Mishra, 2009). Phenolic acids, flavonoids, and anthocyanins extracted from *Hibiscus sabdariffa* exhibited protection against diabetic nephropathy (Lee et al., 2009).

**Cardio protective activity**

Numerous studies have suggested that a correlation between the consumption of polyphenol-rich fresh fruits and vegetables and beverages with the prevention of chronic diseases including cardiovascular diseases and Alzheimer's disease (Heber, 2004; Hertog et al., 1996). Mice with advanced atherosclerosis showed reduction in oxidative stress and atherogenesis when supplemented with pomegranate juice. This activity was associated with reduction in macrophage oxidative stress, macrophage cholesterol flux and even attenuated the development of atherosclerosis. In addition, a tannin-fraction isolated from pomegranate juice had a significant anti-atherosclerotic activity (Kaplan et al., 2001).

Moderate and regular consumption of red wine may be protective in attenuating the thrombogenic response (Soulat et al., 2006). By supplementing red wine extract, or purified (+)-catechin with alcohol, or alcohol alone to drinking water of apoE-deficient (apoE(-/-)) C57BL/6 and wild-type mice, effect on plaque size and/or attenuated thrombotic reactivity at the site of advanced atherosclerotic lesions in mice was evaluated. Red wine extract and (+)-catechin significantly inhibited blood thrombotic reactivity; 64% and 63% reduction in cross-sectional surface area of the *ex vivo* thrombus were observed in relation to red wine extract and (+)-catechin supplementation. Epidemiological studies carried out by de Gaetano et al. (2002) suggested that moderate wine consumption results in antithrombotic role of polyphenols with reduced vascular risk in man. Yang et al. (2008) were of the opinion that ethyl acetate (EtOAc) fraction of *Salvia miltiorrhiza* Bunge (SMB) is a potential
source of inhibitors of platelet dependent thrombosis. They investigated the methanol and ethyl acetate extracts of SMB rich in polyphenols against free radical scavenging activities, and platelet aggregation and adhesion in rats. EtOAc fraction showed better scavenging activity, was potent inhibitor of ADP- and collagen-induced platelet aggregation, also methanol and EtOAc fraction dose-dependently inhibited thrombin-stimulated platelet adhesion to collagen or fibrinogen. Aqueous extracts of Emblica officinalis Gaertn, Hibiscus sabdariffa L, Acacia concinna DC, Xanthium strumarium L., Swertia pulchella Buch Ham, Vitis repens Wight & Arn Prodr, Hizikia fusiforme and Momordica charantia L. were subjected to the analysis of anti-oxidative and anti-thrombotic properties. These plant species exhibited diverse antioxidant, anticoagulant and anti-platelet activities. However, E. officinalis showed strong antioxidant activity and high inhibition of platelet aggregation and hence, could be used as a strong functional food ingredient against oxidative stress and thrombosis (Rao et al., 2014).

Anti-osteoporosis

Polyphenols reportedly exert physiological effects against arteriosclerosis and osteoporosis. However, majority of researches regarding epidemiologic studies of polyphenols consumption and their effects on osteoporosis have recorded mixed results under in vitro, in vivo and clinical studies. Flavonoids present in plant-based foods supports the bone health in humans. In a study carried out for 3160 women participants in the United Kingdom, reported that the dietary intake of anthocyanins and flavones showed their positive association with hip and spine bone mineral density (Welch et al., 2012). 3-methoxyellagic acid, a phenolic compound extracted from the leaves of Feijoa sellowiana Berg. stimulated the mineralization in two human osteoblastic cell lines HOS58 and SaOS-2 (Ayoub et al., 2009).

Effects of three major polyphenols of olive i.e., oleuropein, hydroxy tyrosol and tyrosol on bone formation using cultured osteoblasts and osteoclasts, and on bone loss in ovariectomized mice have been studied (Hagiwara et al., 2011). This study demonstrated that both oleuropein and tyrosol suppressed the bone loss of trabecular bone in femur of ovariectomized mice (6-week-old BALB/c female mice), while hydroxytyrosol attenuated H2O2 levels in MC3T3-E1 cells. Hence, olive polyphenols, oleuropein and hydroxytyrosol can be used as effective remedies in the treatment of
osteoporosis. Stimulation of osteoblast differentiation, reduction in osteoclastogenesis and increase in bone mass were observed by Chen et al. (2010) when young rats were fed with blue berries containing a mixture of phenolic acids.

**Anti-inflammatory activity**

Treatment of RAW 264.7 cell with extract of *Hibiscus sabdariffa* leaf, reduced lipopolysaccharide - induced nitric oxide production dose-dependently in indicates the extract’s potential anti-inflammatory activity (Zhen et al., 2016). This promising anti-inflammatory activities may be due to the combined effect of quercetin, kaempferol and chlorogenic acid, which were the major constituents of the *H. sabdariffa* extract. Polyphenol extracts of *Cynanchi wilfordii* Radix showed anti-inflammatory, antioxidant, and anti-bacterial properties (Jeong et al., 2015). Most potent anti-inflammatory activity was seen in ethanol extract, which reduced levels of nitric oxide, prostaglandin E2, and cytokine (IL-1β, IL-6, IL-10, and TNF-α), as well as inhibited the expression of inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) in a concentration-dependent manner. Tea flowers extract exhibited potent anti-inflammatory effects on acute and immunological inflammation in vivo by markedly suppressing the levels of nitric oxide, tumor necrosis factor-α and interleukin-1β mRNA in mouse liver (Chen et al., 2012). Polyphenols of *Cymbopogon citratus* infusion significantly reduced inflammation and peripheral pain in vivo (Garcia et al., 2015).

**Anti-obesity effect**

Polysaccharide in combination with polyphenols is potential in treating obesity (Xu et al., 2015). Anti-obesity effects of total green tea extracts, polyphenols, polysaccharides, caffeine, and a complex of polysaccharide and polyphenol when supplemented to high-fat diet fed rats was investigated. As per the results obtained, polyphenols and polysaccharides suppressed the increase in the body weight and fat accumulation. Moreover, anti-inflammatory activity, as well as, reduction in serum leptin levels in rats was achieved by polyphenols and polysaccharides. In another study carried out by Shen et al. (2011), supplementation of green tea polyphenols prevented weight gain, promoted bone parameters [femoral bone area, mineral content (BMC) and density (BMD)], liver glutathione peroxidase activity and suppressed the serum leptin in obese female rats. Several cell culture, animal and human studies have
shown the antiobesity potential of green tea catechins, particularly EGCG (Kim et al., 2010; Brown et al., 2011; Chan et al., 2011; Chen et al., 2011; Bogdnanski et al., 2012; Chung et al., 2012). Functional drinks supplemented with catechins and EGCG were potential against obesity, hypercholesterolemia and hyperglycemia in rats (Ahmad et al., 2015).

**Antimicrobial activity**

Disc diffusion assay carried out by Radovanović et al. (2013) observed the antimicrobial effect of polyphenolic extracts of three wild red berry fruit species, viz, European cornel (Cornus mas), blackthorn (Prunus spinosa L.) and wild blackberry (Rubus fruticosus) against Clostridium perfringens ATCC 19404, Bacillus subtilis ATCC 6633, Listeria innocua ATCC 33090, Staphylococcus aureus ATCC 6538, Sarcina lutea ATCC 9341 and Micrococcus flavus ATCC 40240 (Gram (+) bacteria), Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Salmonella enteritidis ATCC 13076, Shigella sonnei ATCC 25931, Klebsiella pneumonia ATCC 10031 and Proteus vulgaris ATCC 8427 (Gram (-) bacteria). Polyphenols obtained from three ethno-medicinal plants, (Momordica charanta, Senna alata and Nauclea latifolia) exhibited the antimicrobial activity against S. aureus, Streptococcus pyogenes, E. coli and Candida albicans (Okoro et al., 2010).

Antibacterial activity of polyphenols obtained from spruce bark, grape seeds, Crataegus monogyna (hawthorn) and Asclepias syriaca (milkweed) in agar diffusion assay against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa species was observed by Ignat et al. (2013). Phenolic compounds obtained from Ginkgo biloba sarcotesta (fleshy seed coat) exhibited the inhibitory activity against several bacteria such as Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Salmonella entericaserovar, Typhimurium, Shigella dysenteriae, Staphylococcus aureus, Streptococcus pyogenes, Vibrio mediterranei, and Vibrio vulnificus (Carraturo et al., 2014).

Nitiema et al. (2012) reported coumarin, a phenolic compound effective in suppressing the bacterial strains Escherichia coli 81nr.149 SKN541, Enterobacter aerogenes CIP 104 725, Salmonella typhimurium SKN533 and Salmonella infantis SKN 557, which are in involved in gastroenteritis diseases. Baicalein, a flavonoid revealed its antiviral activities against the different stages of in vitro replication of
Japanese encephalitis virus, causative agent of Japanese encephalitis, a mosquito-borne viral disease (Johari et al., 2012).

3. Bioavailability of polyphenols

To elucidate the significance of polyphenols in human health, it is important to know the quantity of polyphenols consumed in the diet and their bioavailability. To exert their biological properties, polyphenols have to be available to some extent in the target tissue. Therefore, the biological properties of dietary polyphenols may depend on their absorption in the gut and their bioavailability. Intestinal absorption and metabolism of polyphenols govern their bioavailability (Silberberg et al., 2006). Bioavailability differs greatly between polyphenols. Aglycones and free simple phenolic compounds, flavonoids (quercetin, genistein) and phenolic acids, can be directly absorbed through the small intestinal mucosa (Jung & Fahey, 1983; Wolffram et al., 1995; King et al., 1996; Manach et al., 1997). Some polyphenols such as soy isoflavones are well absorbed through the gut barrier whereas others such as proanthocyanidins, abundant in wine or cocoa, or thearubignins, the main polyphenols in black tea, are hardly absorbed (Manach, 2005). Furthermore, each polyphenol may be found in foods in different forms, which can affect the intestinal absorption.

Germano et al. (2006) have demonstrated the intestinal absorption of phenolic acids using Trichilia emetica extracts and have observed the intestinal absorption of caffeic acid derived from the hydrolysis of chlorogenic acid in the intestinal tract. Free phenolics (cinnamic acid and derivatives such as p-coumaric, ferulic, caffeic, etc.) have been shown to be absorbed through the intestinal tract in both in vivo experiments of rats (Jung & Fahey, 1983) and in vitro experiments of isolated rat jejunum. Chlorogenic acid is most likely metabolized by the colonic microflora (Plumb et al., 1999). Only 11-25% of ferulic acid ingested is known to be excreted in urine as free ferulic acid or as glucuronide conjugate (Bourne & Rice-Evans, 1998).

In plants and most foods, the flavonols exist as glycosides, which on ingestion, are hydrolysed to aglycones by lactase phlorizin hydrolase, and subsequently the aglycone diffuses into the cell (Day et al., 2000; Nemeth et al., 2003). Following cellular accumulation of the aglycone, extensive metabolism occurs within the enterocytes (Petri et al., 2003). This metabolism involves the formation of a number
of phase-II conjugates, most notably sulphates and glucuronides, as well as methylated and mixed conjugates. Conjugation of flavonoids has a major impact on their properties, and the resulting conjugates can be either absorbed or effluxed by active transport.

Fig.2. Schematic representation of bioavailability of polyphenols

Ref: de Souza et al. (2015)
Even though the portal blood facilitates removal of absorbed material to maintain a favourable concentration gradient, flavonols are only partially bioavailable in humans. Metabolism may affect the ability of flavonols to cross the intestinal barrier.

Isoflavone conjugates are metabolised after ingestion either by acid hydrolysis in the stomach or by intestinal bacteria. Flavonols such as catechins are reportedly more bioavailable than quercetin; however, quercetin may reach similar plasma concentrations (high nanomolar to low micromolar) in people who consume large amounts of fruits and vegetables or intentionally supplement their diets with polyphenols (Hollman et al., 1996; Erlund et al., 2002). Quercetin glucosides from onions are much more efficiently absorbed than other quercetin glycosides such as rutin present in tea or apple (Hollman et al., 1997).

Lignans when ingested are converted by gut microflora in the large intestine into two simple phenols: enterolactone and enterodiol. These compounds are known as mammalian lignans and are found to exhibit a number of significant physiological effects. Lignans have been detected in human plasma and urine (Adlercreutz & Mazur, 1997).

Bioavailability of polyphenols in humans are influenced by numerous factors such as variation in food content, matrix and processing, genetic, microbial and dietary factors (Bolca et al., 2010; van Dorsten et al., 2010). As discussed early, polyphenol content, as such, may vary, influenced by several processes before they are bioaccessible. For example, thermal treatment of food material can disrupt the cell wall allowing release of polyphenols to become bioavailable, however, the same treatment would degrade polyphenols and bring down total content of the same from the food. Physical nature of the food also influences the bioaccessibility in turn bioavailability of these compounds.

**Piperine (Spice principle of black pepper) as bioavailability enhancer**

Piperine is an alkaloid present in black pepper and is a popular bioavailability enhancer of many phytochemicals, known to exert several beneficial physiological effects. An enhanced oral exposure of fexofenidine and two-fold increase in its bioavailability after used in combination of piperine in rat studies has been reported (Jin & Han, 2010). This may be due to cellular efflux mediated by P-glycoprotein
during intestinal absorption. By increase in the AUC, $C_{\text{max}}$ in the blood samples, single dose of piperine improved the bioavailability of phenytoin (anticonvulsant drug) in the treatment of uncontrolled epilepsy (Pattanaik et al., 2006). In combination with the piperine, beta lactam antibiotics; amoxycillin trihydrate and cefotaxime sodium in rats recorded significant enhancement in their bioavailability by improving $t_{\text{max}}$, $C_{\text{max}}$, $t_{1/2}$ and AUC. This could be due to the influence of increased bioavailability on enzymes system (Hiwale et al., 2002). Similar study carried out by Janakiraman & Manavalan (2008) in animal model revealed enhanced oral bioavailability of antibiotics; ampicillin and norfloxacin, when used in combination with piperine (20 mg/kg), by improving various pharmacokinetic measurements like $C_{\text{max}}$, $T_{\text{max}}$, AUC and $t_{1/2}$. As observed by Singh et al. (2010), co-administration of piperine increased the peak plasma levels and AUC of metronidazole in male New Zealand white rabbits in comparison to control and other treatments.

A bio-enhancing study was carried out in layer birds to know the effect of the co-administration of piperine on single dose of gatifloxacin (Patel et al., 2011). This study revealed that piperine increased the bioavailability of gatifloxacin by affecting the absorption kinetics and inhibition of the metabolism of gatifloxacin. Enhancement of bioavailability in piperine combined gatifloxacin than only gatifloxacin treated group in broiler birds has been recorded (Devada et al., 2011). Significant improvement in spatial memory and neurodegeneration, including Alzheimer’s disease condition was observed in adult male Wistar rats provided with different doses of piperine (5, 10 and 20 mg/kg BW), which may be due to decrease in lipid peroxidation and acetyl cholinesterase enzyme (Chonpathompikunlert et al., 2010). Significant enhancement of the serum bioavailability of resveratrol in mice by piperine was observed (Johnson et al., 2011). Upon addition of piperine, the degree of exposure to resveratrol (AUC) and the maximum serum concentration ($C_{\text{max}}$), were enhanced to 229% and 1544% respectively.

Co-administrated piperine enhanced bioefficacy of resveratrol in healthy human subjects in relation to cerebral blood flow. However, this treatment (250 mg trans-resveratrol with 20 mg piperine) does not affect on cognitive function, mood and blood pressure (Wightman et al., 2014). In contrast to this, piperine administration to male Wistar rats at different doses of, 5, 10 and 20 mg/kg body weight, once in a day
for 4 weeks, showed anti-depression like activity and enhanced cognitive effect (Wattanathorn et al., 2008). Hepatoprotective effect of piperine against acetaminophen (which is an antipyretic and analgesic drug, whose overdose causes acute liver damage) in mice was evaluated (Sabina et al., 2010). Induction of acetaminophen showed significant increase in the levels of liver marker enzymes such as aspartate transaminase, alanine transaminase and alkaline phosphatase, inflammatory mediator tumour necrosis factor- alpha (TNF-α) and lipid peroxidation but a decrease in antioxidant activity. While, piperine and silymarin (standard drug) treatment to acetaminophen challenged mice showed results contradict to treatment with acetaminophen alone.

Antidepressant-like effects of ferulic acid and piperine in mice has been reported (Li et al., 2015). Reduction in immobility time by 60% in the tail suspension (TST) and forced swimming tests (FST) was observed when mice were administrated with the highest dose of ferulic acid in comparison to control. At 200 mg/kg body weight, ferulic acid and piperine alone administered mice exhibited a maximum and weak antidepressant like effect respectively. However, a combination of low doses of ferulic acid and sub-threshold dose of piperine showed a synergistic effect and also enhancement of monoamine levels in the brain regions (cortex, hippocampus and hypothalamus) and this enhancement was supported by monoamine oxidase activity.

Intragastric co-administration of (-)-Epigallocatechin-3-gallate (EGCG), from green tea (Camellia sinensis) with piperine enhanced the bioavailability of EGCG and plasma C_max and AUC in male CF-1 mice compared to EGCG alone treated mice group (Lambert et al., 2004). Piperine found to increase EGCG bioavailability by inhibiting glucuronidation and gastrointestinal transit but not in hepatic microsomes. Appearance of EGCG in the colon and feces was slower in case of piperine co-treated mice compared to only EGCG treated mice.
4. Scope of the present investigation

Polyphenols from plant-derived foods have caught the highest attention world over owing to their inherent antioxidant potential and thereby many attendant disease preventive and disease curative effects. Red wine (derived from grapes) and green tea both of which provide very high concentrations of polyphenols are now considered to be healthiest drinks because of this reason. Traditional Indian foods being essentially plant derived provide rich amounts of polyphenols, due to the extensive use of fruits, vegetables and whole grains. While Indian traditional cuisines are basically made out of unrefined cereal/legume grains, and that vegetables and fruits are a regular ingredient of them, it is expected that Indian population must be consuming ample amounts of polyphenols in their daily diet. Since diets rich in polyphenols are the preferred meals to derive antioxidant benefits, our population are believed to be already obtaining them naturally enough. Apart from the concentration present in our meals per se, the bioavailability factor is something to be reckoned with to fully understand and assess the strength of our meal in terms of antioxidant polyphenols.

In this context, it is most appropriate that the bioavailability of the polyphenols from Indian foods needs to be understood. In view of the diverse health benefits attributable to the consumption of polyphenols owing to their fundamental antioxidant potential, knowledge on the actual bioavailability of these nutraceutical ingredients from our diet in terms of the extent of intestinal absorption and duration of their presence in body tissues assumes greater significance.

There is absolutely no information on the bioavailability of polyphenols from our grain-based foods. In the absence of any information on the bioavailability of polyphenols from Indian foods, the present research programme aims to study the extent of their intestinal absorption, and the duration for which the absorbed polyphenols reside in the body tissues. For this purpose, food processing on the bioaccessibility of polyphenols from common cereal grains (finger millet, pearl millet, wheat, sorghum), legumes (green gram and chickpea) and a representative vegetable, \textit{viz.}, onion was studied. Also, influence of common food acidulants of Indian culinary on the bioaccessibility of same has been analyzed.
Objectives of the proposed study

In the above context, the present investigation was carried out to address the following objectives:

i. Screening of staple cereals and pulses for polyphenol content and individual profile

ii. Evaluation of the bioavailability of polyphenols from whole grain cereals and pulses commonly consumed in India in vitro and in vivo. Understanding the influence of food processing on the bioavailability of inherent polyphenols.

iii. Evaluation of the bioavailability of polyphenols from Indian plant foods in experimental animals and the influence of co-administered piperine, the known bioavailability enhancer on the same.