General Introduction
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Green leafy vegetables (GLVs) are classified as functional foods because they have health benefits beyond basic nutrition. The dietary value of plant leaves pertains to their position as primary producers in the human food chain. The complex organic compounds manufactured in plant leaves include antioxidant molecules for protection against oxidative solar radiation. GLVs in our diet provide myriad bioactive food components which includes the polyphenols, phytosterols, carotenoids, tocopherols, tocotrienols, organosulfur compounds including isothiocyanates and diallyl-(di,tri)sulfide compounds, soluble and insoluble fibre, and fructo-oligosaccharide. Leafy vegetables are, therefore, important dietary sources of phytochemicals with health-protective and immune-strengthening properties that lower the risks of cancer and cardiovascular diseases through mechanisms that modulate free radical attack on nucleic acids, proteins and polyunsaturated fatty acids. Polyphenols are products of secondary metabolism of plants and are among the most common and widely distributed phytochemicals in fruits and vegetables, with currently ~ 8000 phenolic structures known. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds such as condensed tannins.
Polyphenols are subdivided into groups (Chart 1) by the number of phenolic rings and of the structural elements that link these rings: (1) The phenolic acids with the subclasses derived from hydroxybenzoic acids such as gallic acid and from hydroxycinnamic acid, containing caffeic, ferulic, and coumaric acid; (2) the large flavonoid subclass, which includes the flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols; (3) the stilbenes; (4) the tannins; (5) the lignans and the polymeric lignins and (6) Hydroxycoumarins.

**Phenolic acids**

A major class within the phenolic compounds is the hydroxycinnamic acids, which are widely distributed in plant kingdom. The major hydroxycinnamic acid is caffeic acid, which occurs in foods mainly as an ester with quinic acid called chlorogenic acid (5-caffeoylquinic acid). Chlorogenic acid and caffeic acid are antioxidants *in vitro* and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds for the inhibitory effect on the N-nitrosation reaction *in vitro*.

**Flavonoids**

Flavonoids are the most abundant polyphenols in human diets, and are mainly divided into:

(a) anthocyanins, glycosylated derivative of anthocyanidin, present in colourful flowers and fruits;
(b) anthoxanthins, a group of colourless compounds further divided in several categories, including flavones, flavans, flavonols, flavahols, isoflavones and their glycosides. Flavonols are mainly represented by myrisetin, fisetin, quercetin and kaempferol.

**Dietary Sources - Fruits:** blackberries, black currant, blueberries, black grape, elderberries, strawberries, cherries, plums, cranberry, pomegranate juice, raspberry. Others: red wine.

**Stilbenes**

Stilbenes are structurally characterized by the presence of a 1, 2-diphenylethylene nucleus with hydroxyls substituted on the aromatic rings, and exist in the form of monomers or oligomers. The best known compound is trans-resveratrol, possessing a trihydroxystilbene skeleton.

**Tannins**

Tannins are a group of water-soluble polyphenols having molecular weights from 500 to 3,000 which are subdivided into condensed and hydrolysable tannins, and commonly found complexed with alkaloids, polysaccharides and proteins, particularly the latter. On the basis of structural characteristics there are two groups, gallotannins and ellagitannins of hydrolysable tannins.

Diferuloylmethanes:

Diferuloylmethanes are a small group of phenolic compounds with two aromatic rings substituted with hydroxyls, and linked by aliphatic chain containing carbonyl groups. There are also some other polyphenols such as hydroxytyrosol, a simple polyphenol present in olive fruits and olive oil.
Chart 1. Classification of dietary polyphenols.
Polyphenols exhibit a wide range of properties, depending on their structures. They include yellow, orange, red and blue pigments, as well as various compounds involved in food flavour. Volatile polyphenols such as vanillin are extremely potent odorants, but the major flavours associated with polyphenols are bitterness and astringency. Other major polyphenol characters include their radical scavenging capacity, which are involved in antioxidant properties, and their ability to interact with proteins. The latter is responsible for astringency perception (resulting from interaction of tannins with salivary proteins), for formation of haze and precipitates in beverages, and for inhibition of enzymes and reduced digestibility of dietary proteins.

The chronic diseases constitute the major challenge for medicine and basic biology and will certainly remain so for the next decades. Infectious diseases are replaced by chronic and non communicable diseases as the primary cause of morbidity and mortality. This situation is associated with changes in the diet and lifestyle that contribute to the development of chronic diseases like CVDs, hypertension, diabetes mellitus and some forms of cancer. The ethno botanical reports offer information about the medicinal properties of GLVs which include details of their antidiabetic, antihistaminic, anticarcinogenic and antibacterial activities.

Oxidative stress is the central risk factor for chronic diseases. Oxidative stress, the consequence of an imbalance of prooxidants and antioxidants in the
organism, is rapidly gaining recognition as a key phenomenon in the chronic diseases. Radicals of oxygen (super oxide anion, hydroxyl radical and peroxy radicals), reactive non radical oxygen species such as hydrogen peroxide and singlet oxygen, as well as carbon, nitrogen and sulphur radicals comprise the variety of reactive molecules that can cause damage to cell. These molecules safely interact with free radicals and terminate the chain reaction before the vital molecules are damaged. The phenolic compounds act as antioxidants with mechanisms involving both free radical scavenging and metal chelation. They have ideal structural chemistry for free radical scavenging activities and have been shown to be more effective antioxidants \textit{in vitro} than Vitamin E and C on a molar basis.

The polyphenols inhibit low density lipoprotein (LDL) oxidation in vivo, which is considered a risk factor in atherosclerosis. The phenolic compounds are gaining interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of foods. The importance of natural phenolic compounds from plant materials is also raising interest among scientists, food manufacturer and consumers as functional food with specific health effects.

Several studies have shown that in addition to their antioxidant protective effects on DNA and gene expression, polyphenols, particularly flavonoids inhibit the initiation, promotion and progression of tumours possibly by a different
mechanism. So the attention has been devoted to GLVs, which though unexploited in most cases, possess a tremendous potential to help people overcome the deadly diseases of the modern society.

Rural people consume a wide variety of GLVs in their daily diet depending on their availability. Some of these GLVs grow in the vast area of water bodies in the locality while others are collected from the nearby crop fields. While dietary antioxidants such as Vitamin C and E have received considerable attention, relatively little is known about the similar antioxidant role for the plant derived polyphenols such as flavonoids and phenolic acids. The detail biochemical composition and nutritional facts on the basis of micronutrients present in these GLVs are not explored. The polyphenols present in these GLVs will serve as natural antioxidants and certainly will play an important role in controlling various diseases. So, the knowledge will be very much useful to advocate nutritionally beneficial but low cost diet to the vast economically underprivileged population of rural India.
Objectives of the Study

The objectives are

a. Extraction and quantification of polyphenolic compounds present in various leafy vegetables.

b. Evaluation of the antioxidative properties of the polyphenolic extracts by various *in vitro* methods.

c. Characterization of the polyphenolic components.