REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Beetroot is a vegetable having a beautiful colour and plenty of nutrients. Many like to eat this especially for its colour. Since the carbohydrates in the beetroot are in the form of sugar particles, this quickly digests and mixes in our blood. Beetroot contains water, protein, fat, minerals, fiber and carbohydrate. Minerals and vitamins like calcium, phosphorus, iron, vitamin C, vitamin A together with vitamin B_1, B_2, B_3, B_6, sodium, potassium, sulfur, chlorine, iodine, copper etc. are found in beetroot. Beetroot is also a good tonic for liver problems. If beetroot leaves are cooked like other greens and consumed, diseases like ulcer and jaundice will be cured.

Beets contain antioxidants and anti-inflammatory substances that prevent cancer and fight heart diseases. Red beets provide vitamin A and C, calcium and iron and add fiber to the diet. A single cup serving of plain cooked beets has 50 calories. Red beets also add colour to meals making, eating more enjoyable.

Fruits and vegetables rich in pigments such as anthocyanins, betalains or carotenoids are potent sources of antioxidants. These diverse biological pigments are found as complex mixtures in most fruits and vegetables. Anthocyanins and particularly betalain are heat sensitive, resulting in colour loss during food processing. The ruby-like red colour of table beet (*Beta vulgaris*) has sparked much interest because of its intensity and availability. The colour of beet is a mixture of red betacyanin (BC) and yellow betaxanthin (BX) pigments that belong to a group of compounds collectively known as betalain (Gasztonyi, 2001).

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk.

Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources (Miller, 2000).

The Review of literature pertaining to the present study “A comparative study of antioxidants in beetroot varieties” is discussed under the following headings

2.1 Antioxidants

2.2 Free radicals

2.3 Enzymic antioxidants

- 2.3.1 Superoxide dismutase
- 2.3.2 Catalase
- 2.3.3 Peroxidase
- 2.3.4 Glutathione systems
- 2.3.5 Polyphenol oxidase

2.4 Non enzymatic antioxidants

- 2.4.1 Ascorbic acid
- 2.4.2 α- tocopherol
- 2.4.3 Reduced glutathione
- 2.4.4 Polyphenol

2.1 Antioxidants

Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can involve the production of free radicals, which `can form dangerous chain reactions. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reaction by being oxidized themselves. Antioxidants are often reducing agents such as thiols or phenols.

Although oxidation reaction are critical for life, they can also be damaging, hence, plants and animals maintain complete systems of multiple types of antioxidants such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (http://en.widipedia.org/wiki/antioxidant).

The possible mechanism of action of antioxidants was first explored when it was recognized that a substance with oxidative activity is likely to be one that is itself readily oxidized. Vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agent that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wolf, 2005).

2.2 Free radicals

Free radicals are chemical species possessing unpaired valance electron. These fragments of molecules are very reactive and often very destructive to other molecules present in the vicinity of their production sites. The Free radicals is produced continuously in the cells either as a result of electron byproducts of metabolism viz. during phagocytosis.

Free radicals, the Reactive Oxygen Species (ROS) and the Reactive Nitrogen Species (RNS) are highly reactive due to the presence of one or more mismatched electrons. The oxygen consumed by the body is reduced to generate ATP and water by an oxidative phosphorylation pathway involving multienzyme process and electron transport system. However, only 95% of oxygen is completely reduced to water and produce ATP and less than 4% generate reactive oxygen species, the superoxide anion (O_2), hydroxyl radical (HO), hydrogen peroxide (H_2O_2) etc, (de Grey,1999).

Endogenously produced melatonin has a significant role as antioxidants. Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, it stimulates several antioxidant enzymes which increase its efficiency as an antioxidant. Antioxidative enzymes viz. superoxide dismutase, glutathione peroxidase and glutathione reductase are also stimulated by melatonin (Reiter, 2000).

2.3. Enzymic Antioxidants

The important enzymic antioxidants are catalase, superoxide dismutase, peroxidase, glutathione-S-transferase, glutathione peroxidase, glutathione reductase and polyphenol oxidase.

2.3.1 Superoxide dismutase

Superoxide dismutases (SODS) are a class of closely related enzymes that catalyse the breakdown of the super oxide anion into oxygen and hydrogen peroxide (Zelko, *et al.*, 2002). SOD enzymes are present in almost all aerobic cells and in extra cellular fluids. Superoxide dismutase enzymes contain metal iron cofactors such as copper, zinc, manganese or iron depending on the isozyme. In humans, the copper/zinc SOD is present in the mitochondria. A third form of SOD exists in extracellular fluids, which contains copper and zinc in its active site. Among the three, mitochondrial isozyme seems to be the most biologically important one.

SOD is considered to be fundamental in the process of eliminating reactive oxygen ion by reducing super oxide to form H_2O_2. Catalase and selenium dependent glutathione peroxidise are responsible for reducing H_2O_2 to H_2O. In plants, SOD isozymes are present in cytosol and mitochondria, with an iron SOD found in chloroplasts that is absent from vertebrates and yeast (Van Camp *et al.*, 1997).

2.3.2 Catalase

This enzyme is present in most aerobic cells in animal tissues. Catalase is present in all body organs but low amount in heart and skeletal muscle. Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al.*, 2004).

$$2H_2O_2 \rightarrow 2H_2O+O_2$$

This protein is localized to peroxisomes in most eukaryotic cells (del Rio *et al.*, 1992). Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping pong mechanism, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate (Hinter, *et al.*, 2002). Despite its apparent in hydrogen peroxide removal, humans with genetic deficiency of catalase suffer from "Acatalasemia" (Mueller *et al.*, 1997).

2.3.3 Peroxidase

Peroxiredoxins are peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides, as well as peroxynitrite (Rhee *et al.*, 2005). They are divided into three classes; typical 2 cysteine peroxiredoxins; atypical 2- cysteine peroxiredoxins; and 1- cysteine peroxiredoxins (Wood *et al.*, 2003). These enzymes share the same basic catalytic mechanism in which a redox – active site is oxidized to a sulfinic acid by the peroxide substrate (Claiborne *et al.*, 1999). Peroxiredoxins seems to be important in antioxidant mechanism, as mice lacking peroxiredoxin 1 or 2 have shortened lifespan and suffer from haemolytic anaemia, while plants use peroxiredoxins to remove hydrogen peroxide generated in chloroplasts (Dietz *et al.*, 2006).

2.3.4 Glutathione Systems

The glutathione system includes glutathione, glutathione reductase, glutathione peroxidises and glutathione-S-transferases. This system is found in animals, plants and microorganisms (Creissen *et al.*, 1996). Glutathione peroxidise is an enzyme containing four selenium cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isoenzymes in animals (Brigelius and Traber, 1999). Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. Glutathione peroxidase 1 is dispensable, as mice lacking this enzyme have normal lifespans (Ho *et al.*, 1997), but they are hypersensitive to induced oxidative stress (de Haan *et al.*, 1998). In addition, the Glutathione-S-transferases are another class of glutathione dependent antioxidant enzymes that show high activity with lipid peroxides (Sharma *et al.*, 2004). These enzymes are at high levels particularly in the liver and play a role in detoxification.

2.3.5 Polyphenol oxidase

Polyphenol oxidase or monophenol mono oxygenase is a tetramer that contains four atoms of copper per molecule and binding sites for 2 aromatic compounds and oxygen. The enzyme catalyzes the hydroxylation of monophends to ortho diphenols. They can also further catalyse the oxidation of diphenols to produce ortho quinones (Mayer, 2006).

2.4. Non Enzymic Antioxidants

The important non enzymic antioxidants are ascorbic acid, α-tocopherol, glutathione, polyphenol, total carotenoids and lycopene.

2.4.1 Ascorbic Acid

Ascorbic acid or "Vitamin C" is a monosaccharide antioxidant found in both animals and plants. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase, glutaredoxins (Meister, 1994). Ascorbic acid is reducing agent and can reduce and thereby neutralize reactive oxygen species such as hydrogen peroxide (Padayatty *et al.*, 2003). In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzymes like ascorbic peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka *et al.*, 2002).

$$\text{Ascorbic acid} + 2O_2^- + 2H^+ \rightarrow H_2O_2 + \text{Dehydro ascorbic acid}$$

Ascorbate reacts rapidly with O_2 and even more rapidly with OH to give dehydro ascorbic acid. Dehydro ascorbic acid itself can act as a source of vitamin C.

2.4.2 Vitamin E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat soluble antioxidant vitamins (Herrera and Barbas, 2001). Of these, α-tocopherol has been most studied as it has the highest bioavailability, with in the body preferentially absorb and metabolise this form (Brigelius and Traber, 1999). The α-tocopherol form is also the most important lipid soluble antioxidant and protects cell membranes against oxidation by reacting with the lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing. The oxidized α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by ascorbate, retinol or ubiquinol (Wang and Quinn, 1999). The functions of the other forms of vitamin E are less well studied although α-tocopherol is a nucleophile that may react with electrophilic mutagens and tocotrienols may have a specialized role in neuroprotection (Sen *et al.*, 2006).

α-tocopherol + LOO* → α – tocopherol* + LOOH

α-tocopherol + LOO* → LOO – α tocopherol

Vitamin E also stimulates the immune response. Vitamin E may inhibit cancer initiation through enhanced immune competence. Vitamin E also has a direct chemical function. α-tocopherol act as a prooxidant (reducing agent) or antioxidant depending on whether all of the α-tocopherol becomes consumed in the conversion from ferric to ferrous iron or whether, following this interaction, residual α-tocopherol is available to scavenge the resultant reactive oxygen intermediate.

2.4.3 Reduced glutathione

Glutathione (GSH) is a tripeptide with a gamma peptide linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG), also called L-(−)-glutathione (Valenzuela *et al.*, 2007)

It is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation. Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the nervous system, the gastrointestinal system and the lungs (Park, 2009).

2.4.4 Polyphenol

Poly phenols are a structural class of mainly natural but also synthetic or semi synthetic, organic chemicals characterized by the presence of large multiples of phenol structural units. The number and characteristics of these phenol structures underlie the unique physico chemical and biological properties of particular members of the class (Del Rio, *et al.*, 2010).

2.4.5 Total carotenoids and Lycopene

Red fleshed watermelons contain high quantities of lycopene, carotenoids that imparts the red colour. This compound has powerful antioxidant properties and has been shown to lower the risk of myocardial infarction (Kohlmeier *et al.*, 1997) and some cancers. Few red fruits and vegetables contain detectable quantities of lycopene. The U. S. department of Agriculture (USDA), Carotenoid Database officially considers watermelon, on average, to contain higher levels of lycopene than other fresh fruits and vegetables (Beecher *et al.*, 1999). Because of the potential health benefits of lycopene, there is an interest in increasing its content in commercial cultivars. Red and pink fleshed watermelon also contains carotenoids related to lycopene, such as β-carotene and prolycopene.

2.5 Antioxidants in certain foods

Measurement of antioxidants is not a straight forward process, as this is a diverse group of compounds with different reactivities to different reactive oxygen species. In food science, the oxygen radical absorbance capacity (ORAC) has become the current industry standard for assessing antioxidant strength of whole foods, juices and food additives (Ou *et al.*, 2001). Other measurement tests include Folin – Ciocalteau reagent and the trolox equivalent antioxidant capacity assay (Prior *et al.*, 2005). In medicine, a range of different assays are used to assess the antioxidant capacity of blood plasma and of these, the ORAC assay may be the most reliable (Cao and Prior, 1998).

Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, legumes and nuts. Some antioxidants such as lycopene and ascorbic acid can be destroyed by long term storage or prolonged cooking (Xianquan *et al.*, 2005). Other antioxidant compounds are more stable, such as the polyphenolic antioxidants in food like whole – wheat, cereals and tea (Baublis *et al.*, 2000). In general, processed foods contain less antioxidants than fresh and uncooked foods, since the preparation process may expose the food to oxygen (Henry and Heppell, 2002). The presence of certain antioxidants in various foods is shown in Table I.

Table I: Antioxidants in certain foods

Antioxidant Compounds	Foods containing high levels of these antioxidants
Vitamin C (ascorbic acid)	Fruits and vegetables
Vitamin E (Tocopherol)	Vegetable oils
Polyphenolic antioxidant (resveratol, flavonoids)	Tea, Coffee, Soy, Fruit, Chocolate and red wine
Carotenoids (lycopene, carotenes)	Fruits and vegetables

Some antioxidants are made in the body are not absorbed from the intestine, glutathione which is made from aminoacids and in the gut it is broken down to free cysteine, glycine and glutamic acid before being absorbed, even large oral doses have little effect on the concentration of glutathione in the body. Ubiquinol (Co-enzyme Q) is also poorly absorbed from the gut and is made in humans through the mevalonate pathway (Turunen *et al.,* 2004).

Non polar antioxidants such as eugenol, a major component of oil cloves have toxicity limits that can be exceeded with the misuse of undiluted essential oils (Prashar *et al.,* 2006). Toxicity associated with high doses of water soluble antioxidants such as ascorbic acid are less of a concern, as these compounds can be excreted rapidly in urine.

2.6 Composition of Beetroot

Beetroot is a nutritious vegetable that is an ideal component of a healthy diet. In Britain, the advice is to consume at least five portions of fruit and vegetables a day. More specific advice from some nutritionists is to select fruit and vegetables of different colours to eat as the daily portions: the "red, amber, green". In terms of vegetables, this could include beetroot (red), carrots (amber) and spinach (green). This recognizes the important role played by plant pigments in disease prevention.

Boiling of beetroot increases its carbohydrate and protein content compared to raw beetroot, with a corresponding increase in energy value. Pickling of beetroot decreases the carbohydrate and protein content (to a level below raw beetroot), with a corresponding decrease in energy value. Acetic acid (vinegar) will contribute to the energy value of pickled beetroot, however, if it is eaten undrained (Bender and Bender, 1995).

Beetroot is an excellent source of folates, including folic acid (tetrahydrofolate). Both the greens and roots of beetroot have been recommended for women who are planning to get pregnant, because they provide a good source of folic acid, along with other beneficial vitamins and minerals. Folic acid is a vitamin (in the vitamin B complex) that functions as a carrier of carbon units in a variety of metabolic reactions in the body. It is essential for the synthesis of compounds called purines and pyrimidines, which play an important role in developmental processes. Foods such as beetroot, green-leafy vegetables, liver and kidneys are rich in folates. As noted above, grated raw beetroot is better than cooked beetroot, while pickled beetroot is a much poorer source of folate (McCance and Widdowson, 1995). Table II depicts the composition of beetroot.

Table II: Composition of Beetroot

(per 100g of edible portion)

Contents	Amount	Contents	Amount
Moisture	87.1g	Iron	0.79mg
Protein	1.68g	Copper	0.063mg
Fat	0.18g	Manganese	0.277mg
Fibre	2.0g	Riboflavin	0.027mg
Carbohydrates	9.96g	Folate	80.0µg
Magnesium	23.0mg	Thiamine	0.031mg
Sodium	77.0mg	Nicotinic acid	0.331mg
Potassium	0.31g	Vitamin A	2.0µg
Phosphorus	38.0mg	Vitamin C	3.6mg
Calcium	16.0mg		

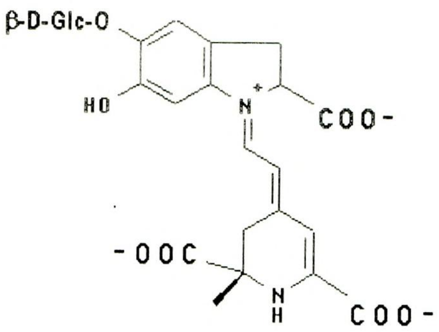
Beetroot is generally considered to prevent illness by bolstering the immune system. Where the immune system is targeted by disease, consuming beetroot as part of a health-promoting diet can help the body fight the severity of the disease (Orr and Patient, 2001).

2.6.1 Betalains

Betalains are alkaloid pigments that are found in some families of plants belonging to the order Caryophyllales, but in no other plants. Betalains are a class of red and yellow indole-derived pigments found in plants of the Caryophyllales, where they replace anthocyanin pigments. Betalains also occur in some higher order fungi. (Strack *et al.,* 2003). They are most often noticeable in the petals of flowers, but may colour the fruits, leaves, stems, and roots of plants that contain them. They include powerful antioxidant pigments such as those found in beets. The name "betalain" comes from the Latin name of the common beet (*Beta vulgaris*), from which betalains were first extracted. The deep red colour of beets, bougainvillea, amaranth and many cacti results from the presence of betalain pigments (Robinson and Trevor, 1963). The particular shades of red to purple are distinctive and unlike that of anthocyanin pigments found in most plants have the highest levels of betalains (a phytonutrient) compared to any other plant food. The anti-inflammatory aspect of betalains helps to prevent many chronic diseases and promotes cardiovascular health. Beets contain lots of lutein and zeaxanthin - both of which help prevent macular degeneration and other age related eye issues. Early research indicates that the betalains support nerve and eye tissue better than most antioxidants.

Betalains are not found in plants containing anthocyanin pigments – structurally they are unrelated. They can be divided into **betacyanins** and **betaxanthins** based upon their molecular structure. **Betacyanins** generally appear red to reddish violet in colour (absorb in the 535-550nm range). **Betaxanthins** generally appear yellow in colour (absorb in the 475-480nm range).

The basic structure of betacyanins



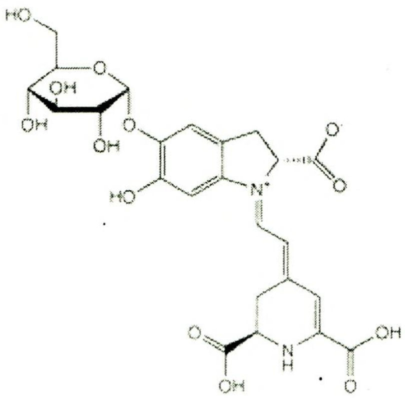
They cause colour in flowers, fruits and sometimes vegetative organs. They are found in the vacuole and they are water-soluble. Beetroot pigment is used commercially as a food dye. It changes colour when heated so can only be used in ice-cream, sweets and other confectionary, but it is both cheap and has no known allergic side-effects. Beetroot itself, of course, is a common salad ingredient when cooked.

2.6.2 Betanin

Betanin or Beetroot Red, is a red glycosidic food dye obtained from beets; it is a aglycone, obtained by hydrolyzing the glucose molecule, the betanin act as a food additive. Betanin degrades when subjected to light, heat and oxygen; therefore, it is used in frozen products with short shelf life or products sold in dry state. Betanin can survive pasteurization when in products with high sugar content. Its sensitivity to oxygen is highest in products with high content of water and/or containing metal cations (e.g. iron and copper); antioxidants like ascorbicacid and sequesters can slow this process down, together with suitable packaging. In dry form betanin is stable in presence of oxygen. Beetroot contains betanin, is a substance that relaxes the mind and is used in other forms to treat depression and contains tryptophan which is also found in chocolate and contributes to a sense of well being.

Betanin is a mood modifier. In the diet, betanin-rich foods are pharmacologically active and can have a positive effect on mood by relaxing the mind. Beetroot, because it contains betanin, is therefore a minor "mood food", alongside ginseng and foods containing caffeine, tryptophan and other pharmacologically-active compounds. More seriously, betanin forms part of the treatment for mood disorders, particularly clinical depression and a range of other medical conditions. Sugar beet is the main source of medicinal betanin. It is sold as a white powder, having a sweetish taste.

Structure of Betanin



Betanin is usually obtained from the extract of beet juice; the concentration of betanin in red beet can reach 300–600 mg/kg. Other dietary sources of betanin and other betalains include the opuntia cactus, swiss chard, and the leaves of some strains of amaranth.

The colour of betanin depends on pH; between four and five it is bright bluish-red, becoming blue-violet as the pH increases. Once the pH reaches alkaline levels betanin degrades by hydrolysis, resulting in a yellow-brown colour.

Betanin can be also used for colouring meat and sausages. The most common uses of betanin are in colouring ice cream and powdered soft drink beverages; other uses are in some sugar confectionery e.g. fondants, sugar strands, sugar coatings, and fruit or cream fillings. In hot processed candies, it can be used if added at the final part of the processing. Betanin is also used in soups as well as tomato and bacon products. Betanin has nearly no potential as allergen (Dean, *et al.*, 2009).

Betanin absorbs well from the gut and acts as an antioxidant. Betanin is a betalain pigment, together with isobetanin, probetanin and neobetanin. Other pigments contained in beet are indicaxanthin and vulgaxanthins.

2.7 Beetroot dyes

Beetroot (*Beta vulgaris*) is the main source of natural red dye, known as "beetroot red". Betanin is the main component of the red colorant extracted from *Beta vulgaris*. Immediately after extraction, betanin is exposed to degradation. The pigment stability is influenced by factors such as enzymes, temperature, oxygen and pH (Stintzing, *et al.*, 2002). Besides betanine, another pigment which is extracted from beetroot is vulgaxanthine.

Betanin can be obtained from beetroot by milling followed by pressing, filtration and evaporation of the resulted juice (Dobre, *et al.*, 1997). The product of this process is a red powder. Solid liquid extraction carried out under conditions which lead to a maximum extraction yield and minimum pigment degradation continues to be a useful method for obtaining beetroot juice.

Betacyanin, the pigment that gives beetroot its colour is an antioxidant, so the humble beetroot could be the key to beating hangover. Betacyanin speeds up detoxification in liver, which enables the body to turn the alcohol into a less harmful substance that can be excreted quicker than normal.