INTRODUCTION

In developing countries like India as well as several developed countries in the world, many persons still are deprived from sufficient food/two meals. Those who are fortunate to have food may not be able to meet the adequate protein and other nutritional requirement. As a result they have poor health status and are prone to diseases very easily. On the other hand in developed countries such as Europe and America excess food availability has laid to increasing obesity and related serious health problems. The food science therefore is facing a challenge to identify niches to counter both the conditions. “Functional Food” is a solution for the problem. Food can be said to be functional if it contains a component that benefits one or more functions in the body in a targeted way that is relevant to either the state of wellbeing and help or the reduction of the risk of the disease or has physiological or psychological effect beyond the traditional nutritional effect. In short, functional food is the one that contains some health promoting component/components beyond traditional nutrients. Functional foods are also known as Neutraceuticals, Medicinal foods, Therapeutic foods, Foodyeuticals, Medifoods and Pharmafoods.

Stephen DeFelice, M.D. the founder chairman of the Foundation for Innovation in Medicine (FIM) Cranford U.S.A., coined the term Neutraceutical from combining the two words nutrition and pharmaceuticals in 1989. According to
DeFelice Nutraceutical can be defined as, “a food or a part of a food that provides medicinal or health benefits including the prevention and/or treatment of disease.”

International food Information Council Foundation (IFIC) in 1998 gave the definition of functional food as, “Food that provides health benefit beyond basic nutrition.” A European commission consulted action program known as, “Functional Food Science in Europe (FUFOSE)” ; in 1998, reached a consensus known as the “European Consensus on Scientific Concepts of Functional Foods.” It says that functional food must remain food and must demonstrate its effect that normally be expected to be consumed in diet; it should not be a pill or capsule. (Shah.2006).

Nutraceuticals i.e. Pharamafoods/Medifoods should not be confused with medical foods. Medical foods are used to feed hospitalised patient or to be a major dietary factor for those with unusual diseases. These some times are also called as ‘enteral formulas’ or ‘enteral foods’.

Several ethnic wild foods used by tribals or ethnic groups over the generations are known to possess medicinal properties apart from the nutritional ones. During the literature survey several such examples were observed where the specific foods are prescribed during some specific health ailment. This has been noted in Part I of the present project.

Since ancient times, plants are known as a source of diverse biologically active chemicals, essential for maintaining health and useful for treating and preventing disease. It is only recently that, several scientific investigations have been performed to discover potential health protective food compounds which might prevent the occurrence of disease, improving general health. It is because we are becoming increasingly aware that, various nutrients in diet play a crucial role in maintaining an optimum immune response. Optimal nutrition is a key factor in influencing the
physiological functions of an individual, therefore, in Ayurveda also, the importance of several plant species in supplementary diet for maintaining sound health has been very well emphasized. Kautilya’s Arthasastra also discusses the food plants that can be used in emergency to give extraordinary strength (Priyadarsan Sensarma 1996).
Despite green revolution, record harvest and overflowing godowns, one fifth of our population still does not have two square meals a day. Obviously the reason is faulty policies of distribution and also the advocacy of cultivation of only few species. It is high time that we should rethink about richness of our heritage food. All India Coordinated Research Project on Ethnobiology (Anonymous 1994) has revealed that about 3900 wild plant species are used as food by tribals.

In Africa where the hunger problem is concentrated, there are actually 2000 species of native food plants. In Africa as well as in the rest of tropics where malnutrition is severest, can be found 3000 different fruits as well as over 1000 vegetables used traditionally. Despite the existence of all these herbaceous heritage hardly any tropical plant is being employed a full advantage to relive the pervasive problems. Of Africa’s 2000 food plants, only Sorghum gets major research and of the 3000 fruits only, Banana and Pineapple got solid global support (Vietmeyer, 1996.).

Traditional vegetables are usually rich in nutrients such as vitamin A and iron. Zennie and Ogzewalla (1977) also found that wild edibles are rich in Ascorbic acid and vitamin A content.

In climatically harsh areas like Ladakh, people are mainly dependent on wild vegetables. Kaul et al. (1985) analysed eleven species for nutritional potential, to promote their domestication.

Tubers mainly constitute the emergency food of tribals.

Cook et al. (1998) screened aqueous extracts of 17 wild edibles of sub-Saharan Africa for their antioxidant contents and compared with those of Spinach and Potato. Out of 17 species, 11 had a greater antioxidant content than Potato. In general,
leaves contained more antioxidants than either fruits or seeds. The total antioxidant capacity of aqueous extracts was relatively high indicating substantial presence of flavonoid glycosides, which are shown to have anticancer properties.

Estimation of carbohydrates of seeds of tree species to evaluate their nutritional status was done by Kadam et al. (1996) and Kadam (2000).

Vaidya et al. (2004) estimated proteins, carbohydrates, starch and reducing sugars of tubers of *Ceropegia hirsute*, *Dioscorea pentaphylla*, *Dioscorea oppositaefolia* and *Vigna capensis*. They found tubers of *Vigna caparis* to be more nutritious than other three species studied.

Problems due to vitamin deficiency were unusual among the Magar tribe of Nepal, due to the high Vitamin A and B content of wild edible leaves (Dietz 2005).

Use of medicinal herbs for vegetable preparation has been noted by earlier workers also. Gajurel et al. 2006 has reported 16 species of medicinal use eaten by Adi tribals of Arunachal Pradesh.

Gehlot (2006) estimated nutritive value of five wild edibles of arid region of Rajasthan. He found them rich in ascorbic acid.

Murugan et al. (2006) studied nutritive and antinutritive characteristics of four wild edible species from South India. They found the species are good source of valuable minerals and essential amino acids compared to the introduced varieties and also important in food security.

Nutritional potential of *Solanum nigrum* var. *virginianum*, used by Igbos and Efik-Ibibio people of Nigeria has been estimated by Akubugwo et al (2007). The leaves are found to be a good source of Vit C, B and folic acid.

Ozbucak et al. (2007) studied nutrition contents of eleven wild edible plants in Central Black Sea region of Turkey.
Aberoumand and Deokule (2009) studied nutritional value of eight wild edibles of Iran and India. They also determined the caloric value and antinutrient like phytates. They found them rich in iron and zinc.

Mensah et al. (2008) has studied phytochemical, nutritional and medical properties of leafy vegetables from Nigeria.

Exhaustive studies on Nutritional potential of some local wild edibles of Amravati Distt. has been carried out by Bhogaonkar and Marathe (2008) and Bhogaonkar et al. (2008a, 2008b).

Kittur et al. (1999) estimated oil content of seeds of seven wild species of Convolvulaceae and Leguminaceae. It was found to be highest in Quamoclit coccinia (22.6%). Linoleic, palmitic and oleic acids are predominant in these seed oil. High protein content was found in five of the species studied.

Nutritional studies comprise of six classes of nutrients-carbohydrates, lipids, proteins, vitamins, minerals and water.,

**Carbohydrates**

Carbohydrates are a varied combination of both very small and very large molecules that comprise about 40 to 45 percent of the energy supply for body. In addition, certain types of carbohydrates, such as fiber and resistant starches are not used by body for energy, but play important health-promoting roles in gastrointestinal tract, supporting digestion and absorption, and helping to eliminate toxins and waste products.

Carbohydrates are composed of carbon, hydrogen, and oxygen, which are arranged into small units called sugars, or monosaccharide’s. Carbohydrate molecules like glucose or sucrose (table sugar) are composed of one or two sugar units, respectively, and are the molecules that give food a sweet taste. These molecules are
sometimes called "simple sugars" because they are small (only one or two units), and are quickly digested, providing immediate energy to the body.

Larger carbohydrate molecules, which include fibers and starches, are composed of at least 10 monosaccharide’s linked together. These polysaccharides may contain up to several hundred monosaccharide’s linked together in different ways. Another term commonly used to describe carbohydrates is *oligosaccharides*, a type of carbohydrate molecule that is in-between polysaccharides and monosaccharide’s in size, and features two to ten monosaccharide’s bonded together.

**The Simple Sugars: Monosaccharides and Disaccharides**

**Monosaccharides**

Monosaccharides are true simple sugars since, as one sugar unit only, they exist in the form in which they can be directly absorbed into the body upon ingestion. Unlike the other carbohydrates, they don't require being broken down during digestion, so these sugars quickly get into bloodstream, increasing blood sugar and providing immediate energy.

Monosaccharides are present in most foods in at least some amount, but are particularly high in foods such as ripe fruit, and honey. Monosaccharides are an important energy source, but when too much of these simple sugars are consumed at once--especially when they are not balanced by complex carbohydrates like oligosaccharides or polysaccharides that take longer to digest and thus help maintain longer-term energy production--monosaccharides can cause a large increase in blood sugar, followed by an abrupt drop. The result is a jolt of energy quickly followed by a feeling of being tired, shaky, or run-down soon afterward. This type of fluctuation in blood sugar, if it occurs frequently, can lead to blood sugar dysregulation conditions such as hypoglycemia and diabetes mellitus. Processed foods often add high amounts
of monosaccharides such as fructose and glucose to promote a sweet taste, which sells more product, but does not sustain health.

**Disaccharides**

Disaccharides contain two monosaccharides bonded together, and include sugars such as lactose (milk sugar), sucrose (table sugar), maltose and isomaltose (sugars formed from the breakdown of starch). Disaccharides are similar to monosaccharides; that is, they provide sweet taste to food and quick energy. Disaccharides also are highly represented in processed foods, and their frequent consumption can lead to blood-sugar disregulation, the same as monosaccharides.

Disaccharides require some digestion to break them into two one-sugar units for absorption, and since each disaccharide is unique, each has its own digestive enzyme. For example, the enzyme *sucrase* for sucrose; *lactase* for lactose etc. For most disaccharides, these enzymes are readily secreted into the intestine after consuming, and digestion of the disaccharides proceeds rapidly. The exception appears to be with lactose (milk sugar).

Many people lack the enzyme *lactase* and are therefore unable to breakdown lactose, a condition called lactose intolerance, which makes the consumption of dairy products problematic for many people. Some studies suggest that *Lactobacillus* supplements are beneficial in this respect as well.

**The Polysaccharides: Starch, Fiber and Resistant Starch**

Plants store their energy by stringing together many glucose units into a long complex of several hundred to several thousand sugar (glucose) molecules. Plant foods that contain stored energy, for example seeds that must provide energy for the young plant when it starts growing, are high in starch. When the young plant starts growing, the starch is broken down into glucose for energy.
**Starch**

After consumption, the body must breakdown this very large molecules to individual sugar units before they can be digested. The digestion of starch takes longer than that of disaccharides; therefore, starch provides an extended, or sustained source of energy. Starches are thought to be better for health and energy.

Starches are called complex carbohydrates because they are so large. Two main types of starches exist in food: amylose and amylopectin. Amylopectin is more quickly digested than is amylose; therefore, foods that contain higher amylose than amylopectin are often suggested as substitutions for people with bloodsugar control problems, like diabetes.

**Protein**

Protein, providing 0.4 calories per gram, is an important source of energy for the body, when carbohydrates and fats are not available. In addition to using protein to generate energy for cellular function whenever necessary, the body uses the amino acids contained in the protein to manufacture its own proteins. (Groff et al. 1995)

The body manufactures several structural proteins, such as myosin, actin, collagen, elastin, and keratin, that maintain the strength and integrity of muscles and connective tissues (ligaments and tendons) (Tipton and Wolfe 2001).

All of the enzymes, that catalyze chemical reactions in the body, are made from protein. In addition, the hormones involved in blood sugar regulation (insulin and glucagon) as well as the thyroid hormones are synthesized from proteins.

Certain proteins are used by the body to carry various substances to body tissues. These transport proteins include haemoglobin (carries oxygen), transferrin (carries iron), ceruloplasmin (carries copper), retinol-binding protein (carries vitamin A), albumin and transthyretin (both carry other proteins) Calcium and vitamin D.
Lipoproteins participate in the transportation of fat and cholesterol. (Mc Williams 1989, Tipton and Wolfe 2001)

Antibodies, which are proteins, play an important role in the immune system by attaching to antigens (viruses, bacteria, or other foreign invaders), thereby inactivating the antigens and making them more visible to the immune cells (called macrophages) that destroy antigens. (Calder and Kew 1988)

Proteins participate in the maintenance of osmotic pressure, which controls the amount of water that is found inside cells. Due to their ability to combine with both acidic and basic substances, proteins help to maintain the normal acid-base balance in the body.

According to the World Health Organization, approximately 300 million children throughout the world suffer from growth retardation due to protein-energy malnutrition. Additionally, children with protein-energy malnutrition have a 40% mortality rate, due to increased susceptibility to infections (Linger 2000).

Deficiency typically occurs in children younger than 4 years old fed diets high in carbohydrates with little or no protein. Symptoms of kwashiorkor include muscle wasting, edema (fluid retention), and an enlarged and fatty liver, with the preservation of visible fat stores. Excessive intake of protein over many years may lead to kidney problems and/or accelerated bone loss eventually leading to osteoporosis (Denke 1988).

**Lipids-**

Lipids are a heterogeneous group of organic compounds such as fatty acids, acylglycerols, phosphoglycerides, steroids, terpenes and prostaglandins which are soluble in non-polar organic solvents, namely chloroform, ether, benzene and hexane but insoluble or only sparingly soluble in water. They are compounds of living
systems consisting basically of carbon, hydrogen and oxygen in addition some classes have nitrogen and phosphorus (Thimmaiah 1999).

Lipids are very wide spread in nature among all vegetable and animal matter.

**Simple lipids**-these are esters of fatty acids with certain alcohols. They are usually further classified according to the nature of the alcohols.

**Fats and oils**-These are esters of fatty acids and glycerol. Oils are liquids at room temperature.

**Compound lipids**- the compound lipids are esters of fatty acids which on hydrolysis yield other substances in addition to the nature of the alcohols.

The role of fat in the body is as essential constituent of the membrane of every cell, energy reserve, regulator of body functions, insulator and protector.

**Crude Fat**-

It includes fats and oils. They are glyceride esters of either saturated or unsaturated fatty acids.

Fats are probably the most complex of the macromolecules in foods because there are so many different types of fats. Unfortunately, fats have been given a bad reputation, in part because fat is the way we store excess calories, and in part because saturated fats, trans-fatty acids, and cholesterol have been associated with health conditions like cardiovascular disease and obesity. The facts are, however, that all fats are not bad, but some fats have been shown to be health-promoting, and some fats are absolutely essential for health.

Fats, are also referred to as lipids. They are composed of carbon, hydrogen, and oxygen like the other macromolecules, but are insoluble in water (hydrophobic). Fats are chemically described as either unsaturated, monounsaturated or polyunsaturated. The saturated fats are straight molecules that form solids at room
temperature, such as butter and the fats found in meat. Monounsaturated fats, like olive oil, are liquids at room temperature but form solids at low temperature. Polyunsaturated fats, found in high amounts in oils from grains and seeds, such as flaxseed oil, are liquid at room temperature and remain liquid even when cooled.

Extremely important role of fats is as major component of cell membranes. Cell membranes contain all kinds of fats -- unsaturated, monounsaturated, and polyunsaturated -- in different amounts. Body cells primarily need polyunsaturated fats along with some monounsaturated fat to keep the cell membranes flexible and moveable. When levels of saturated fat are too high, cell membranes become inflexible and don't function well, so they can't protect the internal parts of the cell, such as its DNA, as well.

**Dietary fiber**

Dietary fiber is undoubtedly one of the most talked about nutrients for health promotion and disease prevention. In fact, dietary fiber is the focus of two FDA-approved health claims that appear on foods labels touting the benefits of high fiber foods for the prevention of heart disease and certain types of cancer.

Characteristic of dietary fiber is that it is derived from the edible parts of plants that are not broken down by human digestive enzymes. Dietary fiber is a mixture of many complex organic substances, each having unique physical and chemical properties. Although a single definition has yet to be agreed upon, dietary fiber is commonly defined as “plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man” (Trowell et al. 1976).

American Association of Cereal Chemists proposed a new definition of dietary fiber that includes the statement "Dietary fibers promote beneficial physiological
effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation."

Institute of Medicine at the National Academy of Sciences (the organization responsible for issuing Recommended Dietary Allowances) has proposed a new definition that differentiates between dietary fiber and added fiber. According to this definition, dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Added fiber, which refers to fiber that is added to foods during food processing, consists of isolated non-digestible carbohydrates that have proven beneficial physiological effects in humans.

Plant cell wall material containing cellulose, hemicellulose, pectic substances, and lignin are the major components of dietary fiber (Selvendran et al 1987) polyfructoses, galactooligosaccharides, gums, mucilages pectins, resistant starches.

In addition mucilages, gums, algal polysaccharides, and synthetic polysaccharides are also considered dietary fiber.

In an attempt to clarify the concept of dietary fiber, many scientists have opted to define individual fiber sources by their physical properties. One widely used classification is that of water soluble or gel-forming viscous fibers and water insoluble fibers.

Soluble fibers are highly fermentable and are associated with carbohydrate and lipid metabolism, while insoluble fibers contribute to fecal bulk and reduce transit times (Madar and Odes 1990). Particle size , water holding capacity, viscosity, cation exchange capability, and binding potential are specific for every fiber source (Dreher 1987).

The chemical structure of the fiber is a major factor in determining its physical properties. Cellulose, for example, contains tightly packed linear polysaccharides that
are insoluble in water and resistant to hydration and swelling. In contrast, pectin is charged viscous polysaccharides that is readily soluble in water and has a high ion binding potential. Lignin and hemicellulose adsorb bile salts, while cellulose alone has a very low capacity for bile salt adsorption (Story 1986). Eastwood and Morris (1992) describe dietary fiber as a “water laden sponge” moving through the intestine. For many years dietary fiber was not considered to have a significant nutritional value. We now know that many fibers are fermented in the large intestine to produce hydrogen, methane, carbon dioxide, and short-chain fatty acids. The short-chain fatty acids are rapidly absorbed from the gastrointestinal tract and contribute to the energy balance of the body (Cummings 1991).

A diet high in fiber may play a role in the prevention and/or treatment of: breast cancer (Cohen 1999), cardiovascular disease, high cholesterol (Fernandez 2001), colon cancer, constipation, diabetes (Zhao et al 2002), diverticulitis, gallstones, irritable bowel syndrome, obesity (Pereira 2001), syndrome X (Dasy and Melby 2003).

**Vitamin A**

Vitamin A, identified in 1913, was the first fat-soluble vitamin to be discovered. Vitamin A is also known as retinol, a name given in reference to the participation of this compound in the functions of the retina of the eye; also been called the "anti-infective" vitamin due to its role in supporting the activities of the immune system (Groff et.al 1998). It stimulates several immune system activities, possibly by promoting the growth, and preventing the stress-induced shrinkage, of the thymus gland. Vitamin A is known to enhance the function of white blood cells, increase the response of antibodies to antigens, and to have anti-viral activity. In addition, retinoic acid is needed to maintain the normal structure and function of
epithelial and mucosal tissues, which are found in the lungs, trachea, skin, oral cavity, and gastrointestinal tract. These tissues, when healthy and intact, serve as the first line of defense for the immune system, providing a protective barrier that disease-causing microorganisms cannot penetrate.

The body can convert certain members of the carotenoid family, including beta-carotene, alpha-carotene, and gamma-carotene, into vitamin A. These carotenoids are sometimes referred to as "provitamin A," and retinol as "preformed vitamin A."

While retinol, or preformed vitamin A, occurs only in foods of animal origin, fruits and vegetables that contain certain carotenoids also provide vitamin A activity. Carotenoids are plant pigments, responsible for the red, orange, and yellow color of fruits and vegetables.

Prolonged vitamin A deficiency can lead to night blindness, due to impaired production of rhodopsin, the compound in the retina responsible for detecting small amounts of light. Xerophthalmia, a condition characterized by changes to the conjunctiva and cornea of the eye, also results from prolonged vitamin A deficiency, and is a major cause of blindness in developing nations. (Smith and Steinemann 2000) Neither cooking nor storage significantly affects the amount or availability of preformed vitamin A in foods.

Since vitamin A is a fat-soluble vitamin, vitamin A deficiency may be caused by a diet that is extremely low in fat and/or the presence of medical conditions that cause a reduction in the ability to absorb dietary fat, such as pancreatic enzyme deficiency, Crohn's disease, celiac sprue, cystic fibrosis, surgical removal of part or all of the stomach, gall bladder disease, and liver disease.
**Vitamin C**

Vitamin C, also called ascorbic acid, is a water-soluble nutrient that is easily excreted from the body when not needed. It's so critical to living creatures that almost all mammals can use their own cells to make it. Humans, gorillas, chimps, bats, guinea pigs and birds are some of the few animals that cannot make vitamin C inside of their own bodies.

In general, an unripe food is much lower in vitamin C than a ripe one, but provided that the food is ripe, the vitamin C content is higher when the food is younger at the time of harvest. Vitamin C is highly sensitive to air, water, and temperature.

Vitamin C serves a predominantly protective role in the body. As early as the 1700's, vitamin C was referred to as the "antiscorbutic factor," since it helped prevent the disease called scurvy. It also protects skin and gums.

The protective role of vitamin C goes far beyond our skin and gums. Cardiovascular diseases, cancers, joint diseases and cataracts are all associated with vitamin C deficiency and can be partly prevented by optimal intake of vitamin C. Vitamin C achieves much of its protective effect by functioning as an antioxidant and preventing oxygen-based damage to our cells. Structures that contain fat (like the lipoprotein molecules that carry fat around our body) are particularly dependent on vitamin C for protection. (Englard and Seifter 1986)

Poor wound healing can be a symptom of vitamin C deficiency. Weak immune function, including susceptibility to colds and other infections, can also be a telltale sign of vitamin C deficiency. Since the lining of our respiratory tract also depend heavily on vitamin C for protection, respiratory infection and other lung-related conditions can also be symptomatic of vitamin C deficiency.
Vitamin C has significant interactions with several key minerals in the body. Supplemental intake of vitamin C at gram-level doses can interfere with copper metabolism. Conversely, vitamin C can significantly enhance iron uptake and metabolism, even at food-level amounts.

Vitamin C also has important interactions with other vitamins. Excessive intake of vitamin A, for example, is less toxic to the body when vitamin C is readily available. Vitamin C is involved in the regeneration of vitamin E, and these two vitamins appear to work together in their antioxidant effect.

Dietary supplements typically contain vitamin C in the form of ascorbic acid. Vitamin C is better absorbed in the presence of flavonoids.

**Chlorophyll**

In many vegetables, there is slightly more chlorophyll ‘a’ than chlorophyll ‘b’, and this slight edge in favour of chlorophyll ‘a’ tends to decrease as the plant ages. However, research studies have yet to clarify what the exact health significance is of this chlorophyll ‘a’-to- chlorophyll ‘b’ ratio.

Some vegetables contain particularly high amounts of total chlorophyll. Best studied of all the vegetables is spinach (*Spinach oleracea*); containing about 300-600 mg per ounce.

The antimutagenic activity of chlorophyll has been well established (Lai 1979; Armito et al. 1980a,b, Kimm et al. 1982)

Research on the health benefits of chlorophyll has focused on the area of cancer. This research got under way when damage to genes (or more precisely, to the genes’ DNA) by carcinogenic substances called aflatoxins (or more precisely, aflatoxin B1, or AFb1), was found to be prevented by chlorophyllin. Chlorophyllin is a derivative of chlorophyll in which the magnesium in its centre is removed and replaced with
copper. (Dashwood et.al.1991, Breinholt 1995, Sarkar et.al 1994 ). Research studies in humans have found that damage to DNA by aflatoxin can be decreased as much as 55% through supplementation with chlorophyllin at 100 mg, three times a day, for four months. This amount of chlorophyllin, 300mg per day, is the same amount of chlorophyll found in one weighted ounce of spinach (Dashwood1992).

Although research is still in the early stage, prevention and treatment of liver cancer, skin cancer, and colon cancer are all being investigated in relationship to intake of chlorophyll-containing vegetables and supplementation with chlorophyllin (Park et.al 1994, Waladkhani 1998).

**Carotenoid**

Carotenoids are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria.

Plant carotenoids are the primary dietary source of pro-vitamin A worldwide, with β-carotene as the most well-known pro-vitamin A carotenoid. There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen).

In humans, carotenoids such as beta-carotene are a precursor to vitamin A, a pigment essential for good vision, and carotenoids can also act as antioxidants. People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illnesses. (Diplock et.al 1998)

Most carotenoid-rich fruits and vegetables are low in lipids.
Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as **xanthophylls**.

The unoxygenated (oxygen free) carotenoids such as alpha-carotene, beta-carotene and lycopene are known as **carotenes**. Carotenes typically contain only carbon and hydrogen.

Carotenoids are efficient free-radical scavengers, and they enhance the vertebrate immune system. There are several dozen carotenoids in foods people consume, and most carotenoids have antioxidant activity. Epidemiological studies have shown that people with high beta-carotene intake and high plasma levels of beta-carotene have a significantly reduced risk of lung cancer.

Some naturally occurring carotenoids are Hydrocarbons, Alcohols, Glycosides, Ethers, Epoxides, Aldehydes, Acids and Acid Esters, Ketones, Esters of Alcohols, Apo Carotinoids, Nor and Seco Carotinoids, retro Carotinoids, Higher Carotenoids.

The structure of **β-Carotene** was deduced by Karrer et al (1930). β-Carotene is an organic compound - a terpenoid, a red-orange pigment abundant in plants and fruits. As a carotene with β-rings at both ends, it is the most common form of carotene. It is a precursor (inactive form) of vitamin A (Susan 1998). Being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic.

In nature, β-carotene is a precursor to vitamin A via the action of beta-carotene 15,15'-monooxygenase. β-Carotenoid is biosynthesized from geranylgeranyl pyrophosphate (Susan 1998).
Lycopene

Lycopene is a member of the carotenoid family of phytochemicals and is a natural pigment responsible for the deep red colour of several fruits, most notably tomatoes. Unlike several carotenoids, lycopene does not have pro-vitamin A activity - in other words, it does not get converted into vitamin A. Consequently, the health benefits of lycopene are attributed primarily to its powerful antioxidant actions. In fact, laboratory experiments indicate that lycopene is a more effective antioxidant than other carotenoids, including beta-carotene. Lycopene is especially effective at quenching a free radical called singlet oxygen. Singlet oxygen is a highly reactive free radical formed during normal metabolic processes that reacts with polyunsaturated fatty acids, which are major constituents of cell membrane. Due to the fact that lycopene is commonly located in cell membranes, it plays an important role in preventing oxidative damage to the membrane lipids, thereby influencing the thickness, strength, and fluidity of the membranes. Cell membranes are the gatekeepers of the cell, allowing nutrients in, while preventing toxins from entering and facilitating the removal of cellular garbage. Maintaining the integrity of cell membranes is a therefore key factor in the prevention of disease.

In addition to its antioxidant activity, lycopene has been shown to suppress the growth of tumors in in vitro (test tube) and in vivo (animal) experiments. One of the ways that lycopene may limit tumor growth is by stimulating cell to cell communication. Researchers now believe that poor communication between cells is one of the causes of the abnormal growth of cells, a condition which ultimately leads to the development of cancerous tumors.

Lycopene is also believed to play a role in the prevention of heart disease by inhibiting free radical damage to LDL cholesterol. Before cholesterol can be
deposited in the plaques that harden and narrow arteries, it must be oxidized by free radicals. With its powerful antioxidant activity, lycopene can prevent LDL cholesterol from being oxidized.

Recent research has suggested that lycopene can boost sperm concentrations in infertile men. Palan and Raz (1996) have shown that lycopene-supplemented diet resulted in a statistically significant improvement in sperm concentration and motility amongst the 30 infertile men being studied with six pregnancies following as a result of the trial.

It is generally accepted that the availability of lycopene is increased when these foods are processed at high temperatures or packaged with oil. (Shi and Maguer 2000) Lycopene is a fat-soluble substance, and as such requires the presence of dietary fat for proper absorption through the digestive tract.

Lycopene may play a role in the prevention and/or treatment of age-related muscular degeneration, breast cancer, cardiovascular disease, cataracts, cervical cancer, exercise-induced asthma, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer.

**Lutein and Zeaxanthin**

Lutein and zeaxanthin are carotenoids, that are not considered to be "provitamin A" compounds, as they are not converted in the body into retinol, an active form of vitamin A. Both of these yellow colored phytonutrients. Both these carotenoids have yellow pigments. They are found concentrated in foods of others colors, notably leafy green vegetables. They are fat-soluble substances, and as such require the presence of dietary fat for proper absorption through the digestive tract. Lutein appears to be sensitive to cooking and storage.
A low dietary intake of carotenoids such as lutein and zeaxanthin is not known to directly cause any diseases or health conditions, at least in the short term, although long-term inadequate intake of carotenoids is associated with chronic disease, including heart disease and various cancers. One important mechanism for this carotenoid-disease relationship appears to be free radicals. Diets low in carotenoids can increase the body's susceptibility to damage from free radicals. Over the long term, carotenoid-deficient diets may increase tissue damage from free radical activity, and increase risk of chronic diseases like heart disease and cancers (Agrawal and Rao 2000).

The eyes are repositories for carotenoids with lutein and zeaxanthin concentrated in the retina and lens. Observational studies have noted that higher dietary intake of lutein and zeaxanthin is related to reduced risk of cataracts and age-related macular degeneration. Researchers speculate that these carotenoids may promote eye health through their ability to protect the eyes from light-induced oxidative damage and aging through both their antioxidant actions as well as their ability to filter out UV light.

Carotenoids may play a role in the treatment and/or prevention of acquired immunodeficiency syndrome (AIDS), age-related macular degeneration, angina pectoris, asthma, cataracts, cervical cancer, cervical dysplasia, chlamydial infection, heart disease, laryngeal cancer (cancer of the larynx), lung cancer, male and female infertility, osteoarthritis, photosensitivity, pneumonia, prostate cancer, rheumatoid arthritis, skin cancer, vaginal candidiasis.

**Anthocyanins**

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue according to pH. Anthocyanins occur in all tissues of higher plants,
including leaves, stems, roots, flowers, and fruits. Anthocyanins are glucosides of anthocyanidins.

Anthocyanins have been shown to act as a "sunscreen", protecting cells from high-light damage by absorbing blue-green and UV light, thereby protecting the tissues from photoinhibition, or high-light stress. In addition to their role as light-attenuators, anthocyanins also act as powerful antioxidants.

Anthocyanins are powerful antioxidants in vitro. This antioxidant property may be conserved even after the plant which produced the anthocyanin is consumed by another organism, possibly explaining why fruits and vegetables with colorful skins and pulp are considered nutritious. Research continues to be underway as to the potential range of health benefits from anthocyanins.

Richly concentrated as pigments in berries, anthocyanins were the topics of research presented at a 2007 symposium on health benefits that may result from berry consumption. Laboratory-based evidence was provided for potential health effects against cancer, (Stoner 2007, Hou DX 2003) aging and neurological diseases, inflammation, (Karlsen 2007) diabetes, bacterial infections (Kong 2003). Results are published in Journal of Agricultural and Food Chemistry February 2008.

**Phenols**

They probably constitute largest group of plant secondary metabolites; wide spread in nature, and to be found in most classes of natural compounds having aromatic molecules. They are important constituents of some medicinal plants and in the food industry they are utilized as colouring agents, flavouring, aromatizer and antioxidants. Some of the phenolic compounds like chlorogenic acid and its polymers especially act as antioxidants. The chlorogenic acid concentration is about 90% of the total phenolic compounds in plants (Ekanayake and Nair1998).
Macro minerals

The minerals present at levels more than 0.05 percent in the human body are defined as macrominerals. Calcium, Phosphorus, Magnesium, Sodium and Potassium belong to this category.

Potassium, sodium and chloride comprise the electrolyte family of minerals. They conduct electricity when dissolved in water. These minerals work together closely. About 95% of the potassium in the body is stored within cells, while sodium and chloride are predominantly located outside the cell.

Calcium

One of the most abundant minerals in the human body, calcium accounts for approximately 1.5% of total body weight. Bones and teeth house 99% of the calcium in the body, while the remaining 1% is distributed in other areas.

Calcium is best known for its role in maintaining the strength and density of bones. In a process known as bone mineralization, calcium and phosphorus join to form calcium phosphate. Calcium phosphate is a major component of the mineral complex (called hydroxyapatite) that gives structure and strength to bones.

Calcium also plays a role in many physiological activities not related to bones including blood clotting, nerve conduction, muscle contraction, regulation of enzyme activity, and cell membrane function. Because these physiological activities are essential to life, the body utilizes complex regulatory systems to tightly control the amount of calcium in the blood so that calcium is available for these activities. As a result, when dietary intake of calcium is too low to maintain normal blood levels of calcium, the body will draw on calcium stores in the bones to maintain normal blood concentrations, which, after many years, can lead to osteoporosis (Groff et al. 1995).
The amount of calcium in foods is not adversely impacted by cooking or long-term storage. Adequate intake of vitamin D is necessary for the absorption and utilization of calcium. Phytic acid, found in whole grains, nuts, and legumes, can bind to calcium to form an insoluble complex, thereby decreasing the absorption of calcium.

Oxalic acid, found in spinach, beets, celery, pecans, peanuts, tea and cocoa, can bind to calcium and form an insoluble complex that is excreted in the feces. While research studies confirm the ability of phytic acid and oxalic acid in foods to lower availability of calcium, the decrease in available calcium is relatively small.

Calcium in food and supplements decreases the absorption of heme and nonheme iron.

Magnesium and calcium compete with each other for intestinal absorption. Consequently, calcium supplements should not be taken at the same time as magnesium supplements.

Calcium may play a role in the prevention and/or treatment of cataracts, colon cancer (Wu 2002), high blood pressure (Bostic 2000, Cappuccio 1995), inflammatory bowel disease, kidney stones, osteoporosis, polycystic ovarian syndrome, pregnancy induced hypertension and preeclampsia, premenstrual syndrome.

**Phosphorus**

Phosphorus is a key element in all known forms of life. Inorganic phosphorus in the form of the phosphate \( \text{PO}_4^{3-} \) plays a major role in biological molecules such as DNA and RNA where it forms part of the structural framework of these molecules. Biological membranes are made from a phospholipid matrix and proteins; typically in the form of a bilayer.
An average adult human contains a little less than 1 kg of phosphorus, about 85% of which is present in bones and teeth in the form of apatite, and the remainder inside cells in soft tissues.

Low phosphate syndromes are caused by malnutrition, by failure to absorb phosphate and by metabolic syndromes which draw phosphate from the blood (such as re-feeding after malnutrition) or pass too much of it into the urine.

Symptoms of hypophosphatemia include muscle and neurological dysfunction, and disruption of muscle and blood cells due to lack of ATP. Too much phosphate can lead to diarrhea and calcification (hardening) of organs and soft tissue, and can interfere with the body's ability to use iron, calcium, magnesium, and zinc.

**Magnesium**

Magnesium is usually referred to as a "macromineral," which means that our food must provide us with hundreds of milligrams of magnesium every day. Inside our bodies, magnesium is found mostly in our bones (60-65%), also in our muscles (25%), and in other cell types and body fluids.

Magnesium is sometimes regarded as a "smoothie" mineral, since it has the ability to relax our muscles. Our nerves also depend upon magnesium to avoid becoming overexcited. (Shiis 1994)

Magnesium and calcium, act together to help regulate the body's nerve and muscle tone. In many nerve cells, magnesium serves as a chemical gate blocker - as long as there is enough magnesium around, calcium can't rush into the nerve cell and activate the nerve. This gate blocking by magnesium helps keep the nerve relaxed. If our diet provides us with too little magnesium, this gate blocking can fail and the nerve cell can become over activated. When some nerve cells are over activated, they can send too many messages to the muscles and cause the muscles to over contract.
This chain of events helps explain how magnesium deficiency can trigger muscle tension, muscle soreness, muscle spasms, muscle cramps, and muscle fatigue. In the heart muscle, magnesium deficiency can result in arrhythmia, irregular contraction, and increased heart rate. (Isert and French 1984)

Over 300 different enzymes in the body require magnesium in order to function. For this reason, the functions of this mineral are especially diverse. Magnesium is involved in the metabolism of proteins, carbohydrates, and fats. It helps genes function properly. Some fluids cannot be stored in our muscle cells unless adequate supplies of magnesium are available. The metabolic role of magnesium is so diverse that it is difficult to find a body system that is not affected by magnesium deficiency. Our cardiovascular system, digestive system, nervous system, muscles, kidneys, liver, hormone-secreting glands, and brain all rely on magnesium for their metabolic function. (Wester 1987)

Because of its role in bone structure, the softening and weakening of bone can also be a symptom of magnesium deficiency. Other symptoms can include: imbalanced blood sugar levels; headaches; elevated blood pressure; elevated fats in the bloodstream; depression; seizures; nausea; vomiting; and lack of appetite. (Abbott and Rude 1993)

Magnesium may play a role in the prevention and/or treatment of alcoholism, angina pectoris, asthma, congenital heart disease, congestive heart failure, coronary artery disease, diabetes, epilepsy, glaucoma, heart attack, HIV/AIDS, hypertension.
Potassium

Potassium is found abundantly in many foods, and is especially easy to obtain in fruits and vegetables. Excellent sources of potassium include chard, button mushrooms, and spinach.

Potassium is especially important in regulating the activity of muscles and nerves. The frequency and degree to which our muscles contract, and the degree to which our nerves become excitable, both depend heavily on the presence of potassium in the right amount (Groff et al. 1995).

Potassium is involved in the storage of carbohydrates for use by muscles as fuel. It is also important in maintaining the body's proper electrolyte and acid-base (pH) balance. Potassium may also counteract the increased urinary calcium loss caused by the high-salt diets typical of most Americans, thus helping to prevent bones from thinning out at a fast rate. (Sellmeyer et al 2002)

Dietary deficiency of potassium is uncommon. Diet that is high in sodium and low in potassium can negatively impact potassium status.

The symptoms of potassium deficiency include muscle weakness, confusion, irritability, fatigue, and heart disturbances. Athletes with low potassium stores may tire more easily during exercise, as potassium deficiency causes a decrease in glycogen (the fuel used by exercising muscles) storage.

Potassium losses from cooking of high-potassium foods can be significant.

Potassium may play a role in the prevention and/or treatment of atherosclerosis, cataracts, dehydration, diabetes, hepatitis, high blood pressure, inflammatory bowel disease, osteoporosis, potassium depletion due to excessive fluid loss from diarrhea, vomiting, or sweating (He and Mc Gregor 2001)
**Micro minerals**

The minerals present at levels less than 0.05 % in the human body are defined as micro minerals. The micro minerals are also known as the trace elements. Some micronutrient minerals are iron, iodine, zinc, copper, fluorine, selenium, chromium, manganese, cobalt and molybdenum.

Certain trace elements are required as cofactors for enzymes with antioxidant-related function, for e.g., glutathione peroxidase (Selenium); superoxide dismutase (copper, zinc and manganese), and catalase (iron). Inadequate dietary intakes of these trace elements may compromise the effectiveness of antioxidant defense mechanisms.

**Iron**

The human body contains approximately 4 grams of iron. Excellent food sources of iron include chard, spinach, turmeric and thyme.

Dietary iron comes in two forms: heme iron and non-heme iron. Heme iron is found only in animal flesh, as it is derived from the hemoglobin and myoglobin in animal tissues. Non-heme iron is found in plant foods and dairy products.

Iron serves as the core of the hemoglobin molecule. The ability of red blood cells to carry oxygen is attributed to the presence of iron in the hemoglobin molecule.

Iron is also an important constituent of another protein called myoglobin. Myoglobin, like hemoglobin, is an oxygen-carrying molecule, which distributes oxygen to muscles cells, especially to skeletal muscles and to the heart.

It is also involved in the production of carnitine, a nonessential amino acid important for the proper utilization of fat. The function of the immune system is also dependent on sufficient iron. (Groff et al. 1995)

Iron deficiency causes microcytic and hypochromic anemia, a condition characterized by underdeveloped red blood cells that lack hemoglobin, thereby
reducing the oxygen carrying capacity of red blood cells. But even before iron deficiency anemia develops, people with poor iron status may experience a variety of symptoms including fatigue, weakness, loss of stamina, decreased ability to concentrate, increased susceptibility to infections, hair loss, dizziness, headaches, brittle nails, apathy, and depression. (Hallberg 2001, Lieu et al. 2001)

In children, iron deficiency is associated with learning disabilities and a lower IQ (Rouault 2001). As a result, the milling of grain, which removes the bran and germ, eliminates about 75% of the naturally occurring iron in whole grains. Refined grains are often fortified with iron, but the added iron is less absorbable than the iron that naturally occurs in the grain.

Several nutrients increase iron absorption including ascorbic acid (vitamin C), copper, cobalt, and manganese. Amino acids also improve iron absorption by stimulating the secretion of hydrochloric acid in the stomach. High dietary intake of calcium may decrease absorption of dietary iron.

**Iodine**

Iodine, a trace mineral, is required for by the body for the synthesis of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Under normal circumstances, body contains approximately 20 to 30 mg of iodine, most of which is stored in thyroid gland, located in the front of neck, just under voice box. Smaller amount of iodine are also found in lactating mammary glands, the stomach lining, salivary glands, and in the blood. As a component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3), iodine is essential to human life. Without sufficient iodine, human body is unable to synthesize these hormones, and because the thyroid hormones regulate metabolism in every cell of the body and play a role in virtually all
physiological functions, an iodine deficiency can have a devastating impact on health and well-being.

Iodine may help inactivate bacteria, hence it is used as a skin disinfectant and in water purification. Iodine may also play a role in the prevention of fibrocystic breast disease, a condition characterized by painful swelling in the breasts, by modulating the effect of the hormone estrogen on breast tissue. Finally, researchers hypothesize that iodine deficiency impairs the function of the immune system and that adequate iodine is necessary to prevent miscarriages. Dietary deficiency of this vital mineral results in decreased synthesis of thyroid hormone.

Goiter, or enlargement of the thyroid gland, is usually the earliest symptom of iodine deficiency. Iodine deficiency may eventually lead to hypothyroidism, which causes a variety of symptoms including fatigue, weight gain, weakness and/or depression. Interestingly, iodine deficiency can also cause hyperthyroidism, a condition characterized by weight loss, rapid heart beat, and appetite fluctuations.

Severe iodine deficiency during pregnancy or infancy causes cretinism.

**Zinc**

Zinc is a micromineral needed in the diet on a daily basis, but only in very small amounts (50 milligrams or less). The other microminerals that all humans must get from food are arsenic, boron, cobalt, copper, chromium, fluorine, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.

Studies on zinc reflected some of the key functions served by this mineral, including regulation of genetic activity and balance of carbohydrate metabolism and blood sugar.
Some nutrients interact with zinc. The most important of these nutrients are copper and calcium. High level of zinc intake can decrease absorption of calcium. Zinc can compromise the body's supply of copper.

**Copper**

Copper is essential in all plants and animals. The human body normally contains copper at a level of about 1.4 to 2.1 mg for each kg of body weight. (http://www.copper.org/consumers/health/papers/cu_health_uk/cu_health_uk.html.)

Copper is distributed widely in the body and occurs in liver, muscle and bone. Copper is found in a variety of enzymes. In addition to its enzymatic role, copper is used for biological electron transport.

It is believed that zinc and copper compete for absorption in the digestive tract so that a diet that is excessive in one of these minerals may result in a deficiency in the other. The professional research on the subject recommends 3.0 mg/day, (Anonymous 1980). Because of its role in facilitating iron uptake. Copper deficiency can often produce anemia-like symptoms.

**Fluorine**

It is an essential element and present in bones, teeth, thyroid gland and skin. There is now no doubt that traces of fluorine in the teeth help to protect them against decay (Srilaxmi . 2002).

**Selenium**

The element has been shown to be necessary in small amounts for promoting growth and maintaining good health. Selenium is essential for the activity of glutathione. Selenium has been associated with reduced cancer risk in several epidemiologic studies (Rao et.al 2001, Virtamo et.al 1987). Several experimental
studies suggest that Se may be one of the most powerful cancer chemo-preventive agents in the human diet. (Pank et al. 1996)

**Manganese**

The human body contains a total of 15-20 milligrams of manganese, most of which is located in the bones, with the remainder found in the kidneys, liver, pancreas, pituitary glands, and adrenal glands.

In the human body, manganese functions as an enzyme activator and as a component of metalloenzymes.

Manganese activates the enzymes responsible for the utilization of several key nutrients including biotin, thiamin, ascorbic acid, and choline. It is a catalyst in the synthesis of fatty acids and cholesterol, facilitates protein and carbohydrate metabolism, and may also participate in the production of sex hormones and maintaining reproductive health. (Groff et al 1995, Keen et al 2000)

**Chromium**

Chromium is component of the low molecular weight protein chromodulin, which potentiates the effects of insulin, presumably by facilitating insulin binding to cell receptor sites. The chief symptom of chromium deficiency is impaired glucose tolerance, a result of decreased insulin effectiveness. Those most likely to have marginal or low intake of chromium are individuals on low caloric intakes or consuming large amounts of processed foods (Thomas 2002).

**Bioactive molecules**

Various secondary metabolites synthesized by plants are biologically active for humans; and thus, they impart medicinal value to the plant species. Mode of action of many such secondary metabolites is known (Evans 1997, Kokate et al. 1998).
Animal tests indicate that **iridoids** are involved in anti-inflammatory and analgesic effects (Evans 1997).

**Alkaloids** a heterogeneous group of natural substances. Their distribution in Angiosperm is uneven. They exhibit diversity of structures and also show an extraordinary spectrum of pharmacological activities. The term alkaloids covers protoalkaloids and also pseudoalkaloids. True alkaloids are toxic in nature. They possess specific physiological action on human body when used in small quantities. They acts as central nervous system depressant.

**Anthraquinones** are laxative in action. They possess anti-retoviral activity. In monocotyledons they are found only in Liliaceae. Among dicotyledons they occur in Rubiaceae, Leguminaceae, Polygonaceae, Rhamnaceae, Ericaceae, Euphorbiaceae, Lythraceae, Saxifragaceae, Scrophulariaceae and Verbenaceae. They are absent from lower plants.

A considerable number of plants scattered throughout the plant kingdom contain $C_{23}$ or $C_{24}$ **steroidal glycosides** which exert on the falling heart a slowing and strengthening effect. They are known as cardiac glycosides. They are used as cardiotonics also having expectorant property. The heart-arresting properties of these glycosides also render them most effective as arrow poisons and a number of tropical plants are better known in this respect than for their medicinal use. These are also called cardenolides. Bufadienolides are condensation products of a $C_{21}$ steroids and a $C_3$ unit.

Glycosides of cardenoloide group are most important medicinally. Synthesis of these compounds has presented many difficulties. All the medicinal preparations are derived from natural resources.
Flavonoids occur both in free state and as glycosides. They are the largest group of naturally occurring phenols. About 2000 flavonoids are known with about 500 occurring in free state. The group is known for its anti-inflammatory and antiallrgic effects, for antithrombitic, antioxidant and vasoprotective properties, for inhibition of tumour promotion and as a protective for gastric mucosa. These effects have been attributed to the influence of flavonoids on arachidonic acid metabolism. Many flavonoids containing plants are diuretic or antispasmodic. Some flavanoids have antitumour, antibacterial or antifungal properties.

Simple phenolics like resorsinol are narcotic in action. Catechol that occurs in cola seeds is stimulant. Fluoroglucinol derivatives of hydroquinone have taenicidal properties; p-hydroxybenzene also shows some activity (Evans 1997).


Setroids exhibit strong anti-inflammatory activity. Steroidal saponins act as harmones.

Volatile oils are secreted in oil cells, in secretory ducts or cavities or in glandular hairs. They are generally mixtures of hydrocarbon and oxygenated compounds derived from these hydrocarbons. Many oils are terpenoid in origin. Trpenoids are antiseptic, stimulant, carminative, diuretic, anthelmintic, analgesic and antirheumatic. They are also used as counter irritants.

The secretion of glandular trichomes of certain genera constitute important materials for perfumery, food and pharmaceutical industries. Some secretions contain narcotic resins and other give rise to skin allergies.
Volatile oils occur as droplets in the cell. They are used for their therapeutic action, for flavouring, in perfumery, for therapeutic purposes they are administered as inhalations, orally, as gargles and mouthwashes and transdermally. Many essential oils are employed in the practice of aromapathy.

**Tannins** are able to combine with animal protein, prevent their putrefaction. They precipitate animal proteins. Simple phenolics like gallic acid, catechins and chlorogenic acid are often present with tannins. They are also known as pseudotannins.

Two main groups of tannins are recognized – hydrolysable tannins and condensed tannins.

i) Hydrolysable tannins – Their solution turns blue with iron salts.

ii) Condensed tannins – (Proanthocyanidins/leucoanthocyanidins) like catechol their solution turn green with iron salt (FeCl₃)

iii) Pseudotannins – compounds of lower molecular weight than true tannins.

Tannin containing drug precipitate protein and have been used traditionally as styptics and internally for protection of inflamed surfaces of mouth and throat. They act as antidiarrhoeals and have been employed as antidotes in poisoning by heavy metals, alkaloids and glycosides. Recent studies have concentrated on the antitumour activity of tannins. Anti-HIV activity has also been demonstrated. They may also behave as antioxidants or as metal chelaters, both properties potentially important in biological systems (Scalbert 1991).

**Saponins** are haemotoxic. They exbits haemolytic property causing haemolysis of erythrocytes.
**Fatty acids** promote smooth contraction of muscle (Evans 1997).

**Emodins** are vasorelaxant. **Polyuronoides** are demulcent and emollient.

**Polyoses** provide tightening effects to the skin; effective as anti-wrinkle agent (Scalbert 1991).
PRESENT ATTEMPT

Ayurveda is not the science dealing only with disease, but is a holistic science of health care. Ayurveda was aware of nutraceuticals. Rice water of any traditional variety is an effective demulcent refrigerant drink in febrile and inflammatory states of intestine and other affections. A local variety of rice from Kerala called ‘Chongelpttu sirumani’ is used immediately after delivery to increase milk (Anil Kumar 2003).

During exploratory phase and literature survey it was found that most of the wild edibles not only are medicinal herbs also but food preparations of some of them are prescribed as remedy for certain diseases and disorders.

As a result of the developments in agricultural techniques and marketing facilities, use of wild edible plants has remarkably decreased. However, many of the wild plants are nutritionally important because of their high vitamin, mineral and fibre contents and they can be used as food and alternatively for the poverty problem. Therefore, it is important to determine nutrition content of wild edible plants and to advocate their use as food.

It was therefore felt necessary to evaluate the nutritional and even medicinal properties of wild edibles.

Out of 125 wild edible plant species recorded during survey 16 were selected for nutritional and nutraceutical evaluation to know their food value as well as medicinal value.
Following 16 species were selected for further study. Nutritional as well as medicinal potential of these species was assessed.

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Name of plant</th>
<th>Family</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bombax ceiba</em> L.</td>
<td>Bombacaceae</td>
<td>Flowers</td>
</tr>
<tr>
<td>2</td>
<td><em>Corchorous trilocularis</em> L.</td>
<td>Tiliaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>3</td>
<td><em>Schleicerha oleosa</em> (Lour)Oken.</td>
<td>Sapotaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>4</td>
<td><em>Bauhinia vahlii</em> Wight and Arn.</td>
<td>Caesalpiniaeae</td>
<td>Seeds</td>
</tr>
<tr>
<td>5</td>
<td><em>Cassia fistula</em> L.</td>
<td>Caesalpiniaeae</td>
<td>Flowers</td>
</tr>
<tr>
<td>6</td>
<td><em>Caesulia axillaris</em> Roxb.</td>
<td>Asteraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>7</td>
<td><em>Goniocaulon indicum</em> (Klein ex Willd.)</td>
<td>Asteraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>8</td>
<td><em>Telosma pallida</em> (Roxb.)Craib</td>
<td>Asclepiadaceae</td>
<td>Flowers</td>
</tr>
<tr>
<td>9</td>
<td><em>Merremia gangetica</em> (L.)Cuford</td>
<td>Convolvulaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>10</td>
<td><em>Rivea hypocratiformis</em> (Desr.)Choisy</td>
<td>Convolvulaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>11</td>
<td><em>Oroxyllum indicum</em> (L.)Vent.</td>
<td>Bignoniaceae</td>
<td>Flower and Fruits</td>
</tr>
<tr>
<td>12</td>
<td><em>Celosia argentea</em> L.</td>
<td>Amaranthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>13</td>
<td><em>Digera muricata</em> (L.)Mart.</td>
<td>Amaranthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>14</td>
<td><em>Chlorophytum tuberosum</em> (Roxb.)Baker.</td>
<td>Liliaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>15</td>
<td><em>Scilla hyacinthiana</em> (Roth.)Mebride.</td>
<td>Liliaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>16</td>
<td><em>Commelina benghalensis</em> L.</td>
<td>Commelinaceae</td>
<td>Leaves</td>
</tr>
</tbody>
</table>
Taxonomic descriptions, recipes of food preparation and review of medicinal properties of these selected species is presented in Part-I (Exploratory phase).

To avoid repetition in experimental phase only results of phytochemical analysis are presented.

Biochemical studies were carried out for edible plant parts of selected species. Plants were screened for nutrients as well as for medicinally important bioactive molecules.

To evaluate the nutritional potential of selected ethnovegetables various macro and micro nutrient contents were estimated following standard prescribed methods. (Thimmaiyah 1999, Johanson 1940, Kokate et. al. 1998, Kulkarni and Apte 2000, Malik and Singh 1980, Sadasivam and Manickam 2005). Tribal and rural communities do not have assured supply of vegetables throughout the year. Routinely many of the vegetables are shade dried and stored for scarcity conditions. Therefore, nutrient content was estimated both for fresh as well as dry tissue.

**Moisture content:-**

10 gm of fresh plant material was shade dried. Plant sample was reweighed after complete drying and moisture content of sample calculated as follows-

\[
\text{% moisture content} = \frac{\text{Loss in Weight}}{\text{Fresh weight of sample taken}} \times 100
\]
Total carbohydrates:-

They include both digestible and non-digestible carbohydrates. Carbohydrates were measured in terms of monosaccharides, produced by hydrolysis.

100 mg of dry plant powder was mixed with 5 ml of 2.5 N HCl and boiled for 3 hours on water bath. Cooled and neutralized by adding sodium carbonate pinch by pinch till no effervescence evolved. The solution was centrifuged. To the supernatant, distilled water was added to make the volume 100 ml. 0.5 ml and 1 ml of solution was pipetted out. To the aliquot 0.5 ml distilled water was added. In each aliquot, 4 ml of ice-cold anthrone reagent was added and the solution was kept in boiling water bath for 8 minutes. Tubes were cooled rapidly and absorbance measured at 630 nm.

Standard graph was prepared with glucose.

Starch:-

500 mg of dry plant tissue was homogenized in hot 80% ethanol, centrifuged and residue collected. The residue was repeatedly washed with hot 80% ethanol to ensure complete removal of sugar and dried over water bath. Dried residue was extracted with 5 ml of water and 6.5 ml of 52% perchloric acid at 0°C for 20 min. Supernatant collected after centrifugation. Extraction was repeated with perchloric acid and supernatants pulled together to make final volume upto 100 ml. 0.2 ml of the supernatant was pipetted out and volume made up to 1 ml with distilled water. To this, 4 ml of anthrone reagent was added (200 mg of anthrone dissolved in 100 ml of ice cold 95% sulphuric acid). The solution was kept on boiling water bath for 8 min. and cooled rapidly under running water. Absorbance measured at 680 nm.

Standard graph was prepared with glucose. The value obtained was multiplied by a factor of 0.9 to estimate starch content.
Reducing Sugars:-

For reducing sugar estimation, Nelson – Somogys method (1952) was followed. 100 mg of dried plant powder was extracted with 10 ml of 80 % ethanol. Supernatant was collected and evaporated to dryness on water bath. Residue dissolved in 10 ml water. Aliquots of 0.1 and 0.2 ml were pipetted out. Volume of each aliquot was made to 2 ml with distilled water. 1 ml of alkaline copper tartarate reagent was added to each tube. Tubes were kept on boiling water bath for 10 min. Tubes were cooled and 1 ml of arsenomolybdic reagent was added to each tube. Volume was made up to 10 ml with water. Absorbance measured at 620 nm after incubating for 10 min at room temperature.

Standard graph was prepared using glucose. Reducing sugar content was calculated from standard graph.

Non-reducing Sugars:-

Non-reducing sugar content was calculated from subtracting the reducing sugar from total carbohydrate content.

Total Nitrogen:-

300 mg dry tissue was taken and digested with a pinch of catalyst and 7.5 ml of conc. H$_2$SO$_4$ for 6-8 hours; till solution became slightly greenish or colourless. Volume was made 50 ml with double distilled water. 10 ml of this solution was transferred to Markham distillation apparatus through the funnel. 10 ml of 40 % NaOH was added to funnel of Markham distillation unit. 10 ml of 2 % Boric acid with mixed indicator was taken in conical flask.(mixed indicator was prepared by dissolving 300
mg of Bromocresol green and 200 mg of Methyl red in 90 % ethanol). Distillation was carried out and ammonia liberated was passed through boric acid solution. Process was continued till volume in conical flask of boric acid became 25 ml. It was then titrated with 0.035 N HCl.

Amount of total nitrogen present in plant tissue was calculated as follows-

\[
\text{(ml HCl in sample - ml HCl in blank)} \times \text{normality of acid} \times 14.01 \times \text{final vol.} \times 100
\]

\[
\frac{\% \text{N}}{\text{Weight of sample} \times \text{vol. of aliquote}} \times 100
\]

**Crude fat:-**

2 gms of dried material was taken in a thimble made of Whatman filter paper and kept in Soxhlet extractor. Preweighed (wt. ‘a’ gm) Soxhlet flask was connected to extractor. Extraction was carried out with petroleum ether (b.p. 40-60°C) for 16 hours. Flask was kept on hot water bath to evaporate the petroleum ether; cooled, dried in desiccator and weighed (wt. ‘b’ gm).

Crude fat content in tissue was calculated as-

\[
\frac{(b-a) \times 100}{\text{Weight of sample}} \times 100
\]

**Food energy:-** Food energy was calculated from the content of the proximate principles assuming that proteins, carbohydrates and fats yield 4, 4 and 9 Kcals respectively per gm.

**Total lipids:-**

For estimation of total lipids Bligh and Dyer (1959) method was followed. 10 gms of fresh tissue was blended in a mixture of chloroform: Methanol (10:2 v/v).
Further homogenized by adding 10 ml chloroform. Again homogenized with 10 ml water. Filtered through Buchner funnel (using vaccum) with the help of Whatman No. 1 filter paper. Filtrate was transferred to a graduated measuring cylinder. Filter paper and blender were washed with 10 ml chloroform, refiltered and transferred to measuring cylinder. After two phases separated; volume of lower chloroform layer was noted (‘x’ ml). Upper methanol layer pipetted out and again volume of chloroform layer recorded (‘y’ ml) and transferred to a weighed conical flask (weight ‘a’ gm). Conical flask was kept in water bath at 40-50°C and filtrate evaporated to dryness. Residue was cooled and dried over phosphoric anhydride in desiccator. The flask was weighed second time (‘b’gms). The residue was cooled and dissolved in 15 ml chloroform. Chloroform again evaporated and residue cooled, dried and weight of flask was taken third time (weight ‘c’ gms).

Total lipid content was calculated as follows.

**Calculation:**

Weight of lipids (gm) : - (b-a) - (c-a) = ‘d’ gm

\[
\text{Total lipids (gm)} = \frac{\text{weight of lipid (d) \times}}{\text{Vol. of chloroform layer (y ml)}}
\]

\[
\% \text{ Total lipids} = \frac{\text{Total lipids (gm)}}{\text{Weight of sample (gm)}}
\]

**Ascorbic acid (Vitamin - C):**

1 gm fresh tissue was homogenized with 25 ml of 4% oxalic acid; centrifuged and filtered to collect the supernatant. 10 ml of extract was transferred to conical flask and bromine was added dropwise with constant stirring till extract turned orange
yellow. Excess bromine was blown off by bubbling the mixture. Volume was made 50 ml with 4% oxalic acid. 0.1-2 ml of brominated extract was pipetted out and volume made 3 ml by adding of distilled water. To this was added 1 ml of DNPH reagent, followed by 1-2 drops of 10% thiourea; (DNPH reagent was prepared by dissolving 2 gm of dinitrophenyl hydrazine in 100 ml of 0.5 N H$_2$SO$_4$) and incubated for 3 hours at 37$^0$C. Crystals of osazone formed during incubation were dissolved by adding 7 ml of 80% H$_2$SO$_4$. Absorbance measured at 540 nm.

**Phenols:-**

100 mg of plant material was extracted in 10 ml HCl in methanol. Supernatant collected and evaporated to dryness on water bath. Residue was dissolved in 5 ml distilled water. Volume made to 7 ml and 0.5 ml Folin-Phenol reagent added with vigorous shaking. Allowed to stand for 3 min. 1 ml of 35% sodium carbonate was added, mixture shaken then allowed to stand for one hour. Absorbance recorded at 630 nm.

Standard graph was prepared with caffic acid.

**Crude fiber:-**

2 gm of dry plant powder was ground with petroleum ether at 38-52$^0$C to remove fat. Residue was dried and boiled with 200 ml of 0.005 N H$_2$SO$_4$ for 30 min. Boiled sample was filtered through muslin cloth and washed with 25 ml of 0.005 N H$_2$SO$_4$, thrice with 50 ml of water and lastly with 25 ml of alcohol. Washed residue was transferred to pre-weighed crucible (W2). Residue was ignited for 30 min at 600$^0$C; cooled in desiccator and reweighed (W3). Percentage of crude fiber in plant tissue was calculated by the following formula.
Anthocyanin:-

One gm of fresh tissue was homogenized in 10 ml alcohol, centrifuged and supernatant collected. 1 ml of extract was pipetted out and to it 3 ml of HCl in aqueous methanol (0.5 N HCl in 80% methanol) was added. To this solution 1 ml of anthocyanin reagent (1 ml of 30 % of H$_2$O$_2$ + 9 ml of methanolic HCl in 5:1 proportion) was added. Incubated for 15 minutes in dark and absorbance read at 525 nm. Anthocyanin content was expressed as A$_{523}$ values.

10µg of Cyanin hydrochloride/ml in methanol-HCl = absorbance of 0.405 in a 1.0 cm cell at A$_{523}$.

Lycopene:-

2.5 gm fresh plant tissue was made to smooth pulp. The pulp was repeatedly extracted with acetone. Acetone extract was transferred to a separating funnel and 20 ml of petroleum ether 40-60$^0$ C (AR) was added with gentle mixing. 20 ml of 5% sodium sulphate solution was added to this mixture in separating funnel with gentle shaking. 20 ml more petroleum ether was added to the separating funnel to produce two clear layers. Petroleum layer was collected. Aqueous layer was re-extracted with 20 ml of petroleum ether. Petroleum ether extract was pulled together and washed with water. Petroleum ether extract was poured over 10 gms of anhydrous sodium sulphate, kept in brown coloured bottle and allowed to stand for 30 minutes. Petroleum ether layer was decanted in 100 ml volumetric flask through cotton wool.
Sodium sulphate slurry washed with petroleum ether till it became colourless, every
time washing collected. Volume made up to 100 ml.

Absorbance was read at 503 nm. Lycopene content was calculated as follows-

\[
\text{mg Lycopene/100 gm sample} = \frac{3.1206 \mu g \times \text{Absorbance}}{\text{Weight of sample (gm)}}
\]

**Carotenoides:-**

2 gms of fresh plant material was ground with 20 ml of distilled methanol. Methanol filtered through Whatman paper on Buchner funnel. Extraction was repeated till the tissue became colourless. Extract was partitioned with ether using separating funnel (if necessary, little distilled water was added to produce separate layers). Ether layer was collected and evaporated to dryness on water bath at 35\(^0\)C. Residue was dissolved in 10 ml ethanol. To this ethanol solution 10 ml of 60% KOH was added to remove lipids and chlorophyll. Mixture was kept in dark at room temperature overnight. Equal volume of water was added to partition the ether layer. Ether layer was collected, evaporated to dryness and residue dissolved in 10 ml ethanol. Absorbance measured at 420 nm.

Standard graph was prepared using \(\beta\)-carotene of high purity (Otto Biochemae). Carotenoid content (\(\mu g/gm\)) in sample was calculated using calibration curve.

**Vitamin A:-** The value of vitamin A was calculated by assuming 0.6 \(\mu g\) of carotene equivalent to 1IU of vitamin A.
**Retinol:**- The value of retinol µg per 100 gm was estimated by taking into consideration that one international unit of vitamin A is equivalent to 0.3 µg of retinol. (Gopalan C.et al 2004)

**Chlorophyll:**-

One gm of fresh material was homogenized with 20 ml of 80% chilled acetone, centrifuged and the supernatant collected in 100 ml volumetric flask. Tissue was repeatedly extracted with pre-chilled 80% acetone till it became colourless. All supernatants pulled together and volume made up to 100 ml. Absorbance was measured at 645,652 and 663 nm.

Chlorophyll content was calculated by following formulas-

\[
V \\
1) \text{mg chlorophyll a/gm tissue} = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \times \frac{V}{1000 \times W} \\
\]

\[
V \\
2) \text{mg chlorophyll b/gm tissue} = 22.9 \times (A_{645}) - 4.68 \times (A_{663}) \times \frac{V}{1000 \times W} \\
\]

\[
V \\
3) \text{mg total chlorophyll/gm tissue} = 20.2 \times (A_{645}) + 8.02 \times (A_{663}) \times \frac{V}{1000 \times W} \\
\]

Where,  
A – Absorbance at specific wavelength  
V – Final volume of chlorophyll extract in 80 % acetone  
W – Fresh weight of tissue extracted.
MINERALS

Ash studies are important both for nutritional and pharmacognostic studies. From nutritional point of view ash yield gives the amount of minerals present in plant tissue.

Ash Yield:-

10 gm of dry plant powder was taken in dry, preweighed crucible and ignited at 750°C for about 6 Hrs in muffle furnace. Cooled at room temperature. Kept in descicator overnight and weighed accurately. Difference in weight was ash yield of the plant material.

Water insoluble Ash:-

About 250 mg of plant ash was dissolved in 10 ml distilled water and filtered through preweighed filter paper. Water insoluble fraction collected on filter paper, thoroughly dried and filter paper reweighed.

\[
\text{Weight of water insoluble ash} = \frac{\text{Weight of water insoluble ash}}{\text{Weight of ash sample}} \times 100
\]

From this water insoluble fraction was calculated.

Acid Insoluble Ash:-

About 250 mg of plant ash was taken in preweighed crucible, dissolved in hot 5 ml 1N HCl and allowed to stand for 5-10 minutes. Excess HCl removed with the help of dropper. Ash was washed repeatedly with distilled water to remove chlorides.
and crucible was kept in oven at 50°C to dry. Crucible was cooled, transferred to descicator and weighed after 24 hrs

\[
\text{Percentage of acid insoluble ash} = \frac{\text{Weight of insoluble ash}}{\text{Weight of sample taken}} \times 100
\]

From this acid soluble fraction was calculated.

**Sulphated Ash:-**

About 250 mg of plant ash taken in preweighed crucible. Ash was moistened with conc. H₂SO₄ and sample ignited till SO₂ fumes ceased. Crucible was kept in furnace at 750°C for one hour. Crucible was cooled, transferred to descicator and weighed accurately.

\[
\text{Percentage of sulphated ash} = \frac{\text{Difference in Weight}}{\text{Weight of ash taken}} \times 100
\]

**Qualitative Analysis:-**

Ash was extracted with HCl and HNO₃ for detection of various elements.

**(A) HCl extract:-**

About 0.5-1 gm of ash was extracted with 10 ml of warm 20% (v/v) hydrochloric acid. Volume was made up to 50 ml and following tests were carried out.

**Sulphur:-**

To 10 ml filtrate few drops of 5% Barium chloride solution were added. Formation of very fine white crystalline precipitate of barium sulphate proves the presence of sulphur.
Calcium:-

20 ml of the filtrate was taken and made slightly alkaline with few drops of dilute ammonium hydroxide (water : ammonium hydroxide, 1:1) and filtered. Few drops of saturated ammonium oxalate solution were added. A white precipitate of calcium oxalate proves the presence of calcium. Solution was saved.

Magnesium:-

Excess of ammonium oxalate solution was added to precipitate all calcium of the above test solution and filtered. Filtrate was then evaporated to a volume of about 5 ml. To the hot filtrate 1 ml of saturated disodium-hydrogen phosphate was added, cooled and allowed to stand. Crystals of ammonium-magnesium-phosphate\((\text{NH}_4)_3\text{Mg}.\text{PO}_4\) formed prove the presence of magnesium. Crystalization can be hastened by rubbing the inside of test tube with a glass rod.

Iron:-

a) To about 10 ml ash solution, few drops of 2% potassium ferrocyanide was added. Development of a dark Prussian blue colour proves the presence of Iron.

b) To about 10 ml of ash filtrate, few drops of 10% solution of potassium thiocyanate or ammonium thiocyanate were added. A blood red colouration due to the formation of iron thiocyanate-\(\text{Fe(CNS)}_6\) proved the presence of iron.
B) HNO₃ extract:-

A second sample of ash was extracted with 10% (v/v) of nitric acid in distilled water, filtered and volume made up to 50 ml. Filtrate was used for following tests.

**Chlorine:-**

To a small portion of filtrate, few drops of silver nitrate solution were added. Heavy white precipitate of silver chloride indicated the presence of chlorine.

**Phosphorus:-**

To 10 ml of filtrate, ammonium molybdate solution was added. Heated for few minutes on steam bath and cooled. A profuse yellow crystalline precipitate of ammonium-phosphomolybdate- \((\text{NH}_4)_3\text{PO}_4\text{(MoO}_3\text{)}_{12}\) proved the presence of phosphorus.

**Sodium:-**

To another portion of the filtrate, solution of uranyl acetate and zinc acetate in acetic acid was added. Pale yellow precipitate confirmed the presences of sodium.

**Aluminium:-**

To about 2ml filtrate, 1 gm solid \(\text{NH}_4\text{Cl}\) was mixed, excess \(\text{NH}_4\text{OH}\) added and thoroughly shaken. Gelatinous white precipitate of \(\text{Al(OH)}_3\) produced indicated the presence of aluminium.

Above solution filtered and filtrate collected for manganese test.
Manganese:-

Filtrate of aluminium test was boiled with 1 ml dilute HNO₃, and 1 gm lead peroxide (PbO₂) added. Contents were boiled again, diluted with 5 ml of water and allowed to stand for few minutes. A pink or violet supernatant (of HMnO₄, acidic manganate) indicated the presence of manganese.

Copper:-

1 ml of filtrate was mixed with few drops of acetic acid. To the solution 5-6 drops of potassium ferrocyanide were added. A chocolate brown precipitate of cupric ferrocyanide indicated the presence of copper.

Nickel:-

1 ml of filtrate was neutralised with few drops of NH₄OH and 6-8 drops of Di-Methyl-Glyoxime (DMG) reagent were added. A scarlet red precipitate of Ni-Dimethylglyoxime complex indicated the presence of nickel.

Quantitative Analysis:-

Iron:-

250 mg plant ash was digested with 10 ml conc. HCl and 10 ml distilled water on heating mantle, till ash completely dissolved. Final volume made up to 100 ml. Different concentrations of digested solution (0.1 - 1 ml) were taken in different test
tubes and volume of each made 8 ml with distilled water. To this 1 ml of conc. HCl and 1 ml of 10% potassium thiocyanate were added.

Absorbance measured at 480 nm.

Standard stock solution was made by dissolving 437.48 mg of ferric ammonium sulphate in 100 ml distilled water. Standard graph prepared for different concentrations.

Amount of iron present in ash calculated from standard graph. Values presented in terms of iron presents per gm dry plant material.

**Phosphorus:**

To one gm dry plant material 10 ml of di-acid digestion mixture (HNO₃ : HClO₄; 9:4) was added with gentle shaking. Mixture was subjected to low heat for 5-10 minutes and then to 100°C till NO₂ was completely fumed off. Digested solution was further concentrated by heating till the volume reduced to 3-5 ml. Colourless solution thus obtained was cooled and volume made up to 25 ml with distilled water, filtered through Whatman no.1 filter paper.

10 ml of the digested solution was mixed with 10 ml ammonium molybdatevanadate solution (25 gm ammonium molybdate in 400 ml distilled water + 1.25 gm ammonium- metavanadate in 300 ml boiling distilled water-cooled + 250 ml of conc. HNO₃ Volume made to 1 liter),20 ml distilled water and volume made up 50 ml. Allowed to stand for 30 minutes.

Absorbance measured at 470 nm.
Standard graph was prepared from standard potassium di-hydrogen phosphate solution (0.2195 gm dissolved in 1 liter distilled water).

Amount of phosphorus present in ash was calculated from standard graph.

**Sodium, Pottasium and Calcium:-**

Sodium, Potassium and Calcium were estimated by Flame Photometer.

To the 100 mg of plant ash 20 ml of 20% HNO₃ was added. Mixture was digested at 60°C overnight. 20 ml of 20% HCl was added to the digestion mixture and allowed to stand for 24 hrs at room temperature. Volume was made up to 50-100 ml by distilled water. The solution used for estimation of sodium, potassium and calcium.

Standard solution of 1000 ppm concentration were prepared as follows-

1. Sodium - 2.5464 gm NaCl dissolved in 1 liter distilled water.
2. Pottasium - 1.7090 gm KCl dissolved in 1 liter distilled water.
3. Calcium - 2.497 gm CaCO₃ in 300 ml distilled water + 10 ml con. HCl; volume made to 1 liter by distilled water.

Air pressure maintained at 5 lb. and gas feeder adjusted to give sharp blue flame. For each solution respective filter was used. Emission scale adjusted to 100 with maximum concentration of standard solution and zero on scale adjusted with distilled water. Different concentrations of working solutions were fed and reading on emission scale noted.

Standard graph prepared by feeding different concentrations of standard solutions. Amount of sodium, potassium and calcium present in ash was calculated from standard graph. Values were presented by conversion in terms of per gm dry weight of plant material.
Many of the ethnovegetables, including the one studied here, also possess medicinal value. The nutrients with medicinal value are termed as nutraceuticals. Here therefore, in addition to nutritive value phytochemical screening for bioactive compounds also has been carried out.

Various bioactive compounds were tested in the fresh as well as dry plant powder of the parts used. Plants were thoroughly washed with water and were shade dried. Dried whole plant/specific parts were powdered and stored in polythene zip lock bags at 30-32°C for further analysis. Detection of bioactive compounds was done by standard prescribed methods. (Anonymous 1966, Evans 1997, Gibbs 1974, Gupta and Varshney 1997, Harborne 1973, Kokate et. al. 1998, Kulkarni and Apte 2000, Peach and Tracey 1979, Trivedi and Goel 1984, Chabra et al. 1984) Responses to various tests were denoted by +, ++ and +++; indicating weak, moderate and strong reactions respectively.

**Iridoids:**

Fresh as well as dry plant powder can be used for this test. To the tissue 5 ml of 1% aqueous hydrochloric acid was added. Extraction was carried for 6 hrs. To 0.1 ml extract 1 ml of Trim-Hill reagent (10 ml acetic acid + 1ml 2% aqueous copper sulphate + 0.5 ml concentrated hydrochloric acid) was added. The tube was heated for short time in a flame and colour change noted. Production of blue, red and violet colour indicated presence of following iridoids.

- Blue colour – Asperulin, aucubin and monotropein.
- Red or violet colour – Harpagide.
Alkaloids:-

Dried plant powder was extracted with 10% acetic acid in aqueous ethanol for about 24 hrs. Extract was further concentrated by boiling in water bath to \(\frac{1}{4}\) of its original volume and cooled. Pigments were removed from extract with chloroform wherever necessary. Pigment free extract was then tested for alkaloids with Mayer’s reagent, Dragendorff’s reagent and Wagner’s reagent.

Mayer’s Reagent:- 13.5 gm of mercuric chloride dissolved in 750 ml water and 200 ml solution containing 50 gm of potassium iodide was added slowly. Volume made to one liter.

Dragendorff’s Regent:- Two stock solution were prepared.

a) 0.6 gm bismuth sub nitrate in 2 ml concentrated hydrochloric acid and 10 ml water.

b) 6 gm potassium iodide dissolved in 10 ml water.

These Stock solutions were mixed together with 7 ml concentrated hydrochloric acid and 15 ml water and whole diluted to 400 ml with water.

Wagner’s Reagent :- 1.2 gm of iodine and 2 gm of potassium iodide dissolved in 5 ml water. Volume made to 100 ml.

Anthraquinones:-

a) Dry plant powder was extracted with 0.5 N KOH and filtered. Extract diluted with distilled water; acidified with acetic acid; re-extracted with benzene and filtered. To benzene extract aqueous ammonia added and shaken gently. Red colour to ammonia layer indicated the presence of anthraquinones.

b) The plant powder was extracted with 80% ethanol. Allowed to stand over night with occasional stirring. Filtered, filtrate collected and evaporated to dryness on water bath. Residue dissolved in water with vigourous shaking; filtered if necessary. 5 ml of benzene added to the solution and benzene layer collected. Aqueous ammonia
added to benzene layer. Development of red colour indicated the presence of anthroquinone.

c) Dry plant powder was boiled with dil.H2SO4 for about 1 hr. Cooled and filtered. Chloroform or benzene was added to filtrate; mixture vigorously shaken and allowed to stand. The chloroform / benzene layer separated, and equal amount of liquid ammonia added. Ammonial layer turning red or pink or violet indicated the presence of anthroquinone. This test is to detect anthroquinone glycosides.

**Simple Phenolics:-**

Plant powder was extracted with aqueous ethanol overnight. To the extract 1-2 drops of 1% aqueous ferric chloride was added. Development of specific color is indicative of the presence of specific phenol as follows-

<table>
<thead>
<tr>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Violet/Purple</td>
<td>Salicylic Acid, O-Cresol (methyl or ethyl esters)</td>
</tr>
<tr>
<td>-Bluish Violet</td>
<td>M-Cresol, alfa naphthol, resorcinol, Phlorglucinol.</td>
</tr>
<tr>
<td>-Blue</td>
<td>p-Cresol</td>
</tr>
<tr>
<td>-Blue Black</td>
<td>Tannic acid, gallic acid.</td>
</tr>
<tr>
<td>-Green</td>
<td>β naphthol.</td>
</tr>
<tr>
<td>-Green(Darkens rapidly changes to red with a drop of NaOH).</td>
<td>Hydroquinone</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>-Reddish brown</td>
<td>Pyrogallol</td>
</tr>
<tr>
<td>-Blue,changes to redbrown</td>
<td>P-nitrophenol</td>
</tr>
<tr>
<td>-Red violet</td>
<td>Hydroxyaldehydes or P-nitrophenol</td>
</tr>
<tr>
<td>-Wine red or red brown</td>
<td>B- ketonic ester or B-diketones.</td>
</tr>
</tbody>
</table>
**Steroids:-**

A) Test for steroid nucleus:-

Tissue was extracted with ethanol. Ethanol extract was dried and defatted with petroleum ether. Defatted extract mixed with chloroform and filtered. To the filtrate 100 mg of anhydrous sodium sulphate was added and shaken gently. Filtered. To the filtrate Libbermann-Burchard reagent was added. Blue or Blue green colour confirmed steroids and red, pink or purple colour, confirms triterpenoid.

**Libbermann-Burchard reagent** :- 10 ml concentrated sulphuric acid + 20 ml acetic anhydride + 50 ml chloroform.

B) Salkowaski test for unsaturated steroids:-

Defatted extract was taken in a test tube. By the walls of the tube concentrated sulphuric acid was slowly added. Ring reaction observed. The sulphuric acid was then mixed slowly and colour change observed immediately and gradually over an hour. Development of cherry red colour confirmed the presence of unsaturated steroids.

**Tannins:-**

Plant material was extracted with rectified spirit. Filtered; evaporated to dryness, dissolved in distilled water and 10% NaOH added, filtered. Extract divided in to two parts.

I- Gelatin-salt reagent added to first part of filtrate. Formation of white precipitate indicated the presence of tannin.

Gelatin salt Regent:- 1% Gelatian + 10% NaCl in 1:1 proportion.
II- Second part of extract was treated with aqueous ammonia and exposed to air. Gradual development of green colour was indicative of the Chlorogenic acid.

**Saponins:-**

One gm dry plant powder was boiled with water for 10 min. Cooled and shaken vigorously. If stable froth up to 2 cm or more developed, it was recorded as positive test.

**Fatty and Organic acids:-**

If in above test stable froth does not produced then aqueous sodium carbonate added and shaken vigorously. If it produces dense stable froth it confirmed the presence of acids like stearic acid, diterpene acids and triterpene carboxylic acid.

**Juglone Test:-**

5 ml of chloroform was added to 1 gm dried plant powder and allowed to stand overnight with occasional stirring. Filtered and evaporated to dryness in water bath. Few ml ether and equal volume of dilute ammonia added and gently shaken. Formation of purple colour indicates the presence of Juglone.

Development of orange colour indicated the absence of Juglone but presence of Lawsone and other naphthaquinones.

Development of yellow colour may be due to flavonoids.

**Emodins:-**

Tissue was extracted with petroleum ether. Residue was saved. Extract evaporated to dryness. Residue divided into two parts, one part dissolved in benzene
and 25% ammonium hydroxide added to it. Appearance of red colour indicated the presence of emodins.

Part of residue collected from above test was extracted with methanol. Extract was collected and kept aside for the test of anthracene glycosides. Residual tissue was extracted with hot water and extract used for testing polyoses, polyuronoids and anthracene glycosides.

**Polyoses:-**

One part of hot water extract was evaporated to dryness and few drops of conc. H₂SO₄ and solution of alcoholic thymol added. Appearance of red colour indicated the presence of polyoses.

**Polyuronoids:-**

To the second part of hot water extract, equal volume of haemotoxylene solution was added. Formation of Violet-purple precipitate which is insoluble in ethanol indicated the presence of Polyuronoids.

**Anthracene Glycosides:-**

Methanol extract saved from above step and third parts of hot water extract were mixed. Few drops of 10% HCl added to mixture and hydrolysed by heating; cooled. Petroleum ether added. To the petroleum ether layer 25% ammonium hydroxide added. Appearance of red colour indicated presence of anthracene glycosides.
Cardenolides:-

a) Cardiac glycosides:- Dry plant material was extracted with rectified sprit overnight. Extract filtered and 10% NaOH and 0.3% nitroprusside solution added to the filtrate. Appearance of transient pinkish red colour indicated the presence of cardiac glycosides.

b) 2-deoxy sugar:- Plant powder was extracted with 80% ethanol. Filtered and evaporated to dryness. Few ml of petroleum ether added to the residue and shaken well; petroleum ether layer discarded. Remains of petroleum ether removed by evaporation. The residue mixed with Keller reagent and transferred to clean test tube. Development of purple ring at the interface indicated positive test.

Keller Reagent:- 0.3% of 10% FeCl3 in 50 ml glacial acetic acid.

Flavonoids:-

Dry plant powder extracted in 70% ethanol overnight. Filtered and 5ml chloroform added. Chloroform layer discarded and chlorophyll-free extract was used for following tests.

A) Shinoda test:- This is general test for flavonoids. To the ethanol extract a piece of Mg ribbon and conc. HCl added drop by drop. Formation of purple pink or orange colour produced if flavonoids present.

B) Flavononols:- If in Shinoda test deep colour developed; instead of Mg ribbon Zn powder was added with HCl. Flavononols produce deep magenta colour.

C) Flavanols:- To the filtrate a pinch of boric acid and few drops of acetic acid added. Formation of bright yellow colour with green fluorescence indicated presence of flavanols.
D) **Flavones and Flavonols:**- To the filtrate few drops of conc. H$_2$So$_4$ added. Formation of yellow colour indicates flavones and flavonols and formation of orange to crimson colour indicated presence of flavonones.

E) **Rao and Sheshadri test:**- To the filtrate few drops of conc. nitric acid added. Formation of brilliant blue colour indicated presence of flavanones.

**Leucoanthocyanin:**-

2N HCl added to the plant powder and heated on boiling water bath for 20 minutes. Cooled at room temperature and filtered. To the filtrate 5 ml iso-amyl alcohol added. Appearance of red colour was indicative of leucoanthocyanin.