INTRODUCTION
Plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties. Several Indian plants are also being used in Ayurvedic and Siddha medicines. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases (Handa et al., 1996). The reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases (Sies, 1993; Halliwell, 1997). Hence search for new synthetic and natural antioxidants is essentially important. Although initial research on antioxidants was mostly on isolated pure compounds, recent focus is more on natural formulations (Hagerman et al., 1998; Haramaki and Packer, 1995). It has been found that compounds in their natural formulations are more active than their isolated form (Khopde et al., 2001).

Mitochondria are regarded as the power houses of the cell. They carry out the most important function, i.e., oxidative phosphorylation. Mitochondria in different cell types vary widely in size, shape and number. Each mitochondrion has an outer membrane that is freely permeable to large molecules and an inner membrane that is relatively impermeable and contains the respiratory chain. The inner compartment of the mitochondria, enclosed by the inner membrane, is the matrix in which Krebs cycle takes place. NADH and FADH$_2$ (that are generated from the Krebs cycle) act as electron donors to the electron transport chain. Proton extrusion across the inner mitochondrial membrane generates an electrochemical proton gradient, which drives ATP synthesis. Oxidative phosphorylation is the process by which the energy of oxidation is coupled to the synthesis of ATP.

The respiratory chain comprises of four enzyme complexes located on the inner mitochondrial membrane (Wallace, D.C., 1992).
Complex I [NADH: Ubiquinone oxido reductase]: It is the largest and contains atleast 45 polypeptides, seven of which are encoded by mitochondrial DNA. NAD linked substrates feed reducing equivalents into the chain via Complex I which passes electrons down the chain to ubiquinone.
Complex II [Succinate: Ubiquinone oxido reductase]: It consists of 5 polypeptides that are encoded by nuclear DNA. It accepts reducing equivalents from succinate and transfers to ubiquinone.
Complex III (Ubiquinone cytochrome C reductase): It consists of 11 subunits with one subunit (cyt.b) encoded by mitochondrial DNA.
Complex IV (Cytochrome C oxidase): It consists of 13 polypeptides 3 of which are encoded by mitochondrial DNA.
ATP Synthase: It is composed of 12 subunits, two of which are encoded by mitochondrial DNA.

The gastrointestinal tract of higher animals and human species is the port of entry of variety of naturally occurring organic plant and animal poisons and a wide variety of chemicals in the form of drugs, pollutants and poisons (Mason et al., 1965). Before being distributed to the body through blood they are first directed into the liver, which plays a key role in the metabolism and elimination of several drugs. Liver cells are equipped with a active detoxification system called the mixed function oxidase which
metabolically alters a variety of xenobiotics and guards the organism against potentially harmful drugs. In chronic liver disease particularly in cirrhosis, hepatic drug metabolism may be altered by changes in hepatic blood flow or in the activity of drug metabolising enzymes resulting in modification of the intensity of therapeutic and toxic effects (Wilkinson and Shand, 1975).

The mitochondrial respiratory chain and free radicals:

Free radicals are the molecules having an unpaired electron in the outer orbit. They are highly unstable and very reactive. Examples are superoxide, hydroxyl, peroxyl, alkoxyl, hydroperoxyl, nitric oxide and nitrogen dioxide. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide, hypochlorous acid, hypobromous acid and peroxynitrite. Hydrogen peroxide reacts with superoxide anions resulting in the formation of hydroxyl radical through the Haber-Weiss Fenton reaction which is highly reactive and would react with biomolecules like proteins, lipids and nucleic acids present in the vicinity (Winston and Cederbaum, 1983). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in animals and humans under physiologic and pathologic conditions (Evans and Halliwell, 2001).

Oxygen free radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. ROS and RNS are well recognised for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial depending upon their extent of generation to living systems (Valko et al., 2006). ROS are produced as byproducts of cellular metabolism, primarily in mitochondria. Small, physiological amounts of ROS are of cellular requirement, because they are involved in signaling pathways such as inducing and regulating a variety of cellular activities, including cytokine secretion, growth, differentiation and gene expression (Halliwell and Gutteridge 1999, Hensley et al., 2000) and in the defense against invading pathogens. However ROS in excess have the potential to induce significant biological damage and hence cell possess many antioxidant systems to scavenge ROS. Under normal physiologic conditions there is a balance between formation and neutralization of ROS. Under conditions of oxidative stress ROS production is increased. ROS induce oxidative damage to all biomolecules like DNA, proteins, and lipids. Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, other diseases and ageing (Dalle-Donne et al., 2006).

The mitochondrial electron transport chain is a source of superoxide. Under physiologic conditions, approximately 1-3% of oxygen consumed by body is converted to superoxide and other ROS. Thus although molecular oxygen is absolutely essential for aerobic life, it can be toxic under certain conditions. This phenomenon is termed as oxygen paradox (Gilbert, 2000). There is a link among mitochondrial metabolism rate and elimination of ROS and age related changes on mitochondrial function (Balaban et al., 2005). A superoxide radical (O$_2^-$) is generated when one electron is
transferred to oxygen. Superoxide radical has characteristics of both anion and radical. At 250 nm, superoxide radical reveals maximum absorption (approximately $\varepsilon=2300$) and the characteristic spectrum of electron spin resonance (ESR). Major sources of superoxide radical in the cells are NADPH oxidase, xanthine oxidase, cytochrome P 450 systems, mitochondrial electron transport chain, and arachidonate metabolism. Superoxide is produced from both Complexes I and III of the electron transport chain. In its anionic form it is too strongly charged to readily cross the inner mitochondrial membrane. Recently, it has been demonstrated that Complex I-dependent superoxide is exclusively released into the matrix and that no detectable levels escape from intact mitochondria (Muller et al., 2004). This finding fits well with the proposed site of electron leak at Complex I, namely the iron–sulphur clusters of the (matrix-protruding) hydrophilic arm. In addition, experiments on Complex III show direct extramitochondrial release of superoxide, but measurements of hydrogen peroxide production revealed that this could only account for $<50\%$ of the total electron leak even in mitochondria lacking Cu, Zn-SOD. It has been proposed that the remaining $50\%$ of the electron leak must be due to superoxide released to the matrix.

When free radicals and other reactive species (e.g., $\bullet$OH, HOO•, ONOO−) extract a hydrogen atom from an unsaturated fatty acyl chain, a carbon centered lipid radical (L•) is produced. This is followed by the addition of oxygen to L• to yield a lipid peroxy radical (LOO•). LOO• further propagates the peroxidation chain reaction by abstracting a hydrogen atom from a nearby unsaturated fatty acid. The resulting lipid hydroperoxide (LOOH) can easily decompose to form a lipid alkoxyl radical (LO•). This series of ROS-initiated lipid peroxidation reactions with the production of lipid peroxy and alkoxyl radicals, collectively called chain propagation, occurs in mammalian cells, such that oxygen free radicals may cause damage far in excess of their initial reaction products. Lipid peroxidation causes changes in the physical and chemical properties of membranes, thus altering their permeability and fluidity.

Oxidative stress is known to cause lipid peroxidation, DNA fragmentation, impaired cellular energy status, and disruption of ion homeostasis. Lipid peroxidation products like 4-hydroxy nonenal and malondialdehyde are highly reactive molecules that can react with $\alpha$-amino group of proteins, RNA and DNA (Sorrell and Tuma., 1987). Lipid peroxidation has also been associated in a various ways with a number of normal and abnormal physiological processes. The normal process includes prostaglandin synthesis, phagocytosis and aging are abnormal conditions in which lipid peroxidation is implicated include haemolytic anemia, reproductive dysfunction, liver necrosis, muscle dystrophy, lung damage, atherosclerosis and testicular atrophy. Extensive lipid peroxidation in biological membranes causes impairment of membrane function, decrease fluidity, inactivation of membrane bound receptors and enzymes, cross linking with sulfydryl groups of enzymes etc. Thus uncontrolled lipid peroxidation mediates a variety of degenerative diseases leading to cell death.

Many different types of protein oxidative modification can be induced directly by ROS or indirectly by reactions of secondary by products of
oxidative stress (Berlett and Stadtman, 1997). Cysteine and methionine are particularly prone to oxidative attack by almost all ROS. Protein modifications elicited by direct oxidative attack on Lys, Arg, Pro, Thr, or by secondary reaction of Cys, His or Lys residues with reactive carbonyl compounds, can lead to the formation of protein carbonyl derivatives (aldehydes and ketones). ROS can cause oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation. Thus protein carbonyl content is the most general indicator and is the most commonly used marker of protein oxidation (Berlett and Stadtman, 1997; Shacter, 2000; Beal, 2002).

Modifications of the respiratory chain Complexes (I–IV) by nitration, carbonylation and HNE formation decrease their enzymatic activity in vitro. The electron leakage from respiratory chain complexes and subsequent ROS formation may cause damage to any specific subunit and contribute to a long-term mitochondrial dysfunction (Choksi et al., 2004).

Mammalian cells possess many defense mechanisms to detoxify free radicals through enzymatic and non-enzymatic reactions (Fig A). The key metabolic steps are the catalysis of superoxide to hydrogen peroxide and oxygen by superoxide dismutase, conversion of H₂O₂ to H₂O by glutathione peroxidase or to O₂ and H₂O by catalase. Since the reaction catalysed by glutathione peroxidase requires GSH as substrate and depends in part on GSSG to GSH ratio, which depends on the redox state of the cell. Radical scavenging antioxidants such as Vitamin E interrupt the lipid peroxidation reaction by transferring its phenolic hydrogen to peroxyl free radical of PUFA. Vitamin C reacts with Vitamin E radical to yield a vitamin C radical and regenerates vitamin E. Like vitamin E radical vitamin C radical is not a reactive species because its unpaired electron is energetically stable. The vitamin C radical is then converted to vitamin C by GSH which is oxidized to GSSG. This is then reduced to GSH by the NADPH dependent glutathione reductase.

Also there are proteins like haemoglobin, transferin and ceruloplasmin that bind ferrous and copper ions and prevent radical generation through Fenton reaction, and proteases, ribonucleases and lipases that preferentially degrade the modified components of protein, DNA and lipids respectively. In addition vitamins, carotenoids, flavinoids can act as chain-breaking antioxidants (Slater et al., 1975).

Fig A: Antioxidant enzymes
One of the most prominent defense systems in the liver is the presence of glutathione (GSH) which is a tripeptide found in high concentrations in all cells. It is synthesized from glutamate, cysteine, and glycine in the cytosol. Due to the presence of cysteine in its backbone, GSH is key in the regulation of disulfide bonds of proteins and in the disposal of electrophiles and oxidants (DeLeve and Kaplowitz, 1991; Hammond et al., 2001).

Glutathione (GSH), the major intracellular non protein thiol, is mainly known as a nucleophilic scavenger and an enzyme-catalyzed antioxidant in electrophilic/oxidative tissue injury. GSH plays an important role in the maintenance of the intracellular redox state. The intracellular level of GSH, which differ from one cell type to another, may be crucial for ROS-induced NF-κB response (Nanxin and Michael, 1999). The extracellular GSH catabolism may be involved in the modulation of cell signalling and activation of transcription factors.

NF-κB is a ubiquitous transcription factor that regulates inflammatory mediators, and several structural proteins that are involved in infection, inflammation, stress responses and apoptosis (Baeuerle and Henkel, 1994). The exact and complex molecular mechanisms involved in the regulation of NF-κB remain to be elucidated (Nanxin and Michael, 1999). Reactive oxygen species have been implicated as second messengers involved in the activation of NF-κB via tumour necrosis factor (TNF) and interleukin-1 (Poli et al., 2004 and Valko et al., 2006). H₂O₂ possess several properties that make it ideal for to act as second messenger. They are, of small size and therefore diffuse rapidly through biological membranes, and their synthesis and degradation are fast (Baeuerle et al., 1996).

Superoxide dismutases constitute the only mammalian antioxidant enzymes converting superoxide to H₂O₂. There are 3 types of mammalian SODs i.e., copper–zinc SOD (CuZnSOD), manganese SOD (MnSOD), and extracellular SOD (ECSOD). CuZn-SOD is mainly a cytosolic enzyme, but it has also been detected in cellular organelles. The distribution of CuZnSOD positions it to be the primary enzyme protecting cells against cytosolic-generated superoxide. MnSOD is synthesized in the cytosol as a precursor molecule and is transported to the mitochondria [Matsuda et al., 1990]. Being mitochondrial, probably it has an important role in the oxidant resistance and apoptosis of rapidly growing cancer cells. ECSOD is synthesized in the cytosol and has a secretory leader sequence and four heparin-binding domains (one per each of four subunits) that contribute to the binding of ECSOD to extracellular matrix proteins (Marklund et al., 1982; Oury et al., 1994).

MnSOD is essential to the vitality of mammalian cells. MnSOD is a homotetramer, which contains one manganese ion per subunit (Weisiger and Fridovich, 1973). The MnSOD gene is located in chromosome 6q25. Total knockout of the MnSOD gene is perinatally lethal, leading to neurological manifestations and cardiotoxicity (Lebovitz et al., 1996; Tsan, 2001). Heterozygous mice with lowered MnSOD activity have increased mitochondrial oxidative damage [Tsan, 2001; Williams et al., 1998]. The familial form of amyotrophic lateral sclerosis, or FALS, has been shown to
be associated with defects in the gene encoding Cu,Zn-SOD (sod 1) (Rosen, D. R. et al., 1993; Deng et al., 1993; Siddique, 1990).

CuZnSOD, especially genetic variations thereof, been linked to degenerative neuronal diseases. CuZnSOD is a homodimer with a molecular weight of 32 kDa and its gene is located in chromosome 21q22 (McCord and Fridovich, 1969; Sherman et al., 1984). CuZn-SOD knockout animals are viable (Ho et al., 1998), but fibroblasts derived from those animals proliferate more slowly (75%) than control cells and their resistance to paraquat toxicity is decreased (Huang et al., 1997), emphasizing the importance of CuZn-SOD in cell growth and survival. In mammals the highest levels have been detected in the liver, kidney, erythrocytes, and central nervous system.

Gluthathion peroxidase (GSH-Px, EC 1.11.1.9) is a selenoprotein including selenocystein residue in the active site. Three types of GSH-Px have been confirmed. Cellular glutathione peroxidase is a tetrameric protein, and is composed of four subunits containing one atom of Se. Plasma glutathione peroxidase exhibits a tetrameric as well as cellular form. A third glutathione peroxidase, namely phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px), is a monomeric membrane-associated enzyme. These enzymes catalyze the reduction of H₂O₂ and a variety of organic hydroperoxides (ROOH). The decomposition efficiency of the substrates of glutathione peroxidase depends on the concentration of the reduced glutathione as a coenzyme. It is suggested that the physiological roles of GSHPx include reduction of H₂O₂ and prevention of lipid peroxidation as well as catalase (Little, 1972).

Catalase (EC 1.11.6.1) is a tetrameric hemeprotein, and is mainly confirmed in peroxysome. It is ubiquitously distributed in animal and plant cells and catalyzes the reduction of various substrates such as H₂O₂, methylperoxide (CH₃OOH), and ethylperoxide (C₂H₅OOH). Under optimum condition (Km = 1.1 M), this enzyme activity is higher than GSHPx. Patients with acatalasemia, a rare congenital disorder with abnormalities in erythrocyte catalase activity and lowered catalase levels in other tissues, appear to live a fairly normal life (Ogata, 1991).

### Allyl alcohol:

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Allylalcohol/vinyl carbinol/2-Propan-1-ol</th>
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<tbody>
<tr>
<td>Molecular formula</td>
<td>C₃H₇O/CH₂=CHCH₂OH</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>58.1 gms</td>
</tr>
<tr>
<td>Physical state,</td>
<td>Colour liquid with pungent odour</td>
</tr>
<tr>
<td>appearance</td>
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Allyl alcohol is a toxic, colorless liquid. It is an extremely hazardous substance. It is mainly used as herbicide and is used in the manufacture of food flavorings, allyl compounds, war gas, resins, plasticizers and fire retardants, allyl esters. It is an intermediate for pharmaceuticals, other organic chemicals and military poison.
Short-term (acute) effects

Severe irritation and burns of the eyes, nose and skin. Inhalation of allyl alcohol causes a build-up of fluid in the lungs (pulmonary edema), causing a severe shortness of breath and death if not treated. Ingesting allyl alcohol can cause abdominal pain, nausea, vomiting, diarrhea and/or liver damage, headache, dizziness, weakness and loss of consciousness, coma, cardiovascular failure and death.

Long-term (chronic) effects

Liver or kidney damage, depending on the route of exposure. Inhalation of allyl alcohol causes respiratory conditions such as asthma, bronchitis or emphysema, coughing, shortness of breath and lung irritation and/or damage.

Allyl alcohol (AA) is a chemical used in manufacturing processes in food flavoring and in agriculture as a weed killer (Beauchamp et al., 1985; Atzori et al., 1989). It irritates mucous membranes and is especially harmful in the liver, producing cell necrosis selectively in the periportal zone. It is metabolized by cytosolic alcohol dehydrogenase to acrolein, an unsaturated aldehyde (Rees and Tarlow, 1967). Acrolein is a powerful electrophile and reacts with nucleophiles, such as sulphhydryl groups. The thiol group of glutathione is a favored target and so glutathione is primarily involved in the reaction which leads to GSH depletion. The reaction is markedly accelerated by the activity of cytosolic glutathione S-transferase. The nucleophilic glutathione is an important protective factor of hepatic cells in the detoxification of acrolein.

The effect of acute exposure of rats to allyl alcohol (0.05 ml/kg, ip) on the activity of enzymes of hepatic phase I (cytochrome p450-linked microsomal monooxygenases, epoxide hydrolase) and phase II (glucuronyl-, glutathione-, acetyl- and sulfo transferases) biotransformation were studied in rats. Allyl alcohol reduced hepatic cytochrome p450 in liver, and the activities of ethylmorphine demethylase, benzphetamine demethylase, benzo[a]pyrene hydroxylase, and ethoxyresorufin deethylase. No significant decreases in epoxide hydrolase or glucuronyltransferase activities were observed. The activities of cytosolic conjugating enzymes (glutathione, sulfo- and acetyl transferases) also were minimally affected by toxic liver injury (Gregus et al., 1982).

Acute Exposure of allyl alcohol administered to Fischer 344 rats produce cell specific injury in centrilobular hepatocytes, perportal hepatocytes, and bile duct cells, respectively. Allyl alcohol administration increased serum alanine aminotransferase activity but had no effect on serum gamma-glutamyl transferase activity (Leonard TB et al., 1984). Acute Exposure 0.05 ml/kg of allyl alcohol administered orally to mice caused depletion of hepatic glutathione (Sieger’s et al., 1977).

It has been suggested that the alkylation of nucleophilic groups of cellular macromolecules affected by acrolein after glutathione depletion is the event actually leading to cell injury (Ohno et al., 1985). However other
reports have suggested that a major role in allyl alcohol induced hepatotoxicity play lipid peroxidation (Miccadei et al., 1988; Pompella et al., 1991). Acrolein depletes glutathione content of the hepatocytes there by sensitizing the cells to the constitutive flux of active oxygen species (Miccadei et al., 1988).

Acrolein is a highly toxic aldehyde involved in a number of diseases as well as drug-induced toxicities. Formed during the combustion of organic matter, it is implicated in the pathogenesis of smoke inhalation injury to the lung (Hales et al., 1988). Acrolein also forms via hepatic biotransformation of the chemotherapeutic drugs like cyclophosphamide and Ifosfamide (Ludeman, 1999). In addition, there is growing recognition that endogenous acrolein, formed via lipid peroxidation, mediates cell damage in various diseases of old age, including Alzheimer’s disease (Uchida et al., 1998a; Uchida, 1999; Lovell and Markesbery, 2001). Acrolein’s pronounced toxicity reflects its reactivity as a bifunctional electrophile, ensuring that it readily attacks electron dense-centres in DNA and protein (Esterbauer et al., 1991). This reactivity underlies most of the cellular effects of acrolein, including alterations in the activity of transcription factors such as AP-1, nuclear factor kB, and Nrf2 (Horton et al., 1999; Biswal et al., 2002; Tirumalai et al., 2002); inhibition of cytokine production (Li et al., 1997); and cell death (Li et al., 1997; Kern and Kehrer, 2002).

Glutathione S-transferases (GSTs):

The glutathione-s-transferases (GSTs, EC 2.5.1.18) are an integral part of phase I (oxidation)/ phase II (conjugation) system, multifunctional proteins involved in the detoxication of reactive electrophilic compounds from exogenous (xenobiotics and their metabolites) or endogenous origin (organic peroxides, product of oxidative stress) in aerobic organisms (Jakoby, 1978).

Structural multiplicity of GST:

Although GSTs are distributed ubiquitously, they have been studied most extensively in mammalian livers, mainly because of their exceptional abundance there. In the rat liver, the GSTs constitutes up to 10 % of the total extractable proteins whereas they represent approximately 3% in the rat brain and human liver (Jakoby, 1978).

So far, seven different classes of cytosolic isozymes of GSTs are identified i.e., alpha, Mu, Pi, Theta, Sigma, Kappa and Zeta (Coggan et al., 1998; Hayes and Pullford, 1995; Mannervik et al., 1992; Board et al., 1997) and three different forms of Microsomal GSTs (Andeersson et al., 1994; Jackobsson et al., 1996). They are active as homo- or hetero- dimers containing subunit belonging to the same class (Carne et al., 1979), with the exception of microsomal GSTs that exists as homotrimer ranging from 17 to 28 kDa. Each subunit consists of two binding sites designated as G-site, for GSH binding at N-terminus, and an H-site for hydrophobic or electrophilic substances binding at the C-terminus of the protein (Armstrong, 1997). The binding domain of GSTs to GSH is exploited for the purification of GSTs employing a GSH-affinity column.
Armstrong in 1994 proposed that binding of GSH to G-site results in formation of thiolate anion (GS-) that lowers the pKa of GSH from 9.0 in aqueous solution to 6.5 in bound state. Binding of GSH to G-site is primarily facilitated by conserved tyrosine residue that stabilizes thiolate anion (GS-) by hydrogen binding.

**Catalytic and Non-catalytic functions of GSTs:**

Various actions of GST can be classified into two categories: (1) catalytic functions and (2) binding, transport and storage functions. The common feature of all GSTs is their ability to increase the nucleophilicity of the sulfhydryl group of GSH, which participate in various reactions. The well known catalytic functions of GST are conjugation, peroxide reduction and isomerisation.

**Catalytic Functions:**

1. **Conjugation:**
   
   All GSTs conjugate the sulfhydryl group of GSH to the electrophilic centers of second substrates, forming a GSH thioether product. This reaction is the first step of the mercapturic acid pathway and has been reviewed extensively (Chasseaud, 1979; Jakoby and Habig, 1980; Smith et al., 1977). The second substrates include a wide variety of xenobiotics, most of which are synthetic chemical such as halonitrobenzene, or endogenously generated electrophilic intermediates, such as arene oxides, a phase one biotransformation product. The thioether product itself is excreted directly into the bile, and finally into the faeces after further metabolism in the gut (Chasseaud, 1976; Neilsen and Rasmussen, 1977). The conjugates that reach the kidney are further converted to mercapturic acid and then excreted into the urine (Kozak and Tate, 1982; Tateishi and Shinizu, 1980; Thompson and Meister, 1977). The purpose of the mercapturic acid formation appears to be inactivation of the potentially toxic electrophilic center of the substrate molecule and, at the same time, formation of a more hydrophilic conjugate for excretion. Therefore GSTs serve a key role in preventing the interaction of highly reactive chemical compounds and activated carcinogens with macromolecules such as proteins or nucleic acids (Chasseaud, 1979).

2. **Oxidation-reduction:**
   
   In 1976 it was reported that some GSTs catalyze GSH-dependent reduction of organic hydroperoxides (Lawrence and Burk, 1976; Prohaska and Ganther, 1976). This glutathione peroxidise activity is referred to as glutathione peroxidise II to distinguish it from glutathione peroxidise I (EC.1.11.1.9), which was originally reported in 1957 to be tetrameric protein containing four atoms of selenium (Se) per 84,000 dalton molecular weight mass (Mills, 1957). The major biochemical difference between GSHPX I and GSHPX II and the basis for their analytical distinction is the ability of Se-GSH-PX to catalyze the reduction of both organic hydro peroxides and hydrogen peroxide, whereas the GSTs with GSHPX II activity, also referred to as non Se-GSH-Px, can only catalyze the reduction of organic hydroperoxides (Lawrence and Burk, 1976). In the selenium deficient rats there is an increased GPX II activity. The GSHPX II
catalyses the conversion of organic peroxides to corresponding alcohols. This type of reaction is thought to represent nucleophilic attack by GSH on electrophilic oxygen. It is believed to involve two steps, one of which is catalytic and proceeds via the formation of sulfenic acid of glutathione. The substrates that GSTs reduce include fatty acids phospholipids, and DNA hydroperoxides. As these compounds are generated by lipid peroxidation and oxidative damage to DNA, it is proposed that GSTs as well as other GSH-dependent enzymes help to combat oxidative stress. Detoxification of lipid peroxides by microsomal GSTs can occur in situ whereas detoxification by cytosolic GSTs requires prior release of FA hydroperoxides by phospholipase A2.

3. Isomerization:
The GSTs were also discovered to have isomerase activity (Benson et al., 1977). The reaction can be carried out with two physiologically important substrates, Δ5- androstene-3, 17-dione and maleyl acetone (Benson et al., 1977; Keen and Jakoby 1978). A transient GSH adduct is presumed to be formed in these reactions, which rearranges to GSH and the more stable isomer of the organic substrates, GSH is, therefore not consumed.

Non-catalytic functions

Ligand binding properties:
Many GST enzymes exhibit a ligand binding functions, which involves the non-covalent binding of hydrophobic ligands such as heme, bilirubin, various steroids, and conceivably some lipophilic anticancer drugs that are not the usual substrates (Bhargava et al., 1980; Homma et al., 1985). GST-mediated ligand bindings are often associated with the inhibition of GST activity by the bound ligand and appear to facilitate the intracellular transport of these lipophilic compounds (Boyer and Kenny, 1985). For example, binding of thyroid hormone to GST controls their intracellular transfer to receptors and components involved in their metabolism. It was shown previously that bile acids are capable of binding to certain forms of GSTs with affinity constants in the order of $10^{-4}$-$10^{-5}$ M$^{-1}$. Bile acid binding was found to inhibit 50% of cytosolic GST activity (Vessey and Zakim, 1981).

Induction of toxicity by GST

GST binding to foreign compounds does not always result in detoxification. For example, a few GSH conjugates are relatively unstable and the reaction product is either cleaved to liberate an unconjugate metabolite that requires further detoxification (Thiolyis), or the reaction is reversible-allowing regeneration of the original electrophile (Reversible conjugation).

Thiolyis

This occurs with certain ethers, esters and organic phosphates when conjugation leads to cleavage of the substrate with only one of the two products being conjugated. In case of p-nitrophenol acetate, the herbicide fluorodifen and insecticide EPN results in the release of p-nitrophenol; presumably, the p-nitrophenol is metabolized by UDP-glucuronosyl transferase and phenol sulfotransferase. Thiolyis represent incomplete
detoxification because the conjugated cleavage product still provides threat to the cell.

**Reversible conjugation**

This occurs with certain cytotoxic isothiocyanate. Following reaction of benzyl, allyl, phenethyl isothiocyanate with GSH, their respective conjugates are not stable and yield the parental thiocyanate in mildly acidic media. In the case of benzyl and phenethyl isothiocyanate, GST can catalyze both forward and backward reactions but at high concentrations, the equilibrium is shifted in favour of formation of the GSH conjugates. The reversibility of this reaction means conjugates may not represent detoxification products rather, temporary storage or transport forms.

GSTs exist in multiple forms in rat liver (Habig et al., 1974; Mannervik, 1985). The major cytosolic forms consist of homo or hetero-dimers of the subunits Ya, Yc, Yb and Yb₂ (Hayes & Mantle, 1986). These subunits have also been named with the arabic numerals 1, 2, 3 and 4 respectively, (Mannervik, 1985). Heterodimers can only be formed between subunits that have extensive sequence homology, and the subunits that can hybridize are thought to arise from the same gene family (Ketterer et al., 1983; Hayes, 1984). The major GST isoenzymes are GST YaYa, GST YaYc, GST YcYc, GST YbYb, GST YbYb₂ and GST Yb₂Yb₂ (Hayes, 1983). The GSTs have differing non-Se-GSHpx activities, dependent upon the activity of their individual subunits, with the Ya and Yc subunits having the highest activity (Meyer et al., 1985; Mannervik, 1985). Ya and Yc catalyse the reduction of hydro peroxides, isomerisation of prostaglandin. Yb subunit involved in the formation of leukotrienes.

**Medicinal plants:**

**Phyllanthus fraternus:**

**Family:**
Euphorbiaceae

**Habitat:**
Annual herb, the stem is non-erect and 30cm tall, leafy shoot is 5-10cm long, oblong and joined to the brachlets of the stem, six sepals in the flower, distributed in India, pakistan and introduced into Saudi Arabia, Africa and West indies (Abedin et al., 2001). It is widely distributed in the northern region of India.
**Phyllanthus fraternus**

Properties and uses ascribed to *P. fraternus* in traditional medicine:

The plant is bitter in taste, astringent, stomachic, diuretic and antiseptic. It is used in gastric complaints including dyspepsia, colic, diarrhoea and dysentery; also employed in dropsy and diseases of urinogenital system. The plant is also said to be useful in diabetes. A decoction of the leaves is used as a refrigerant for scalp; leaves and roots are made into poultice with rice water for application on oedematous swellings and ulcers. The latex is also applied to offensive sores and ulcers, mixed with oil, it is used in opthalmia. The fresh leaves are also considered as a remedy for jaundice.

Chemical studies of the plant have reported that the leaves of *P. fraternus* contain a number of lignans and alkaloids. Phyllanthin (a bitter constituent) and hypophyllanthin (a non-bitter compound) were isolated from the leaves of *P. fraternus* and identified as lignans. Phyllanthin was found to be (+) 3, 4, 3′, 4, 9, 9′-hexamethoxy-8, 8′-butyrolignan with absolute (8s, 8′s) configuration (Row et al., 1964). The hexane extract of the leaves gave three additional lignans viz. niranthin, nirtetralin and phyltetralin (Anjaneyulu et al., 1973). The distribution of the lignans in the leaves varied considerably with geographic location of the plant (Anjaneyulu et al., 1973). The aerial parts of the *P. fraternus* yielded four alkaloids, 4-methoxy securinine (phyllanthine) and 4-methoxy-norsecurinine. The ethyl acetate portion of the water soluble fraction of the ethanolic extract of the *P. fraternus* roots yielded 2 new glycoflavones, which are characterised as 3,5,7-trihydroxy flavonal-4′-o-L-rhamnopyranoside (Chauhan et al., 1977). Another new compound viz., lintetralin was also isolated from *P. fraternus* (Ward et al., 1979).
Pharmacological studies on *P.fraternus*:

The aqueous extract of *P.fraternus* leaves was reported to produce hypoglycaemic action in normal as well as alloxan-diabetic rabbits. The extract lowered the blood sugar even when it was administered one hour after glucose administration. The hypoglycaemic activity of the leaf extract appeared to be higher than that of tolbutamide (Ramakrishnan et al., 1982). Petrol extract of *P.fraternus* (whole plant) showed antifungal activity against *Helminthosporium sativum* (Bhatnagar et al., 1961). An aqueous extract of the plant inhibits DNA polymerase of wood chuck hepatitis virus (WHV) and binds to the surface antigen of WHV in vitro (Venkateswaran et al., 1987). *P.fraternus* has been shown to be effective as an adjunct with other siddha drugs in the treatment of jaundice due to infective hepatitis (Ramanan and Sainani, 1961). It was reported earlier from our laboratory that mitochondrial dysfunction caused by the administration of alcohol (Sebastian and Setty, 1999) or thioacetamide (Padma and Setty, 1997) or carbon tetra chloride (Padma and Setty, 1999) could be prevented by prior administration of aqueous extract of *P. fraternus*.

*Hemidesmus indicus* (Linn.) R.Br.

**Family:**
Asclepiadaceae

![Hemidesmus indicus](image1)

**Roots**

![Roots](image2)
Habitat:
Throughout India

Classical and Common Names:

Ayurvedic:
Saarivaa (White variety), Ananatmuula, Gopavalli, Upalasaarivaa, Kapuuri. Krishna Saarivaa (black variety), Jambuupatraasaarivaa Ayurvedic Formulary of India accepts Hemidesmus indicus and Cryptolepis buchananii Roem and Schytt as the white and black varieties of Saarivaa.

Siddha:
Ninnari

English:
Indian Sarsaparilla

Telugu:
Sugandhipala, muttapulgam

Parts Used:
Root

Properties and Uses:
The drug is sweet, demulcent, diaphoretic, diuretic and tonic and is useful in the loss of appetite, fever, skin diseases, diarrhoea, and nutritional disorders and is a blood purifier.

The root is included in the Indian pharmacopoeia (1966) and the Indian pharmaceutical vodex (1955). It is sweet, demulcent, alternative, diaphoretic, diuretic, tonic and is useful in the loss of appetite, fever, skin diseases, diarrhoea, and is a blood purifier. It forms an important ingredient of some Ayurvedic preparations such as Aswagandhadi churnam, Aswagandhadi leham, chandanasava and others.

The plant has been mentioned in Indigenous System of Medicine as Mutravirecham, Balya, Raktashodhak (blood purifier), Dahaprashaman (soothes burning sensation) and useful in treatment of jwar (fever) kushtha, pradar, agnimandya, prameha and others.

The methanolic extract of Hemidesmus indicus roots was found to inhibit lipid peroxidation and scavenge hydroxyl and superoxide radicals in vitro. The amount required for 50 % inhibition of lipid peroxide formation was 217.5 µg / ml. The concentrations needed to scavenge hydroxyl and superoxide radicals were 73.5 and 287.5 µg / ml, respectively (Mary et al., 2003).

Active Principles and Pharmacology:

- Two novel pregnane glycosides, denicunine and heminine have been isolated from the dried stem of Hemidesmus indicus. Twigs of the plant gave a pregnane ester diglycoside named desinine.
- Roots gave β-sitosterol, 2-hydroxy-4-methoxy-benzaldehyde, α-amyrin, β-amyrin and its acetate, hexatriacontane, lupeol actacosonoate, lupeol and its acetate.

- Leaves, stem and root cultures produce cholesterol, campesterol, β-sitosterol and 16-dehydroprogrenolone.

- Leaves and flowers also gave flavonoid glycoside: rutin, hyperoside and iso-quercitrin.

- The essential oil (2-hydroxy-4-methoxy-benzaldehyde) isolated from the plant possesses anti bacterial property against Gram-positive and Gram-negative bacteria.

- The aqueous ethanolic extract of the whole plant showed anti-viral activity against Ranikhet disease virus.

- A saponin from the drug was found to possess an anti-inflammatory activity in experimental animals. The fresh decoction of roots was found to possess a blood-purifying property.

**Use in Western Herbal:**

*Hemidesmus indicus* has been successfully used in the cure of venereal disease. The drug is used as an infusion, as boiling dissipates its volatile principle, for rheumatism, skin diseases and thrush. Also used in nephritic complaints and for sore mouth in children.

Sarsaparilla is used as an anti-inflammatory and cleansing agent, which can bring relief to skin problems such as eczema, psoriasis and general itchiness, and helps in rheumatism and gout treatment. It has a tonic and specifically testosterogenic action on the body leading to increased muscle bulk and has a potential use for impotence.