Chapter 1
Review of Literature
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1.11.1. Dasamula plants Pseudarthria viscida L. and Premna serratifolia L. selected for the present study
1.1 Introduction

For thousands of years humanity has relied on diverse plant resources for health benefits and to cure ailments. Written evidences on the extensive use of herbal medicines in treating diverse illnesses have been unearthed from remnants of ancient civilizations of India, China, Mesopotamia, Egypt, etc. Even before that, primitive cave men started using different plants available in their locality to cure many ailments which affected them (Bensky and Gamble, 1993). They passed on the knowledge acquired from trial and error to next generations as traditional knowledge which later through methodical collection of information, refinement, and consolidation processes gave life to a number of traditional herbal therapy systems (Cassileth, 1998). Tropical countries are blessed with their rich biodiversity which also nurtured herbalism, i.e. use of herbal products known for medicinal values. Any part of a plant such as roots, leaves, bark, flower and seeds could be a potent source for herbal products and active constituents can be isolated from them via purification procedures. Herbal medicines, according to WHO, are those final commercial products containing active components of plant origin (WHO, 1993). It is not to be surprised that almost twenty five percent of modern medicines are directly derived from traditional medicinal plants (Akerele, 1993). Even some of the essential compounds for drug making are still sourced from plants in Allopathic system.

1.2 The tradition of herbal treatment

Traditional medicinal practices which use plant based drugs are categorized into a few basic systems. Traditional Chinese and Sri Lankan medicines along with India’s own Ayurveda are counted as the most refined among traditional medicinal practices. These traditions are found to be better evolved than contemporary African and Western herbal medicines which remained as the best options for the people of the respective geographical regions for centuries. Majority of the population in Asia, Africa and Latin America still relay upon traditional medicinal systems, especially medicines based on plant
Figure 1.1. Different varieties of plants used in therapeutic systems

- Trees: 36%
- Herbs: 28%
- Shrubs: 21%
- Herbaceous climbers: 15%
- Lianas: 8%
- Climbers: 7%
products, for primary health care. In China 30 to 50 percentage of medicine consumption is contributed by herbal products. In countries such as Ghana, Mali, Nigeria and Zambia majority of children affected with malaria fever get immediate treatment via traditional herbal remedies prepared in their home itself. Even in certain regions of Western Europe and Northern America locally available herbal remedies were prevalently used. In Canada about 70% of population prefers to incorporate herbal supplements in their day today life (WHO, 2002).

As one third of the population in developing nations lack proper health care facilities, traditional medicines, ensured with safety and effectiveness, could help in making proper healthcare accessible to all sections of population. Emerging economies such as China and South Korea have already incorporated traditional herbal treatment methods in their public health care system. China effectively used Artemisia annua based traditional herbal remedies to fight malarial disease which was found resistant to modern treatment regime. Twentieth century saw resurgence of interest towards traditional medicinal practices. The alternative medicine (CAM), a collective term which include all types of healthcare provisions outside the official health sector, became popular when developed and industrialized countries begun adopting traditional medicines. Herbal medicines are nowadays grabbing more attention and respect from modern health care systems in the developed nations. The new trend has been favoring not only herbal medicines but also other herbal products which include nutraceuticals, health food, etc. Moreover, reports on use of herbal medicines showed that they have the lowest level of side effects among all CAMs (Chan, 2003). In several parts of the world people use CAMs even in the treatment of HIV/AIDS infected patients (WHO, 2002).

1.3 Indian system-based herbal medicines

Ayurveda, Unani, Siddha and Amchi are very much rooted in India and are considered among some of the most significant therapeutic systems of Asian
Medicinal System. Together with folklore medicines they form our rich heritage of traditional medicinal system. Different varieties of plant species are used by all these therapeutic systems (Singh, 2003). Several of these medicinal plants were mentioned in one of oldest anthology of human knowledge, the Rigveda. The Atharvaveda still remains a rich source of our traditional medicinal practices. Later inscriptions of “Charaka Samhita” and “Sushruta Samhita ” provided vivid picture of wide range of medicinal plants prevalently used in therapeutics (Chopra et al.,1953). Indian subcontinent is well known for rich diversity of medicinal plants and is considered as one of the major mega diversity hubs of the world. Tropical and subtropical forests in India contribute about seventy percent of medicinal plants followed by temperate forests and other high altitude forest types which contribute remaining less than thirty percent of medicinal plants (Kumar et al.,1997).

1.4 Free radicals and living system
These are molecular species having an unpaired electron in atomic orbits; capability to exist freely is another important characteristic feature to be noted about free radicals. They are highly reactive and can donate or capture electrons from other molecules hence to behave like oxidative or reductive agents. Free radicals can generally exist only for a fraction of seconds; though there are a few exceptions as well. Among them oxygen derivatives have very significant role in causing several diseases. Both internal as well as environmental factors assist the mechanism of free radical formation in human body. However free radicals have significant role in several metabolic pathways and signal transduction pathways. WBC acts as a mediator in destroying pathogenic invaders; free radicals involve in this process in a significant manner, having a very crucial role to play. Free radicals are known to influence inflammatory responses and tissue repair advancements (Handelman, 2000). When the balance between free radical generation and active prevention by endogenous antioxidant system get toppled, oxidative
Figure 1.2. Major sources of free radicals and the consequences

Endogenous sources
- Reactions of enzymes
- Autoxidation reactions
- Respiratory burst

Exogenous sources
- UV radiation and other ionizing radiations
- Cigarette smoking
- Xenobiotics

Free Radical

$O_2^{\cdot-}$ and $H_2O_2$

$Fe^{2+}$ and $Cu^{+}$

$OH$

Lipid Peroxidation
Changes in DNA bases
Protein damage

Tissue Damage
stress become a reality. This further leads to starting of chain reactions which facilitate generation of more free radicals. These free radicals attack vital macromolecules such as proteins, DNA, lipids and carbohydrates and cause destruction to tissues. This ultimately brings adversities to the health condition of the organism (Small et al., 2012). There are several kinds of free radicals found in biological system which are capable of inducing oxidative stress.

1.4.1 Superoxide (O$_2^{-}$)

Superoxide is formed when a single electron is added to oxygen and this can occur due to several mechanisms in a living system (Halliwell and Gutteridge, 1992). Free radical intermediates are formed in mitochondrial membrane where reduction of oxygen to water happens as part of electron transport chain. However a few electrons often leak into mitochondrial matrix leading the formation of superoxide (Becker and Hoek, 1999). Activity of enzymes such as cytochrome p450 oxidase could also result in the leakage of electrons to liver tissues that may lead to superoxide production. Superoxides may be produced in different cells according to the kind of role they have to play. This include vascular endothelial cells which neutralize nitric oxide (Tsao et al., 1998; Barbacanne, 1999), cells that regulate growth and differentiation of other cells (Masters, 1998) and finally phagocytotic cells which take part in respiratory burst (Kemp, 1997).

1.4.2 Hydrogen Peroxide (H$_2$O$_2$)

Spontaneous dismutation reaction by superoxide produces H$_2$O$_2$ in biological systems. Moreover, there are direct formation of H$_2$O$_2$ by the enzyme actions such as catalysis by glycolate oxide and D-amino acid oxidase. These Reactive Oxygen Species (ROS) are proven as weak oxidizing agents so far; still H$_2$O$_2$ is capable enough to cause destruction of proteins and enzymes containing thiol groups. It plays very significant role in the destruction of bacteria by phagocytes via production of ROS in presence of myeloperoxidase. Unlike Superoxide, H$_2$O$_2$ could pass through cell membranes, move far away from
location of origin before decomposing into hydroxyl radical (Valko et al., 2005) which is a highly havoc creating molecule.

1.4.3 Hydroxyl radical (OH•)

Hydroxyl radical is formed in living body due to several reasons. Disintegration of either superoxide or hydrogen peroxide or hydroperoxides (Valko et al., 2005) catalyzed by transition metals like that of iron and copper lead to the formation of hydroxyl radicals. Though they have very short life span, hydroxyl radicals are extremely reactive free radicals and capable of causing destruction to nuclear components, proteins, carbohydrates and lipid membranes. Macrophages are known to produce hydroxyl radicals in the event of microbial invasion. Since hydroxyls cannot be removed enzymatically, endogenous antioxidants such as GSH play greater role in controlling proliferation of hydroxyl radicals.

1.4.4 Organic hydroperoxide (ROOH)

These are organic compounds formed by unstably bonded oxygen molecules. These bonds could easily break up to generate free radicals. Organic hydroperoxides usually form when free radicals attack membrane lipids or nucleobases. Protein hydroperoxides formed during oxidative stress act as source of free radicals causing destruction to macromolecules. Peroxiredoxins are thiol-dependent peroxidases which are effective in inhibiting the activities of aromatic hydroperoxides (Flohé et al., 2011). Seleno-organic compound ebselen was found to be effective against membrane lipid peroxides (Zhao et al., 2002) by acting like a weak glutathione peroxidase.

1.4.5 Peroxyl (ROO•) and alkoxy (RO) radicals

These are highly reactive oxygen species playing important roles in lipid peroxidation process. Peroxyl radicals are usually derived from azo compounds. They are instrumental in producing highly reactive singlet oxygen (1O2). Alkoxy radicals are formed by dissociation of lipid hydroperoxides in the presence of transition metal species. (Halliwell and Gutteridge, 1989). Lipid peroxy radicals (LOO•) are formed by peroxidation of lipids, especially the polyunsaturated fatty acids. Peroxyl radicals are cause
hydroperoxide related oxidative damage which results in several diseases (Porter and Wujek, 1988). Lipid peroxys can snatch hydrogen from other lipids and results in the formation of lipid hydroperoxides (LOOH). The advancement of lipid peroxidation can be prevented by antioxidant agents such as vitamin E, α-tocopherol etc. (Yoshikawa et al., 2000).

1.4.6 Nitric oxide (NO•) and Peroxynitrite (ONOO−)
Nitric oxide is a colorless gas under normal conditions. Nitric oxide is distinctively behaves as a free radical; as we could see that it possess an unpaired electron. It has to play a significant role in cell signaling mechanism and has the ability to diffuse freely through cell membranes. Nitric oxide induces vasodilation and thereby helps in preventing ischemic injury. Biosynthesis of nitric oxide is mediated by nitric oxide synthase enzyme by the utilization of substrates such as arginine. Phagocytosis, as part of immune response, also generates nitric oxide (Azzi et al., 2002). This mechanism causes toxicity to pathogens by destroying their genetic material and by destabilizing iron and sulfur components (Hibbs et al., 1988). However during inflammatory conditions nitric oxide, along with several other free radicals, become a source for oxidative stress. When nitric oxide reacts with superoxide radicals, formation of peroxynitrite occur; considered to be a highly reactive nitrogen species, which is a conjugate acid and not a free radical. The unavailability of active nitric oxides results in the depletion of vascular endothelial cells and chronic disorders such as hypertension, atherosclerosis, myocardial anomalies etc. start getting unveiled (Kojda and Harrison, 1999). Though not a free radical, peroxynitrite has the ability to act as a powerful oxidant and cause oxidative stress in living system. Such a condition results in the elevated expression of transcription factor NF-κB, which in turn induce expression of nitric oxide synthase gene and a number of inflammatory cytokines (Pall, 2013).

1.5 Effect of oxidative stress on disease conditions
Checks and balances, if not properly placed, may lead to oxidative stress and leads to destruction of several biomolecules. Membrane lipids, on free radical
attack, undergo a process known as lipid peroxidation, a complex process, and the byproducts of this process such as malondialdehyde are turned out to be useful markers to measure extent of lipid peroxidation (Romero et al., 1998). Free radical attack on proteins and nucleic acids cause unwanted modifications of amino acids and nucleic acids which may lead to cellular dysfunction and consequently lead to several disease conditions which include different types of cancers, atherosclerosis, inflammatory conditions, diabetes, neurodegenerative diseases, chronic inflammatory responses, infertility and aging (Begin, 1990; Spiteller, 2001; Silva et al., 2015). Oxidative modification of LDL cholesterol play very important role in the formation of atherosclerosis plaques and it mainly occurs in the arterial wall where antioxidant presence is scares and subsequently lead to cardiovascular diseases. Moreover LDL on oxidation can be lethal to arterial endothelial cells and cause alteration in vasomotor responses. In several occasions oxidized LDL functions to induce formation of autoantibodies. A number of research works established the fact that the supplementation of antioxidants such as vit E, vit C and β-carotene have positive effect in coronary heart disease patients (Esterbauer, 1992). This also stands as a testimony on how important it is becoming to address the oxidative stress related angle to tackle a disease condition.

1.5.1 Inflammation

Inflammation is an important segment of defensive response mechanism of body and is necessary for the survival strategy. Inflammatory response becomes necessary to have the repair mechanism start functioning during tissue injury. Acute inflammatory reactions are related to the response of body’s innate immune system to pathogenic attack. Immune cells such as neutrophils, macrophages etc. start secreting cytokines as well as chemokines as a signal for other immune cells to march towards site of infection. Inflammatory response also results in the dilation of vascular system. ROS act as mediators for inflammatory response; also adores a significant role in cell
signaling. However prolongation of free radical chain reaction leads to disease conditions which are mediated by inflammatory responses. Mitochondrial leakage of superoxide induces free radical chain reactions and cause inflammatory responses. NADPH oxidases produce reactive oxygen species in phagocytes which cause respiratory burst and destruction of pathogens (Clark and Kupper, 2005; Mittal et al., 2014). Inflammation can also be induced by peroxynitrites which is formed when superoxide react with nitric oxide produced by macrophages, epithelial cells, and neutrophils. Inflammation related hypernociception involve two stages. In the first stage the local immune cells along with those migrated to this site, start generating an array of hypernociceptive inflammatory mediators initiated by tumor necrosis factor alpha (TNF-α), a cytokine which can cause apoptosis. This in turn causes the release of interleukin-1β and chemokines; the process consequently stimulates the release of various prostaglandin molecules which include hypernociceptive mediators (Verri et al., 2006). In first stage of carrageenans induce inflammatory response histamine, serotonin etc. are formed. Meanwhile prostaglandin production around the injured tissues also starts elevating. Later inflammatory phase is mediated by polymorphonuclear cells, leukotrienes and bradykinin along with prostaglandins (Brito and Antonio, 1998).

1.5.2 Carcinogenesis
Cancer development in living system is a multidimensional process which is influenced by several factors. Oxidative stress is known to cause alterations in the structure of macromolecules such as protein, DNA, membrane lipids etc. Changes in DNA strands lead to mutations and chromosomal aberrations and any unwanted mutations can cause cancer. Hydroxylation of bases causes alterations in transcription process and thereby affecting normal cell growth process (Valko et al., 2004). Changes such as breakage of DNA strand sugar, alterations in DNA-protein interactions etc. can be induced by oxidative stress. Prevalence of different types of cancers among smokers and those who
exposed to radiation are testimony to the influence of free radicals in carcinogenesis (Droge, 2002). The alteration in the redox environment is found to support proliferation and survival of cancer cells. Some oncogenes such as Ras, when expressed, were found to reduce the level of GSH and cause imbalances to prevailing redox environment (Giorgio, 2015).

1.5.3 Liver toxicity

Hepatocytes are susceptible to oxidative stress. There are several chemical agents which are known to cause oxidative stress related hepatotoxicity. Carbon tetrachloride is known to cause free radical induced toxicity to liver. Its metabolism in liver starts with formation of the trichloromethyl radicals (CCl\textsubscript{3}) in presence of cytochrome P450 (CYP) enzymes. Trichloromethyl radicals on reaction with oxygen produce trichloromethylperoxy (CCl\textsubscript{3}O\textsuperscript{2}\textsuperscript{-}) free radicals which are highly reactive. These two radicals act in tandem and cause damages to macromolecules like nuclear components, protein, carbohydrates etc. which may leads to cell destruction (Weber, et al. 2003). The oxidative stress becomes too heavy that the components of endogenous antioxidant mechanism such as GSH get sharply depleted. The destruction was evident when serum showed elevated level of enzymes such as GOT, GPT and ALP in carbon tetrachloride exposed animals (Klingensmith and Mehendale, 1982). Paracetamol, when encountered with cytochrome P450 enzymes produce toxic metabolite N-acetyl-p-benzoquinone imine, a potent liver toxin (Udem et al., 1997). When N-acetyl-p-benzoquinone imine is present in very small quantities, liver is capable enough to manage it. However, high intake of paracetamol causes imbalance by the increasing concentration of N-acetyl-p-benzoquinone imine and this consequences in the GSH depletion in liver tissues (Kalinec et al., 2014). The auditory cells are known to get affected by N-acetyl-p-benzoquinone imine; this may further lead to unmanageable damage as well to the auditory system.
1.5.4 Cardiovascular diseases
Oxidative stress is considered to cause several kinds of cardiovascular diseases. Any imbalance in the redox condition due either external factors or aging could favor disease conditions such as heart failure, ischemic reperfusion injury, atherosclerosis, hypertension etc. As aging progress production of reactive oxygen species also get accelerated. At the same time detoxification and repair functions start weakening slowly. Studies show that about 80% death among people of above 65 years age is due to cardiovascular diseases (Karavidas et al., 2010; Skibska and Goraca, 2015). Increase in free radical generation affect the normal functioning of vascular endothelial cells which is followed by accumulation of inflammatory cells and lipids on the wall of vascular system and emergence of atherosclerosis which further weaken or damage blood vessels. Atherosclerosis along with vascular endothelial dysfunction promotes vasoconstriction which in turn elevates blood pressure. This impairs free flow of oxygenated blood to heart muscles and this deprivation makes heart muscles less tolerant to ischemic conditions (Lesnefsky, 2001) and possibility for cardiac failure becomes higher.

1.5.5 Gastro intestinal ailments
Several external factors, including consumption of alcohol, infection of the stomach with Helicobacter pylori, use of non-steroidal anti-inflammatory drugs (NSAIDs) and other external stress factors may harm our digest system; paving the way for oxidative stress further leading to gastric disorders. Free radicals such as superoxide anions, hydroxyl etc. induces gastric ulcer. Increased level of lipid peroxidation was also found in similar conditions along with indications for higher rate of depletion of GSH like endogenous antioxidants (Franke et al.,2005). Ethanol causes erosion to gastric mucosa by destroying integrity of mucosal matrix which is evident from the formation of gastric ulcer lesions and altered blood circulation in mucosa. This destructive process mainly associated with generation of free radicals which destroy vital macromolecules in tissues and also lead to cell membrane destruction, lesions in epithelial lining and cell death. These conditions create imbalance in the
secretion of hydrochloric acid secretion in stomach which is, in normal circumstances, remain under the control of neural and hormonal signals (Franco et al., 2015).

1.5.6 Nephrotoxicity
Oxidative stress is considered as one of the major factors for renal failure. More than 20% of population above 65 years old is found with some kind of renal abnormalities (Nasri, 2013). Cisplatin is a platinum based anticancer drug; known to cause nephrotoxicity. Cisplatin activity in body induces formation of free radicals such as superoxides and hydroxyls and thereby causes oxidative stress. Renal toxicity is marked by increased level of lipid peroxidation and reduced level of GSH and similar protein thiols. There is a noteworthy decrease in the activity of transport proteins. Cisplatin inhibit deoxyribonucleic acid synthesis and induce apoptosis. Cisplatin induced tubular damage is marked by loss of water reabsorbing tubules consequently lead to dehydration as well as loss of body weight (Mahran et al., 2011; Noroozi et al., 2015). Such anomalies affect blood circulation and lead to heart related disorders. Antioxidant treatments were found to reduce the level of oxidative stress and bring normal renal functioning. Tocotrienol has shown the ability to reduce proximal tubular injury and lipid peroxidation and also increased the level of GSH and catalase activity (Rafieian-kopaei, 2013)

1.6 Endogenous defence mechanism and oxidative stress.
As the free radicals could cause far reaching impacts on living body, efficient antioxidant defense systems of both exogenous and endogenous nature are present to protect cellular components.

1.6.1 Superoxide dismutase
It catalyzes dismutation of superoxide to hydrogen peroxide
\[ \text{O}_2^{\cdot-} + \text{O}_2^{\cdot-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]
Hydrogen peroxide thus formed gets scavenged by either catalase or glutathione peroxidase. The important types of superoxide dismutase enzymes found in membrane tissues include extracellular superoxide
dismutase, manganese superoxide dismutase, copper zinc superoxide dismutase etc.

1.6.2 Catalase
Catalase is credited with being first ever antioxidant enzyme to be characterized. Catalase converts hydrogen peroxide into water which takes place in two stages.

catalase–Fe(III) + 2H₂O₂ → catalase–Fe(III) + 2H₂O + O₂

Catalase usually found in the peroxisomes of cells and is highly active in hepatocytes and erythrocytes (Valko et al., 2004).

1.6.3 Glutathione peroxidase and Glutathione reductase
Glutathione peroxidases (GPx) catalyze the oxidation of glutathione by utilizing a hydrogen peroxide or a lipid hydroperoxide (Eklow et al., 1984) and thereby help to reduce the damages by oxidative stress. Though widely distributed in all tissue types, highest concentration of them can be found in hepatocytes. GPx is the main scavenger in cytosol and mitochondria. Here GSSG is the oxidized form of GSH.

ROOH + 2GSH → GSSG + H₂O + ROH

Since activity of Glutathione peroxidase is related to concentration of reduced glutathione, it should be made available by glutathione reductase. The latter is a flavin nucleotide dependent enzyme.

GSSG + NADPH + H⁺ → 2GSH + NADP⁺

1.6.4 Glutathione (GSH)
It is the most abundant endogenous antioxidant molecule. Reduced glutathione (GSH) is one of the foremost providers of thiol groups in the cellular environment. It has a direct role as free radical scavenger and also acts as essential factor for glutathione peroxidase (Rahman and Mac Nee, 2000). GSH not only scavenge reactive oxygen species like superoxides but also help in rejuvenating other antioxidants. Hence it helps in the reverting of α-tocopheroxyl radical to α-tocopherol. It also control proliferation of reactive nitrogen species produced excessively during inflammatory response (Espinosa-Diez et al., 2015).
1.6.5 Other chain breaking antioxidants

These are very small molecules which can form stable products with free radicals either by receiving or donating electrons. Co-enzyme Q10 on reduction forms Ubiquinol-10 and scavenges lipid peroxyl radicals even precisely than α- tocopherol or carotenoids. It also helps in the recovery of membrane bound α- tocopherol from tocopherol radical (Shi et al., 1999). There are certain chain breaking antioxidants which are in aqueous phase and can scavenge harmful radicals in aqueous compartments. Uric acid is effective in scavenging free radicals while getting converted into allantoin (Ames et al., 1981). Urate can provide protection against dangerous oxidative agents such as ozone and play major role in increasing the life span of human being. Bilirubin when bound to albumin act as an efficient radical scavenger and studies show that it play very important role in preventing oxidative damage in neonate. Protein bound thiol groups when bound on plasma proteins act as antioxidants by providing an electron to neutralize the free radicals. Such reactions end up with formation of thyl radical, further activity of which can be prevented by ascorbate and retinol (Riedl, 1996). Cysteine residue in albumin helps in neutralizing peroxyl radicals. By allowing copper ions to bind on it, albumin blocks copper dependent lipid peroxidation and hydroxyl radical formation. It also plays a major role in scavenging hypochlorous acid formed during phagocytosis (Plessinger et al., 2000). By exposing itself to the attack of free radicals and further cellular damage, albumin acts as sacrificial molecule. Melatonin is an important endogenous hormone known to perform free radical scavenging activity and also act as inhibitor to cancer cell growth. Melatonin cause alterations in the TNF-α gene expression and significant reduction in DNA synthesis in murine cancer models (Farriol et al., 2000; Sanchez et al., 2015).

1.7 Exogenous antioxidants as supplements

Vitamin E is one of the best examples for such antioxidants. They actively interact with peroxyl radicals and thereby helping in curtailing further
progression of lipid peroxidation (Niki, 1987). Ascorbate (vitamin C) is an essential factor for several enzymes that involve in the catalysis of hydroxylation reactions. It has been known to scavenge superoxides, hypochlorous acid, hydroxyl radicals, hydrogen peroxide, aqueous peroxyl free radical, and oxygen free radicals in singlet forms (Cathcart, 1985). Carotenoids are another kind of antioxidants with remarkable features. Among them β-carotene is most important. It is efficient in scavenging oxygen radicals as well as trapping peroxyl radicals (Kennedy and Liebler, 1992). Flavanoids are category of polyphenolic antioxidants mainly isolated from plant products. Thousands of them are identified so far and classified into different groups according their chemical structure. They are flavanols (eg: catechins), flavonols (eg: kaempferol), flavones (eg: apigenin) and isoflavons (eg: genistein). Emerging evidences of several experiments show that flavonoids in diet could help recover from the adversities of oxidant stress (Sanz et al., 1994; Cotelle, 2001).

1.8 Herbal products as therapeutic agents

Diverse phytotherapeutic regimes which remained as the reliable sources for health care for thousands of years entered into a new phase in the 20th century. Advancement in science made it possible to isolate active ingredients from medicinal plants. This lead to the differentiation of phytotherapy into traditional phytotherapy and the newly emerged rational phytotherapy; latter is mainly based on herbal products. Rational phytotherapy follow appropriate pharmacological investigations and clinical patient studies which provide documented evidence on the efficiency of herbal products in question (Schulz et al., 2004). Even after being continued as one of the most reliable therapeutic methods in practice for such a long time period, traditional phytotherapy systems were looked at by modern science as handicapped because of the difficulty the former faces in passing through different modern pharmacognosy analysis methods to generate sufficient data on its efficiency in therapy. The difficulties faced in standardizing multicomponent drugs still
prevails, even though they have found a place in world market as dietary supplements. However plant derived phytochemicals saw a sharp growth in their use in pharmaceuticals and carved out their own niche in modern health care regime all over world. Day by day new herbal compounds having therapeutic values are isolated from diverse plant resources. It is to be noted that about eighty percent of existing higher plants are yet to be surveyed for their therapeutic values.

1.9 Challenges and measures

1.9.1 Quality control of herbal products

Plant based drugs are getting popular day-by-day. Traditional medicinal practices, based on herbal products, already exist in different parts of world especially among rural populations in developing world and remain highly diverse in their methods and practices. This is absolutely true in the case of India; diverse medicinal practices such as Ayurveda, Unani, Siddha etc. have a number of regional variations and use diverse and unusual herbal combinations. Prevalence of the use of substitutes for certain herbs is becoming a matter of concern. As this field is witnessing rapid commercialization, allegations of adulterations are unearthed frequently. Active compounds present in the herbal preparations individually or together responsible for desired therapeutic effect which could be adversely affected by any deterioration in quality of the raw materials or due to any compromises in preparation and quality control stages. In this respect quality control has a major role to play to alleviate many such concerns. It should be noted that phytochemical analysis of the herbal samples to be conducted in several steps. The plant should be identified with the help of a taxonomist. Extracts are to be prepared using suitable solvents and subjected to purification processes of multiple stages. Purity and concentration of constituents of the preparations can be analyzed via modern analytical methods such as High Pressure Thin Layer Chromatography (HPTLC), Gas Chromatography (GC) and Mass Spectrophotometry (MS) (Watson, 1999).
Herbal drug industry still at large depended upon traditional plant collectors who gather medicinal plants from forests, fields, country sides etc. and chances of contaminations are very high during this phase. Presence of contaminants such as microbes, radioactive substances, pesticides and aflatoxin like materials are to be determined. However the deterioration of quality of herbal drugs due to prevalence of adultery is a very serious issue which threatens the credibility of traditional medicinal practices such as Ayurveda. To face such challenges modern analytical methods should come in handy. Profiling of phytochemicals each medicinal plant and the proportional presence of such active components in the drugs prepared from medicinal plants can be prepared as data base and can be used as standard references to detect adulteration or compromises in established procedures in drug preparation. Difference in the season of cultivation, collection, and storage of plants has impacted on their chemical composition and the availability of active compounds. Rather than collecting directly from wild, growing the medicinal plants in uniform conditions can have more desirable outcomes on quality and safety of drugs produced (Eskinazi et al.1999). Lack of uniform standard procedures and strict guidelines acceptable throughout the world and unavailability of several reference compounds which is to be used as standards in quality checking processes also cause hindrances in the progress of the herbal drug industry (Robersts et al.,1997). Huge disparities in the availability of pharmacopeial monographs of medicinal plants cause challenges in shaping quality standard checks for herbal drugs. Principles of chromatographic fingerprinting and photo equivalence come in handy to find remedies for such hitches. Governments should come out with stringent laws and guidelines compliant with frame works proposed by organizations such as the Food and Drug Administration of US and International Conference on Harmonization. WHO Model List of Essential Drugs provides a set of procedures to develop official standards in each nation considering existing legislations and market conditions (WHO, 1979; WHO, 1980).
1.9.2 Toxicity and Adverse Effects of final products
Prevalent use of herbal drugs without conducting proper toxicity studies has invite larger debates. Most of the developing nations, through frequently depending on herbal drugs for health care, are not only notorious for lack of any; efforts were seldom taken to retain or update any data on the side effects of such drugs. There is a misnomer that as herbal drugs are natural, they are safe (Jowell, 1999). However, what modern science experienced in several instances is counter to this argument. Cultural influence also plays very significant role in in the attitude of people of several third world countries; they are ready to blindly believe and follow prescriptions by traditional practitioners without considering the side effects of such drugs. Severity of adversity would be very high on high risk groups such as children, pregnant women and elderly people and such instances could also turn out to be life threatening. Those who take medication for chronic ailments such as diabetes, depression, hypertension, hypercholesterolemia, cardiac failure etc. should be extra cautious before taking any herbal drugs (Moore and Adler, 2000; Lopez et al., 1995; D’Arcy, 1991). Compromises on the good manufacturing practices perhaps happen to be the greatest threat to safety of herbal drugs as this may lead into the formation or unintentional addition of toxicants. In rare cases poisonous herbs are used in some traditional medicinal practices and it has to be mandated that authorized persons with adequate experiences only should handle such materials (Winston and Nvwoti, 1992). Presence of heavy metals in several drug preparations from Asian countries including India resulted in rejection of such drugs in US and several western countries (Shaw et al., 1997). This scenario persuades Indian government to have a rethink and consequently Ayush has introduced guidelines to be followed by all herbal medicines intended to be exported.

1.9.3 Threat of adulteration during different stages of production
Ever increasing demand for herbal drugs, stiff competition for raw materials, unavailability of several rare medicinal plants due to overexploitation and habitat depletion have paved way for unhealthy practices of adulteration. In
such cases genuine herbal products or ingredients are partially or fully substituted with inferior substances which could be natural or artificial (Mukherjee, 2002). It has to be emphasized that these inferior substitutes lack the active ingredients which provide therapeutic property to a particular herbal drug and may contain compounds harmful to human body. Such toxic substances, together with microbial and chemical contaminants present in raw materials, possibly cause adversarial effect on human health instead of providing therapeutic effect. There are frequent reports on renal failure of persons who frequently use herbal drugs of spurious quality (Nelson, 1995). In Canada, about seventy people were forced to undergo renal transplantation or dialysis when they got affected by interstitial fibrosis in kidney, as they consumed herbal preparations for body slimming treatment, containing wrong species of plants (WHO, 2002).

1.9.4 Reproducibility of Biological Activity of Herbal Extracts
According to Cordell (2000) about forty percent of plant extracts lack reproducibility of their activity. There are several factors which cause such a difficult scenario. The biochemical profile of same species may vary according to the season and locality of collection. Different varieties of same plants, in several occasions, have shown slightly different chemical profile and laxity in proper evaluation by considering such factors could lead to the production of substandard drugs. Synergistic activities of several compounds in such undesired conditions could result causing adverse reactions in the body of the consumer. There are arguments for incorporating advanced genetic marker tests together with modern biochemical analysis for identification of plant materials in accurate manner, especially when the whole plant is not unavailable for the verification of the taxonomist. DNA can be isolated from any parts of the plant and with suitable markers DNA fingerprinting analysis can be conducted to establish the identity of plant material.

1.9.5 New opportunities in medicinal plant culture
Plant science has grown in such a pace to develop efficient methods to cultivate food crops in surplus so that food can be provided cheap for those
who are in need. New varieties of plantation crops are developed to suit the needs of industry. However the attention of researchers, on scientific cultivation of medicinal herbs in large scale, was laid only in recent time. Ever growing herbal drug industry needed medicinal plants which are uniform in genetic makeup and chemical profile. This would ensure reproducibility of the therapeutic properties as well. Mediculture, which emphasize on scientific cultivation of medicinal plants, could be the most suitable response to the need of the hour.

1.10 Tissue culture propagation of Medicinal plants
Modern tissue culture techniques have the potential to facilitate the needs of the natural medicinal product industry. Ninety percent of herbal plant needs are met from forests, of which seventy percent are collected by destructive harvesting due to the demand for different parts of plants such as leaf, root, stem bark, seed, wood, whole plant etc. (Ved et al., 1998). Unfortunately only about twenty percent of the demands are met through large scale cultivation. Furthermore, getting the formulations standardized is quite a challenge unless there is uniformity in the chemical constituents of the plant material. These challenges could be overcome by propagating medicinal plants via tissue culture (Chaturvedi, 2007). Clonal multiplication through tissue culture facilitates preservation and propagation of true gene pool of plants with desired characteristic features. Likewise, the in situ production of desired compounds can be further enhanced in controlled environment with suitable tissue culture techniques.

1.11 Dasamula Plants
Ayurveda, the science of life, has brought true health and wellness to millions of individuals throughout the ages by suggesting simple changes in their daily living practices. Integrating just a few of such proven systems into lifestyle can bring about radical changes in an individual’s life. The natural ingredients of herbs help in bringing “Arogya”, the healthy body and mind,
Table 1.1. List of Dasamula plants

<table>
<thead>
<tr>
<th>BOTANICAL NAME</th>
<th>FAMILY</th>
<th>SANSKRIT</th>
<th>MALAYALAM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Desmodium gangeticum</em> DC</td>
<td>Fabaceae</td>
<td>Prishnaparni</td>
<td>Orila</td>
</tr>
<tr>
<td><em>Pseudarthria viscida</em> L</td>
<td>Fabaceae</td>
<td>Salaparni</td>
<td>Moovila</td>
</tr>
<tr>
<td><em>Solanum xanthocarpum</em> Schard and Wendle</td>
<td>Solanaceae</td>
<td>Kantakari</td>
<td>Velvazhuthana</td>
</tr>
<tr>
<td><em>Solanum indicum</em> L</td>
<td>Solanaceae</td>
<td>Brihati</td>
<td>Cheruvazhuthina</td>
</tr>
<tr>
<td><em>Tribulus terrestris</em> L</td>
<td>Zygophyllaceae</td>
<td>Gokshura</td>
<td>Njerinjil</td>
</tr>
<tr>
<td><em>Aegle marmelos</em> Correa</td>
<td>Rutaceae</td>
<td>Bilva</td>
<td>Koovalam</td>
</tr>
<tr>
<td><em>Premna serratifolia</em> L</td>
<td>Verbanaceae</td>
<td>Angnimantha</td>
<td>Munzja</td>
</tr>
<tr>
<td><em>Oroxylum indicum</em> Vent</td>
<td>Bignoniaceae</td>
<td>Shyonaka</td>
<td>Payyazhantha</td>
</tr>
<tr>
<td><em>Gmelina arborea</em> L</td>
<td>Verbanaceae</td>
<td>Kashmiri</td>
<td>Kumbil</td>
</tr>
<tr>
<td><em>Stereospermum suaveolens</em> DC</td>
<td>Bignoniaceae</td>
<td>Patala</td>
<td>Pathiri</td>
</tr>
</tbody>
</table>
Figure 1.4. Dasamula plants

Oroxylum indicum  

Stereospermum suaveolens  

Gmelina arborea  

Aegle marmelos
Figure 1.5. Dasamula plants

*Solanum xanthocarpum*  
*Desmodium gangeticum*

*Solanum indicum*  
*Tribulus terrestris*
Figure 1.6. Dasamula plants selected for the present study

*Pseudarthria viscid*a

*Premna serratifolia*
to human being. Renowned Ayurvedic texts such as Charaka Samhita, Ashtanga Hridayam, Sushruta Samhita etc. guide as through the rich knowledge we have on medicinal plants. In Ayurveda, Dasamula comprises of a combination of ten medicinal plants and their roots, essential in preparing different formulations used to treat heart ailments, pain, arthritis, fever, hysteria, rheumatism and asthma (Sharma, 2006; Hemlal and Subban, 2012). They are known to have rejuvenating and immuno-modulatory effects; also reliable in treating urinary calculi.

Dasamula plants comprised of roots of five large trees or Bruhat Panchamula and five minor herbs or Laghu Panchamula. It is to be noted that roots are not the only parts for which Dasamula plants are depended upon. Other plant parts like root bark, leaf, flower, seeds etc. are also in use for preparation of different formulations. Some of the most sought after Dasamula formulations comprise Dasamulakwatha, Dasamularishta, Dasamula satpalaghrutha, Dasamula ghrita, Dasamularasayanam, Dasamula Kaduthrayamkashayam. Dasamulakwatha is an aqueous concentrate of Dasamula plants. Dasamularishta is a fermented preparation in which alcoholic content is retained even during consumption. On the other hand Dasamula satpalaghrutha and Dasamula ghrita are crude drugs in semi-solid forms (Ayurvedic Formulary of India, 1978). Most of the Dasamula preparations are intended to pacify Vatha and Kapha imbalances and consequences.

*Desmodium gangeticum* DC is an undershrub, found in different parts of India. Several active compounds were obtained from the roots of this plant which include gangetinin, pterocarpanoids, gangetin, and desmodin (Purushothaman *et al.*, 1971; 1975). Stem and leaves contain Indole alkaloids (Ghosal and Banerjee, 1968). The root extract was used against inflammation, fevers, urinary discharges, piles asthma, bronchitis, thirst, vomiting, dysentery and biliousness. Root extracts, in combination with other drugs, used for snake bite and scorpion sting.
**Solanum xanthocarpum** Schard and Wendle is a very prickly, diffuse, bright-green, perennial herb and grows throughout India, Sri Lanka, South Asia and Tropical Australia. The major chemicals present in its berries include solasonine, solamargine and solasurine, caffeic acid and solasodine (Siddiqui et al., 1983). The other phytochemicals isolated are galactoside of β-sitosterol, apigenin, quercetin etc. (Dubey and Gupta, 1978). Berries are in use to treat inflammations, chronic bronchitis, different fevers, muscular pains, bladder stone, dysuria, asthma and sterility in women. The seeds used to treat boils, scabies, and asthma cough. Root is found effective against urinary concretions, asthma, different fevers, bronchitis, pains, piles, thirst, heart diseases etc. Gulcoalkaloid from *S. xanthocarpum* inhibited the anaphylactic bronchoconstrictor response in sensitized guinea pig lungs (Gupta et al., 1978). *S. xanthocarpum* was also effective in treating bronchial asthma and non-specific cough (Bector and Puri, 1971).

**Solanum indiucm** L. is an under-shrub found in warmer regions of India. The chemical examinations of the fruits and stems showed presence of alkaloids and saponins (Varshney and Aftab, 1971). It is utilized in the treatment of leucoderma, fever, asthma, pain, bronchitis and vomiting. It is also employed in difficult parturition and in toothache. Juice of the leaves along with ginger is effective in profuse vomiting conditions. The root and fruits are prescribed for treating snake bite (Kirthikar and Basu, 1974). The major compounds found to be present in *S. indiucm* are diosgenin, β-sitosterol, lanosterol, solasonine, solamargin and solasodine (Rathore et al., 1978).

**Tribulus terrestris** L. is a herb, found mostly in tropical and warm temperate regions of India. Diosgenin, ruscogenin, gitogenin and 25-D-spirostan-3, 5-diene were isolated form root extracts. Glycone and traces of arabinose were also isolated from this herb (De Kock et al., 1958). The dried fruit contain diastase, peroxidase and traces of glucosides. Harmine is another compound reported from its seeds. The leaves reported with presence of quercetin, D-
glucose and L-rhamnose (Panova et al., 1970). Fruits are regarded as cooling, diuretic, tonic and aphrodisiac and they are used in painful urination and related urinary disorders. An infusion made from the fruit has been found very useful as a diuretic in gout, renal dysfunctions and gravel. The roots are cooling, tonic, fattening and aphrodisiac, improve appetite, alleviate burning sensation and reduce inflammation (Wealth of India, 1954).

*Aegle marmelos* Correa is found in the sub-Himalayan tract, in Central and South India and also in Burma and Ceylon. Traditionally it is considered as a sacred tree. The phytochemical screening of the root, stem, leaves and fruits revealed the presence of carbohydrates, glycosides, tannins, sterols and flavonoids. Stem, leaves and fruits contain β-sitosterol, α and β-amyrin, marmin, umbelliferone, xanthotoxin, imperatorin and skimmianine (Govindachari et al., 1983). Coumarins such as marmelosin, marmesin, imperatorin, marmelide, methyl ether, xanthotoxol, scoparone, scopoletin, umbelliferone, alloimperatorin and marmenol were also reported from *A. marmelos* (Farooq, 2005; Kokate et al., 2002). Lupeol, rutin and marmesin were isolated from leaves. The effect of the root extract showed appreciable antimicrobial activity against *Vibrio cholerae*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Candida albicans*, *Aspergillus fumigatus* etc. (Pitre et al., 1987).

*Oroxylum indicum* Vent, a small tree, found mainly in India. Chen et al. (2005) isolated four flavonoids, baicalein-7-o-diglucoside, baicalein-7-o-glucoside, baicalein and chrysin, from seeds of *O. indicum*. Oroxylene, pinostrobin and stigmast-7-en-3-ol were isolated from bark (Luitel et al., 2010). The root bark is cooling, aphrodisiac, tonic and appetizer and useful in diarrhea and dysentery. It is very useful in treating biliousness, fevers, bronchitis, intestinal worms, vomiting, dysentery, leucoderma, asthma etc.

*Gmelina arborea* L. is a moderately sized, deciduous tree found commonly in South Asia and Indo-China regions. Important phytochemicals isolated from
it includes gmelofuran-a, gmelinol, umbelliferone, clutylferulate, arboreol, gmelofuran, apigenin, luteolin, quercetin, paulownin (Shafaq et al., 2010). Root preparations can play a major role as laxative, anthelmintic and improves the appetite. It is used to treat hallucinations, thirst, piles and abdominal pain, burning sensations, fevers and urinary discharges. Pulverized roots are applied locally in gout. A decoction, prepared with roots and bark is taken internally for snake bite.

*Stereospermum suaveolens* DC is a deciduous tree and is found mainly in the moist parts of India. Palmitic acid, stearic acid, oleic acid lapachol and β-sitosterol were detected in root extracts (Tandon et al.,1969). Sitosterol and betulin were isolated from the leaves and stem bark (Rastogi and Mehrotra, 1991). From fresh leaves 5, 7, 3’, 4’-tetrahydroxy-6-o-β-glucosyl flavones and 6-o-β-D-glucosyl-scuellarein were isolated (Nair et al., 1988). The root is useful in treating ‘kapha’ and ‘vata’ imbalances, inflammations, vomiting, asthma, fevers, blood disorders, thirst and loss of taste. The infusion of bark is considered diuretic, tonic and cooling.

Apart from the eight medicinal plants discussed above, there are two more medicinal plants as Dasamula namely *Pseudarthria viscida* L. and *Premna serratifolia* L. (*Premna integrifiolia* L.). Extracts of these two plants were considered for the present study.

### 1.11.1 Dasamula plants *Pseudarthria viscida* L. and *Premna serratifolia* L. selected for the present study

*Pseudarthria viscida* L. Wight & Arnis of Fabaceae family is an important Laghu Panchamula among Dasamula plants. *P. viscida* is a perennial viscid undershrub with slender branches and trifoliate leaves. Rapid decline in forest cover is threatening the existence of this species and hence considered as near threatened (Natarajan et al., 2004). The plant is found in peninsular India and Srilanka. Dashamularistam, Mahanarayana Talia and , Anutailam
Figure 1.3. Various ailments on which Lupeol was found effective

- Inflammation
- Renal diseases
- Arthritis
- Cardiovascular diseases
- Cancer
- Liver toxicity
- Microbial attack
- Diabetes
have *P. viscida* as one of the ingredient (Sivarajan and Balchandran, 1994; Kirtikar and Basu, 1987). The roots are used as astringent, sweet, bitter, emollient, digestive, anthelmintic, anti-inflammatory, diuretic, cardio-tonic, aphrodisiac, febrifuge, rejuvenating and tonic. Its therapeutic effect can be witnessed while treating vitiated conditions of vata and pitta, cough, bronchitis, asthma, tuberculosis, dyspepsia, diarrhoea, fever, food poisoning, vomiting and general debility. To treat rheumatism, asthma, heart diseases and piles, root decoction is used. In case of headache and hemicranias, the root juice is given as a nasal drop (Warrier *et al.*, 1996). Its root was reported with both antioxidant and antiulcer properties (Gincy and Sasikumar, 2007; Babu *et al.*, 2008). Ethanol extract of *P. viscida* was found reliable in treating castor oil induced diarrhoea of Wistar albino rats in dose dependent manner (Vijayabaskaran *et al.*, 2010). Aqueous extract of *P. viscida* was effective in treating streptozotocin induced diabetes in rats (Rajendran *et al.*, 2012). Stem, root and leaf extracts of *P. viscida* were found to be acting against several pathogens which cause destruction to several food crops (Deepa *et al.*, 2004). Extracts of areal parts and roots were useful in treating inflammation in Wistar rats (Saravanan *et al.*, 2010; Khatale *et al.*, 2011).

Studies by Rajendran *et al.* (2007) could prove the presence of lupeol, a triterpionid in *P. viscida* root extracts. Gas Chromatography-Mass Spectrometry analysis of methanolic extract of *P. viscida* root showed the presence of active constituents such as 3-O-Methyl-d-glucose, n-Hexadecanoic acid, Oleic acid and 9, 12-Octadecanoic acid (Muthukrishnan and Thinakaran, 2012). Other active compounds isolated from roots include Butane-1,1 Diethoxy-3-methyl, d-Mannitol-1-decyl sulfonyl, n-Hexadecanoicacid, Oxirane tetra decyl, Tetradecanoic acid, Undecanoic acid and phenolic compounds such as ferulic acid, rutin, quercetin, gallic acid, caffeic acid etc. Phospholipase A2 play significant role in the formation of inflammatory intermediaries. All the above said compounds were subjected to comparative docking analysis for Phospholipase A2 and 70-80% of ligands
were found docking in correct mode (Muthukrishnan and Thinakaran, 2011). These findings once again emphasize the ameliorative role *P. viscida* extracts can play in inflammatory conditions. Alkaloid fraction separated from aqueous extract of *P. viscida* root was found cytotoxic against HeLa (Human cervical cancer line) and NIH 3T3 (mouse embryonic fibroblasts) cell lines in MTT assay (Hemlal *et al.*, 2011).

*Premna serratifolia* L. (*Premna integrifolia* L.) is widely found throughout the tropical Asia. Stem bark contains alkaloid premnin, which decreases contraction forces of heart and produces dilation of pupils (Chopra *et al.*, 1956). *P. serratifolia* has tremendous potential in treating of vata as well as kapha conditions, flatulent, cough, asthma, leprosy, skin disorders, inflammations, dyspepsia, constipation, bronchitis, cardiac disorders, different fevers, diabetics, neuralgia and anorexia (Sudo *et al.*, 2000). Root, leaf, and bark of *P. serratifolia* have therapeutic values. The roots are astringent, sweet thermogenic, anti-inflammatory, cardiotonic, digestive, stomachic, carminative, antibacterial and tonic. Bark juice has the potential to treat malarial infection (Chopra, 1969). Traditionally the leaves along with pepper are administered in the treatment of cold and fever. Decoction is used in treating ailments related to inflammation and arthritis (Rathore *et al.*, 1977). *P. serratifolia* is known to have antibiotic and antihyperglycemic properties (Natkarni, 1976) and is found to be useful in preventing blood coagulation (Gopal and Purushothaman, 1984). Stem bark and stem wood extracts were found to be reliable as a cardioprotector in animal models (Rajendran *et al.*, 2008).