Review of literature

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine (Owolabi et al., 2007)

*A. racemosus* willd. (Liliaceae) is an ascending, spinous, much branched, perennial climber. It is indigenous to tropical and subtropical India and quite commonly met within most states from sea level to about 1, 4000m elevation (Quality standards of Indian medicinal plants, Vol 1: 29-33). *A. racemosus* is considered to be medicinally important because of the presence of major constituents like steroidal saponins (Satavarin I-IV) and sapogenin in the roots. The root extract of *A. racemosus* has been reported to exhibit a wide range of cytotoxic, antioxidant, tyrosinase inhibitory and antimicrobial activities (Potduang et al., 2008). Satavarin V, a new steroidal saponin was also isolated from the roots by RP-HPLC and its structure was determined by 1D and 2D NMR studies (Hayes et al., 2006). Other active compounds such as quercetin, rutin and hyperoside are found in flowers and fruits while diosgenin and quercetin-3 glucuronide are present in the leaves (Anonymous, CSIR. 1987). Several methods of HPLC with ELS detector are currently in use for the analysis of marker compounds such as steroidal saponin and sapogenin. The most commonly protocols include a HPTLC (Jain and Agrawal, 2009 and Wang et al., 2011) and HPLC with ELS detector (Patricia et al., 2006, Kite et al., 2007 and Penumajji et al., 2010). One of the general problems in the detection of saponins is the lack of ultraviolet (UV) chromophores and low signal
with UV detection. For this reason, ELSD is used as an alternative and effective tool for the identification of the compound which does not contain strong chromophores. Due to lack of chromophore groups sarsasapogenin, a saturated steroidal sapogenin does not absorb in the λ UV range hence, Negi et al., (2011) has developed the RP-HPLC method for the investigation of derivatized sapogenin.

The genus Vitex belongs to the family Verbenaceae. It includes 80 genera and about 800 species. Vitex is a genus of trees and shrubs widely distributed in the tropic and warm region. V. negundo Linn is a shrub quite prolific in India, occurring up to an altitude of about 1500 m. It is one of the common plants used in traditional medicine and reported to have a number of biological activities (Cushnie and Lamb, 2005). Flavonoids, iridoids, terpenes and steroids are the major classes of compounds isolated from V. negundo (Kumar et al., 2010). A large number of compounds with varying structural classifications including glucononitol, camphene, α-pinene, citral, β-carphylene, cascin,orientin,isorientin,corymbosin, flavonoid glycosides, diterpenoid, triterpenoids, long chain unsaturated fatty acids, iridoid glycosides and lignin have been reported from the leaves, twigs, seeds and roots of  V. negundo (Sharma et al., 2009). Decoction of the leaves of V. negundo is used as a bath in the puerperal state of women in India. Also the dried leaves of V. negundo are smoked for the relief of headache and catarrh (Kirtikar and Basu, 1980).The leaf extract of V. negundo has been reported to exhibit a wide range of pharmacological activities. The methanolic extract of V. negundo had been found to exhibit very potent antifeedent, anti-inflammatory, analgesic and anti convulsant activities (Sharma et al., 2009). V. negundo is used in several commercial formulations and in the Ayurvedic System of Medicine.
leaves of *V. negundo* is used as a drug of choice for pain, inflammation and related diseases (Tiwari et al., 2012, Lokhande and Verma, 2009). *Vitex trifolia* is a shrub or small tree, its leaves contains flavonoids, sterols and terpenoids. The leaves are considered useful as an external application to all rheumatic pain, sprains etc. The petroleum and ethanol extracts of leaves of *V. trifolia* exhibited moderate inhibiting activity against most of the tested Gram positive and Gram negative bacteria (Sharma et al., 2009, Hossain et al., 2001). Significant hepatoprotective activity of aqueous and ethanol extracts of *V. trifolia* in carbon tetrachloride induced liver damage of male wistar rats was reported. (Manjunatha et al., 2009). Genus *Vitex* is a rich source of iridiods. Negundoside (NGN), an iridoid O-glycoside isolated from *V. negundo* was reported to protect human liver cells against calcium mediated toxicity induced by carbon tetrachloride via inhibition of lipid peroxidation, followed by an improved intracellular calcium homeostasis and inhibition of Ca$^{2+}$ dependent proteases (Tasduq et al., 2008). Agnuside (AGN), another iridoid glycoside, composed of aucubin and p-hydroxybenzoic acid, is a chemotaxonomic marker of the genus *Vitex* and has been isolated from *V. negundo*, *V. cymosa*, *V. agnus castus* (Sharma et al., 2009, Boros and Stermitz, 1990 Hansel et al., 1965). p-hydroxy benzoic acid (PHBA) is an another compound present in the *Vitex* species. Agnuside is reported to be linked with the occurrence of PHBA. PHBA has a high absorbance in UV region (Hoberg et al., 2000). NGN and AGN led to increase in osteoblast differentiation and mineralization in *in vitro*, supporting the use of *V.negundo* in traditional medicine (Kumar et al., 2010). Although, all parts of *V. negundo* and *V.trifolia* are used in traditional system of medicines, the leaves are the most potent for medicinal uses. A lot of research work has been reported for isolation,
characterization and pharmacological activities of biologically active molecules from the Vitex species available in India and worldwide, only a few validated HPLC methods has been reported for the simultaneous identification and quantification of PHBA and AGN in Vitex species. Hoberg et al., (2000) reported a HPLC method for the simultaneous determination of PHBA and AGN with a total run time of 23 minutes using a gradient elution mode with acetonitrile and O- phosphoric acid (0.5%) in V. Agni- Casti Fructus. Lokhande et al. also reported a HPTLC method for quantification of NGN in Vitex negundo Linn. leaf. Sing et al., (2011) reported a HPLC method for the determination of negundoside, an irodiod from leaves of V. negundo. Recently, Tiwari et al., (2012) reported a validated high performance thin layer chromatography method for simultaneous quantification NGN, AGN and 6’-p-hydroxy benzoyl mussaenosidic acid in V.trifolia and V. negundo.

T. chebula belongs to the family Combretaceae and is found throughout India chiefly in deciduous forests. It occurs abundantly in North India and range extends southwards at 300 to 900 m attitude (Aiyer and Kolamal, 1963). It is a popular traditional medicine not only used in India but also in other countries of Asia and Africa. T. chebula has long been used because a number of phytochemical constituents have been found to be associated with the plant extract that include mainly the different types of chebulic acid, gallic acid, ellagic acid, tannic acid, amino acids, flavonoids like luteolin, rutins and quercetin etc. These compounds found to be responsible for many of pharmacological activities (Surya Prakash et al., 2012). It is extensively used in unani, ayurveda and homeopathic medicine. It is used for the treatment of number of diseases like cancer, paralysis, cardiovascular diseases, ulcers, leprosy, arthritis, gout, epilepsy
etc. It has been reported as antioxidant (Suchalata S et al., 2009), antidiabetic (Rao N.K et al., 2006), antibacterial (Kannan P et al., 2009), antiviral (Kim TG et al., 2001), antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc. It is used extensively in the preparation of many Ayurvedic formulations for infectious diseases such as chronic ulcers, leucorrhoea, pyorrhoea and fungal infections of the skin. It is used to prevent aging and impart longevity, immunity (Aher et al., 2011) and body resistance against disease. It has beneficial effect on all the tissues. T. bellerica plant is also common throughout India in the plains and lower hills, chiefly in the deciduous forests, at 900 tannin (Wealth of India, Raw Materials, 1956). A reversed-phase preparative HPLC method with UV spectrophotometric detection has been developed by Mahajan and Pai, 2010 for the simultaneous isolation of eight hydrolysable tannins from dried fruits of T. chebula. Although, all parts of T. chebula and T. bellerica are used in traditional system of medicines, the fruits are the most potent for medicinal uses.

*Emblica officinalis* (Fam: Euphorbaceae) is a deciduous tree nearly 12 m tall. Leaves small narrowly linear and resemble pinnate leaves. Flowers are fleshy, globose and pale yellow and blooming in month of March to continue up to May. It is distributed throughout tropical and sub tropical India, chiefly in the dry deciduous forest ascending up to 1350 m in the Himalaya. It is a rich source of vitamin C. seeds contain fixed oil, phosphatides and essential oil. Fruits and leaves contain tannins, polyphenolic compounds, corialgin and ellagic acid. It is diuretic, laxative cardiac, astringent and liver tonic (Medicinal Plants of India- an Encyclopedic, 2003, Sharma, R. 96- 97). The fruits of *E. officinalis* have been reported to exhibit a wide range of pharmacological activities.
It had been found to exhibit potent antidiabetic, antioxidant, anti-inflammatory (Zhao et al., 1999), immunomodulatory (Srikumar et al., 2005), antipyretic, analgesic (Perianayagam, et al., 2004), antiulcer (Bandyopadhyay et al., 2000) cytoprotective (Biswas et al., 1999) antitussive, hypolipidemic, hepatoprotective (Jose and Kuttan, 2000, Bhattacharya et al., 2000) and gastroprotective activity (Nain et al., 2012, Manjumdar et al., 2011, Gopa et al., 2012) It is also reported to useful in the treatment of cancer (Rajeshkumar et al., 2001) diabetes, liver treatment, heart disease, ulcer, anemia and various other diseases (Khan, 2009). Four hydrolysable tannins, the novel emblicanins A and B, and the known punigluconin and pedunculagin, were isolated from fresh pericarp of fruits (Ghosal et al., 1996), whereas, three other ester glycosides, named phyllaemblicins A, B and C, methyl ester of a highly oxygenated norbisabolane and phyllaemblic acid, were isolated and reported from the roots of P. emblica, along with 15 tannins and related compounds (Zhang et al., 2000). The fruit contains a series of diterpenes referred to as gibberellins, as well as the triterpenes lupeol, flavonoids, polyphenols, phyllantine and zeatin alkaloids and a number of benzenoids, including amlaic acid, corilagin, ellagic acid, 3-6-di-O-galloyl-glucose, ethyl gallate, 1,6-di-O-galloyl-β-D-glucose, 1-di-O-galloyl-β-D-glucose, putranjivain A, digallic acid, phyllembic acid, embolicol and acactaric acid. Six new phenolic constituents, L-malic acid 2-O-, mucic acid 2-O-, mucic acid 1,4-lactone 2O-, 5-O-, 3-O-, and 3,5-di-O- gallates, were isolated from the fruit juice of P. emblica together with their methyl esters and their structures were determined by spectral and chemical methods (Zhang et al., 2001) The fruit also contains considerably higher concentration of most minerals like calcium. Zinc, copper, manganese iron (Dahiya et al., 1992) and amino acids like
glutamic acid, proline, aspartic acid, alanine, and lysine, the dried fruits contain gallic acid, tannin, sugar, gum, albumin, crude cellulose, mineral matter and moisture, etc. A sensitive, rapid, reverse phase HPLC method was reported for analysis of Triphala curma using gallic acid, ellagic acid, chebulagic acid and chebulinic acid as markers (Madhavi et al., 2010. Pawar et al., 2009). An activity-directed fractionation and purification process was used by Li et al., 2008 to identify the antioxidative components of *emblica* fruit. Dried fruit of *emblica* was extracted with methanol and then partitioned by ether, ethyl acetate, butanol and water. The ethyl acetate fraction showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity among four fractions. Six compounds were identified to be geraniin, quercetin 3- beta -D-glucopyranoside, kaempferol 3- beta -D-glucopyranoside, isocorilagin, quercetin, and kaempferol, by spectral methods, $^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible (UV-Vis) spectrophotometry and mass spectroscopy (MS).

Medicinal plants provide abundant natural antioxidants which are virtually important for human health. Oxidants and antioxidants in humans are maintained in balance in a normal physiological state. However, the overproduction of oxidants in certain conditions may cause oxidative stress leading to oxidative damage to bio molecules and cells (Temple, 2000, Willett, 2002). Reactive oxygen species (ROS) are formed during normal cellular metabolism but when present in high concentration they become toxic. Oxidative stress or excessive production of ROS is being implicated in many diseases such as cancer, artherosclerosis, aging, diabetes etc (Finkel and Holbrook, 2000; Halliwell and Gutteridge, 1985). The potential targets for ROS in cells are membrane
lipids, DNA and proteins. Antioxidants are very important for human health. Natural antioxidants or other compounds such as vitamins, minerals or proteins can neutralize free radicals. Antioxidant supplementation is recommended to provide cellular protection from the deleterious effects of excessive reactive oxygen species concentration (Yanai et al. 2008). Antioxidants are our first line of defense against free radical damage and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoking, drugs, illness, stress, and even exercise can increase free radical exposure (Ablise et al., 2011). Free radical scavenger may prevent the oxidative stress by peroxidation, inhibiting free radicals and also by other mechanism they prevent diseases. In addition to endogenous antioxidant defense systems, consumption of dietary and plant-derived antioxidants appears to be a suitable alternative as the synthetic antioxidants are reported to be harmful for human health. Further, synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have restricted use in food because of their carcinogenic properties (Ito et al., 1986). Also , the use of the most potent synthetic food antioxidant tertiary butylated hydroquinone (TBHQ) is not permitted in Japan and Europe (Suja et al., 2005). Therefore, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years (Lobo et al. 2010). Medicinal plants contain substantial amounts phytochemicals with capacity to scavenge the free radical and can serve as a potential source of natural antioxidants (Kratchanova et al. 2010). The majority of antioxidant belongs to flavonoids, isoflavones, anthocyanins, coumarins, lignans, catechins and isocatechins class of phytochemicals.