People tend to rely on traditional and other forms of complementary and alternative medicine for chronic conditions which do not respond well to conventional or modern drug treatments. Among these most common are neurological disorders such as anxiety, pain and epilepsy (Spinella, 2001). Centuries before the advent of modern medicine, synthetic chemistry and the pharmaceutical industry, virtually all medicines came from plants (Agosta, 1997). These medicinal plants have been an important source for the discovery of novel bioactive compounds which served and continue to serve as lead molecules for the development of new drugs (Cragg et al., 1997). Aspirin, atropine, scopolamine, taxol, theophylline, d-tubocurarine, vincristine and vinblastine are a few examples of such invaluable therapeutic tools for today’s physicians (Cox et al., 1994; Jones, 1996).

Stress has become a part of the modern world and lifestyles. Persistent stress leads to anxiety. In small quantities stress and anxiety are good as they can motivate and help one be more productive but people with persistent stress feel anxious quite often and anxiety interferes in their daily lives and is a matter of concern. The term anxiety covers four aspects of experiences an individual may have: mental apprehension, physical tension, physical symptoms and dissociative anxiety (Healy, 2008). Anxiety disorder is divided into generalized anxiety disorder, phobic disorder, and panic disorder; each has its own characteristics and symptoms and they require different treatment (Gelder et al., 2005). The emotions present in anxiety disorders range from simple nervousness to bouts of terror (Barker, 2003). The different neurotransmitters proved for their role in stress are four biogenic amines i.e. two catechol amines (NE norepinephrine and DA dopamine), indoleamine (5-HT serotonin) and a quaternary amine (Ach), some other neurotransmitters e.g. amines like epinephrine and histamine, amino acids like GABA, glutamate, aspartate, glycine, taurine, tryptophan neuroactive peptides including those widely
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known as pituitary hormone releasing factors, certain prostaglandins, angiotensine, opiate, peptides like endorphins, enkhephalins etc. in brain have also been reported to be under the influence of stressor and with an additive role in the precipitation of various psychosomatic events of stress (Ganong and Lorenzen, 1967; Udupa, 1978; Solomon et al., 1985; Takada et al., 1996). Serotonin, an important brain neurotransmitter, is key in the regulation of appetite, mood, and melatonin production. The presence of serotonin in the brain is associated with a balanced emotional state. This is achieved in part by decreasing the activity of certain excitatory hormones, including dopamine and noradrenaline (Birdsall, 1998). These evidences suggest anxiety to be caused by dysfunction of one or more neurotransmitters and their receptors and have emerged to be a very important area of research.

Anxiety disorders are the most common class of neuropsychiatric disorders in USA (Kessler et al., 2005) and many other countries (Alonso and Lepine, 2007). The life time prevalence of panic attacks (a form of anxiety disorder) is around 7-9% in most countries and 1% alone in india with the prevalence of generalized anxiety disorder is very high i.e. 8.5% in the general population (WHO, 2001). Anxiety disorders affect 16.6% of population worldwide (Somers et al., 2006) and numerous efforts have been made to understand the pathophysiology of the disease and treatments. The strategies employed for treating these anxiety disorders are strongly influenced by local socio-economical factors and traditional practices. In more developed areas of the world, the most common approach for treating anxiety is pharmacotherapy (Cloos and Ferreira, 2009; Sheehan and Sheehan, 2007). More specifically, the most widely prescribed drugs for treating anxiety belong to benzodiazepines which are known to act through the GABAergic system (Dinan, 2006; Lader, 1984) but their side effects are prominent, including sedation, muscle relaxation, anterograde amnesia and physical dependence (Kaplan and Sadock,
2005). In more recent decades, busiprone which acts primarily through the serotonergic system is also being used (Dinan, 2006). Other pharmacological tools to treat anxiety disorders include monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and selective serotonin re-uptake inhibitors (SSRI) (Baldwin and Polkinghorn, 2005; Sheehan & Sheehan, 2007a).

Now, at the start of a new millennium, it is estimated by the World Health Organization that 80% of the world’s inhabitants must rely on traditional medicines for health care (Farnsworth et al., 1985). These traditional medicines are primarily plant-based. Even in the remaining population, natural products are important in health care. It is estimated that 25% of all prescriptions dispensed in the USA contained a plant extract or active ingredients derived from plants. It is also estimated that 74% of the 119 currently most important drugs contain active ingredients from plants used in traditional medicine (Newman et al., 2003). Another study of the most prescribed drugs in the USA indicated that a majority contained either a natural product or a natural product was used in the synthesis or design of the drug (Cragg et al., 1997). All of these investigations demonstrate the importance of natural products in drug discovery.

Herbal remedies (HRs) constitute a strong component of traditional, complementary and alternative medicine. In most developing countries, HRs play a critical role in the management of various diseases owing to the challenges confronting the appropriate delivery of official health care to millions of people in remote and rural communities. In realization of the inherent value of HRs to primary health care and the fact that over three quarters of the world’s population rely mainly on plants for health care, the World Health Organization (WHO) has advocated for the proper identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies in medicare (Wambebe, 1998).
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Pharmacological and toxicological evaluations are therefore critical in drug and standardized phytomedicine development.

Plants, as a source of medicine, have been used throughout history for treatment of mood disorders (anxiety, depression, sleeplessness, and physiologically related conditions) (Schmidt et al., 2008). Today there are over 10 medicinal plants that are used commercially as regulated Natural Health Products (NHPs), or EU phytomedicines to treat mood disorders related to anxiety (Blumenthal et al., 2000). These are used in North America as over the counter medications by both the general public and diagnosed patients. There is now growing interest in these products among some physicians in North America because patient compliance is high and evidence of efficacy is available. The two most widely used botanicals are St-John’s Wort (SJW) (Hypericum perforatum) and Kava Kava (Piper methysticum) (Linde, 2009; Sarris et al., 2009). These medicinal plants have been well studied pharmacologically and phytochemically and there is extensive animal behavior and clinical research supporting their efficacy. Unfortunately, both of these NHPs have come under scrutiny for unrelated toxicology issues (Linde, 2009; Sarris and Kavanagh, 2009). SJW has recently been found to have potentially life threatening drug interactions with certain types of drugs, while Kava has been withdrawn from some markets due to idiosyncratic hepatotoxicity in a small number of individuals. Therefore, there is an opportunity and potential need to replace these NHPs. Thus, there is a need of robust anxiolytic compounds from medicinal plants that have lesser side effects and a more immediate onset of anxiolytic action than the currently available therapeutics. On the basis of these considerations, it was the purpose of this dissertation to explore medicinal potential and develop standardization of three selected medicinal plants i.e. Dalbergia sissoo Roxb., Citrus limon Linn. and Elaeocarpus sphaericus, used traditionally for their anxiolytic activity but poorly studied scientifically.
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*Dalbergia sissoo* Roxb., also called Indian Rosewood, belongs to the legume family (*Fabaceae*). Plants of the genus *Dalbergia* are medicinally important and have been used for the treatment of gonorrhoea, arthritis, and rheumatic pains. It has been reported in folk medicine and is used mainly as CNS stimulant, antibacterial, aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic (Anonymous, 1950; Nadkarni, 1982; Singh and Chaturvedi, 1966). Phytochemical examination of genus *Dalbergia* has provided a large number of compounds, which include flavonoids, furans, benzophenones, coumarins, styrenes and terpenoids (Salwa *et al.*, 2001; Chihiro, 2003; Ramireddy *et al.*, 2008; Rana and Kumar, 2011).

*Citrus limon* Linn. (Lemon) is an important member of *Citrus* genus belonging to family *Rutaceae*. In the traditional systems of medicine the fruit, peel, pulp and juice have been used for treating different disorders like peel as appetizer, cardiac tonic, elixir, hygienic, pulmonary sedative, stomachic and mood disorder (Arias and Laca, 2005). Pulp is antihelminthic, antilithic (Limyati *et al.*, 1998). The bark and root has been used as a febrifuge and the seeds as a vermifuge (Kirtikar and Basu, 1993). Best remedy for scurvy, used in dysentery and diarrhea (Pullaiah, 2006). The main chemical constituents in *Citrus limon* are carotenoids in pulp and peel, coumarins and psoralens in fruits, stem and root bark, flavonoids and alkaloids in fruit and leaves (Anonymous, 1992; Kawaii *et al.*, 2000).

*Elaeocarpus sphaericus* (*Elaeocarpus ganitrus*) commonly known as Rudraksha, belonging to family *Elaeocarpaceae*. The seeds, bark and leaves of Rudraksha, are used to cure various ailments like mental disorders, headache, fever, skin diseases, curing cough and breathing problems, treating stomach pain, liver problems, treat high blood pressure, heart diseases, cure small pox *etc* (Khare, 2004; Sakat *et al.*, 2009; Swami *et al.*, 2010). The main chemical
constituents in *Elaeocarpus sphaericus* are alkaloids, flavonoids, tannins and fatty acid (Johns *et al.*, 1970 and 1971; Chand *et al.*, 1977).

The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant, through additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process (Briskin, 2000). This fact has a basis in the sense that medicinal actions of plants are unique to particular plant species or groups, consistent with the concept that combinations of secondary metabolites in a particular plant are often taxonomically distinct (Wink, 1999).

Some plant products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules, or neurotransmitters and thus have beneficial medicinal effects on human due to similarities in their potential target sites (e.g. CNS, endocrine system, *etc.*) (Kaufman *et al.*, 1999). The presence of alkaloids, tannins, cardiac glycosides, steroids, terpenoids, flavonoids, anthraquinones, phlobatannins, reducing sugars, and saponins in the plants extracts as secondary metabolites are responsible for various biological activities. The presence of flavonoids, alkaloids, terpenoids and fatty acids in the plant extract have been reported to be responsible for anxiolytic and sedative effects observed in different plant extracts (Houghton, 1999; Dhawan *et al.*, 2001; Carlini, 2003).

The behavioural tests of anxiety are useful to better understand the potential activity in humans and the mechanism of action of the drugs. Behaviour can be both an event or a process and observable behaviours are the result of the integration of all of the processes ongoing in underlying organ systems, in interaction with the external social and physical environment. Animal models can allow the study of mechanisms of specific behaviours and their pathophysiology. It can aid in developing and predicting therapeutic responses to
pharmacological agents (Bourin et al., 2007). Ethologically based animal models of fear and anxiety attempt to approximate the natural conditions under which such emotional states are elicited. Ethological models of anxiety such as the Elevated Plus Maze (EPM) are more susceptible to climate/environmental changes, even though all experiments were carried out in the same experimental conditions. EPM is most popular behavioural tests for research on anxiety and frequently used mouse models of anxiety (Lister, 1987). There has also been the development of several derivatives of the EPM including the elevated T-maze, zero maze and the unstable elevated exposed plus maze (Jones, 2001), a recently established model of extreme anxiety in rats which has all four arms exposed and oscillated in the horizontal plane. But EPM has been widely used as a tool in the investigation of the psychological and neurochemical basis of anxiety, for screening anxiety-modulating drugs on mouse genotypes (Bourin, 1997; File, 2001; Holmes, 2001). The EPM is in the form of a ‘plus’ with two open elevated arms facing opposite to each other and separated by a central square and two arms of the same dimensions, but enclosed by walls.

Montgomery (1955) reported that rodents consistently spend greater time in the closed arms when placed in mazes comprising of open and closed arms. Avoidance of the open arm portrays a manifestation of fear and anxiety. Based on these assertions, the elevated plus-maze tests are reliable means of identifying selective anxiolytic effect of drugs. Handley and Mithani (1984) further demonstrated that rodents avoid the open arms while also reporting that open arm avoidance is reduced by diazepam (anxiolytic agent) and enhanced by picrotoxin (anxiogenic agent). The open arm – closed arm approach for screening for anxiolytic effect has worked well in identifying the anxiolytic potential of benzodiazepine/GABA_A receptor related agents while not being reliable in detecting anti-anxiety effects through unrelated mechanisms, e.g. 5-HT_{1A}
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partial agonists like buspirone (Rodgers et al., 1994).

Extraction is the main step for the recovery and isolation of bioactive phytochemicals from plant materials, before component analysis (Karimi and Jaafar, 2011). It is influenced by their chemical nature, the extraction method employed, sample particle size, as well as the presence of interfering substances. Additional steps may be called for if the removal of unwanted phenolics and non-phenolic substances such as waxes, fats, terpenes, and chlorophylls is of interest.

Liquid-liquid and solid-liquid extraction are the most commonly used procedures prior to analysis of polyphenolics and simple phenolics in natural plants. They are still the most widely used techniques, mainly because of their ease of use, efficiency, and wide-ranging applicability.

Commonly used extraction solvents are alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate. However, very polar phenolic acids (benzoic, cinnamic acids) could not be extracted completely with pure organic solvents, and mixtures of alcohol–water or acetone–water are recommended. Less polar solvents (dichloromethane, chloroform, hexane and benzene) are suitable for the extraction of nonpolar extraneous compounds (waxes, oils, sterols and chlorophyll) from the plant matrix. Other factors, such as pH, temperature, sample to-solvent volume ratio, and the number and time intervals of individual extraction steps, also play an important role in the extraction procedure. Extractions are, almost invariably, repeated two to three times and extracts are combined. Soxhlet extraction is frequently used to isolate step wise secondary metabolite from solid samples by using different solvents based on their increase in order of polarity (Sticher, 2008).

The crude extracts having biological activity are used as a source for isolation of active molecule(s) using different methods of purification. The extract is subjected to several rounds of fractionation to separate other molecules from bioactive molecule. The highly pure fraction with
potent biological activity is then subjected to structural analysis. One of the popular approaches is to use combination of methods of purification, such as HPLC separation and other chromatographic methods followed by determination of the structure by Mass spectroscopic analysis, Nuclear magnetic resonance (NMR), Infrared (IR) and X-ray crystallography. Majority of the biologically active natural products have been isolated using bioactivity-guided fractionation (Pezzuto et al., 1997; Sasidharan et al., 2011). In bioactivity-guided fractionation, the extract of an organism or a mixture of unknown molecules is fractionated and simultaneously biological activities of purified fractions are tested to determine the active fraction in each step of purification. In this process, extract of an organism having large number of molecules is initially separated into two or major parts based on their solubility in aqueous and organic solvents or a combination of organic and aqueous solvents. Then the bioactive sample is further purified into small fractions using chromatographic methods \textit{i.e.} HPLC, etc. Purified fractions in each step of purification are subjected to biological activity testing. This procedure is also useful to detect any modifications in the nature of bioactive component due to the purification, which may lead to the loss of its bioactivity (Cannell, 1998). This procedure is also useful to select and make changes in the process of purification to purify the active molecule without significant changes in its activity. The first two phases in elucidation of the structure of molecules are identification of functional groups and carbon skeleton. The main objective of spectroscopic analysis is to identify functional groups in the molecule and molecular fragments. In this, majority of structural correlations are obtained empirically by examining the spectra of known compounds. Mass spectroscopy is useful to calculate the accurate mass of small molecules, peptides and proteins etc. as well as to identify chemical entities in the structure of the molecule. NMR is used to give information on detailed environment of the nucleus and its relationship to its neighbors.
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In this, chemical shift of a proton resonance is a reflection of magnetic environment of the nucleus. IR spectrum is important to reveal details of functional groups and also interaction between functional groups in the molecule. However, structural conclusions are drawn from various methods using complementary information regarding the structure of the molecule. Use of two or more complementary methods of structural analysis provide valuable structural details necessary for definitive assessment of identity and to ensure a high degree of experimental confidence (Sasidharan et al., 2011).

The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance.
Citrus

Antioxidant activity
Antistress activity
Antibiotic activity
Antacid activity
Antimicrobial activity
Decrease bone loss
Antiallergic activity
Antianxiety activity
Antidote for poison
Analgesic & Anti-inflammatory activity
Antifeedent activity
Cardiovascular activity
Hypoglycemic activity

Fig. 1: Scheme for Biological activities of Citrus