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Natural sources have provided us with an excellent hunting ground for discovering newer therapeutically active moieties and plant kingdom is one of these sources for giving us natural drugs. The history of plants to be utilized as medicines is thousands of years old (Samuelsson, 2004). These plant materials initially took in the form of crude drugs such as poultices, teas, powders tinctures, and many other herbal formulations (Balick and Cox, 1997; Samuelsson, 2004). From near past it has been discovered that properties of medicinal plants are due to its active chemical compounds. In the early 19th century morphine has been isolated as an active compound from opium (Kinghorn, 2001; Samuelsson, 2004). The discovery of drug from medicinal plants has been started from the era when the isolation of primarily drugs such as digitoxin, quinine, cocaine, and codeine has begun. Like morphine some are still in use for different purposes (Newman et al., 2000; Butler, 2004; Samuelsson, 2004). Now days, number of scientists have been working in order to isolate and characterize the pharmacologically active compounds from medicinal plants. Drug discovery techniques have been discovered and applying for the standardization of herbal medicines to obtain analytical marker compounds.

Drug discovery from medicinal plants are not simple but it has evolved to include numerous fields of inquiry and take advantages of different analytical procedures. The process initiated with a botanist especially with ethnobotanist, ethnopharmacologist, or plant ecologist that can easily collects and identifies their desired plant(s). Collection may involve those species with known biological activity which need to be study for their active compound(s) and new for isolation (e.g., traditionally used herbal remedies) or may also involve those taxa that have been collected randomly for a large screening purposes. It is also important to take care and respect the intellectual property rights of a given area, country where plant(s) of interest are collected (Baker et al., 1995).
Phytochemists are also called natural product chemists. These phytochemists after proper collection, identification and cleaning processes, make crude extracts from the selected parts of the plant materials, subject these crude extracts to biological screening of their desire assays, and commence the process of isolation and characterization of the active chemical compound(s). The whole processes are called bioassay-guided fractionation. Molecular biology is very important and taking essential part in drug discovery from medicinal plant. It determines and implements appropriate screening technique that directed towards physiologically relevant molecular targets.

2.1 ANXIETY

When researchers are observing maladaptive emotional behaviors in an individual, be it human or any other mammal, they are often confronted by an array of overlapping symptoms such as depression and anxiety (Lydiard et al., 1996). This is because of the fact that many neuropsychological diseases, most notably anxiety disorders, are in fact almost never expressed exclusively. This is due to the comorbid nature of these diseases or states of behavior.

The prevailing way to characterize anxiety is that its nature is linked to a state of chronic fear that persists in the absence of a direct threat (Coutinho et al., 2010). Although anxiety that occurs when subjected to an aversive external stimulus, it is in essence an adaptive tool for motivating the animal or human to quickly find a way of escaping or terminating this source of initial anxiety, it becomes maladaptive if it persists without reason. In fact, the symptoms associated with anxiety can become quite discomforting and can effectively interfere with a person's ability to function properly. Anxiety is a common emotional phenomenon in humans (Clement et al., 2002). It is an emotional state, unpleasant in nature and is associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat (Gupta et al., 2010). It is considered to be a normal reaction to stress and is characterized by heart palpitations, fatigue,
nausea and shortness of breath. It can aggravate many physical and mental ailments and also impede recovery from any other problems. Classically, anxiety is distinguished into the ‘state’ and the ‘trait’ anxiety. “State anxiety” is anxiety a subject experiences at a particular moment and is increased by the presence of an anxiogenic stimulus. In contrast, “trait anxiety” does not vary from moment to moment and is considered to be an “enduring” feature of an individual (Spielberger et al., 1970; Lister, 1990; Beuzen and Belzung, 1995).

2.1. A FORMS OF ANXIETY DISORDERS -

Anxiety disorders comprise clinical conditions of Generalized Anxiety Disorder, Obsessive-Compulsive Disorder, Panic Disorder, Post-traumatic Stress Disorder, Social Anxiety Disorder and Phobias.

Generalized Anxiety Disorders: It involves a broad presentation of anxiety. It is characterized by long-lasting anxiety (for over 6 months) that is not focused on anyone object or situation. Those suffering from this disorder experience non-specific persistent fear and worry and become overly concerned with everyday matters like health, work, money or family and experience these symptoms even when there are no signs of trouble in their life (Barchas and Altemus, 1999; Gupta et al., 2010).

Obsessive Compulsive Disorder: This is a particularly important form of anxiety disorder which is characterized by obsessions i.e. recurrent thoughts that may not be about real-life problems and which the person fails to ignore or suppress. Compulsions are repetitive behaviours that the person feels driven to perform in response to an obsession. The compulsive behaviours attempt to reduce the distress from the obsessions (Merikangas and Pine, 2002; Gupta et al., 2010).

Panic Disorder: In this type of disorder the person suffers from brief attacks of intense terror and apprehension which is often characterized by trembling, shaking, confusion, dizziness,
nausea, and difficulty in breathing, lasting for a few minutes. The person also believes that he or she is seriously ill or about to die and this feeling can leave the person depressed or shaken for quite a while afterwards (Gupta et al., 2010).

**Post-traumatic Stress Disorder** : Post-traumatic stress disorder is an anxiety disorder which results from a traumatic experience. The symptoms include flashbacks or nightmares about what happened, hyper vigilance, startling easily, with drawing from others, and avoiding situations that remind the person of the event. This disorder can continue for a sustained period of time with marked impairment in function (Barchas and Altemus, 1999).

**Social Anxiety Disorder** : Is a marked and persistent fear of social or performance situations (Gupta et al., 2010).

**Phobias** : A phobia is an unrealistic or exaggerated fear of a specific stimulus, such as heights, enclosed places or other situations. The phobic individual may experience full panic attacks when exposed to such stimuli. Phobias tend to be the most common form of anxiety disorder whereas panic disorders are fairly rare in the general population (Merikangas and Pine, 2002)

**2.1.B SYMPTOMS DURING ANXIETY -**

The human brain is the centre of human nervous system and is a highly complex organ. The part of the brain that triggers a response to danger is the locus coeruleus and the area of the brain responsible for the acquisition and expression of fear conditioning is the amygdala (Pare et al., 2004). Once the neurotransmitters pick up over activity/hyperactivity in the locus coeruleus, the amygdala senses danger and instructs us to run from danger. Hence, once the amygdala gets activated it sends an alarm to the heart to beat faster, breathing to become rapid and in turn activates all the biological components of fight/flight response. The symptoms experienced during an anxiety attack include:
• Rapid heartbeat and rapid breathing
• Twitching or trembling
• Muscle tension
• Headaches
• Sweating
• Dry mouth and difficulty in swallowing and
• Abdominal pain

Sometimes other symptoms accompany anxiety, such as:
• Blurred vision and Dizziness
• Diarrhea or frequent need to urinate
• Irritability, including loss of temper
• Sleeping difficulties and nightmares
• Decreased concentration and
• Sexual problems.

All these physical symptoms are felt when one is anxious or having a panic attack. These are part of a system that is designed to keep one safe and do not cause any harm. They cause a problem only when they occur in response to situations where one is not physically threatened (Khanum and Razack, 2010).

2.1.C EPIDEMIOLOGY OF ANXIETY -

Anxiety is implicated in a number of psychiatric disorders, such as depression, panic attacks, phobias, generalized anxiety disorder, obsessive-compulsive disorder and post-traumatic stress disorder (Gross and Hen, 2004). The lifetime 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 \textit{i.e.} 8.5\% in the general population (WHO, 2004). Anxiety disorders affect 16.6\% of population worldwide (Somers \textit{et al.}, 2006) and have become an important area of research interest in psychopharmacology during this decade. Anxiety disorders are psychiatric disorders affecting nearly 25\% of the adult population at some point in their life. The prevalence of anxiety disorders is 30.5\% and 19.2\% in women and men respectively. The prevalence of anxiety disorders is remarkably high in young people. Children aged 7 to 11 years reported a 15.4\% prevalence rate of anxiety disorders. Anxiety disorders are the most common class of neuropsychiatric disorders in USA (Kessler \textit{et al.}, 2005) and many other countries (Alonso and Lepine, 2007). Approximately 18\% of the US adult population will suffer from any anxiety disorder during their life. However, of those 18\% diagnosed with an anxiety disorder, 22.8\% are classified as severe, 33.7\% as moderate, and 43.5\% as mild (NIMH, 2012). The highest incidences occurred for special phobias, social phobias, and generalized anxiety disorder. A survey has also stated that less than 14\% of people with such psychiatric disorders only receive appropriate treatment due to reasons such as lack of appropriate screening, lack of access to medication, being non-responders to medication, and exhibiting subclinical symptoms (Leon \textit{et al.}, 1997).

2.1.D PATHOPHYSIOLOGY OF ANXIETY -

2.1.D.a Neuro-Pharmacology of Anxiety - There are many anatomical structures involved in generating, maintaining and dampening a state of anxiety, one example is within the endocrine system. The hypothalamic pituitary adrenal axis (HPA) plays a major role in the acute stress response. If not kept in balance, for example in the case of people suffering from a prolonged state of anxiety, it can over time cause anatomical changes such as enlarged adrenal glands (Kessing \textit{et al.}, 2011). In the central nervous system (CNS), researchers have identified many
sites that are involved with anxiety, however there are three particular structures that stand out, including the amygdala, the hippocampal complex and the prefrontal cortex (Engin and Treit 2007a; Engin and Treit, 2007b). All three structures are anatomically and functionally connected. The amygdaloid complex is located within the medial temporal lobes of the brain and comprises different nuclei including the basolateral, medial, and central nucleus (LeDoux, 1998). The exact purpose of each of the nuclei is not yet known, but the complex has multiple connections with almost all other regions of the brain. The amygdala has been linked to emotional states and conditioned learning and memory. In the case of an auditory stimulus, the amygdala receives input from the thalamic nuclei and the auditory cortex which reaches the lateral nucleus and are passed on and amplified either directly to the central nucleus or by intra-amygdaloid pathways through the basolateral and other nuclei (Kapp et al., 1992; Campeau and Davis, 1995). The central nucleus then passes the information to higher brain regions for processing, but also triggers an immediate reaction causing behavioral, autonomic nervous system, and hormonal (through activation of the hypothalamic-pituitary-adrenal axis) responses. The basolateral nucleus is therefore the main integrator for incoming stimuli since its removal renders rodents less affected by a threatening stimulus (Campeau and Davis, 1995). The central nucleus seems to be the efferent site of the amygdala in eliciting conditioned fear responses (Kapp et al., 1992). The exact functions of each nucleus are not fully understood, but the amygdala has both multiple afferent and efferent connections with most other areas of the brain and does play a major role in the affective evaluation of stressful events (Davis and Whalen, 2001).

Although for many years researchers have suggested that the hippocampus only plays a peripheral role in the regulation of anxiety, more recent theories have suggested that the hippocampus system may play a more central role than previously envisioned (Lydiard et al.,
1996). As with most cortical areas of the brain, the prefrontal cortex has multiple reciprocal connections with other areas of the brain. More specifically, many studies have shown that the medial prefrontal cortex is involved in a variety of anxiety responses (Blanco et al., 2009; Canteras et al., 2010; Etkin, 2010). Since all three structures have complex interactions with other parts of the brain and the neuroendocrine system, it is difficult to focus on a single structure to develop a solution for treating anxiety disorders. This has led to extensive research in the identification of neurotransmitter systems and their respective synaptic receptors utilized by potential anxiolytic compounds (Sheehan and Sheehan, 2007). As well, there are other CNS structures, than the well-studied ones mentioned above, that play a role in the expression of anxiety, such as the nucleus accumbens (Lopes et al., 2007).

The most widely researched target for reducing anxiety is the GABAergic neurotransmitter system. The main inhibitory neurotransmitter in the brain, Gamma Amino Butyric Acid (GABA) having dampening effect in overall brain activity. It has been contribute to the anxiolytic effect of benzodiazepines in different nuclei of the amygdala, predominantly the basolateral and the central nuclei as well as a band of GABA receptors between the central and medial nuclei of the amygdala (Swanson and Petrovich, 1998; Shekhar et al., 2005). While GABA, especially the widely distributed GABA_A receptors play a pivotal role in serving as major output for the central and basolateral nucleus is linked to the main excitatory neurotransmitter glutamate (Sah et al., 2003). Since both nuclei are interconnected and specific lesions to the central and basolateral nuclei have been linked to a loss of fear in animals, it seems likely that feedback projections from the central to the basolateral nucleus serve as inhibitors for a fear stimulus that then projects back to the central nucleus via the basal and accessory basal nuclei (Treit et al., 1993).
The general receptor hypothesis for anxiety is a major part of the pharmacology of anxiolytics currently used in treatment. One of the main targets of this anxiolytic treatment is the GABA$_A$ receptor. The GABA$_A$ receptor is a ligand-gated chloride channel (Figure-1). Binding of GABA in most cases and other positive allosteric modulators such as diazepam to this receptor increases the influx of negatively charged chloride ions. Thus by increasing local synaptic GABA secretion, this usually causes, in most of the synaptic clefts, local hyperpolarizing pulses in the post-synaptic neurons. This in turn implies that the increased GABA presence in the synaptic clefts has thus an overall hyperpolarizing effect on the neurons of the CNS since the GABA$_A$ receptors are distributed throughout the CNS (Sieghart and Sperk, 2002). It is hypothesized that people who are suffering from non-adaptive sustained states of anxiety have unbalanced hyperactive neuronal activity which might be a result of insufficient inhibitory control in the overall CNS neurotransmission. This is the reason that the GABAergic system has been identified as the logical culprit responsible for the dysfunctional state of anxiety (Sah et al., 2003).

Figure-1: GABA$_A$ heteropolymeric structure showing the 5 subunits forming an ionotropic chloride channel (Campagna-Slater and Weaver, 2007)
Both the amygdala and the prefrontal cortex have important roles in emotional processing and expression. These regions play a major role in affective disorders and have been shown repetitively to be involved in anxiety (Davis, 1992; Dudai, 2003). When benzodiazepines, which are GABA\(_A\) specific agonists and are commonly used as psychopharmacological anxiolytics, are administered, a notable reduction in anxiety symptoms is observed. It is thought that by increasing the GABAergic response in the amygdala, abnormal expression of anxiety can be reduced to a “normal” state by altering amygdala neuronal activity (Pesold and Treit, 1995; Shekhar et al., 2003). Neurons from the amygdala have reciprocal projections to various cortical and subcortical regions including the prefrontal cortex (Sah et al., 2003). Deregulations in neuronal activity of any of these regions can produce anxiogenesis (Denver, 2009).

The serotonergic system is another neurotransmitter system which has been shown more recently to be involved in anxiety. The neurotransmitter, 5-hydroxytryptamine (5-HT, serotonin) has also been linked to anxiety and treatment options target a specific subreceptor, 5-HT\(_{1A}\), to induce anti-anxiety effects (Martinez et al., 2002). For example, Buspirone agonists of the 5-HT\(_{1A}\) subreceptor induce anti-anxiety effects (Barrett and Vanover, 1993). There are several efferent serotonergic projections to the amygdaloid complex and the ventral hippocampus, which target presynaptic GABAergic nerve terminals. These neuronal inputs have been shown to inhibit the synaptic GABA release, leading to a disinhibition of neuronal activity in the amygdala. Thus by administrating a 5-HT\(_{1A}\) agonist, a downstream regulation of amygdaloid GABAergic expression is thus modulated (Almada et al., 2009).

Of course, the above explanation only offers a brief summary of the underlying neurobiology implicated in anxiety. There are other neurotransmitter systems like norepinephrine, dopamine, acetylcholine which have shown to be involved in modulating anxiety-like behavior in rodents.
(Durant et al., 2010). In addition, there is evidence that many neuropeptides such as urocortin, neuromedin-B, cholecystokinin and endocannabinoids are involved in anxiety but these systems are less studied than the classic neurotransmitters (Thorsell et al., 1999; Merali et al., 2006a; Merali et al., 2006b; Bedard et al., 2007; Alldredge, 2010).

2.1.D.b Neuro-Chemistry of Anxiety (Neurotransmitters in the Brain)

Anxiety is recognized as one of the most important emotional processes with firm neurobiological roots. The neurochemistry of anxiety although not well understood has emerged to be a major area of research leading to new approaches in the treatment of anxiety. It is caused due to too many or too few neurotransmitters in the brain. Brain synthesizes several neurotransmitters such as acetylcholine, adrenaline, dopamine, endorphins, serotonin, gamma amino butyric acid, glutamate etc. Most information has come from studying the action of anxiety-reducing or anxiolytic drugs. The evidences suggest anxiety is because of dysfunction of one or more neurotransmitters and their receptors. The major thrusts of current work dealing with anxiety disorders have centered around the gamma amino butyric acid mechanisms, the serotonergic system, the noradrenergic mechanisms and neuropeptides (Barchas and Altemus, 1999). New evidences suggest a role for adenosine and cholecystokinin in the development of anxiety; drugs interactions with these neurotransmitters also may have anxiolytic effects.

**Gamma Amino Butyric Acid (GABA)**: It is one among the chief inhibitory neurotransmitters in the mammalian brain and an increasing wealth of information suggests that GABAergic mechanisms have a special role in the neurophysiology of anxiety (Nestoros, 1984). GABA works to regulate the neuronal excitation and thereby serves as a ‘brake’ on the neuronal circuitry during stress and is the brain’s natural stress reliever (Weeks, 2009). Brain has three different types of GABA receptors GABA$_A$, GABA$_B$, GABA$_C$ (Deckers et al., 2003). GABA$_A$
receptors are ligand-gated ion channels (ionotropic receptors) and GABA_B receptors are the seven trans membrane spanning G-protein coupled receptors (metabotropic receptors). The physiologic role of GABA_C receptors is yet to be described.

![GABA formation diagram](image)

**Figure-2 :** GABA is formed by the decarboxylation of L-glutamate.

The GABA_A receptors mediate fast inhibitory synaptic transmissions and regulate the neuronal excitability and are responsible for rapid mood changes (e.g. anxiety, panic and stress response). GABA_A receptors are targets of sedating drugs such as benzodiazepines, barbiturates, neurosteroids and ethanol. Alteration of the influx of chloride ions within this receptor complex is associated with development of anxiety. All of the most commonly used anti-anxiety drugs (benzodiazepines, barbiturates, and ethanol) selectively enhance only GABA mediated transmission (Nestoros, 1984), thereby elevating GABA levels. The GABA_B receptors mediate slow inhibitory potentials and are known to play an important role in memory, depressed mood and pain. The GABA_B receptor ligand/agonists include baclofen, phenibut *etc* among others.
Thus, these GABA agonists/analogues elevate GABA levels thereby exerting anti-anxiety, relaxing and anti-convulsant effects.

**Serotonin**: It has long been viewed as a neurotransmitter involved in regulating emotional states. Of the 14 or so mammalian serotonin receptor subtypes that have been described, at least four have been implicated in anxiety in various animal models (Tallman et al., 2002). It has been reported that reduced levels of serotonin can produce anxiolytic effects (Lucki, 1996). The brain serotonin receptors have been divided into a wide range of subtypes based on their pharmacological specificities, anatomical distribution and function (Barchas and Altemus, 1999). One of the receptor subtypes implicated in anxiety is the serotonin 1A receptor subtype (5-HT$_{1A}$), an autoreceptor located presynaptically on serotonin neurons. When stimulated, this receptor inhibits the synthesis and secretion of serotonin (Barchas and Altemus, 1999). The 5-HT$_{1A}$ receptor agonist buspirone exhibits anxiolytic effects in animals and is useful in the treatment of generalized anxiety disorder but not in panic disorder. In contrast to benzodiazepines, buspirone has a delayed onset of action and must be administered for up to several weeks before a significant reduction in anxiety is observed and has no sedative, anti-convulsant or muscle-relaxant activity and no significant addiction liability (Batool, 2008; Barchas and Altemus, 1999). Other serotonin receptors potentially involved in anxiety include the 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_{3}$ receptors. Antagonists for 5-HT$_{2A}$ receptor like ritanserin exhibit anxiolytic effects in some animal models (Critchley and Handley, 1987; Gleeson et al., 1989). Likewise, blockage of the 5-HT$_{2C}$ receptor produces anxiolytic effect in animals (Kennet et al., 1985). In humans 5-HT$_{2A}$ receptor agonist m-chlorophenyl piperazine (m-CPP) has been shown to generate anxiety in control subjects and in patients with a wide variety of anxiety disorders (Barchas and Altemus, 1999). The 5-HT$_{3}$ receptor antagonist ondansetron has been reported to
be anxiolytic in some animal models (Costall and Naylor, 1991). The selective serotonin reuptake inhibitors (SSRIs) have proven useful for panic and obsessive-compulsive disorder. Thus, the finding that a number of drugs that are useful in panic disorder are not useful in generalized anxiety disorder and vice versa suggests that the fundamental mechanism of these processes are different (Barchas and Altemus, 1999).

**Norepinephrine**: Elevated levels of norepinephrine are helpful in situations of emergencies or in fight/flight response. However, continuously elevated levels even when not in situations of danger put the person in states of anxiety, fear, irritability etc. Thus, the role of catecholamines in anxiety is being studied using adrenergic receptor agonists and antagonists (Barchas and Altemus, 1999).

**Neuropeptides**: Neuropeptides have been implicated in the regulation of complex behaviour including anxiety related behaviors and psychopathology (Landgraf, 2001; Landgraf, 2005). There is increasing evidence suggesting that neuropeptides including substance-P, corticotropin-releasing factor, neuropeptides-Y, vasopressin, oxytocin, somatostatin, cholecystokinin, galanin have relevance in anxiety (Gorman, 2003; Madaan and Wilson, 2009). Behavioural effects of these peptides also have been studied using molecular biology techniques, including the central administration of anti sense sequences that block translation of peptides or peptide receptor proteins, over expression of peptides in intact animals and generation of knockout mice lacking particular peptides or peptide receptors (Barchas and Altemus, 1999).

**2.1.E PHARMACOTHERAPEUTIC APPROACHES FOR ANXIETY**

The main therapeutic approaches for the treatment of anxiety disorders are benzodiazepines and selective serotonin reuptake inhibitors (SSRIs), which act on the GABA$_A$ receptor or the serotonergic neurotransmission, respectively (Uhlenhuth et al., 1999). Use of SSRIs has
increased over the last decade due to lesser side effects and a higher effectiveness especially for obsessive-compulsive disorders compared to the long-established benzodiazepines, which have been linked to hepatotoxicity (Sheehan and Sheehan, 2007). One disadvantage of SSRIs and many other anti-anxiety drugs are the delayed onset of action and limitation of use based on interaction with co-medication or other side effects. Another treatment option is the azapirones, mainly represented by buspirone, which have a benefit-over-risk advantage in the treatment of specific anxiety disorders over benzodiazepines (Cadieux, 1996). However, the current non-responder rate to pharmacotherapy is as high as 40% and necessitates the search for new therapeutic approaches (Denys and Geus, 2005).

2.2 MEDICINAL PLANTS AS A SOURCE OF IMPORTANT DRUG

For at least five thousand years humankind has relied on natural products as the primary source for medicines (Mann, 1992). There was little significant change over much of this time period; however, the last two centuries have brought an explosion of understanding how these natural products are produced and how they interact with other organisms. 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. These traditional medicines are primarily plant-based (Farnsworth, 1985). According to survey in 2001 and 2002, approximately one quarter of the best-selling drugs in the world were natural products or derived from natural products (Butler, 2004). It has also been reported that approximately 28% of new chemical entities (NCEs) between 1981 and 2002 were natural products or natural product-derived natural products (Newman et al., 2003) and another survey during this period 20% of NCEs were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products. On the bases of this report it has been assumed that research on natural
products accounts for approximately 48% of the NCEs reported from 1981–2010 (Newman et al., 2003).

Furthermore, it has been known that natural products also provide a starting point for laboratory syntheses with diverse structures and often with multiple stereo centers that can be challenging synthetically (Clardy and Walsh, 2004; Peterson and Overman, 2004; Nicolaou and Snyder, 2004; Koehn and Carter, 2005). Natural products shows many structural features in common (e.g., aromatic rings, chiral centers, degree of molecule saturation, complex ring systems, and number ratio of heteroatoms) which have been shown to be very important to drug discovery efforts (Feher and Schmidt, 2003; Piggott and Karuso, 2004; Clardy and Walsh, 2004; Koehn and Carter, 2005). Some natural products that are isolated from medicinal plants can serve not only as new drugs themselves but can also be made useful by further necessary modification by medicinal and synthetic chemists.

Sometime new chemical structures are very difficult to found during drug discovery from medicinal plants, in such cases known compounds with new biological activity can provide important drug directions. Molecular target play important rule in drug discovery, since the sequencing of the human genome, a lot new molecular targets have been identified as important and useful in various diseases (Kramer and Cohen, 2004). It has also be known that the compounds isolated from traditionally used medicinal plants shown to act on newly validated molecular targets, one example is indirubin, which targeted and inhibit cyclin dependent kinases (Hoessel et al., 1999; Eisenbrand et al., 2004) and another example is kamebakaurin, which has been shown to target and inhibit NF-nB (Hwang et al., 2001; Lee et al., 2002). There are many known compounds which shown to act on novel molecular targets, this development leads to produce interest in members of these frequently isolated plant compound classes. There are many
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examples but some are like cucurbitacin-I, is found to be highly selective in inhibiting the JAK/STAT3 pathway in case of tumors with activated STAT3 (Blaskovich et al., 2003), another example is H-lapachone, which also selectively kills cancer cells over normal cells by direct activation of checkpoint during the cell cycle (Li et al., 2003), and betulinic acid is also the same type of compound, with selective melanoma cytotoxicity which control the cell cycle by the activation of p38 (Pisha et al., 1995; Cichewicz and Kouzi, 2004).

According to a review article by (Balunas and Kinghorn, 2005), Four new drugs which have been derived from medicinal plants, and have been introduced recently to the U.S. market i.e. Compound I-IV (Figure-3). The drugs are, Arteether (I, or Artemotil®) is an effective antimalarial drug which is derived from artemisinin, which is a sesquiterpene lactone and isolated from Artemisia annua L. (Asteraceae) (Van et al., 1999). Galantamine or galanthamine (II, Reminyl®) is isolated from Galanthus woronowii Losinsk. (Amaryllidaceae) (Pirttila et al., 2004; Heinrich and Teoh, 2004). It has been approved for the treatment of Alzheimer’s disease. An other compound, Nitisinone (III, or Orfadin®) shows a characteristic to control the rare inherited disease, tyrosinaemia (Frantz and Smith, 2003). Nititsinone in actual is the modified form of mesotrione, which is an herbicide based on the natural product leptospermone, isolated from Callistemon citrinus Stapf (Myrtaceae) (Mitchell et al., 2001; Hall et al., 2001). Tiotropium (IV, Spiriva as a trade name) is another drug which has been released recently to the United States market and has been used for the treatment of chronic obstructive pulmonary disease (COPD) (Frantz, 2005; Mundy and Kirkpatrick, 2004).
Compounds V-VII (Figure-4) which are in Phase III clinical trials or registration and are in process of modifications of drugs that currently in clinical use (Butler, 2004). A metabolite of morphine \( i.e \) morphine-6-glucuronide (V), isolated from \textit{Papaver somniferum} L. (\textit{Papaveraceae}), which have very little side effect as compared to morphine and will be used as an alternate pain medication (Lotsch and Geisslinger, 2001). A modified vinblastine \( i.e \) Vinflunine (VI), isolated from \textit{Catharanthus roseus} (L.) G. Don (\textit{Apocynaceae}) can be use as an anticancer agent with high efficacy (Bonfil \textit{et al}., 2002; Okouneva \textit{et al}., 2003). Exatecan (VII) is developed as an anticancer agent and very close similarity with camptothecin that have been isolated from \textit{Camptotheca acuminata} Decne. (\textit{Nyssaceae} (Cragg and Newman, 2004; Butler, 2004). The drug, Calanolide A (VIII) is dipyranocoumarin natural product, isolated from Malaysian rainforest tree \textit{Calophyllum lanigerum} (Yang \textit{et al}., 2001; Yu \textit{et al}., 2003; Kashman...
et al., 1992). It has been investigated that Calanolide A which shows an anti-HIV drug with a very unique and high specific mechanism of action particularly as a non-nucleoside reverse transcriptase inhibitor (NNRTI) of type-1 HIV and is very high effective against AZT-resistant strains of HIV (Yu et al., 2003; Currens et al., 1996; Buckheit et al., 1999). The drug Calanolide A is in Phase II clinical trials process (Creagh et al., 2001).

![Morphine-6-glucuronide (V)](image1)
![Vinflunine (VI)](image2)
![Exatec (VII)](image3)
![Calanolide A (VIII)](image4)

Figure-4 : Examples of Some Drug Isolated from Medicinal Plants are under Clinical trails

2.2.A COMMON HERBAL REMEDIES FOR ANXIETY -

Ayurveda, the Indian traditional system of medicine uses herbs and their preparations to treat various neuropsychiatric disorders. Numerous herbs have been used for centuries in folk and other traditional medicine to calm the mind and positively enhance mood. Herbal medicine which plays an important role in developing countries, are once again becoming popular throughout developing and developed countries. Use of herbal medicine is increasing
enormously in the Western world (Sparreboom et al., 2004). In spite of the large number of animal studies evaluating the potential anxiolytic effects of plant extracts, very few controlled studies have been conducted in a clinical setup. The efficacy and safety of utilizing these natural drugs to treat anxiety, has only just begun to be exactly tested in clinical trials within the last 10 to 15 years (Saeed et al., 2007; Garcia-Garcia et al., 2008; Kinrys et al., 2009). For instance, both Kava-kava (Piper methysticum) and St. John’s wort (Hypericum perforatum) showed beneficial effectiveness in double blind, randomized placebo controlled trials to treat anxiety and depression (Ernst, 2002). Also, extracts of valerian, hops, lemon balm and passion flower preparations have been employed for the prevention and treatment of psychiatric disorders such as anxiety, sleep disorders, convulsions, cognitive impairment and depression (Beaubrun and Gray, 2000). The commonly used herbal remedies for treating anxiety disorders are described below.

**Passiflora incarnata (Passion Flower)** - It is a folk remedy for anxiety. The anxiolytic effects of passionflower are well documented in rodents (Dhawan et al., 2001; Dhawan et al., 2002). In randomized double blind study, passion flower extract was effective in generalized anxiety disorder (GAD) outpatients as compared to oxazepam (Akhondzadeh et al., 2001). In another double-blind placebo-controlled study, preoperative oral Passiflora incarnata reduces anxiety in ambulatory surgery patients (Movafegh et al., 2008).

**Piper methysticum (Kava kava)** - There is substantial evidence that kava has a positive effect on the symptoms of anxiety disorders. Animal studies have demonstrated anti-anxiety activity of kava (Garrett et al., 2003; Bruner and Anderson, 2009). Several randomized double-blind clinical studies in GAD patients showed beneficial effect of kava-kava in reducing anxiety (Watkins et al., 2001; Connor and Davidson, 2002; Boerner et al., 2003; Sarris et al., 2009).
another 8-week randomized, double-blind multi-center clinical trial, the efficacy of *Piper methysticum* was compared with two anxiolytic drugs opipramol and buspirone in GAD patients (Boerner et al., 2003).

**Hypericum perforatum (St. John’s wort)** - It is a popular supplement for treating depression but is much less popular for treating anxiety disorders. Chronic administration of *Hypericum perforatum* induced an anxiolytic effect in the elevated T-maze and the light/dark transition test (Flausino et al., 2002; Singewald et al., 2004). It inhibits the reuptake of serotonin, noradrenaline, dopamine and modulates neuronal excitability via glutamatergic and GABAergic mechanisms (Wonnemann et al., 2000; Langosch et al., 2002). The evidence of positive effects of St. John’s wort on anxiety disorders is weak. An open-label uncontrolled observation with 500 subjects showed beneficial effect of St. John’s wort extract in reducing anxiety disorder symptoms in patients diagnosed with depression comorbid with anxiety (Muller et al., 2003). No placebo-controlled, randomized, double-blind trials have shown St. John’s wort to be effective in treating generalized anxiety disorder, post-traumatic stress disorder, obsessive-compulsive disorder (OCD), or phobias.

**Valeriana officinalis (Valerian)** - Valerian is one of the most popularly used herbal medicines for insomnia and is also used to treat anxiety (Donath et al., 2000). Hydroalcoholic and aqueous extracts of valerian roots have shown affinity for the GABA<sub>A</sub> receptor in the brains of rats (Benke et al., 2009). In humans, valerian has been successful in the treatment of insomnia and tension (Vorbach et al., 1996; Donath et al., 2000; Stevinson and Ernst, 2000; Ziegler, 2002).

**Ginkgo Biloba** - In the elevated plus maze, senescent mice treated with extract of *Ginkgo biloba* spent more time in open arms than those treated with vehicle control (Ward et al., 2002). A standardized extract of *Ginkgo biloba* in doses of 240 mg and 480 mg compared with placebo for
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four weeks, involving patients with GAD and adjustment disorder with anxious mood (Woelk et al., 2007). Both the doses of extract of Ginkgo biloba showed a statistically significant reduction in somatic symptoms compared to baseline (which was not observed in the placebo group).

**Galphimia glauca** - It is a plant used in Mexican traditional medicine as a "nerve tranquilizer". Many studies have demonstrated anxiolytic effect of methanolic extract from this plant species. A controlled study comparing the extract of *Galphimia glauca* Cav. with lorazepam in patients with GAD and the result of both the groups showed a significant reduction in anxious mood and somatic symptoms, without any difference between treatments (Herrera-Arellano et al., 2007).

**Matricaria recutita** (Chamomile) - Chamomile is one of the most popular single ingredient of herbal teas. Chamomile contains flavonoids, which exert benzodiazepine-like activity (Avallone et al., 2000). A recent study evaluated the efficacy of a standardized extract of *Matricaria recutita* (L), compared with placebo for eight weeks in patients with mild to moderate GAD. There was a statistically significant reduction in anxious mood and somatic symptoms with extract of *Matricaria recutita* (L) compared to placebo treated group (Amsterdam et al., 2009).

**Astragalus membranaceus** - *Astragalus membranaceus* is a useful Korean herb that has been clinically prescribed for stress-related illness. *Astragalus membranaceus* significantly restores learning and memory deficits in chronically stressed rats. In the elevated plus maze, *Astragalus membranaceus* treatment significantly increases the time spent in the open arms compared to control group. It also enhanced choline acetyltransferase (ChAT) expression in stressed rats (Park et al., 2009). No clinical data is available for its anxiolytic effect.

There is limited clinical data on the efficacy of herbal medicines for the treatment of anxiety disorders because of limited knowledge regarding chemical composition of the products, lack of standardization of these preparations and the paucity of well controlled studies. Preliminary
evidence suggests that herbal medicines may have a role in the treatment of anxiety disorders so need further research to isolate main active constituents and standardized it for clinical research.

2.3 BIOASSAY GUIDED ISOLATION OF NATURAL PRODUCTS

Natural sources have many useful and important bioactive compounds and many have been discovered using bioactivity directed fractionation and isolation (BDFI). Majority of the bioactive compound have been isolated using bioactivity-guided fractionation (Pezzuto et al., 1999). 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.

**Bioguided Fractionation:** It is commonly used to elucidate possible pharmacological active compounds from a complex mixture of an extract. One assumption for the usefulness of bioguided fractionation is that the extract actually exhibits the pharmacological activity in question. The other approach would be to start with a single compound that is known from an extract and evaluate its pharmacological activity. However, if the compound alone does not show any activity, this does not exclude a possible contribution of this substance to the activity seen with the whole extract since many extracts resemble the pharmacological response as a synergistic effect of active compounds and facilitating compounds, that enhance absorption or bioactivation of the active substances. Thus, bioguided fractionation provides a powerful tool to identify active compounds from pharmacological active extracts with smaller chances of loosing a possible active substance when testing single compounds (Butterweck and Schmidt, 2007). The research of pharmacognosy or isolation of natural products facilitated by newly development of new bioassay methods. It has been found that the bioactive compounds are mostly plant secondary metabolites, which become medicine after processing to pure compounds; some are
very useful dietary supplements, and many useful commercial products. Further modification of the active compounds lead to enhance the biological profiles and a large number of such compounds which are approved or undergoing clinical trials for clinical uses against different diseases like pulmonary diseases, cancer, HIV/AIDS, malaria, Alzheimer’s and other diseases (Newman et al., 2003; Butler, 2004). Crude herbs are used as drugs in different country of the world and therefore it take a basic part of many traditional medicines worldwide. In Asia, traditional Chinese medicine (TCM), Korean Chinese medicine, Japanese Chinese medicine (kampo), Ayurvedic medicine (India) and Jamu (Indonesia), phytotherapy and homeopathy in Europe, Alternative medicines are typically named when herbal therapies use with various other traditional remedies in America. Integrative medicine came into being when the alternative medicine, mainly the aforementioned traditional and folk medicines used worldwide, with conventional medicine (Western medicine).

2.4 TECHNIQUE USED IN PHYTOCHEMISTRY

Due to the fact that plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different chromatographic separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, should be used to obtain pure compounds.

2.4.A CHROMATOGRAPHY - The term chromatography is used for a set of laboratory techniques which involve the separation of mixtures of different constituents. The mixture is dissolved in a solvent or a "mobile phase" which pass through a stationary phase, which
separates the different constituent of the solution and allows it to be isolated. Chromatography may be classified as :

**Preparative:** This type of chromatography is used when the separated components of a mixture is applied for further use (and is thus a form of purification).

**Analytical:** This type of chromatography is use just for measuring the relative proportions of analytes in a mixture and therefore is done normally with smaller amounts of material.

Chromatographic technique can be classified in two ways :

i) Techniques by difference in bed shape.

ii) Techniques by difference physical state of mobile phase

2.4.A. (i) **Techniques by difference in bed shape** - It includes column chromatography and planar chromatography.

(a) **Column Chromatography** - It is a separating method which is used to purify every chemical compounds from mixtures of different compounds. This type of chromatography is used for from micrograms up to kilograms of separating samples. In this, a glass tube of different diameter and length are used as column. A glass tube with a diameter from 50 mm and a height of 50 cm to 1 m with a tap at the bottom can be used as a classical preparative chromatography column. Slurry of the eluent with the stationary phase powder is prepared and then carefully poured into the column. A special precaution should be taken in order to avoid air bubbles. The slurry is then pipetted on top of the stationary phase. This layer of slurry is usually protected with a small layer of sand or with cotton or glass wool in order to protect the shape of the separating slurry mixture from the pouring of newly added eluent or solvent. The eluent is slowly passed through the column by opening the tap to move the component of the slurry of organic compounds. It always useful to use a spherical eluent reservoir or an eluent-filled and stoppered separating funnel is put
on top of the column. The stationary phase differently retained the individual components from each other and separates them while they are running at different velocities through the column with the eluent and therefore one compound can be elute at the end of the column at a time. A series of fractions is collected during the entire chromatography process. The composition of the eluent flow can be monitored thoroughly and therefore each fraction is analyzed for dissolved compounds. For this purpose analytical chromatography, UV absorption, or fluorescence technique can be used. Coloured compounds (or fluorescent compounds with the help of an UV lamp) can be seen through the column glass wall as moving bands. Column chromatography divided into two phases i.e. Stationary phase or adsorbent and mobile phase or eluent.

Stationary Phase: The stationary phase or adsorbent is a solid material in column chromatography. Mostly silica gel is used as stationary phase for column chromatography and another is alumina which is second used stationary phase. In the past cellulose powder has often been used. Also possible are affinity chromatography or expanded bed adsorption (EBA) and ion exchange chromatography, reversed-phase chromatography (RP). The finely ground powders or gels are used as the stationary phases and/or are microporous for an increased surface, while in EBA a fluidized bed is used.

Mobile Phase: It is either a pure solvent or of different solvents mixture. It is very precisely studied so that the retention factor value of the compound of interest is roughly around 0.2 - 0.3, it can be minimizing the time and the amount of eluent to run the chromatography. The chosen of good eluent system is very important so that the different compounds can be separated easily and effectively. The eluent system is optimized in small scale pretests, in each case often using thin layer chromatography (TLC) providing the same stationary phase. The time required to run a column can be minimizes by maximizes the flow rate of the eluent and thereby minimizes
diffusion, which results a better separation. Although there are many techniques to maximize the
column run rate, for example a simple laboratory column can be runs by gravity flow which can
be increased by extending the fresh eluent filled column above the top of the stationary phase or
negatively controlled with the tap controls. A pump can also be used for better achievement of
flow rates or compressed gas (e.g. air, nitrogen, or argon) can also be used to push the solvent
through the column (flash column chromatography) (Still et al, 1978).

(b) Planar Chromatography - Planar chromatography is also a separation technique in which
the stationary phase is a plane or present as a plane. A paper can be used as a plane, which may
serves as such or impregnated with stationary bed (paper chromatography), Glass plate can also
be used on which a layer of solid particles spread (thin layer chromatography). The traveling of
different compounds in the sample mixture travel with different velocities according to how
strongly they interact with the stationary phase as compared to the mobile phase. The
Retardation factor (Rf), which are very specific for each chemical and can be used to aid in the
identification of an unknown substance. Planar Chromatography divided into paper
chromatographic and thin layer chromatography.

Paper Chromatography - The technique of paper chromatography is very simple in which a
small dot or line of sample solution placed onto a strip of chromatography paper. There is a jar
containing a shallow layer of solvent in which the chromatography paper placed and sealed the
jar. The solvent rises through the capillary action of the paper, it reach the sample mixture which
starts and travel along with the solvent toward the upper side of the paper. As the paper is made
of cellulose which is a polar substance, and the compounds within the mixture travel farther in
case if they are non-polar. While the polar substances bond with the cellulose paper more
strongly and therefore do not travel as far.
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*Thin Layer Chromatography (TLC)* - It is very important technique for qualitative study in both small and large scale and therefore widely-employed laboratory technique and it is very closely related with paper chromatography. The only difference between thin layer and paper chromatography is to used a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate while in the other paper are used as stationary phase. The TLC as compared to paper has the advantage of faster runs rate, better separations of the component, and the choice between different adsorbents *etc.*

2.4.A. (ii) **Techniques by physical state of mobile phase** - It includes gas chromatography and liquid chromatography.

(a) **Gas Chromatography (GC)** - In other words Gas-Liquid chromatography, (GLC), is also a separation technique in which gas is use as the mobile phase. Gas chromatography is always carried out in a particular type of column, which is typically "packed" or "capillary. Stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium) are used in Gas Chromatography (GC). Partition equilibrium of analyte is based on both stationary and mobile phase. The material of stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). Such system is always used for in analytical chemistry. GC due to its high temperature unsuitable for high molecular weight biopolymers or proteins (because heat denature protein molecule), frequently encountered in biochemistry. Such type of chromatography is well suited for use in industrial chemical, the petrochemical, environmental monitoring. GC is very important technique and largely used in chemistry research.

(b) **Liquid Chromatography (LC)** - It is another separation technique for organic compounds in which the mobile phase is always a liquid. It can be performed both in a column or a plane. In
the recent research liquid chromatography that generally utilizes very small packing particles along with a relatively high pressure, such technique is named as high performance liquid chromatography (HPLC).

In order to use the HPLC technique, the sample is accelerated by a liquid (mobile phase) at high pressure through a column that is packed with irregularly or spherically shaped particles or a porous monolithic layer (stationary phase). HPLC is further divided into two different sub-classes which are based on both the polarity of the mobile and stationary phases. Such GC technique in which the mobile phase is less polar than stationary phase (e.g. toluene use as the mobile phase, and silica use as the stationary phase) is known as normal phase liquid chromatography (NPLC), while in cases where the mobile phase is polar than stationary phase (e.g. water-methanol mixture use as the mobile phase and C18 = octadecylsilyl use as the stationary phase) is known as Reversed Phase Liquid Chromatography (RPLC). It has been known that the "normal phase" has very few applications as compared to RPLC which has been used considerably more. Such technique in which no pressure is used to accelerate the mobile phase through the stationary phase are named as fast protein liquid chromatography which come under the broad heading of chromatography. The above mentioned chromatographic techniques are always used in Phytochemistry research. There are different other chromatographic techniques are also used e.g., Supercritical fluid chromatography, Affinity chromatography, Size exclusion chromatography, Chiral chromatography, Ion exchange chromatography, Countercurrent chromatography etc.

2.4.B SPECTROSCOPIC TECHNIQUES - Spectroscopy is terms used to refer to the measurement of radiation intensity as a function of wavelength and are often used to describe Experiment spectroscopic methods. It is a sufficiently broad field that many sub-disciplines exit,
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each with numerous implementations of specific spectroscopic techniques.

2.4.B.(i) **Ultraviolet-Visible Spectroscopy** - UV-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) related to the spectroscopy of photons in the UV-visible region. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges \(i.e.\) near ultraviolet (UV) and near infrared (NIR) ranges. The colour of the chemicals involved is directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum. This technique apposite the fluorescence spectroscopy, in that fluorescence involved with transitions of molecule from the excited state to the ground state, while in UV-visible spectroscopy the absorption measures transitions from the ground state to the excited state (Skoog, 2007).

**Application** : UV/Visible spectroscopy is widely used in the quantitative analysis of transition metal ions and highly conjugated organic compounds solutions. It has also been used for the detector for HPLC. The presence and absence of an analyte gives an indication which can be considered to be proportional to the concentration. For perfect results, the instrument's indication about an analyte in the unknown should be compared with the indication of a standard; this is identical to the use of calibration curves. The response or indications \(e.g.,\) peak height) for a particular amount of concentration is known as the response factor.

2.4.B.(ii) **Infrared Spectroscopy** - Infrared spectroscopy (IR spectroscopy) is also a part of spectroscopy that studies the infrared region of the electromagnetic spectrum. There are different techniques which are related with IR spectroscopy, the most common one is absorption spectroscopy. As with all other spectroscopic techniques, it can also be useful in identifying compounds or examination of sample composition. Infrared spectroscopy related tables are easily available in literature.
Fourier transform infrared (FTIR) spectroscopy is a form of IR spectroscopy and it is a measurement technique for collecting infrared spectra. Instead of recording the intensity of energy absorbed when the frequency of the infra-red light is non-constant (monochromator), the infra-red light is guided through an interferometer. After passing through the sample under investigation, the measured signal is the interferogram. Performing a mathematical Fourier transform on this signal results in a spectrum identical to that from conventional (dispersive) infrared spectroscopy. FTIR spectrometers are very cheaper than other conventional spectrometers because building of interferometers is very easier as compared to the fabrication of a monochromator. It has been noted that the measurement of a single spectrum is much faster for the FTIR technique due to simultaneous collection of the information at all frequencies. These are the usefulness of the multiple samples to be collected and calculated the averaged together which results an improvement in sensitivity. Due to the various advantages of FTIR, virtually all latest infrared spectrometers are FTIR instruments.

**Uses and Applications**: Applications of infrared spectroscopy for both organic and inorganic chemistry have been highly successful (Lau, 1999). The applications of IR spectroscopy in the field of semiconductor microelectronics are much beneficial. IR spectroscopy is useful in both research and industry as very reliable and simple technique for dynamic measurement, quality control and measurement. IR spectroscopy is useful technique in forensic analysis for both criminal and civil cases and also useful to find out the degree of polymerization in polymer synthesis. Due to the development in the instruments the infrared measurements became easy across the whole range of interest as fast as 32 times a second. IR spectroscopy techniques have been developed to analyze the quality of tealeaves. It has been understood that a well trained manpower can be used more sparingly, at a significant cost saving (Luypaert et al., 2003).
2.4.B.(iii) NMR Spectroscopy - Nuclear magnetic resonance spectroscopy or which is also known as NMR spectroscopy, which has been named due to which the magnetic properties of certain nuclei used in this technique. Both $^1$H NMR and $^{13}$C NMR spectroscopy are important applications for the organic chemist. In principle, NMR is applicable to that entire nucleus which possessing spin. NMR spectrum gives us many types of information. Functional groups can be determined by using infrared spectroscopy similarly analysis of a 1D NMR spectrum gives information on the type and number of chemical entities which is present in a molecule. However, NMR is much useful as compared to IR because a lot of information obtained from NMR. NMR can be applied to a wide variety of samples, both in the solution and the solid state. Therefore its impact on the natural sciences has been substantial. NMR is also used to the mixtures of analytes. It can also be used to understand the dynamic effects like temperature and reaction mechanism and can also provide useful information regarding protein and nucleic acid structure and function.

2.4.B.(iv) Mass Spectrometry - Mass spectroscopy is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules.

It is used for determining the elemental composition of a sample, the masses of particles and of molecules, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds (Edmod and Stroobant, 2001).

**Basic Principle** - A mass spectrometer generates multiple ions from the sample under investigation, it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type.

The complete process involves in conversion of the sample into gaseous ions, with or without
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fragmentation. The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio.

Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.

**Analysis of Biomolecules using Mass Spectrometry** - Mass spectrometry is the only analytical techniques which provided similar information with electrophoretic, chromatographic or ultracentrifugation methods. The results were not absolute as they were based on characteristics other than the molecular weight. Thus the only possibility of knowing the exact molecular weight of a macromolecule remained its calculation based on its chemical structure.

The development of desorption ionization methods based on the emission of pre-existing ions such as plasma desorption (PD), fast atom bombardment (FAB) or laser desorption (LD), allowed the application of mass spectrometry for analyzing complex biomolecules (William, 1947; Phil, 1991).

2.5 **ANIMAL MODELS USED FOR SCREENING ANXIOLY蒂CS**

Animal tests of anxiety are used to screen for novel compounds for anxiolytic or anxiogenic activity, to investigate the neurobiology of anxiety. There is a diversity of animal models of
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anxiety currently available. These behavioural models involve exposure of animals to stimuli (exteroceptive or interoceptive) that appear capable of causing anxiety in humans. Despite their apparent diversity, animal anxiety models may be grouped into two general categories involving either conditioned \((e.g.\) Geller-Seifter conflict, potentiated startle) or unconditioned (social interaction and light/dark exploration tests) responses (Rodgers and Dalvia, 1997). An ideal model of anxiety should have predictive, face and construct validities. A model that has predictive validity should display reduced anxiety when treated with anxiolytics, while in a model with face validity, the response of an animal to a threatening stimulus should be comparable to the response known for humans, and the mechanisms underlying anxiety should be exhibited by a model with construct validity (McKinney \emph{et al.}, 1969). Naturally, one or more models usually combine to achieve these parameters. Conditioning models require considerable training of subjects, food or water deprivation and/or the use of electric shock as an aversive stimulus. However, some of these procedures, such as conditioned defensive burying, take advantage of the natural tendency of rodents to make faster stimulus response associations when faced with ecologically relevant (versus arbitrary) environmental challenges (Treit, 1990). The study of unconditioned responses to various forms of external threat represents a logical extension of this refinement of laboratory methods, providing a high degree of ecological validity for the research and allowing for a very much more complete behavioural characterization of the effects of experimental manipulations. Hence models involving unconditioned behavior were adopted in this study as discussed below:

\textbf{2.5.A ELEVATED PLUS MAZE TEST} - The elevated plus-maze (EPM) is the most popular of all currently available animal models of anxiety, and affords an excellent example of a model based on the study of unconditioned or spontaneous behaviour (File, 2001; Handley \emph{et al.}, 1993;
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Rodgers and Dalvia, 1997; Carobrez et al., 2005). It has been initially described by Pellow and co-workers (Pellow et al., 1985) as a simple method for assessing anxiety responses by rodents. It as made of four arms (two open and two closed) that are arranged to form a plus shape (Handley et al., 1984). These authors described the assessment of anxiety behaviour of rodents by using the ratio of time spent on the open arms to the time spent on the closed arms. In the test, mice or rats are placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm are recorded by a video-tracking system and observer simultaneously for 5 minutes. Other ethological parameters (i.e. rears, head dips, and stretch-attend postures) reflect antianxiety behaviour (Walf and Frye, 2007). Unlike other behavioural assays used to assess anxiety responses that rely upon the presentation of noxious stimuli (i.e. electric shock, food/water deprivation, loud noises, exposure to predator odour etc) that typically produce a conditioned response, the EPM relies upon rodents proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance) (Walf and Frye, 2007). However, the focus of this study using the EPM was to assess the anxiolytic effect of the plant extract. This model was chosen because it has face validity, which is the ability of a task to appear to measure what it is supposed to measure. For instance, in the EPM, the anxiety or fear of open spaces/heights of rodents seems to be measured. In this task, the open arms are avoided and rodents spend the majority of the time in this task in the closed arms of the maze. Other anxiety-related behaviours of rodents, such as freezing/immobility and defaecation, are increased on the open arms of the maze compared to the closed arms (Pellow et al., 1985). The EPM also has construct validity, which refers to whether an observable dependent variable, such as time spent in the open arms of the EPM, used measures an unobservable construct, such an anxiety. This is demonstrated by anxiogenic drugs reducing time spent on the open arms and
anxiolytic drugs increasing the time spent on the open arms of the EPM (Pellow et al., 1985).

2.5.B. LIGHT/DARK BOX TEST - Apart from the elevated plus-maze, the light/dark box and the open field are other test models that provide unconditioned anxiety-like behaviour. All of these tests do not require conditioning, do not cause physical discomfort and are considered to have ecological validity (Rodgers and Dalvia, 1997). This test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light (Crawley and Goodwin, 1980). A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects (Bourin and Hascoet, 2003). The light/dark box apparatus is based on the initial model described by Crawley et al. (Crawley and Goodwin, 1980). However, many structural modifications have been made to it. The typical dimensions of the compartment are generally one third for the dark compartment and two third for the light compartment. The model is based on the observation that although nocturnal rodents such as mice will naturally tend to explore a novel environment, open fields appears to have aversive properties which inhibit exploratory behaviour (Bourin and Hascoet, 2003). Here, the safe area is the small dark compartment (one third) and the aversive area is the large illuminated compartment. When a rodent is placed in the box, the conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). Thus in the light/dark test, drug induced increase in behaviours in the light part of a two compartment box, in which a large white compartment is illuminated and a small compartment is darkened, is suggested as an index of anxiolytic activity.
2.6. PLANTS SELECTED FOR ANXIOLYTIC ACTIVITY

*Dalbergia sissoo* Roxb.

*Dalbergia sissoo* Roxb., also known as Indian Rosewood, belongs to the legume family *Fabaceae*. It is a large perennial tree found in the lowland region throughout India and is also indigenous to Pakistan, Bangladesh, Afghanistan and Nepal. It is used as timber or firewood and for the treatment of a variety of ailments by different ethnic groups (Kritikar and Basu, 1975; Ministry of Health & Family Welfare 2001).

**SYNONYMS -**

<table>
<thead>
<tr>
<th>Language</th>
<th>Synonyms</th>
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<tbody>
<tr>
<td>English</td>
<td>Bombay blackwood, Indian rosewood,</td>
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<tr>
<td>Hindi</td>
<td>Shisham, Sisam, Sissoo, Kara</td>
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<td>Tamil</td>
<td>Sisuitti, Sisso, Nukku kattai, Yette, Gette</td>
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<td>Dalbertia sissoo</td>
</tr>
<tr>
<td>Nepali</td>
<td>Sissau, Sisham</td>
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</tbody>
</table>

**CLASSIFICATION -**

*Dalbergia sissoo* has the following taxonomy (Wunderlin and Hansen 2002):

```
Kingdom       - Plantae (plants),
Subkingdom    - Tracheobionta (vascular plants),
Super division- Spermatophyta (seed plants),
Division      - Magnoliophyta (flowering plants),
Class         - Magnoliopsida (dicotyledons),
Subclass      - Rosidae,
Order         - Fabales,
Family        - Fabaceae
Genus         - *Dalbergia*
Species       - *sissoo*
```
**DESCRIPTION** - *Dalbergia sissoo* as a deciduous tree, up to 25 m high, bark grayish-brown, leaves pale green, alternately arranged, compound and pinnate with 3-5 leaflet, broadly ovate to sub-orbicular, entire, acuminate, terminal leaflet long stalked; pale yellow flowers appear in spring, sub-sessile, in short axillary panicles; white to dull yellow and fragrant. calyx bell shaped, with 5 short teeth; petals much longer than the calyx; ovary hairy, style glabrous, strap shaped. Root- taproot and an extensive lateral root system, often at the soil surface and producing suckers (PIER, 2006; Gilman and Watson, 1993). The pods of *sissoo* when ripe contain 1-3 seeds, indehiscent, reniform flat, light brown, with delicate papery testa (Zabala, 1990). Fruit is 5-7x0.08x1.2 cm, strap-shaped, pale brown, mostly 1-seeded, less often 3-seeded (Alam *et al.*, 2001).

**CHEMICAL CONSTITUENTS** - Phytochemical examination of *Dalbergia sissoo* has provided a large number of compounds, which include glycosides, coumarins, essential oils, phenols, terpenes, neoflavonoids, dalbergin, dalberginone, dalbergichromene, dehydroamorphigenin, isoflavones, irisolidone, biochanin-A, muningin, tectorigenin, prunetin, genestein, sissotrin, rotenoid, naringenin and prunetin-4-O-galactoside. The flavone norartocarpotin, amyrin, sitosterol and stigmasterol (Krishnamurty *et al.*, 1963; Banerjee *et al.*, 1966; Mukerjee *et al.*, 1971; Sharma *et al.*, 1979; Salwa *et al.*, 2001; Chihiro, 2003; Rami, 2008; Rana *et al.*, 2009).

<table>
<thead>
<tr>
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<td>Leaves</td>
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<td>Flavonoids, Flavanones, Naringenin</td>
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<tr>
<td>Stem bark and Heartwood</td>
<td>Neoflavenes (Dalbergichromene), Flavonoids, Cinnamylphenols, Chalcones (Isoliquiritigenin), Isosalipurposide, Amino acids (Glycin, Alanine, Threonine, Isolucine, Phenylalanine), Myristic</td>
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<table>
<thead>
<tr>
<th>Plant Parts</th>
<th>Chemical Components</th>
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<tbody>
<tr>
<td>Pods and Flower</td>
<td>Tannins, Caviunin-7-O-gentiobioside, Tectorigenin, Biochanin-A</td>
</tr>
<tr>
<td>Roots and Root bark</td>
<td>Cardiac Gycosides, Atmraquinones, Saponins, Isoflavone (7-γ,γ-dimethylallyloxy-5-hydroxy-4’methoxyisoflavone), biochanin-A, Chalcone (2,3-dimethoxy-4’-γ,γ-dimethylallyloxy-2’hydroxy chalcone flavone), 7-hydroxy-6-methoxy flavone, Rotenoid, Dehydroamorphigenin</td>
</tr>
</tbody>
</table>

(Hilditch and Williams, 1964; Asaad et al., 2000; Wang, 2009; Rana and Kumar, 2011)

ETHNOPHARMACOLOGY - Plants of the genus *Dalbergia* are medicinally important and have been used for the treatment of gonorrhoea, arthritis, and rheumatic pains (Anonymous. 1950; Nadkarni, 1982; Singh and Chaturvedi, 1966). In folk medicine, it has been used mainly as aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic. It is also used in conditions like emesis, ulcers, leucoderma, dysentery, stomach troubles and skin diseases (Kirtikar and Basu, 1975; Nadkarni, 1954; Chopra et al., 1956; Duke and Wain, 1981). In Arabic countries the aqueous leaves extract of *Dalbergia sissoo* has been used for the treatment of gonorrhea (El-Dagwy, 1996). The hard wood *Dalbergia sissoo* which is very heavy and durable, widely used for the manufacturing of boats furniture, wheels and carts etc. In Ayurvedics prescribe, the leaf juice for eye ailments. The wood and bark used for abortifacient, anthelmintic, antipyretic, aphrodisiac, expectorant, refrigerant, dysentery, dyspepsia, leucoderma, skin ailments, for anal disorders, for blood diseases, burning sensations, eye and nose disorders, scabies, scalding urine, stomach problems, and syphilis. The alterative wood is used in India for boils, eruptions, leprosy and nausea (Kirtikar and Basu, 1975). Its leaves are boiled and given to animals for bilious disorders (Baquar, 1989; Duke, 1983). Oil obtained from the seeds is used to cure skin diseases. (Mbuya et al., 1994).
Wood: The wood of *Dalbergia sissoo* is highly durable with excellent finishing colour and smoothness; used for veneer, furniture, cabinets, panelling, carving, small timber, plywood, ornamental turnery, tool handles, sprouting goods and musical instruments (Lowry and Seebeck, 1997). The sawdust works in the absorption of nickel ions and has the potential of removing these heavy metals from industrial and commercial waste water sources (Habib et al., 2006). The wood has a high caloric content and is an important fuel wood and charcoal source (Sheikh, 1989). The wood fibers are processed into a pulp that is further made into paper. Heartwood yields 5.4% of oil, which approaches the texture of vaseline after cooling. It is suitable as a lubricant for heavy machinery (Browne, 1968). The wood is excellent for cooking. The calorific value of the sapwood is about 4900 kcal/kg, and that of heartwood is about 5200 kcal/kg. The sapwood is also used for pulp making (White, 1994). Its wood is well and does not warp or split, extremely durable and is one of the timbers least susceptible to dry-wood termites in India. Wood offers resistance to sawing and cutting but is excellent for turnery, takes a good polish and finishes to a smooth surface. Its root wood is used for tobacco pipes. *Dalbergia sissoo* wood is popular for doors and windows. The heartwood is extremely durable (the specific gravity is 0.7–0.8 g cm$^3$). It is a useful source of honey, but the flowers are only lightly attached to the flower branch and fall easily. The bees are therefore not able to take full advantage of the large number of flowers. The honey produced is dark amber with a strong flavour. Sulphate pulp from wood is used in producing writing and printing paper (Parkash and Hocking, 1986).

Leaves and Young Shoot: The leaves, young shoots and green pods are used as good fodder for livestock and grazing animals, typically in winter seasons when other fodder is not available (Sheikh, 1989; Tewari, 1994). April to May is the best time for the production of high quality fodder (Jackson, 1987). Based on dry weight, the leaves of *Dalbergia sissoo* in India contain up
to 24.1% crude protein, 4.9% fat, 26.1% crude fiber and 12.0% ash (Gohl, 1981). Some ethnic groups in Cameroon are said to relish eating fresh young leaves of *Dalbegia sissoo* (Nadkarni, 1954).

**ECOLOGICAL IMPORTANCE** - *Dalbergia sissoo* provides numerous services to the landscape and environment and is commonly employed in agro-forestry (Lowry and Seebeck, 1997; Kayastha, 1985). It is used as a windbreak and shelter belt and as a shade tree in intercropping of orchards, mango, tea and coffee plantations. Since it has an aggressive root system and is prone to suckering, it is commonly used for erosion control and soil stabilization along stream and river banks. It is widely planted in its native countries for reforestation programs (Sharma *et al.*, 2000). It is also valued for its ability to increase soil fertility through nitrogen fixation and is intercropped for these reasons as well. Due to its fragrant flowers and shade, it is planted in urban areas along roadsides and in gardens as an ornamental plant (El-Hadidi and Boulos, 1988; El-Sheikh, 1989 and 1996; Gilman and Watson, 1993). The leaf litter that accumulates and decomposes also contributes to soil fertility by adding additional nitrogen, potassium, iron, manganese and organic carbon (Keay, 1989). It is found in a variety of wastelands, like in south Asia, where it is known as a colonizing species. In sub-Himalayas, the homeland of *Dalbegia sissoo*, abound with a variety of orchids, many of which are known throughout the world for their beauty (Parrotta, 1989). The ease of propagation by self-seeding, coppice, root suckers and stumps and the many environmental and socio-economic benefits makes it one of the most valued tree species by farmers in the region (Tewari, 1994). *Dalbegia sissoo* is one of the most trees used in greenbelt, which is defined as the mass plantation of pollution tolerant trees and shrubs in an area for the purpose of minimizing air pollution by filtering, intercepting and absorbing pollutants in an effective manner for improvement of the
environment. The importance of greenbelt can be ascertained from the estimate of cleaning capacity of 3.7 ton of CO₂ from atmosphere and supply of 2.5 ton of oxygen from one hectare of *Dalberia sissoo* woodland (Sharma and Roy, 1999).

REPORTED BIOLOGICAL ACTIVITIES - *Dalbergia* genus possesses immense traditional application. So far, this species have been screened for their biological activity and experimental results have shown a wide spectrum of such effects, the important ones are as follows:

**Analgesic, Antipyretic and Anti-inflammatory Activity** : Alcoholic extract of *Dalbergia sissoo* leaves have shown peripheral analgesic activity and central analgesic activity in various models *viz*; acetic acid induced writhings, hot plate method, tail-clip test in mice and Randoll-selitto assay. Leaves extract also showed antipyretic activity in Brewers yeast induced pyrexia in rats (Misar *et al*., 2005). The ethanolic extract of *Dalbergia sissoo* leaves significantly inhibited carragenin, kaolin, and nystatin induced paw edema as well as the weight of granuloma induced by the cotton pellet. It also inhibited dye leakage in acetic acid -induced vascular permeability test in mice (Hajare *et al*., 2001). The bark extract of *Dalbergia sissoo* showed significant antinociceptive activity as evidenced by an increase in reaction time to the pain stimulus (Asif and Kumar, 2011). Seed extract of *Dalbergia sissoo* showed significant analgesic and antipyretic activity (Mallinath *et al*., 2010). Methanolic leaves extract of *Dalbergia sissoo* shows analgesic and antinflamatory activity (Sidana *et al*., 2011). The bark extract of *Dalbergia sissoo* showed significant analgesic activity as evidenced by the increase in reaction time to the pain stimulus (Islam and Elhddad, 2012).

**Antidiabetic Activity** : Ethanolic leaves and bark extract of *Dalbergia sissoo* showed significant antidiabetic activity (Pund *et al*., 2012).
Osteogenic Activity: Oral doses of butanolic leaves and bark extract of *Dalbergia sissoo* in the preclinical setting are effective in preventing estrogen deficiency-induced bone loss by dual action: inhibition of bone resorption and stimulation of new bone formation (Khedgikar *et al.*, 2012). Ethanolic extract of *Dalbergia sissoo* leaves showed significant osteogenic activity (Dixit *et al.*, 2012).

Anti-Spermatogenic Activity: A significant decrease (p<0.01) in sperm motility and sperm count in the epididymis were observed with ethanol stem bark extract of *Dalbergia sissoo* Roxb. (Vasudeva and Vats, 2011).

Antidiarrhoeal Activity: The decoction of dried leaves of *Dalbergia sissoo* possesses anti-diarrhoeal activity (Mujumdar *et al.*, 2005). Ether extract of *Dalbergia sissoo* bark showed significant and dose dependent anti-diarrhoeal activity. The extracts also significantly reduced the intestinal transit time in charcoal meal when compared with atropine sulphate (1 mg/kg ip) (Kalaskar *et al.*, 2010). *Dalbergia sissoo* leaves extract is antidiarrhoeal as it affects bacterial virulence (Brijesh *et al.*, 2006).

Larvicidal and Mosquito Repellant Activity: The oil extracted from wood scrapings of *D. sissoo* has shown dose dependent larvicidal activity, growth inhibitor and repellant action against *Anopheles stephensi, Ades aegypti* and *culex quinquefasciatus* (Ansari *et al.*, 2010).

Antimicrobial Activity: Cow urine extract of *Dalbergia sissoo* and *Datura stramonium* showed antibacterial potential against pathogenic strains of gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria. So it can be a potent antiseptic preparation for prevention and treatment of chronic bacterial infections (Yadav *et al.*, 2008).

Antioxidant Activity: Aqueous alcoholic bark extracts of *Dalbergia sissoo* Roxb. showed
antioxidant activity (Kumari and Kakkar, 2008). Methanolic extracts of trunk exudates of *Dalbergia sissoo* showed nitric oxide production inhibitory activity (Shrestha et al., 2008). Aqueous and methanol stem bark extracts of *Dalbergia sissoo* Roxb. shows antioxidant activity (Roy et al., 2011). In different extracts of stem bark of the plant *Dalbergia sissoo*, chloroform extract possesses marked antioxidant activity, where as methanol extract shows moderate activity in different in vitro antioxidant assays. Strong positive correlation was observed between the total phenolic content and different anti-oxidant assays which helped to conclude that phenolic compounds in the extracts of *Dalbergia sissoo* are able to scavenge DPPH, ferrous and nitric oxide and have reducing potential in addition to their ability to chelate metals such as iron (Kaur et al., 2011). *Dalbergia sissoo* root extract is effective in scavenging free radicals and has the potential to be a powerful antioxidant (Pooja et al., 2010).

**Molluscicidal Activity**: The crude ethanolic extracts of *Dalbergia sissoo* fruits and roots exhibited promising molluscicidal activities (LC90 values<100 mg⁻¹) against adult *Biomphalaria pfeifferi* with additional toxicities towards its 0-24 hr old egg masses (Adenusi and Odaibo, 2008)

**Anthelmintic and AntiSchistosomiasis Activity**: Different leaves extracts of *Dalbergia sissoo* showed anthelmintic activity (Hood et al., 2011). Ethanolic extracts of *Dalbergia sissoo* fruits, leaves, roots and stem bark showed antischistosomiasis activity. Ethanolic extract of the fruits was the most active with 100% mortality at 50 mg/l followed by those of the leaves ( at 100 mg/l), roots (at 200 mg/l) and stem bark (at 400 mg/l) (El-Din et al., 2011).
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_Citrus limon_ Linn.

_Citrus limon_ Linn. is an important member of genus _Citrus_ belonging to family _Rutaceae_. It is native to India, Australia, New Zealand, Mexico, America, Portugal, Italy, France and Spain (Bhattacharjee, 1998). Lemon varieties were first introduced into the Mediterranean countries when Romans navigated through the Red sea to India. Now Greece is one of the main exporters of _Citrus_ (Vekiari et al., 2002).

Lemon grows in the regions with temperate summers and mild winters, particularly in the Mediterranean countries, Southern California and Argentina (Lota et al., 2002). In India, it is cultivated in home gardens and small sized orchards in Uttar Pradesh, Bombay, Madras and Mysore.

SYNONYMS - (Agrawal et al., 2005)

<table>
<thead>
<tr>
<th>Language</th>
<th>Synonym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hindi</td>
<td>Jambirinibu, Nimbu</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Limbu</td>
</tr>
<tr>
<td>Marathi</td>
<td>Idlimbu</td>
</tr>
<tr>
<td>Telugu</td>
<td>Pedda nimba</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Jambir</td>
</tr>
<tr>
<td>Tamil</td>
<td>Periya elimichan</td>
</tr>
<tr>
<td>English</td>
<td>The lemon of India</td>
</tr>
</tbody>
</table>

BOTANICAL CLASSIFICATION -

<table>
<thead>
<tr>
<th>Classification Level</th>
<th>Taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom</td>
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</tr>
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<td>Devison</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
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</tr>
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</tr>
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</tr>
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<td>Family</td>
<td>Rutaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Citrus</td>
</tr>
<tr>
<td>Species</td>
<td>limon</td>
</tr>
</tbody>
</table>

(http://en.wikipedia.org/wiki/lemon)
The important lemon varieties grown in India are Nepali round, Nepali oblong, Seedless lemon or Baramasia, Eureka, Lisbon, Villofranca, Meyer and Malta.

**DESCRIPTION** - A tree up to 6 m in height, of spreading habit, the trees possesses spines, small, stout; leaves light green, oblong to elliptic ovate, lanceolate, sharp pointed, sub serrate, petioles narrowly winged; flowers purple in the bud, large; fruits fleshy, ovoid or oblong with terminal nipple, very acidic, seeds few, small (Anonymous, 1992).

**CHEMICAL CONSTITUENTS** - Lemon contains a number of important constituents (Table - 2) and (Figure-5).

### Table-2 : Chemical constituents in *Citrus limon* Linn.

<table>
<thead>
<tr>
<th>CAROTENOIDS IN PULP AND PEEL</th>
<th>FLAVONOIDS</th>
<th>COUMARINS AND PSORALENS IN FRUITS, STEM AND ROOT BARK</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhDIOFLUENNE, ❯ CAROTENE, ❯ CAROTENE, CRYPTOXANTHIN, VIOLAXANTHIN, AUROXANTHIN</td>
<td>NARINGIN, ERIOCITRIN, Hesperitin, Hesperidin, Neohesperidin, Diosmin, Apigenin, Chrysoeriol,isorhamnetin, limocitrin,isolimocitrin, limocitrol,isolimocitrol, quercetin, isovitexin, phlorin, eryodictyol</td>
<td>ERIOCITRIN, DIOSMIN, RUTIN, ISORHOIFOLIN, NEOPONCITRIN, NOBELETIN, NATSUIDDAIN, DEMETHYLNOBILETIN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BERGAPTEN, BERGAMOTTIN, BYAKANGELICIN, CITROPTEN, IMPERATORIN, ISOIMPERATORIN, ISOPIMPINELLIN, PHELLOPTERIN, PRANGOL, SCO PARON, SCOPOLETIN, UMBELLIFERONE, UMBELLIPREMIN, XANTHYLETIN</td>
</tr>
</tbody>
</table>


### Table-3 : Chemical constituents composition of leaf and peel oil of *Citrus limon* Linn.

<table>
<thead>
<tr>
<th>PEEL OIL</th>
<th>LEAF OIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-PINENE, B-PINENE, SABINENE, MYRCENE, LIMONENE, A-TERPINENE, P-CYMENE, NERAL, GERANIAL</td>
<td>A-PINENE, B-PINENE, SABINENE, 3-CARENE, MYRCENE, LIMONENE, CINEOLE, OCIMENE, 6-METHYLHEPT-5-EN-2-ONE, CITRONELLAL, LINALOOL, A-TERPINEOL, NERAL, NERYLACETATE, GERANIAL, GERANYL ACETATE, CITRONELLOL, NEROL, GERANIOL</td>
</tr>
</tbody>
</table>

(Lota *et al.*, 2002)
Review of Literature

Bergamottin

Vanillin

Bergapten

Limonene

<table>
<thead>
<tr>
<th>CHEMICAL CONSTITUENTS</th>
<th>R</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>C₂-C₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eriocitrin</td>
<td>RUTINOSYL</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>SINGLE</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>RUTINOSYL</td>
<td>OH</td>
<td>OCH₃</td>
<td>H</td>
<td>SINGLE</td>
</tr>
<tr>
<td>Rutin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>O</td>
<td>DOUBLE</td>
</tr>
<tr>
<td>Diosmin</td>
<td>RUTINOSE</td>
<td>OH</td>
<td>OCH₃</td>
<td>H</td>
<td>DOUBLE</td>
</tr>
</tbody>
</table>
Review of Literature

Figure-5: Chemical Structure of constituents present in *Citrus limon* Linn.

ETHNOPHARMACOLOGY - In the traditional systems of medicine the fruit, peel, pulp and juice have been used for treating different disorders.

**Fruits**: Fruits are digestive when boiled with sugar or honey (Limyati *et al*., 1998). They are used as remedy against plague; in the form of pickles they are useful in hypertrophy of spleen, as carminative, antiseptic and in dyspepsia (Pullaiah, 2006).

**Peel**: Peel as appetizer, cardiac tonic, elixir, hygienic, pulmonary sedative, stomachic (Arias and Laca, 2005). Tinctures and fluid extracts made from dried peel are used in the formulation of liqueres (Atal and Kapur, 1989).

**Pulp**: Pulp is antihelminthic, antilithic (Limyati *et al*., 1998), remedy against drunkenness (Ajaiyeoba *et al*., 2003) antipyretic, appetizer, cholagogue, cures boils and throat, tonsil abscesses, anti-inflammatory, vascular stimulant, antiemetic and prevents dizziness (Arias and Laca, 2005).

**Oil**: Oil of lemon is stimulant and rubifacient when applied externally, used in the large quantities for flavouring candies, pastry and baked goods (Atal and Kapur, 1989).

**Juice**: Juice is used as an antidote against poison; juice and gum powder used topically for scabies and in rheumatic gout. The bark and root has been used as a febrifuge and the seeds as a vermifuge in the West Indies (Kirtikar and Basu, 1993). Best remedy for scurvy, used in dysentery and diarrhea (Pullaiah, 2006).

**ACTIONS RECOGNISED IN CURRENT LITERATURE**

**Fruits** - Analgesic, antianaemic, antiemetic, antisclerotic, antipyretic, antiseptic, demulcent, moisturizing (Arias and Laca, 2005), kill intestinal worms (Aiyeloja and Bello, 2006), used for
treatment of snake bites in North-Western Colombia (Otero et al., 2000a; Otero et al., 2000b) used to treat abundant menses (Ososki et al., 2002).

**Pulp** - Antidiarrhoeic, intestinal mucosa protector, local haemostatic, vascular stimulant.

**Peel** - Antiseptic, carminative, diuretic, eupeptic (Arias and Laca, 2005) and antimalarial (Ali et al., 2004).

**REPORTED BIOLOGICAL ACTIVITY** – According to many scientific studies of *Citrus limon* shows broad spectrum of biological activities.

**Antifertility Activity**: Alcoholic extract and ethyl acetate fraction of *Citrus limon* seeds exerted reversible anti-fertility action on male albino rats by halting the development of sperms at spermatocytic level (Kulkarni et al., 2012).

**Antioxidant Activity**: Citrus fruits show antioxidant activity. *Citrus limon, Citrus reticulate* and *Citrus sinensis* exhibited stronger scavenging potential. The antioxidant activity of fruit juices was shown to be directly related to the content of ascorbic acid and total phenolics (Rekha et al., 2012). The leaves, fruits and peel of *Citrus limon* serve as the potential source of natural antioxidants (Mohammadian et al., 2012).

The flavone eriocitrin is abundant in lemon (Fuster, 1997). It is used in numerous multivitaminic complexes, in which flavonoids are used due to their antioxidant property for maintaining capillary integrity and peripheral circulation. Eriocitrin has the greatest antioxidant activity of all the glycoside flavonoids present in lemon fruits (Miyake et al., 1997; Miyake et al., 1998).

**Anticancer Activity**: Hesperidin present in *citrus limon*, is also an effective antioxidant since it is able to quench the oxygen free radicals which are involved in cancer (Galati et al., 1994; Monforte et al., 1995; Tanaka et al., 1996; Berkada et al., 1998; Koyuncu et al., 1999). Citrus limonoids may have potential for the prevention of estrogen-responsive breast cancer (MCF-7)
Review of Literature

via caspase-7 dependent pathways (Kim et al., 2013). Furocoumarins like bergamottin (5-geranyloxy- psolaren), 8-geranyl oxypsolaren and 5-geranyloxy 7 methoxy coumarin which are obtained from peel of lemon fruit inhibit the in vitro tumor promotion, superoxide and nitric oxide generation, thus act as chemopreventive agents (Miyake et al., 1999).

**Antihelmentic Activity:** Methanolic seed extract of *Citrus limon* showed significant antihelminthic activity at 50 mg/ml concentrations whereas Chloroform, showed moderate activity and Petroleum ether extract is having least antihelmintic activity (Munne et al., 2011). The ethanolic extracts exhibited varying degree of activity against the worms followed by death at all tested concentrations. The extract of *Citrus limon* Linn. peels was found to show potential anti-helmintic activity when compared to standard drug (Bairagi et al., 2011).

**Antibacterial Activity:** The antimicrobial activity of silver nanoparticles derived from lemon leaves showed enhancement in activity due to synergistic effect of silver and essential oil components of lemon leaves (Vankar et al., 2012). Citrus juices of *lemon* and *bitter orange* (*C. limon* and *C. auran-tium*) showed good antibacterial activities against gram positive and gram negative microorganisms (Waidulla et al., 2010). Citrus lemon has greater activity against *Vibrio cholerae* compared to the antibiotic erythromycin. It also indicates that the extracts is very active and possess anticholeric properties (Preetha and Judia, 2011). Ethyl acetate and acetone extract of *Citrus limon* peel showed antibacterial activity (kumar et al., 2011). The ethanol peel extract of *Citrus limon* shows antimicrobial activity against tested microorganisms (Maruti et al., 2011).

**Pediculicidal Activity:** *Citrus limon* juice showed significant pediculicidal activity. Decrease in morbidity of lice was observed with increasing dilutions of lemon juice (Shrivastava et al., 2010)
Hypolipidimic Activity: The *Citrus lemon* juice (1ml/kg/day) revealed a significant reduction in serum cholesterol, triglycerides; low density lipoprotein levels and resulted in an increase in high density lipoprotein. These results suggest that the hypocholesterolemic effects of *Citrus lemon* Linn. juice may be due to its antioxidant effect (Khan et al., 2010).

Antiobesity Activity: Hyperlipidemia, hyperglycemia and insulin resistance were significantly suppressed by lemon polyphenols. Supplementation with lemon polyphenols also significantly up-regulated the mRNA level of the peroxisome proliferator activated receptor-α (PPARα) compared to the low fat diet and high fat diet groups in the liver (Fukuchi et al., 2008).

Analgesic, Antiinflammatory and Antigout Activity: Hesperidin, the principal flavonone in lemon (Fuster, 2004) influences vascular permeability, increases capillary resistance and has analgesic and anti-inflammatory properties. Diosmin, a flavone present in *Citrus limon* improves muscular tone and vascular resistance to inflammatory processes, hence it is used against illnesses such as chronic venous insufficiency and rheumatic arthritis. It posses antihemorrhoidal, antioxidant and antilipid peroxidation properties (Berqvist et al., 1981; Darmon et al., 1987; Gabor, 1988; Loncampt et al., 1989; Jean and Bodinier, 1994). The leaves and peel extracts of *Citrus aurantium*, *Citrus limetta* and *Citrus limon* possess xanthine oxidase inhibitory activity that might be helpful in preventing or slowing the progress of gout and related disorders (Muthiah, 2012).

Antifeedant Activity: *Citrus* limonoids (Limonin, nomilin and obacunone) which are obtained from seeds of *Citrus limon* showed antifeedent activity against *Spodopetra frugiperda* thus confirming their probable role as chemical defense agents in *Citrus*-herbivore interactions (Ruberto et al., 2002).

LEMON OIL
Review of Literature

- Heart rate changes have been reported after exposure to lemon essential oil (Kikuchi et al., 1991).

- Lemon oil was found to decrease the stress induced behavioral effect and to decrease the pentobarbital sleeping time (Tsuchiya et al., 1991).

- Lemon oil odour can improve mood, creativity and perceived health (Knasko, 1992).

- Inhalation of lemon oil vapour induced anxiolytic and antidepressant like effect on EPM (Komori et al., 1995; Komiya et al., 2006).

- Lemon oil was also reported to protect the skin as antioxidant (Calabrese et al., 1999).

- Lemon oil vapours posses analgesic effect and have ability to modulate the behavioral and neuronal responses related to nociception and pain (Aloisi et al., 2002).

- Lemon oil inhibits the heat shock induced apoptosis of astrocytes. It suggests that lemon oil can promote neuroprotective effect on brain (Koo et al., 2002).

- The essential oil exhibited strong antioxidant activities as well as antiproliferative activity against HeLa cell line (Yan et al., 2010).

- Essential oil from the leaves of Citrus limon exhibits an antioxidant action in preventing lipoperoxidation (probably due to hydroxyl radical scavenging activity) and a clear antinociceptive activity (Campelo et al., 2011).

- Essential oil from leaves of Citrus limon exhibits antidepressant and anticonvulsant activity (Campelo et al., 2011).

- The Lemon peel oil has shown antimicrobial activity, but was active against both Gram+ve and Gram-ve organism (Roy et al., 2012).

- The use of essential oil from Citrus lemon as antibacterial agents will be suitable for applications on the food industry (Viuda et al., 2008)
Lemon oil shows larvicidal property (Zayed et al., 2009).

Lemon essential oil is used for personal protection against mosquito vectors of disease (Oshaghil et al., 2003).

Citrus peel essential oils shows biocidal activity against some food spoilage bacteria (Javed et al., 2011).

Essential oil of peel of Citrus limon shows cytotoxic effects (Monajemi et al., 2005).

Essential oil of leaves from Citrus limon shows sedative, anxiolytic and antidepressant effects (Lopes et al., 2011).

**Lemon Juice By-Products** - Lemon possesses the highest antioxidant potential among Citrus fruits and it is the most suitable fiber for dietary prevention of cardiovascular and other diseases (Gorinstein et al., 2001). High density fiber powder may be used for the enrichment of usually consumed food or for the production of dietary fiber tablets (Fernandez et al., 2003). High dietary fiber lemon powder obtained from lemon by products has good functional and microbial quality, as well as favorable physicochemical characteristics to be used in food formulations (Lario et al., 2004).

**TOXICOLOGY**

Lemon oil contains furocoumarin derivatives and is known to cause phototoxicity. Oxypeucedanin and bergapten were the substances which cause phototoxicity. Oxypeucedanin was found to elicit photopigmentation on coloured guinea pig skin without preceding visible erythema (Naganuma et al., 1985).
**Elaeocarpus sphaericus**

*Elaeocarpus sphaericus* (syn. *Elaeocarpus ganitrus*) commonly known as *Rudraksha* in sanskrit and Rudraki in Hindi. It is grown in Assam and Himalayan region of India for its attractive fruit stones and medicinal properties (Asolkar, 1992).

**SYNONYMS** - (www.ayushveda.com/herbs/Elaeocarpus ganitrus.htm.)

- Hindi - Rudraksha
- Gujarati - Rudrakshi Mara
- Bengali - Rudraky
- Marathi - Rudraki
- Sanskrit - Rudraksha
- Kannada - Chatt Sampangi
- Tamil - Bhootnshan
- English - *Elaeocarpus ganitrus*

**BOTANICAL CLASSIFICATION** - (www.ayushveda.com/herbs/Elaeocarpus ganitrus.htm.)

- Kingdom - Plantae
- Division - Magnoliophyta
- Class - Magnoliopsida
- Order - Oxilidales
- Family - Elaeocarpaceae
- Genus - *Elaeocarpus*
- Species - *sphaericus/ganitrus*
**DESCRIPTION** - *Elaeocarpus sphaericus* commonly known as *Rudraksha* is a large evergreen broad leaved tree found in tropical and subtropical areas at the attitude ranging from seacoast to 2,000 meters above the sea level (www.rudrakshanepal.com). The tree is considered as a threatened plant in North eastern region of India. The leaves of *Rudraksha* tree are shining green on the upper side with dull leathery on the dorsal side. They are ovate in shape. The leaves are 5-6 inch in length and 2-3 inch in width. The main trunk of *Rudraksha* tree is cylindrical with a greyish white & rough textured bark. The fruits of *Rudraksha* appear in June and ripen by August & October. They are globular in shape and sour in taste. The bead present inside is hard. Fruits are 1cm. in diameter and dark blue. The flowers of *Rudraksha* are white with fringed petals and they appears in April- May. The flowers are ½ inch in diameter. Flowers arise from axils of fallen leaves, nodding, white, about 1cm across, anther bristled at the apex (Khan et al, 2004).

**COMPOSITION OF RUDRAKSHA SEED** - *Rudraksha* beads is a plant product. It contains carbon, hydrogen, nitrogen, oxygen and trace elements in combined form. Percentage composition of gaseous elements in Rudraksha beads (Handicraft Indiamart.com).

**Table -4 : Composition of Rudraksha Seed**

<table>
<thead>
<tr>
<th>Gaseous elements</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>50.031</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.95</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>17.897</td>
</tr>
<tr>
<td>Oxygen</td>
<td>30.53</td>
</tr>
</tbody>
</table>

**CHEMICAL CONSTITUENTS** - Alkaloids of the plant are of Indolizidine type: From the leaves isomeric alkaloids of molecular formula, C$_{16}$H$_{21}$NO$_2$, have been isolated such as: (+) Elaeocarpidine, Isoelaeocarpidine, (+)-Elaeocarpine, (+)-Isoelaeocarpine, Elaecarpiline,
Isoelaeocarpiline and Alloelaeocarpiline. Five new indolizidine alkaloids, Grandisines C, D, E, F, and G, and one known indolizidine alkaloid, (−)-isoelaeocarpiline, were isolated from leaves of *Elaeocarpus grandis*. Rudrakine is also example of non aromatic Indolizidine (Johns et al., 1970; Johns et al., 1971; Gribble et al, 1988).

**Tannins (Leaf)** : Gallic acid and Ellagic acid (Chand et al., 1977; Ray et al., 1979)

**Flavonoids (Leaf)** : Quercetin (Chand et al., 1977).

**Fatty Acids (Seed)** : Palmitic acid, isopalmitic acid and linoleic acid (Rastogi and Mehotra, 1991).

**TRADITIONAL AND AYURVEDIC PROPERTIES OF RUDRAKSHA SEED** -

Ayurveda refers to this wonderful bead and gives details of *Rudraksha* for strengthening body constitutions. The seeds of *Rudraksha*, its bark and leaves all are used to cure various ailments like mental disorders, headache, fever, skin diseases etc. *Rudraksha* may be worn either on wrist, arm or other parts of the body for the curing of many diseases such as:

**Blood Purifier**: *Rudraksha* is used for treating the blood impurities and strengthens the body substance (Khare, 2004).

**Blood Pressure**: *Rudraksha* is used to treat high blood pressure (Sakat et al., 2009).

**Brain diseases**: *Rudraksha* is used for treating all brain diseases like brain fever. This also helps in increasing memory (Khare, 2004).

**Controlling Epilepsy**: The pulp of *Rudraksha* fruit or bark is used for controlling epilepsy. It is also good for womens who suffering from hysteria and coma (Gupta et al., 1984).

**Jaundice and Stomachache**: *Rudraksha* is used for treating stomach pain and liver problems

**Anxiety**: *Elaeocarpus sphaericus* imposes positive effect on stress, anxiety, depression, palpitation and lack of concentration (Rama, 2010).
**Review of Literature**

**Temperature:** It cools down the body temperature and brings calm to mind (Khare, 2004).

**Small pox:** Powdered seeds of *Elaeocarpus sphaericus* with equal quantity of black pepper should be taken with water to cure small pox (Swami et al., 2010).

**OTHER USES OF RUDRAKSHA SEED:** Unripped and riped fruits of *Elaeocarpus sphaericus* shows different uses:

- *Elaeocarpus sphaericus* is used to make prayer beads.
- The fruits of this plant are cleaned, polished, sometimes stained and used for making bracelets and other ornamental objects (Anonymous, 2001).
- *Elaeocarpus sphaericus* also possess antiageing property (http://rudraksha.mht,2009).
- *Rudraksha* is used in cosmetics to bring skin glow, also brings in a charming face.

**RUDRAKSHA AND HINDU MYTHOLOGY**

All legends pertaining to the origin of Rudraksha describe them as tears shed by Lord Shiva. According to one story, Lord Shiva once entered a profound state of meditation for the benefit of mankind. When he emerged from this state and opened his eyes, the deep joy and peace that he felt were expressed as tears, which ran down his cheeks and fell on the earth. From his tears emerged the Rudraksha tree. The word Rudraksha, in fact, comes from two Sanskrit words – ‘rudra’, a synonym for Lord Shiva and ‘aksha’ meaning eyes (Ramadurai, 2007).

Most of power of *Rudraksha* seems to be associated with no. of mukhis that the bead has *Rudraksha* available in 1 to 38 mukhis but *Rudraksha* of 1-14 mukhis are commonly found. The five faced *Rudraksha* are found easily & abundantly. One mukhi *Rudraksha* is rare. Depending upon the availability & production of *Rudraksha* different prices have been allocated for different mukhis *Rudraksha* (Indiamart.com).

**Table-5 : Different Mukhis of Rudraksha**
RUDRAKSHA

MAJOR BENEFITS

1 Mukhi | Enlightens the super consciousness, provides improved concentration and mental structure changes.

2 Mukhi | Blesses the wearer with ‘UNITY’. It could be related to Guru- Shishya, parent- children or friends.

3 Mukhi | The wearer gets free from sins or wrongs from his life and returns to purity. Ideal for those who suffers from guilt and depression.

4 Mukhi | The wearer gains power of creativity when blessed, increases memory power and intelligence.

5 Mukhi | Wearer gains health and peace, increases memory also.

6 Mukhi | Saves from emotional trauma of worldly sorrows and gives learning, wisdom and knowledge.

7 Mukhi | It should be worn by peoples who are suffering from finance and mental set-up.

8 Mukhi | Removes all obstacles and brings success in all undertakings.

9 Mukhi | Wearer is blessed with lot of energy, powers, dynamisms and fearlessness which are useful to live a life of success.

10 Mukhi | It works like a shield on one’s body and drives evils away.

11 Mukhi | Blesses wearer with wisdom, right judgment, fearlessness and success.

12 Mukhi | Wearer gets the quality of sun- to rule and to move continuously with brilliant radiance and strength. Removes worry, fear. Increases self image and motivation.

13 Mukhi | It gives honor and fulfills all the earthly desires. It is helpful for meditation.

14 Mukhi | It awakens the sixth sense organ by which the wearer foresees the future happenings.

RUDRAKSHA OIL

It is cold compressed 100% pure oil extracted from Rudraksha seeds. It is used as a dietary supplement. Two drops of oil once a day is required for internal healing. It is also used as externally as hair oil daily, removes dandruff and acts as hair conditioner, reduces acne & pimples. It also pacify skin condition such as eczema, ringworm removes itching and helps to heal faster. It is also used as body massage oil (www.rudraksha-ratna.com).

REPORTED BIOLOGICAL ACTIVITIES
Antihypertensive Activity: Aqueous extract of *Elaeocarpus ganitrus* Roxb. seeds powder at the dose levels of 25, 50 and 100 mg/kg, I/V., shows antihypertensive activity in renal artery occluded hypertensive rats (Sakat *et al*., 2009; Lakshmi *et al*., 2011).

Antidepressant Activity: Ethanol extract (90%) of the fruits of *Elaeocarpus ganitrus* showed central nervous system antidepressant effect, characterized by typical behavioural actions, potentiation of hexobarbitone hypnosis and morphine analgesia, anticonvulsant and anti-amphetamine effects. In addition the extract showed a cardiotonic, depressor, smooth muscle relaxant and hydrocholeretic activities, part of these being mediated through beta adrenoreceptor stimulation and in part through a direct musculotropic effect (Bhattacharya *et al*., 1975).

The benzene (BE), chloroform (CE), and acetone (AE) extracts of *Elaeocarpus sphaericus* fruits at a dose of 200 mg/kg, i.p. showed a tendency to decrease swim stress immobility (Singh *et al*., 2000a and 2000b).

Analgesic and Antiinflammatory Activity: *Elaeocarpus sphaericus* fruits at a dose of 50-200 mg/kg orally shows analgesic activity against both acute and sub-acute models (Singh *et al*., 2000a). Sequential Petroleum ether (PE), benzene (BE), chloroforms (CE), acetone (AE) and ethanol (EE) extracts (50-200 or 200 mg/kg, ip, or 200 mg/kg, po) of dried *Elaeocarpus sphaericus* fruits, showed significant anti-inflammatory action against both acute and sub-acute models in rats (Singh *et al*., 2000a and 2000b).

Antioxidant Activity: The ethanolic extract of leaves of *Elaeocarpus ganitrus* at 500 ug/ml shows maximum iron chelating activity (76.70%) followed by the scavenging of the ABTS$^+$ radical (55.77%) at the same concentration. The extract showed only moderate hydroxyl radical scavenging activity (13.43%). This activity is due to phenolic and flavonoids in the leaves provide substantial antioxidant activity (Kumar *et al*., 2008).
Antimicrobial Activity: Petroleum ether, benzene, chloroform, acetone and ethanol extracts of dried *Elaeocarpus sphaericus* fruit showed significant antibacterial activity against some pathogenic bacteria. Fruit juice of this plant has been used in treatment of diarrhoea and dysentery (Nath and Singh, 1999; Shazid et al., 2010). Aqueous extract of *Elaeocarpus ganitrus* leaves exhibited excellent antibacterial activity (Kumar et al., 2011).

Anxiolytic Activity: *Elaeocarpus ganitrus* (*E. sphaericus*) seeds shows anxiolytic activity in a polyherbal formulation (*Nordostachys jatamansi* (root) 100 mg, *Rauwolfia serpentina* (root) 100mg, *Acorus calamus* (rhizome) 75 mg, *Elaeocarpus ganitrus* (seed) 75 mg, *Withania somnifera* (rhizome) 75 mg and *Tinospora cordifolia* (stem) 75 mg) i.e., Tensarin tablet (Rauniar et al., 2007). Ethanolic and chloroform extract of seeds of *Elaeocarpus sphaericis* shows significant antianxiety activity (Singh et al., 2012a). Methanolic extract of *Elaeocarpus sphaericus* fruits at the dose of 200 mg/kg increased the percentage of time-spent and the percentage of arm entries in the open arms of the Elevated Plus-Maze (EPM) and decreased the percentage of time-spent in the closed arms of EPM (Shah et al., 2010).

Antiasthamatic Activity: The petroleum ether (PE), benzene (BE), chloroform (CE), acetone (AE) and ethanol (EE) extracts of *Elaeocarpus sphaericus* fruits shows mast-cell stabilizing activity against bronchial asthma (Singh et al., 2000a).

Antiulcerogenic Activity: The petroleum ether (PE), benzene (BE), chloroform (CE), acetone (AE) and ethanol (EE) extracts of *Elaeocarpus sphaericus* fruits shows significant antiulcerogenic activity in a dose of 200 mg/kg, p.o. (Singh et al., 2000b).

Hypnotic Activity: The petroleum ether (PE), benzene (BE), chloroform (CE), acetone (AE) and ethanol (EE) extracts of *Elaeocarpus sphaericus* fruits showed significantly enhanced pentobarbitone sleeping time (Singh et al., 2000a).
Antifungal Activity: Chloroform and ethanol extracts of *Elaeocarpus ganitrus* showed high antifungal activity against *Candida albicans*. Whereas, chloroform, ethanol and water extracts showed moderate inhibition against *Aspergillus niger* (Singh et al., 2010a and 2000b).

Immunostimulant activity: The alkaloidal fraction of *Elaeocarpus ganitrus* has shown significant stimulation of NBT reduction and non-significant increase of lysosomal enzyme activity of PEC indicating enhanced phagocytic capability of these cells (Amolkar and Archana, 2009).

Antidiabetic activity: In normoglycemic rats, aqueous extract of *Elaeocarpus ganitrus* (EGA) showed a significant (p<0.05) hypoglycemic effect at 2 hr. In STZ-induced diabetic rats, the EGA showed a significant (p<0.05) decreased the blood glucose level in a dose-dependent manner during the 30 days of treatment period. EGA modulated lipid profile changes in STZ-diabetic rats in a dose-dependent manner (Kumar et al., 2011).

Leaf extract of *Elaeocarpus ganitrus* produced hypoglycaemic effect in normal rats. The study indicates clinically significant antidiabetic activity of *Elaeocarpus ganitrus* in diabetic rats. The chitosan based extract improved the anti-diabetic activity of *Elaeocarpus ganitrus* clearly indicating synergism (Rao et al., 2012).

Other Activities

Ethanolic extract of fruits exhibit sedative, hypnotic, tranquillizing, anticonvulsive, antiepileptic and antihypertensive properties (Bhattacharya et al., 1975; Pandey and Bhattacharya, 1985).