1. INTRODUCTION

1.1 Anxiety disorders: An overview

Global scenario of persons afflicted by mental disorders is alarming (Barbotte et al., 2001). About 500 million people suffer from neurotic, stress related and somatoform problems, 200 million from mood disorders, 83 million from mental retardation, 30 million from epilepsy, 22 million from dementia, and 16 million from schizophrenia. These disorders are more common in developed countries. In the US, about 44.3 million people suffer from diagnosable mental disorder (NIMH, 2001). A survey of ‘Self-reported work-related ill-health’ (SWI 2003/2004) estimated that work-related stress, depression or anxiety affected 5,57,000 people in Great Britain, with an estimated 12.8 million lost working days (HSE, 2005).

Anxiety disorders are serious medical illnesses that have affected 1/8th of total population worldwide irrespective of gender, age, religion, nationality and profession (NIMH, 2002). Anxiety Disorders Association of America (ADAA) described anxiety disorders as the most common mental illness in the US, that have affected 19.1 million (13.3%) of the adult (18-54 years) US population (ADAA, 2003). A study commissioned by ADAA on ‘The Economic Burden of Anxiety Disorders’ revealed that anxiety disorders cost the US more than $42 billion a year, almost one-third of the $148 billion total mental health bill for the US. In India, prevalence rate for all mental disorders is 65.4 per 1000 population, and that for anxiety neurosis is 18.5 per 1000 population (Madhav, 2001). The Global Research on Anxiety and Depression (GRAD) network, a consortium of world’s leading psychiatric epidemiologists and clinical researchers, during the 154th annual meeting of ‘American Psychiatric Association’ (APA) has observed that, “a significant number of world’s population is plagued by chronic and excessive anxiety, also known as generalized anxiety disorder (GAD), which is more serious than those of lung disease, sleep disorders and major depression, and affects more than 5% of the world population” (GRAD, 2001). Following is the categories of anxiety disorders (ADAA, 2003; NMHA, 2005):
1. **Panic disorder (PD)** is characterized by panic attacks, sudden feeling of terror that strike repeatedly and without warning. Physical symptoms include chest pain, heart palpitations, sweating, trembling, shortness of breath, dizziness, abdominal discomfort, fear of losing control, fear of dying, tingling sensations, and hot flushes. Panic disorders have affected 2.4 million (1.7%) adult US population. Women are twice more likely to be afflicted than men.

2. **Obsessive–compulsive disorder (OCD)** is characterized by uncontrollable obsessions (recurring thoughts or impulses that are intrusive or inappropriate and cause the sufferer anxiety) and compulsions (repetitive behaviours or rituals). It has affected 3.3 million (2.3%) adult US population. It is equally common among men and women.

3. **Post-traumatic stress disorder (PTSD)** is characterized by persistent symptoms (nightmares, flashbacks, numbing of emotions, depression, feeling angry and irritable) that occur after experiencing a traumatic event such as war, rape, child abuse and natural disaster. It has affected 5.2 million (3.6%) adult US population. Women are more likely to be afflicted by this disorder.

4. **Social phobia or Social anxiety disorder (SAD)** is characterized by an intense fear of situations where embarrassment may occur. Physical symptoms include palpitations, tremors, sweating, diarrhoea, confusion and blushing. It has affected 5.3 million (3.7%) US adult population. It is equally common among men and women.

5. **Specific phobia (SP)** is characterized by the excessive fear of an object or a situation, exposure to which causes an anxious response. Specific phobias affect an estimated 6.3 million (4.4%) US adult population and are twice as common in women as in men.

6. **Generalized anxiety disorders (GAD)** are characterized by chronic, exaggerated worry about everyday routine life events and activities, lasting at least six months. Physical symptoms include fatigue, trembling, muscle tension, headache or nausea. It has affected an estimated 4 million (2.8%) US adult population and is twice as common in women as in men. Though, GAD is the most frequent anxiety disorder, yet only 20% of patients...
receive proper treatment (Robins and Regier, 1991; Kendler et al., 1992). GAD results loss of 6 for every 30 work-impairment days.

1.2 Causes of anxiety disorders

Various factors causing anxiety disorders are described below (APA, 1999; NHS Direct, 2004; The Health Center, 2004; Repich, 2005; UMMC, 2005).

1.2.1 Heredity/Genetic factors

Anxiety disorders (PD and OCD) tend to run in families. Studies have shown that if one of the twins has an anxiety disorder, the second is more likely to have an anxiety disorder.

1.2.2 Brain chemistry

The symptoms of long term social anxiety disorder can be attributed to the improper chemical balance in the brain. Several neurotransmitters namely serotonin, norepinephrine, gamma-aminobutyric acid (GABA), which are produced in the brain, directly affect one’s feelings about a given situation. Thus brain, too, appears to play a role in the onset of anxiety disorders because symptoms of anxiety disorders are often relieved by medications that alter the level of chemicals in the brain.

1.2.3 Personality

People with low self-esteem and poor coping skills are more prone to anxiety disorders. Conversely, an anxiety disorder that begins in childhood may itself contribute to the development of low self-esteem.

1.2.4 Life experiences

Long term exposure to abuse, violence, poverty or stressful experiences (the early death of a parent, bad marital or family relationships, or traumatic experiences) may affect individual’s susceptibility to anxiety disorders.
1.2.5 Stress overload/Lifestyle factors
Excessive stress over time, and poor lifestyle habits such as overwork, lack of sleep, poor diet and lack of regular exercise promote anxiety.

1.2.6 Thought patterns
Negative thoughts can actually create physical symptoms of anxiety.

1.3 Management of anxiety disorders
Such a horrid emergence of mental disorders has attracted the attention of researchers towards various pharmacotherapeutic approaches for the management of these ‘modernization borne diseases’ (Barbotte et al., 2001). Barbiturates, benzodiazepines (BZDs), azaspirones, norepinephrine and serotonin-reuptake inhibitors, monoamine oxidase inhibitors and phenothiazines are some of the commonly used psychotropic drugs (Baldessarini, 2001). Among these, BZDs are the most widely prescribed synthetic chemical drugs for the treatment of anxiety, insomnia, epilepsy, and stress. Regular use of BZDs causes deterioration of cognitive functioning, addiction, physical dependence and tolerance (Council Report, 1997; Longo and Johnson, 2000; Baldessarini, 2001). Abrupt cessation of chronic treatment with BZDs causes the appearance of withdrawal effects comprising re-bound anxiety, restlessness, epilepsy, and motor agitation (Busto et al., 1986; Ashton, 1995). In the light of adverse effects associated with the synthetic drugs, researchers have been exploring natural resources to find out safer and effective drugs. Investigating plants, based on their use in traditional systems of medicine, is a sound, viable and cost effective strategy to develop new drugs (Dhawan, 1995). Plants like Valeriana officinalis, Nardostachys jatamansi, Withania somnifera and Panax ginseng have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Inclusion of these well-established CNS affecting plants in the arsenal of modern therapeutics has revived the faith of researchers in the plants (Bloom and Kupfer, 1994).
1.3.1 Plants having anti-anxiety activity

Following review has been compiled on anxiolytic plants using references from major databases such as Chemical Abstracts, Medicinal and Aromatic Plants Abstracts and Pub Med, and covers the publications during the period 1998 to 2005.

*Abies pindrow* Royle Syn. *A. webbiana* (Talispatra; Pinaceae) leaves have been useful Ayurvedic remedy for fever, inflammatory conditions, bronchitis, asthma, expectorant, and as carminative. The ethanolic extract of *A. pindrow* leaves did not exhibit behavioural effects upon administration of single dose, but when administered orally once daily for three consecutive days at different dose levels (50 or 100 mg/kg, p.o.), the extract showed significant dose dependent anxiolytic effects on all the paradigms of anxiety viz., OFB, EPM and EZM (Kumar et al., 2000a). *A. pindrow* induced a significant increase in the open field ambulation and slight increase in rearings but did not affect grooming and fecal dropping. It also increased the time spent in open arms, head dips and stretch attend posture in rats on EPM apparatus.

*Achillea millefolium* Linn. (Yarrow milfoil; Asteraceae) has been traditionally used in the treatment of sore throat, hemoptysis, haematuria, diabetes, spasmodic disorder and menorrhagia. In Mexico, the plant has been used to alleviate hemorrhoids, hypertension, indigestion, insomnia, migraine, inflammation, diarrhoea, anxiety, and as a sedative. The aqueous extract of *A. millefolium* flowers was evaluated for anti-conflict effects in female Wistar rats during late proestrus or diestrus (Molina-Hernandez et al., 2004a). It reduced conflict behaviour during late proestrus at doses of 8, 10 or 12 mg/kg, p.o., but only higher dose (12 mg/kg) was found to be effective to reduce conflict behaviour during diestrus.

*Acorus calamus* Linn. (Vach; Aroidaceae) / *Bacopa monnieri* Linn. (Brahmi; Scrophulariaceae) have been used in Indian system of medicine as nerve tonic, stimulant, and in atonic dyspepsia and flatulent colic. The combined effect of *A. calamus* and *B. monnieri* was assessed at a dose of 500 mg TDS for 6 weeks in
patients suffering from anxiety neurosis (Singh et al., 2003). Among 81, 47 adult subjects showed a significant improvement in nervousness, restlessness, irritability, poor concentration, sleep and loss of appetite. Encouraging results have been observed on certain electrophysiological parameters like EEG, EMG, GSR etc.

*Albizia* spp. (Mimosaceae) have been used to treat depression, anxiety, insomnia, restlessness, pain, and to invigorate blood circulation. The aqueous extract of *A. julibrissin* Durazz. (Nemunoki, momosa) stem bark (100 or 200 mg/kg, p.o.), upon single or repeated treatment for seven days, exhibited significant anxiolytic activity in rats using EPM apparatus (Kim et al., 2004). Anti-anxiety activity of the plant was suggested via the serotonergic system as pindolol (10 mg/kg, i.p.) abolished anxiolytic effects.

Saponins rich *n*-butanolic fraction (25 mg/kg, p.o.) extracted from *A. lebbeck* Benth. (Siris) leaves exhibited significant anti-anxiety activity in dose dependent manner in mice using EPM model of anxiety (Une et al., 2001).

*Aniba riparia* (Nees) Mez (Leuro; Lauraceae). Riparin III (25 or 50 mg/kg, i.p.), isolated from unripe fruits of *A. riparia*, significantly increased the number of entries in the open arms of the EPM, increased the number of head dips in the hole board test, reduced sleeping latency and prolonged pentobarbital-induced sleeping time, decreased the immobility time in the tail suspension and forced swimming tests in mice (Sousa et al., 2004). These observations suggested that riparin III possesses antidepressant and anxiolytic like effects.

*Casimiroa edulis* Llave & Lex (White sapota; Rutaceae) leaves have been used in Indian system of medicine as a remedy for diarrhoea, and as anthelmintic. In Mexico, aqueous extract of the dried leaves of *C. edulis* are claimed to possess anticonvulsant and anxiolytic activity. The aqueous extract of *C. edulis* leaves significantly increased open arms exploration in the EPM test, increased the number of squares crossed in the OFB test in male Wistar rats at doses of 25 or 35 mg/kg, i.p., and decreased locomotion in the EPM and OFB at higher doses (45 or 55 mg/kg) (Molina-Hernandez et al., 2004b). The extract when co-administered with desipramine cancelled its antidepressant effects.
**Cecropia glazioui** Sneth (Embauba; Moraceae) has long tradition of use as antiasthmatic, anxiolytic, antihypertensive and cardiotonic in tropical and subtropical Latin America. Rocha et al. (2002) reported that *C. glazioui* exhibits calming effects in mice. Repeated administration (three times over a 24 h period) of aqueous extract (0.5 or 1.0 g/kg, p.o.) of *C. glazioui* leaves increased the frequency of entries in the open arms of EPM three-fold when compared to that of single dose administration. Exactly similar profile of action was observed with the butanolic fraction (25-100 mg/kg, p.o.) of aqueous extract but not with remaining aqueous fraction.

**Celastris paniculatus** Willd. (Jyotishmati; Celastraceae). In Ayurveda, leaves of *C. paniculata* have been reported as emenagogue and antidote for opium poisoning; bark as abortifacient; seeds as laxative, emetic, tonic and stimulant. Petroleum ether extract of seeds of *Celastrus paniculatus* exhibited antianxiety activity at a dose of 3.2 g/kg/day for 5 days in mice (Jadhav et al., 2003).

**Centella asiatica** (Linn.) Urban (Gotu Kola; Apiaceae). The earlier eclectic physicians had prescribed Gotu Kola to treat emotional disorders such as depression and fears (Gotu Kola, 1999). Sedative and anxiolytic properties of *C. asiatica* have been tested on small animals and are believed to be associated with its saponin glycosides brahmoside and brahminoside constituents (Cauffield and Forbes, 1999). A double-blind, placebo-controlled study was performed on 20 healthy subjects to evaluate anxiolytic activity of *C. asiatica* (Bradwejn et al., 2000). *C. asiatica* (12g, single oral dose) significantly attenuated the peak of acoustic startle response amplitude 30 and 60 minutes after treatment.

**Ceratonia siliqua** Linn. (Carob; Leguminosae) is mainly used in food industry for the high amount of gum in the seeds. It has been reported that leaves and pods of the plant contain compounds which showed high affinity for both central and peripheral BZD receptor sites (Avallone et al., 2002). Authors have suggested that methanol extract of *C. siliqua* leaves and pods can be used to obtain anxiolytic and sedative effects.
Citrus aurantium Linn. (Sour orange; Rutaceae) is used in Brazilian folk medicine and other countries to treat insomnia, anxiety and epilepsy. In an experiment, essential oil from peel (EOP), and hexane (HF), dichloromethane (DF) and aqueous fractions (AF) of hydro-ethanolic (70% w/v) extract of C. aurantium leaves were evaluated for sedative/hypnotic activity by observing increase in sodium pentobarbital induced sleeping time, anxiolytic activity using EPM apparatus, OFB and rotarod test, and anticonvulsant activity in PTZ- and MES-induced convulsions in mice at doses of 0.5 or 1.0 g/kg, p.o. (Carvalho-Freitas and Costa, 2002). EOP exhibited anticonvulsant activity at the dose of 0.5 g/kg as it increased the latency period of tonic seizures in both convulsing experimental models. HF and DF exhibited only sedative activity at the dose of 1 g/kg. This effect was not dose dependent. Group of mice treated with EOP (1 g/kg) increased the sleeping time induced by barbiturates (sedative activity) and the time spent in the open arms of EPM (anxiolytic activity), but failed to respond in OFB and rotarod tests. Finally, authors concluded that the anxiolytic effect observed with EOP (1 g/kg) in EPM test was due to motor dysfunction.

Clitoria ternatea Linn. (Butterfly pea; Fabaceae) has long tradition of use for its cathartic action. The methanol extract of C. ternatea (100-400 mg/kg, p.o.) roots has been reported to increase occupancy in the open arms of EPM by 160% and in the lit box of the light/dark exploration test by 157% (anxiolysis), the duration of immobility in tail immersion test (antidepressant activity), reduce stress-induced ulcers and reduce the convulsive action of PTZ and MES in mice and rats (Jain et al., 2003). The extract also decreased the time required to occupy the central platform in the EPM and increased discrimination index in the object recognition test, indicating its nootropic activity.

Coriandrum sativum Linn. (Dhaniya; Umbelliferae) has been recommended for the relief of anxiety and insomnia in Iranian folk medicine. The aqueous extract of C. sativum seeds (100 mg/kg, p.o.) exhibited significant anxiolytic activity using EPM apparatus in mice (Emamghoreishi et al., 2005).

Davilla rugosa Poiret (Cipo-Caboclo; Dilleniaceae). Aqueous infusion of D. rugosa stems has been traditionally used as anti-inflammatory, antiulcer,
purgative, stimulant, aphrodisiac and tonic. In an experiment, the hydro-alcoholic extract of stems (HE) was fractionated with chloroform (CF), chloroform/ethyl acetate (CAF), ethyl acetate (EF) and ethanol/water (EWF) (Guaraldo et al., 2000). Rats were treated orally with HE (7.5, 15, 30 or 60 mg/kg, p.o.) or fractions (15 mg/kg, p.o.). In the OFB, HE (15 mg/kg, p.o.), AEF, EF and EWF (15 mg/kg, p.o.) increased locomotion frequency and decreased immobility time. An opposite effect, i.e., decrease in locomotion frequency was observed at higher doses, i.e., 30 or 60 mg/kg, p.o. of HE. In the EPM, total number of entries into the open and closed arms, and time spent in the open arms were increased only with 15 mg/kg, p.o. dose of HE.

**Echium amoenum** Fisch. Et Mey. (Viper’s bugloss; Boraginaceae). In Iran, the decoction of dried violet-blue petals of *E. amoenum* has long been known to have tranquilizing effects. Ethanolic extract of the plant flowers at a dose of 50 mg/kg, i.p. increased the percentage of time spent and the percentage of the entries in open arms of EPM (Rabbani et al., 2004). Another group of researchers found similar effects in the aqueous extract of *E. amoenum* (5, 10, 30, 62.5, 80 or 125 mg/kg, i.p.) (Shafaghi et al., 2002). The extract exhibited significant dose dependent increase in time spent in the open arms of EPM.

**Erythrina mulungu** Linn. (Mulungu; Leguminosae-Papilionaceae). In herbal medicine, a leaf/bark decoction or tincture from the plant is considered to calm agitation and other disorders of the nervous system, including insomnia. The hydro-alcoholic extract from the inflorescence of *E. mulunga* was evaluated for anxiolytic activity in defensive behaviours related to generalized anxiety and panic disorder in rats using different anxiety models such as the ETM, the light/dark transition, and the cat odor test (Onusic et al., 2002; Onusic et al., 2003). The extract exhibited anti-anxiety activity upon acute (200 mg/kg, p.o.) as well as chronic (50 mg/kg, p.o. for 7 days) administration. The extract increased avoidance latency in T maze test in a way similar to diazepam. It also increased significantly the number of transitions between the two compartments and the time spent in the lightened compartment of the light/dark transition apparatus. On the other hand, escape latencies (T-maze test), locomotor activity and behavioural measurements in response to cat odor test were not altered by the treatments.
From these observations, authors have suggested that *E. mulunga* exerts anxiolytic-like effects on a specific subset of defensive behaviours, particularly those that have been shown to be sensitive to low doses of BZDs.

*Eschscholzia californica* Cham. (Californian poppy; Papaveraceae) exhibits anxiolytic activity but is devoid of myorelaxant or anticonvulsant activities (Rolland et al., 2001). Hydro-alcoholic extract (60%) significantly increased the time spent by mice in the lit box of light/dark model at a dose of 25 mg/kg, i.p. These anxiolytic effects seemed to be mediated through BZD receptors as flumazenil suppressed these effects.

*Euphoria longana* Lamarck (Longan Arillus; Sapindaceae) pulp or flesh has been used as a tonic, and for the treatment of amnesia, insomnia, various palpitations due to fright, etc., in herbal remedy prescriptions of Chinese and Japanese traditional medicine. The aqueous extract of *E. longana* exhibited significant anti-anxiety activity at a dose of 2 g/kg, s.c. in mice using Vogel type anti-conflict method (Okuyama et al., 1999). Adenosine, isolated from *E. longana*, produced significant anti-conflict effect at a dose of 30 mg/kg, s.c.

*Eurycoma longifolia* Jack (Tongkat Ali; Simaroubaceae). The decoction of roots of this plant has been used as a health tonic and antistress remedy. The chloroform, n-butyl alcohol and water fractions obtained from methanol extract of *E. longifolia* roots, when administered separately for 5 days twice daily at a dose of 0.3 mg/kg, p.o., produced a significant increase in the number of squares crossed and decreased immobility in the OFB test, increased the open arms entries and time spent in the open arms of the EPM, and significantly decreased the fighting episodes (footshocks of 2-mA intensity) in the treated mice, thereby, confirming anxiolytic activity of the plant (Ang and Cheang, 1999).

*Ginkgo biloba* Linn. (Ginkgo; Ginkgoaceae) has been traditionally used worldwide for cerebrovascular insufficiency, cardiovascular malfunction and bronchitis. It has been reported that single oral dose (0.5 or 1.0 g/kg) administration of *G. biloba* extract (GBE), standardized to contain 24% ginkgo-flavoglycosides and 6% ginkgo-terpenoid lactones, shortened the time spent in the
open arms of elevated plus maze, while sub-acute administration (7 days) of GBE prolonged the time spent in the open arms (Kuribara et al., 2003). Significant anxiolytic effects were produced at a dose of 125 mg/kg. Ginkgolide A-C and bilobalide, isolated from the plant, were also evaluated for anti-anxiety activity. Only ginkgolide A exhibited anxiolytic activity upon daily administration (1 or 2 mg/kg, p.o.) for five days. Ginkgolic acid conjugates (GAC) isolated from the leaves of Indian *G. biloba*, and its two market preparations, i.e., EGb 761 (containing 24% Ginkgo flavone glycosides and 6% terpenes) and Ginkgocar were evaluated for anxiolytic activity in rats using variety of experimental models viz., EPM, OFB, novelty-induced feeding latency and social interaction (Satyan et al., 1998). GAC (0.6 mg/kg, p.o.), on acute administration, augmented open arm entries, the open arm/closed arm entries ratio and increased time spent in the open arm of the EPM. In the OFB, it increased ambulation and reduced immobility time. GAC significantly attenuated the increased latency to feed in novel environment. EGb and Ginkgocar did not evoke significant activity. These observations suggested GAC are the anxiolytic constituents of *G. biloba*.

*Glycyrrhiza glabra* Linn. (Liquorice; Leguminosae). The hydro-alcoholic extract of *G. glabra* roots and rhizomes was evaluated for anxiolytic activity using different paradigms like EPM, foot shock induced aggression, etc., at dose levels between 10-300 mg/kg, i.p. (Ambawade et al., 2001). The extract increased the duration of occupancy by mice in open arms of EPM and also increased latency to foot shock-induced aggression in dose dependent manner.

*Hypericum perforatum* Linn. (St John’s wort; Guttiferae) has been traditionally used as antidepressant, anti-anxiety, anti-inflammatory, sedative, antistress and analgesic. The standardized extract of the whole plant, containing 0.54% total hypericins (0.11% hypericin and 0.43% pseudohypericin) and 0.09% protoforms, was found to increase the number of crossings and rearings in the OFB test at a dose of 2778 mg/kg, p.o. (containing 15 mg/kg total hypericins), and enhance significantly the latency time in light/dark model at a dose of 1852 mg/kg, p.o. (Vandenbogaerde et al., 2000). A similar study performed by Kumar et al. (2000b) reported that the hydro-alcoholic extract of *H. perforatum* whole plant at doses of 100 or 200 mg/kg, p.o., once daily for three consecutive days, exhibits significant
anxiolytic activity in various paradigms of anxiety viz., OFB, EPM, EZM, novelty-induced suppressed feeding latency (FL) and social interaction test (SI). The anxiolytic effects observed in different experimental models are summarized below:

(i) OFB: a significant increase in open field ambulation; slight increase in rearings and activity in centre.
(ii) EPM: a significant augmentation of open arm entries; open arm/closed arm entries ratio and time spent in open arms.
(iii) EZM: a significant increase in time spent on open arms and entries in open arms; slight decrease in head dips and stretched attend posture.
(iv) FL: a significant attenuation of the novelty-induced increase in feeding latency.
(v) SI: a significant increase in social interaction in novel environment.

The lyophilized aqueous extract of the plant has been reported to induce significant rise in immobility time and diminution of rearings, suggesting clear sedative effects at doses ranging from 10-100 mg/kg (Coleta et al., 2001). At lower dose (5 mg/kg), it increased time spent in open arms of the EPM, thus indicating anxiolytic effect. Since the aqueous extract was devoid of hyperforin, these effects can not be attributed to this substance. Beijamini and Andreatini (2003a) reported that repeated administration of standardized *H. perforatum* extract LI60 (Hyp LI 60; 300 mg/kg, p.o.) exerted anxiolytic like effects (decreased inhibitory avoidance) and an antipanic effect (increased one way escape) in ETM apparatus. Chronic oral administration (380 mg/kg/day) of Hyp LI 60 abolished depression and anxiety-related behaviour in Mg-depleted mice in various paradigms of depression and anxiety viz., rotarod test, home cage activity, OFB, light/dark and forced swimming tests (Singewald et al., 2004). Repeated administration of Hyp LI 60 (300 mg/kg, p.o.) for 21 days showed anti-anxiety and antipanic activities in the mouse defense test battery (Beijamini and Andreatini, 2003b). This extract reduced flight reactions (number of avoidance distance and overall flight speed) to the presence of the predator and the number of upright postures. Various modes of actions have been postulated for anxiolytic activity of *H. perforatum*. It was reported that these anxiolytic effects of *H. perforatum* were via β-receptor activation (Zanoli et al., 1998a). The anxiolytic
Effect of *H. perforatum* could be partly linked to facilitatory activity of pseudohypericin on GABA evoked currents and to the inhibitory influence on glutamatergic transmission mediated by NMDA receptors (Vandenbogaerde, et al., 2000). Single administration of ethanol extract of Indian *H. perforatum* [IHp] (50 or 200 mg/kg, p.o.) was also reported to affect monoamines concentration in rats’ brain (Kumar et al., 2001). IHp treatment significantly decreased the levels of serotonin, but augmented the levels of norepinephrine and dopamine.

*Kielmeyera coriacea* Mart. (Pau Santo; Guttiferae) has been used for the treatment of several tropical diseases including schistosomiasis, leishmaniasis, malarial, fungal and bacterial infections. The hydro-alcoholic extract of the leaves (120 mg/kg/day, p.o.) exhibited significant anti-anxiety activity in mice using OFB and EPM tests (Audi et al., 2002).

*Leptospermum scoparium* J.R. et G. Forst. (Myrtaceae). The white flowers of the plant have been reported to contain the methylated and methoxylated flavonoids, which have a high binding affinity to BZD receptor (Haberlein and Tschiersch, 1998). On the basis of this observation, authors have suggested that the plant might have anxiolytic potential.

*Lippia alba* (Mill.) N.E. Brown (Cidreira; Verbenaceae). The tea obtained from *L. alba* leaves has been largely used as a tranquilizer, and in gastrointestinal problems. Three chemotypes of essential oil from *L. alba* were responsible for anxiolytic activity of the plant in mice (Vale et al., 1999). The main constituents for the activity are citral, β-myrcene and limonene present in essential oil I, citral and limonene present in essential oil II, carvone and β-myrcene in essential oil III. In EPM test, maximum effects were presented by essential oil II (25 mg/kg, i.p.) followed by essential oil I and essential oil III (100 mg/kg, i.p.) with respect to control for all parameters (% number of entries, % time spent in the open arms) studied. Essential oil I (200 mg/kg, i.p.) decreased only the number of rearings as compared to control whereas essential oil II and III (100 and 200 mg/kg, i.p., respectively) decreased both the number of rearings and grooming as compared to
control in OFB test. In rotarod test, only essential oil II at a dose of 200 mg/kg, i.p., decreased the time of permanence on the bar.

*Magnolia* (Magnoliaceae) bark has been used in traditional Chinese medicine for centuries for reduction of stress and muscle tension. Honokiol, main constituent isolated from the *Magnolia* bark, exhibited anxiolytic activity at a dose of 0.2 mg/kg, p.o. (seven daily treatments) using EPM apparatus (Kuribara et al., 1999). Anxiolytic activity of two Chinese polyherbal formulations – Hange-Koboku-to (composed of 5 plants including *M. officinalis* Rehd. Et Wils.) and Saiboku-to (composed of extracts of 10 plants including *M. obovata* Thunb.) has been attributed to honokiol (Kuribara et al., 2000). *M. officinalis*-free preparation of Hange-Koboku-to or Saiboku-to did not have any anxiolytic effect.

*Marticaria* (Chamomile; Compositae) has been used traditionally as sedative, spasmylytic and anxiolytic. The flavonoid apigenin isolated from *M. chamomilla* Linn. and *M. recutita* Linn. have been evaluated for behavioural effects on rats (Zanoli et al., 1998b; Zanoli et al., 2000). The results showed that flavonoid reduced the locomotor activity at a dose of 25 mg/kg, when injected intraperitoneally, but did not demonstrate anxiolytic, myorelaxant or anticonvulsant effects. Avallone et al. (2000) reported that the inhibitory activity of apigenin on locomotor behaviour in rats could not be ascribed to an interaction with GABA<sub>A</sub>/BZD receptor, but to other neurotransmission systems. In contrast to above reports, it has been reported that apigenin exhibits anti-anxiety activity as it enhanced the modulatory action of diazepam at low doses (Goutman et al., 2003; Campbell et al., 2004).

*Passiflora* spp. (Passion flower; Passifloraceae) have been traditionally used as sedative, nerve tonic and anxiolytic. The hydro-alcoholic extracts of *P. alata* Dryand. and *P. edulis* Sims were evaluated for anxiolytic activity using EPM apparatus (Petry et al., 2001). Both plant extracts showed an increase in the time spent in the open arms of EPM at doses of 50,100 or 150 mg/kg, i.p. *P. caerulea* Linn. has been reported to exhibit anxiolytic activity but not myorelaxant or amnesic effects (Viola et al., 1998). *P. incarnata* has been traditionally used as sedative, nerve tonic and anxiolytic. Methanol extract of
*P. incarnata* aerial parts was reported to exhibit anxiolytic activity in mice at a dose of 125 mg/kg, p.o. using EPM model of anxiety (Dhawan et al., 2001a; Dhawan et al., 2001b; Dhawan et al., 2001c; Dhawan et al., 2001d). Various plant parts of *P. incarnata* viz., aerial parts (leaves, stems, flowers), underground parts, and whole plant were also evaluated for anti-anxiety activity, and exhibited significant anxiolytic activity in order of leaves>stems>flowers>whole plant. Underground parts were found to be devoid of anxiolytic activity. A double-blind randomized trial, performed on 36 outpatients diagnosed with GAD using DSM IV criteria, compared the efficacy of aqueous extract of *P. incarnata* with oxazepam in the treatment of GAD (Akhondzadeh et al., 2001). Eighteen patients received *P. incarnata* extract (45 drops/day) plus placebo tablet group, and 18 received oxazepam (30 mg/day) plus placebo drops for a 4-week trial. Both were found effective in the treatment of GAD. Subjects on oxazepam encountered the problems related to impairment of job performance, while patients treated with *P. incarnata* extract showed low incidence of the problems.

**Piper** (Kava, piperaceae). Kava-Kava, a traditional psychoactive beverage prepared from *P. methysticum* Forster, has been used in the South Pacific for tranquilizing and anxiolytic effects. Kava-Kava preparation has been reported to exhibit anti-anxiety activity in rats using EPM apparatus (Rex et al., 2002). Ethanolic extract of the plant exhibited dose dependent anxiolytic activity in the mirrored chamber avoidance assay and elevated plus maze assay (Garrett et al., 2003). ED$_{50}$ for Kava induced increase in time spent in mirrored chamber and on the open arms of the EPM were 125 mg/kg and 88 mg/kg, i.p., respectively. These activities were not mediated through the BZDs binding site on the GABA$_A$ as fluamzenil could not block the behavioural actions of Kava. Earlier reports on the therapeutic potential of Kava in the treatment of anxiety disorders have been covered in two review articles (Pittler and Edzard, 2001; Singh and Singh, 2002). Kavalactones, a mixture of compounds, have been considered responsible for biological effects of Kava, and its pharmacological properties have been postulated to include blockade of sodium ion channels, enhanced ligand binding to GABA$_A$ receptors, diminished excitatory neurotransmitter release due to calcium ion channel blockade, reduced neuronal reuptake of norepinephrine, reversible inhibition of MAO-B and suppression of the synthesis of the eicosanoid.
thromboxane A (2) which antagonizes GABA<sub>A</sub> receptor function. *P. methysticum* samples containing 12.8-100% total kavalactones, and fractions containing kavalactones 1-6 in varying concentration (0.1-67.5%) were evaluated for anxiolytic activity, and compared against a 5 mg/kg dose of chlordiazepoxide using the chick social separation-stress model (Feltenstein et al., 2003). *P. methysticum* extract samples attenuated distress vocalization in a concentration-dependent manner. The fraction containing highest concentration of dihydrokavin attenuated distress vocalizations in a manner equivalent to that of chlordiazepoxide. The extract samples and fraction exhibited anxiolytic activity but were devoid of sedative activity.

A randomized double-blind placebo-controlled clinical trial, performed on 37 adults with DSM-IV GAD using Kava for 4 weeks, ruled out the effectiveness of Kava in treating GAD (Connor and Davidson, 2002). In this study, weekly efficacy assessments were made on the basis of Hamilton Anxiety Scale (HAMA Scale), Hospital Anxiety and Depression Scale (HADS), Self Assessment of Resilience and Anxiety (SARA). Kava was found to be superior on the SARA in low anxiety, and placebo was superior on the HADS and SARA in high anxiety. This study suggested that Kava is not superior to placebo in treating GAD.

In contrast to afore-mentioned report, three different randomized placebo-controlled, double-blind clinical studies were performed on about 230 patients suffering from neurotic anxiety using standardized Kava-Kava special extract WS 1490 (Malsch and Kieser, 2001; Gastpar and Klimm, 2003; Geier and Konstantinowicz, 2004). The patients were given WS 1490 (50-300 mg/day) for four weeks, followed by two-week observation. The results were established on the differences between baseline and end of treatment of the HAMA and on a subjective well being scale (Bf-s) as well as the BZD withdrawal symptoms. The secondary measures were analyzed by measuring the changes in the Erlanger Anxiety, Tension, Aggression Scale (EAAS) and Clinical Global Impression (CGI), The Brief Personality Structure Scale (KEPS) and The Adjective Checklist. Safety of the treatment was checked by interviews, adverse event reports and laboratory investigation. WS 1490 was found to be superior to placebo regarding HAMA, Bf-s total scores and all secondary efficacy measures.

An eight-week randomized, reference-controlled, double-blind, multi centre clinical trial investigated Kava Kava LI150 (400 mg/day) in 129 patients...
suffering from GAD (Boerner et al., 2003). The results were established on the basis of primary measure HAMA Scale and secondary measures including Boerner Anxiety Scale (BoEAS), CGI, Bf-s, a sleep questionnaire (sf-13), and a quality of life questionnaire (AL). Seventy five percent patients responded with the treatment and 60% achieved full remission. A 37-year-old female outpatient with GAD, SP and SAD was treated with phytotherapy (Kava-kava) (Boerner, 2001). Within 4 weeks, symptoms had improved by 75% and by 6 months, an almost total remission of symptoms was observed. The herbal medicine was well tolerated. Kava has considerable potential in the treatment of anxiety disorders.

Emulsion of the essential oil, extracted from \textit{P. solmsianum} C. DC. aerial parts, and of sarisan, a myristicin analogue, isolated from the essential oil, (5 or 10% v/v) exhibited anti-anxiety activity in mice (Moreira et al., 2001).

\textbf{Rubus brasiliensis Martius} (Amora branca; Rosaceae) has been traditionally used as anticonvulsant, hypnotic, muscle relaxant and anxiolytic. Nogueira et al. (1998a; 1998b) carried out the study to evaluate the anxiolytic effect of \textit{R. brasiliensis} infusion and various fractions of ethanolic extract in male Wistar rats and Swiss mice. The treatment was administrated 30 minutes prior to behavioural evaluation in the EPM at doses of 50, 100 or 150 mg/kg, p.o. Both the infusion and hexane fraction of ethanolic extract significantly increased number and percentage of open arm entries of rats and mice at a dose of 150 mg/kg whereas aqueous and butanolic fractions (from ethanolic extract) did not show any effect. The anxiolytic effect of the extract in mice was blocked when flumazenil, a specific GABA\textsubscript{A} receptor antagonist, was administered 15 minutes prior to administration of the extract. Nogueira and Vassilieff (2000) reported that hexane fraction exhibited hypnotic, anticonvulsant and muscle relaxant effects in barbituric-hypnosis test, PTZ-induced seizures, and muscle relaxant studies in the inclined plane, respectively at a dose of 300 mg/kg. Reversal of these effects by flumazenil treatment supported that a liposoluble principle with low toxicity may be acting as an agonist on GABA, a BZD receptor complex.

\textbf{Saussurea lappa} C.B. Clarke (Kuth; Compositae). The essential oil of \textit{S. lappa} allayed the anxiety in a woman in labour when inhaled, and showed no adverse effect on mother and foetus (Huntsoe et al., 1999).
**Scutellaria** (Huangqin; Labiatae) species have been used traditionally as antibacterial and sedative in Chinese system of medicine. A monoflavonoid wogonin, isolated from *S. baicalensis* Georgi roots, exhibited significant dose dependent anxiolytic activity in EPM through positive allosteric modulation of the GABA<sub>A</sub> receptor complex via interaction at the BZD receptors (Hui et al., 2002). These anxiolytic effects were not accompanied by sedative and myorelaxant effects. Later, it was reported that flavonoid baicalin and its aglycone baicalein are responsible for anxiolytic activity of *S. baicalensis* (Liao et al., 2003). Baicalein (10 mg/kg, i.p.) and baicalin (20 mg/kg, i.p.) significantly increased number of shocks accepted in the Vogel shock conflict test over 9 minutes thereby confirming their anxiolytic activity. These effects were found to be antagonized by flumazenil but not pindolol, 5 HT<sub>1A</sub> receptor antagonist. Later, 26 flavonoids were isolated from *S. baicalensis* and tested for their affinity for BZD binding sites of GABA<sub>A</sub> receptors (Wang et al., 2002). Only 2'-OH flavones exhibited most potent binding affinity. A flavone 5,7,2'-trihydroxy-6,8-dimethoxy flavone (4 or 8 mg/kg, p.o.) exhibited significant anxiolytic activity in the EPM apparatus, which was abolished by co-administration of flumazenil (Huen et al., 2003a). It did not exhibit sedative or myorelaxant activities in any of the paradigms (rotarod and horizontal inclined wire test). Another flavone 5,7-dihydroxy-6-methoxy flavone showed BZD receptor antagonistic properties (Huen et al., 2003b).

Oral administration (1 ml) of aqueous extract (100 mg/ml) of *S. lateriflora* Linn. (Skullcap) significantly increased the number of entries into the centre of an open field arena, number of unprotected head dips, number of entries and length of time spent in the open arms of the EPM in rats (Awad et al., 2003). Authors have suggested that baicalin and its aglycone baicalein, present in *S. lateriflora*, might play a role in anxiolytic activity as they are known to bind to the BZD sites of the GABA<sub>A</sub> receptor.

**Sesbania grandiflora** (L.) Poiret (Agati; Leguminosae) has been traditionally used as antitumour, anthelmintic and contraceptive. In Ayurveda, the leaves of *S. grandiflora* are used as a medicine. The juice of leaves and flowers is a popular remedy for nasal catarrh and headache. Leaf juice, mixed with honey, is administered for curing congenital bronchitis or cold in babies. Benzene:ethyl acetate (BE) fraction of the acetone soluble part of petroleum ether extract from
S. grandiflora leaves exhibited anticonvulsant activity in a variety of animal models (PTZ-, strychnine- and MES-induced convulsions), and anti-anxiety activity in EPM test (Kasture et al., 2002). BE significantly delayed the onset of convulsions in PTZ- and strychnine-induced seizures in mice, and reduced the duration of tonic hind leg extension in MES test in dose dependent manner. Maximum effects were observed at a dose of 100 mg/kg, p.o. BE also inhibited electrically induced hind leg seizures in mice and lithium-pilocarpine induced status epilepticus in rats. It prolonged the duration of sleep induced by pentobarbital and antagonized the effects of D-amphetamine at a dose of 45 mg/kg, p.o. Mice treated with BE preferred to remain in the open arms of the EPM. These activities have been attributed to a triterpene.

*Stachys lavandulifolia* Vahl (Lavendelblaettrige; Lamiaceae) has been used as an anxiolytic and sedative in Iranian folk medicine. The anxiolytic effect of hydro-alcoholic extract and essential oil of *S. lavandulifolia* aerial parts was studied on the EPM model of anxiety in mice (Rabbani et al., 2003). The hydro-alcoholic extract (100 mg/kg, i.p.) increased the percentage of time spent, the percentage of arm entries in the open arms of the EPM, and decreased the percentage of time spent and the percentage of arm entries in the closed arms of the EPM. Essential oil was found to be devoid of anti-anxiety activity.

*Valeriana* (Valerian; Valerianaceae). Although the use of *Valeriana* root and rhizome extracts to cause sedation and relieve sleep problems dates back to 18th century, yet exact composition of the preparation was not clear. Multiple substances in the *Valeriana* extracts are held responsible for their effects, and most important among them are valepotriates, their decomposition products the baldrinals and various components of the essential oil, in particular, the valerenic acid (Houghton, 1999). The hydro-alcoholic extract of *V. edulis* var. *procera* Meyer roots (100 or 300 mg/kg, p.o.) exhibited dose dependent anticonvulsant and anxiolytic activity in mice (Oliva et al., 2004). At higher dose (1000 mg/kg), it decreased rotarod performance and traction force, and prolonged the pentobarbital-induced sleeping time. It has been reported that 6-methylapigenin and hesperidin, isolated from roots and rhizomes of *V. wallichii* DC and *V. officinalis* Linn., are responsible for anxiolytic and sedative properties of the
plants in rats (Wasowski et al., 2002; Marder et al., 2003). Hesperidin (4 mg/kg, i.p.) decreased the ambulatory locomotor activity, reduced the exploration of holes and the number of rearings performed in the hole board test and increased the sodium thiopental-induced sleeping time (sedative activity). 6-methylapigenin (1 mg/kg, i.p.) increased % open arm entries and time spent in open arms of the EPM (anxiolytic activity), but was devoid of sedative activity even at 10 fold higher dose. 6-methyl apigenin was proved to have affinity for the BZD sites of GABA$_A$ receptor (Ki = 0.5 μM). Later, it was reported that a flavonoid linarin, isolated from *V. officinalis*, has sedative and sleep enhancing properties that are potentiated by valerenic acid (Fernandez et al., 2004). Linarin (4 or 7 mg/kg, i.p.) significantly reduced the number of rearings performed in the hole board test. At higher doses (7 or 14 mg/kg, i.p.), it significantly augmented the sleeping time induced by thiopental. Co-administration of valerenic acid (5 mg/kg, i.p.) with linarin (4 mg/kg, i.p.) showed sleep-enhancing effects as evidenced by reduction in head dippings in hole board test, and a striking increase in the sleeping time induced by sodium thiopental.

A parallel, double-blind, flexible-dose, placebo-controlled study (4 weeks) was performed on 36 patients with GAD DSM III-R using valepotriates (mean daily dose – 81.3 mg), diazepam (mean daily dose – 6.5 mg) or placebo (Andreatini et al., 2002). All the three groups presented significant reduction in the total HAMA scores, but only the diazepam and valepotriates groups showed significant reduction in the psychic factor of HAMA. Authors suggested that the valepotriates may have potential anxiolytic effect on the psychic symptoms of anxiety.

*Withania somnifera* Linn. (Ashwagandha; Solanaceae), a commonly used herb in Ayurvedic medicine, possesses anti-inflammatory, antitumour, antistress, antioxidant, immunomodulatory, hemopoietic, anxiolytic, nervine, sedative and rejuvenating properties (Mishra et al., 2000; Dhuley, 2001). Glycowithanolides, isolated from the roots of the plant, are considered to be the bioactive constituents. Glycowithanolides when given orally once daily for 5 days to rats, exhibited anxiolytic activity similar to that of diazepam in EPM, SI and FL models of anxiety (Bhattacharya et al., 2000).
Zingiber officinale Linn. (Ginger; Zingiberaceae) is used in folk medicine for relief from many ailments, especially nausea, motion sickness and other gastrointestinal disorders. It has been reported that the benzene fraction of acetone soluble part of petroleum ether extract of dried rhizomes of ginger significantly decreased occupancy in the closed arms and increased the time spent in the open arms of the EPM at doses of 15 or 30 mg/kg, i.p., suggesting the presence of anxiolytic principles in the benzene fraction (Vishwakarma et al., 2002).

Ziziphus jujuba Miller (Desi ber; Rhamnaceae) has been used traditionally for its action on insomnia and anxiety. The ethanolic extract of Z. jujuba seeds was evaluated for anxiolytic activity using black/white test, EPM apparatus, and ambulatory behaviour test, and sedative activity in hexobarbital-induced sleeping time test (Peng et al., 2000). The extract (0.5, 1.0 or 2.0 g/kg, p.o.) was orally administered to male ICR mice, 30 minutes prior to behavioural evaluation. The ethanolic extract increased the first entry, total time spent in the white chamber at a dose of 0.5 g/kg, increased the percentage of time-spent and the percentage of arm entries in the open arms of the EPM. At higher dose (1.0 g/kg), it prolonged the hexobarbital-induced sleeping time. The results suggested that the plant possess anxiolytic activity at lower dose and sedative activity at higher dose.

Miscellaneous:
Sho-ju-sen (SK), a Japanese herbal medicine, contains a water extract of Sasa kurinensis Makino et Sibata (Kumazasa; Poaceae) leaves (SS), ethanol extract of Pinus densiflora Siebold et Zucearini (Japanese red pine; Pinaceae) (PN) and Panax ginseng C.A. Meyer (Ginseng; Araliaceae) (PX) in the ratio of 8:1:1 (Kuribara et al., 2001). Single dose administration of SK (10 or 20 ml/kg, p.o.) did not show anxiolytic activity in the elevated plus maze. SK (1%, 3% or 30% solutions for 7 days) developed anxiolytic effects in mice. SS (8% solution), PN (1% solution) or PX (1% solution) were separately screened for anxiolytic activity. Among these constituents of SK, only SS exhibited anxiolytic activity. Flumazenil completely reversed the anxiolytic effects of SK and SS. Thus, BZD receptors are involved in the anxiolytic activity of SK and SS.
Kami-Shoya-San (TJ-24) is one of the traditional Chinese herbal medicine used for the treatment of menopause anxiety (Mizowaki et al., 2001). The components of this medicine are *Bupleurum scorzoneraefolium* Willd. (Bupleuri Radix; Bupleuraceae), *Paeonia lactiflora* Pallas (Paeoniae Radix; Paeonaceae), *Atractylodes lancea* (Thunb.) DC. (Atractylodis Lanceae Rhizoma; Compositae), *Arctanglica officinalis* Hoffm. (Angelicae Radix; Umbelliferae), *Porc cocos* (Schw.) Wolf (Hoelen; Polyporaceae), *Gardenia jasminoides* Ellis (Gardeniae Fructus; Rubiaceae), *Paeonia suffruticosa* Andr. (Moutan Cortex; Paeonaceae), *G. glabra* (Glycyrrhizae Radix; Leguminosae), *Z. officinale* (Zingiberis Rhizoma; Zingiberaceae), *Mentha arvensis* Malinvaud (Menthae Herba; Labiatae). A dose dependent increase in social interaction time in male mice was observed with acute administration of TJ-24 (25-100 mg/kg, p.o.). These effects were blocked by flumazenil as well as finasteride (5α-reductase inhibitor), thereby, suggesting involvement of neurosteroid synthesis followed by GABA receptor stimulation for anxiolytic effects for TJ-24.

Indole alkaloid alstonine containing plants have been traditionally used in Nigeria to treat mental illnesses (Costa-Campos et al., 2004). Alstonine (1 mg/kg, i.p.) significantly increased the number of head dips, but did not increase locomotion (squares crossed) in hole board test. In light/dark model, it exhibited significant anxiolytic activity as evident from the increase in the latency for the first crossing from light to the dark compartment and number of crossings between compartments.

Essential oils. Rose oil, extracted from various rose flower species, inhalation (1.0, 2.5 or 5.0% w/w) exhibited significant anxiolytic activity in male rats by inducing increase in the time spent in the open arms of elevated plus maze with respect to diazepam (De Almeida et al., 2004). In similar studies, intraperitoneal administration of rose oil produced an increase in response rate during alarm period in the Geller conflict test and increased the number of electric shocks mice received in Vogel’s conflict test (Umezu et al., 2002). These effects were attributed to citronellol and 2’-phenethyl alcohol.
Various essential oils frankincense (FRA), juniper (JUN), cypress (CYP), geranium (GER), Jasmine (JAS) and lavender (LAV) extracted from *Boswellia thurifera* Roxb. (Burseraceae), *Juniperus communis* Linn. (Cupressaceae), *Cupressus sempervirens* Linn. (Cupressaceae), *Pelargonium adoratissimum* Linn. (Geraniaceae), *Jasminum officinale* Linn. (Oleaceae) and *Lavandula angustifolia* Miller (Labiatae), respectively, were evaluated for anxiolytic activity in mice at a dose of 1600 mg/kg, i.p. using Geller type conflict test (Umezu, 2000). Among all essential oils, only lavender oil exhibited a significant anticonflict effect in a dose dependent manner.

**Botanical extracts.** Various botanical extracts viz., NPS00032, a hydro-alcoholic extract from the Rutaceae family; NPS00033, an aqueous extract of the Rutaceae family; NPS00034, a hydro-alcoholic extract of *Acorus gramineus* Soland (Acori graminei; Acoraceae); NPS00035, an aqueous extract of Acori graminei; NPS00038, an aqueous extract from the Magnoliaceae family; NPS00039, a hydro-alcoholic extract of *Alchemilla erythropoda* Juz. (Ladies Mantle; Rosaceae); and NPS00068, an alcoholic extract of *Primula veris* (Cowslip; Primulaceae) were evaluated for their anxiolytic activity using chick social separation-stress procedure (Sufka et al., 2001). NPS00033 (28 or 56 mg/kg, i.p.) and NPS00039 (12.5 or 25 mg/kg, i.p.) exhibited significant anxiolytic activity without causing sedation.

**Dietary products.** Dietary soy phytoestrogens have been reported to produce anxiolytic effects in both male and female rats (Lund and Lephart, 2001). The animals fed with phytoestrogen-rich Phyto-600 diet spent more time on and made more entries into the open arms of the EPM compared to animals fed the phyto free diet.

**1.4 The genus *Turnera***

The literature review on *Turnera* emphasizes the traditional use and clinical potential of *Turnera* species, especially *T. aphrodisiaca*. Additionally, it raises a question on traditional claims of these species, which have not been proven scientifically.
This review has been compiled using references from major databases such as Chemical Abstracts, Medicinal and Aromatic Plants Abstracts, PubMed, King’s American Dispensatory, Raintree Nutrition Incorporation, Henriette’s Herbal Homepage, National Agricultural Library (AGRICOLA), Duke’s Phytochemical and Ethnobotany database, UK Cropnet Ethnobotany database, Archives of American Folk Medicine and USPTO Patent Full Text and Image database.

The available information on *Turnera* has been divided into six sections, i.e., ethnopharmacology, morphology and microscopy, phytoconstituents, pharmacological studies, clinical studies and toxicology, covering prominent species of *Turnera*. The ethnopharmacological section has been further subdivided into two sections, i.e., traditional uses, and alternative and complementary medicinal uses. The reports, in which *Turnera* species have been used as domestic remedy by common men without any prescription for the treatment of various ailments, have been discussed under traditional uses. The subhead “alternative and complementary medicinal uses” highlights *Turnera* species as medicine prescribed by medical practitioners for the treatment of various ailments. It also mentions uses for which *Turnera* species or their preparations are available in the market. Under every section, *Turnera* species have been arranged in alphabetical order.

The genus *Turnera* belongs to the family Turneraceae, and comprises about 85 species of tropical and sub-tropical American, African and Madagascar plants that vary in habit from herbs, sub-shrubs, shrubs or, rarely, to trees (Osol et al., 1947; Lawerence, 1951; Willis, 1957; Prain, 1981; The Wealth of India, 1996).

1.4.1 Ethnopharmacology
1.4.1.1 Traditional uses

Only a few species of *Turnera* have been used as traditional remedies in various ailments. *Turnera aphrodisiaca* Ward {Syn.: *T. diffusa* Willd. (Jackson, 1946)}, commonly known as Damiana, was used by the Aztecs for its aphrodisiac properties, and has been known for its sexual stimulant properties for about 300 years (Lowry, 1984). It was noted that the missionary Jesus Maria de Salvatierra
in his chronicles of 1699 recommended Damiana. *T. aphrodisiaca* has been in use in the United States since 1874 as aphrodisiac, stimulant, mood elevator and tonic (Cohen et al., 2001). The plant has been used as a stimulant, aphrodisiac, tonic, diuretic, nerve tonic, laxative, urinary antiseptic, testosterone mimetic, and in kidney, menstrual and pregnancy disorders (Peterson, 1905; Timothy, 1954; Hocking, 1955; Dominguez and Hinojosa, 1976; Braun and Malone, 1978; Stuart, 1979; Hoffman, 1991; Bradley, 1992; Grieve, 1994; Mills, 1994; Ellingwood, 1999; Parfitt, 1999). A tea, made from Damiana, is used as a tonic beverage (UCLA Folklore Archives, 2001). Damiana is a folklore restorative remedy for curing masculine insufficiency (Young, 1967). Israel and Youngkin (1997) collected information on selected herbal therapies for perimenopausal and menopausal complaints based on scientific sources, and reported that *T. aphrodisiaca* has a primary reputation as an aphrodisiac due to testosterone like properties of the alkaloids present in the plant.

*T. aphrodisiaca* has been used extensively in the traditional system of therapeutics of many countries (Raintree Nutrition Incorporation, 1999). In the ancient Maya civilization, Damiana was used for “giddiness and loss of balance”, and as an aphrodisiac. In the Bahamas, the plant is used for headache and enuresis. Damiana is applied as a plaster for lumbago, and ingested as a decoction for parturition in California (Campa, 1950). In France, the plant is used as an aphrodisiac. In Germany, the leaves of the plant are used to relieve nervous debility, and for a tonic action on the hormonal and central nervous system. In Haiti, the plant is used as an aphrodisiac, tonic, liqueur, and in intestinal diseases. In Holland, Damiana is known for its sexual enhancing properties and tonic effect on the reproductive organs. In Mexico, the plant is used in amaurosis, stomachache, diabetes, dysentery, dyspepsia, malaria, paralysis, rhinitis, syphilis, intestinal diseases, and as an aphrodisiac, astringent, diuretic and tonic. The plant has been used by Mexicans to treat gastrointestinal diseases (Hernandez et al., 2003). In the US, the plant is used as an aphrodisiac, astringent, expectorant, laxative, stimulant, tonic, and in dysmenorrhea (Raintree Nutrition Incorporation, 1999). The midwives and women of loose morals of western Mexico also attribute emmenagogue properties to it (Ellingwood, 1999). Damiana has achieved some repute in the treatment of sexual impotence, but it is always given in conjunction with strychnine, phosphorus or some other stimulant (Culbreth, 1927; Osol, et al.,
Damiana has been described as a tonic, diuretic and aphrodisiac, especially when combined with extracts of Kola (*Kola vera* Schum.) and Saw palmetto (*Serenoa serrulata*) berries (Lowry, 1984). Damiana is one of the common ingredients of traditional herbal remedies used as alternatives for treating menopausal symptoms (Duke, 1985; Zava et al., 1998). It also maintains normal menstruation at puberty (Priest and Priest, 1982). The leaf infusion of Damiana has been used in diseases related to the gastrointestinal, respiratory systems (Caceres, 1996) and reproductive organs (Saggese, 1959; Tilgner, 1999), and for the treatment of gonorrhea (Koch, 1936). Aerial parts of *Turnera* species have been used for the treatment of diseases related to gastric systems (Weniger et al., 1986).

The infusion of fresh leaves of *T. guianensis* Aubl. is indicated to treat inflammatory diseases, and as an immunomodulator, while the decoction of its dried leaves is employed to treat furunculosis (Pio Correa, 1984). In Mexico, *T. pringei* has been used for hangovers (UK Cropnet, 2003). *T. subulata* Smith has been used to treat boils. Poultices made from the roots of *T. trioniflora* Sims are applied to boils (The Wealth of India, 1996). *T. ulmifolia* Linn. has been used in indigestion, bronchitis, and as a tonic (Bradley, 1992). The plant is used for the management of weakness, cold, fever and boils (Beckwith, 1928). In India, it has been used for chest ailments, indigestion, biliousness and rheumatism (The Wealth of India, 1996). In the Bahamas, it is used for melroenia, sore throat, cold, and as an emmenagogue (UK Cropnet, 2003). In Haiti, it is used in vertigo, dysmenorrhea, hemorrhage, toothache, metrorrhagia, lumbago, dyspepsia. In Java, the plant has been used for dysentry. In Mexico, the plant is used as tonic, and for dyspepsia.

### 1.4.1.2 Alternative and complementary medicinal uses

Amongst various species of the genus *Turnera*, *T. aphrodisiaca* is the only one which is extensively used clinically throughout the world. *T. aphrodisiaca* was first introduced as a drug in 1874 by Dr. F.O. St. Clair, and first appeared in the form of a tincture from the firm of Messers Helmick and Co. of Washington, D.C. (Felter and Lloyd, 1983). The product was promoted as a powerful aphrodisiac, and to give increased tone and activity to all secretions in that vicinity.
Damiana has been prescribed in all conditions where a general tonic is needed, especially for weakness of the central nervous system (Hoffman, 1992; Ellingwood, 1999). The drug is useful in some cases of chronic cystic and renal catarrh. It relieves irritation of the urinary mucous membranes, improves digestion, and overcomes constipation. In respiratory disorders, it is employed to relieve irritation and cough, and due to its tonic properties, to check hypersecretion from the broncho-pulmonary membranes. In reproductive disorders, it is used to treat dysmenorrhea, menopausal headache, bad complexion, rough or discolored patches on the skin with acne, especially of severe type, depending upon uterine irritation, delayed or suppressed menstruation, and amenorrhoea in very young girls. Damiana has been prescribed in young girls with menstrual complaints. The remedy must be given in full doses, i.e., 5 to 10 grains of the extract 3 or 4 times a day, to accomplish these results. As a nerve tonic it is often used with oats. Depending on the situation, it combines well with Kola or Skullcap (Scutellaria lateriflora). Damiana has proved of value in all the cases of sexual debility in both sexes, and even in those cases where strychnine and phosphorus failed to give the desired benefit (Anonymous, 1885; Fenwick, 1887). The water extract, a teaspoonful three times a day for two or three months, has been prescribed to treat nervous affections, renal disease and diabetes.

Balch and Phyllis (1999) report that Damiana relieves headaches, controls bed-wetting, and stimulates muscular contractions of the intestinal tract, and, further, comment that Damiana interferes with iron absorption when taken internally.

The British Herbal Pharmacopoeia (1983) lists the specific indications for Damiana as anxiety neurosis associated with impotency, and lists other indications as depression, nervous dyspepsia, atonic constipation and coital inadequacy. Homoeopathic materia medica mentions use of Damiana tincture in sexual neurasthemia, impotency, renal and cystic catarrh, nervous prostration, frigidity of females, and to maintain normal menstrual flow in young girls (Boericke, 1988).

It has been reported that herbal narcotic distillates, which are used for smoking purposes, contain T. aphrodisiaca (Huang et al., 1982). Ingestion of an infusion of Damiana leaves, or alternatively smoking the leaves, produces a feeling of euphoria characterized by relaxation and increased imagination (Tyler, 1980). A herbal preparation containing Damiana as one of the ingredients has
been reported to exhibit favorable effects on the symptoms of irritable bladder 
associated with functional and neurohormonal disorders, and on bacterial bladder 
infections (Westendorf, 1982). These reactions are supposedly more pronounced 
in women. A Mexican liqueur called ‘Damiana, a liqueur for lovers’, containing
small amount of *Turnera*, has been commercially exploited as aphrodisiac
(Lowry, 1984). Veritin-516, a proprietary formulation containing Damiana, is sold
for the relief of the devastating humiliation of sexual weakness from sexual excess or alcohol.

Damiana is one of the constituents of a breast enhancing oral formulation, 
that is used as a dietary supplement (Ernest and Smith, 2001; Michael and Allen,  
2001; Fugh-Berman, 2003). Damiana is included in a number of herbal 
formulations which are used to treat menopausal symptoms (Morrow, 1998; Dantas, 1999),
diabetic male sexual dysfunction (Shlyankevich, 1996), impotence (McLeod, 1993) and to improve sexual response and psychological effects
(Vermillion and Holmes, 1997; Heleen, 2002).

1.4.2 Morphology and microscopy

A survey of the literature revealed that detailed accounts of morphology and microscopy of the genus *Turnera* are missing. Preliminary information on habits of the following species is available: *T. acuta* Spreng. (Brittonia, 1957), *T. amapaensis* Cowan (Brittonia, 1957), *T. angustifolia* Mills. (Curtis, 1794), *T. trioniflora* (The Wealth of India, 1996). However, somewhat detailed notes on morphological aspects of *T. aphrodisiaca* and *T. ulmifolia* have been reported. *T. aphrodisiaca* (Plate 1, p 62) is a robustly growing, spreading plant which consists of herbaceous stems, and is indigenous to South-Western Texas and Mexico (The British Pharmaceutical Codex, 1911; Culbreth, 1927; Felter and Lloyd, 1983; Perez et al., 1984; Hoffman, 1991; Hoffman, 1992; Raintree Nutrition Incorporation, 1999). *T. aphrodisiaca* stem is small, reddish-brown, woody; leaves pale green or yellow green in color, 10-25 mm long, 4-10 mm broad, broadly lanceolate, short petioled, acute tip, cuneate base, serrate margin, smooth surface, lower surface glabrous with few hairs on ribs, and prominent veins on the under surface; branches have reddish-brown bark; flowers yellow,
globose pods, 8-12 mm long with 5 yellowish petals and 5 styles; fruits small capsules.

*T. ulmifolia* (Plate 1, p 62) is a polymorphic perennial herb, often woody at base, up to 1.5 m in height. Stem bright green, covered with short hairs; buds small, vegetative, borne singly at the nodes; leaves alternate, 5-8 cm long, broadly lanceolate-oblong, short thick petiole, tip acute to acuminate crowded at the shoot apex, base cuneate, margins very coarsely, lobately serrated, rather flaccid and pendent, pinnately veined, two glands located near the intersection of the blade and petiole; stipules small, subulate and deciduous; flowers fragrant, solitary, axillary, on a short peduncle about the length of petiole; calyx of five, deep, lanceolate segments; corolla full yellow, of five, nearly rotundate, shortly-unguiculated; petals spreading; stamens five, yellow; filaments short; anthers subulate; ovary ovate, one celled, with three parietal placentae and many ovules; styles three, erect; stigmas penicillate; fruits small capsule filled with tiny seeds (Hooker, 1845; Brouwer and Stahlin, 1955; Arnold, 2002).


### 1.4.3 Phytoconstituents

Eighty species of Turneraceae have been tested and found to contain cyanogenic constituents (Spencer et al., 1985). Analysis by HPLC, NMR and paper chromatography data showed all to possess tetraphyllin A (1) and B (2), epitetraphyllin B, and deidaclin (3). Table 1 summarizes phytoconstituents reported from various species of *Turnera*.
Table 1: Phytoconstituents of various species of *Turnera*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Phytoconstituents</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. angustifolia</em> Mills.</td>
<td>Cyanohydrin glucoside linamarin (4) (Olafsdottir et al., 1990; Jaroszewski et al., 1995); cyanogenic glucoside deidaclin (3) (Olafsdottir et al., 1992).</td>
</tr>
<tr>
<td><em>T. aphrodisiaca</em> Ward (Syn. <em>T. diffusa</em> Willd.)</td>
<td>Cyanogenic glucoside tetraphyllin B (2) (Spencer and Seigler, 1981); flavonoid gonzalitosin I (5) (Dominguez and Hinojosa, 1976); arbutin (6) (Auterhoff and Hackle, 1968; Piacente et al., 2002); damianin (Steinmetz, 1960); tricosan-2-one (Fryer, 1965); hexacosanol-1; volatile oil containing α-pinene, β-pinene, p-cymene, 1,8-cineole, opopienone, cadalene, epicubenol (Auterhoff and Hackle, 1968; Bicchi et al., 2003), δ-cadinene and calamenene (Bordoloi et al., 1989); caffeine (Huang, et al., 1982); β-sitosterol (Jaroszewski, et al., 1995); resin; tannin (Steinmetz, 1960).</td>
</tr>
<tr>
<td><em>T. caerulea</em> Moc. &amp; Sesse</td>
<td>Cyanohydrin glucoside linamarin (4) (Olafsdottir, et al., 1990); essential oil containing γ-cadinene and β-caryophyllene (Morais et al., 1994).</td>
</tr>
<tr>
<td><em>T. diffusa</em> Willd.</td>
<td>See <em>T. aphrodisiaca</em></td>
</tr>
</tbody>
</table>
Table 1: Continued.

<table>
<thead>
<tr>
<th>Species</th>
<th>Compounds and Additives</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. subulata</em> Smith</td>
<td>Proteins (Bahadur and Swamy, 1983; Athanasiou and Shore, 1997); sugars; nucleic acids</td>
</tr>
<tr>
<td></td>
<td>(Bahadur and Swamy, 1983).</td>
</tr>
<tr>
<td><em>T. ulmifolia</em> Linn.</td>
<td>Cyanogenic glucosides deidaclin (3) (Spencer and Seigler, 1980; Tober and Conn, 1985),</td>
</tr>
<tr>
<td></td>
<td>tetraphyllin A (1), epi-tetraphyllin B and tetraphyllin B (2) (Tober and Conn, 1985);</td>
</tr>
<tr>
<td></td>
<td>essential oil containing α-elemene, trans-caryophyllene, lineol, pinene (Lima et al.,</td>
</tr>
<tr>
<td></td>
<td>2000); caffeine (Tarar and Patil, 1979); fatty acids uemolic acid, malvalic acid,</td>
</tr>
<tr>
<td></td>
<td>sterculic acid (Hosamani, 1993).</td>
</tr>
</tbody>
</table>

1
\[
\text{NC} \quad \text{O-glu} \\
H \quad H
\]

2
\[
\text{NC} \quad \text{O-glu} \\
\text{HO} \quad \text{H}
\]

3
\[
\text{glu-O} \quad \text{CN} \\
H \quad H
\]

4
\[
\text{HOH}_2 \text{C} \\
\text{HO} \quad \text{HO} \quad \text{HO} \quad \text{O-C-CN} \\
\text{CH}_3 \quad \text{CH}_3
\]
1.4.4 Pharmacological studies

An aqueous extract of *T. diffusa* has been reported to exhibit sexual stimulating activity in sexually sluggish male rats at a dose of 1 ml/kg (Arletti et al., 1999). The plant extract improved copulatory behaviour of impotent rats, and also increased the percentage of rats achieving ejaculation. Aqueous extract of whole plant of *T. diffusa* has been reported to exhibit significant hypoglycaemic activity in alloxan-diabetic male mice (Perez, et al., 1984). A glucose tolerance test performed on rabbits by Alarcon et al. (1998) showed significant hypoglycaemic activity in the orally administered decoction of leaves of *T. diffusa*. Contrary to these reports, Alarcon et al. (2002) recently reported that hydro-alcoholic extract of *T. diffusa* is devoid of any hypoglycaemic activity. Dichloromethane and methanol extracts of *T. diffusa* exhibited relaxant effect on the smooth muscle of the corpus cavernosum of guinea pig (Hnatyszyn et al., 2003). Hexane extract of *T. aphrodisiaca* exhibited antibacterial activity against...
Gram-positive and Gram-negative bacteria (Hernandez et al., 2003). A review on frequently used medicinal plants in Baja California Norte compiled by Winkelman (1986) added few preliminary pharmacological reports (antidiabetic, diuretic and CNS depressant) on *T. aphrodisiaca*. In the light of these preliminary pharmacological reports, author suggested that Damiana requires proper scientific investigation to establish its biologically active chemical constituents profile and mode of action.

An exhaustive pharmacological study was performed on *T. ulmifolia* aerial parts by two Brazilian researchers (Antonio and Brito, 1998). They have attributed anti-inflammatory as well as antiulcerogenic activity of the plant to hydro-alcoholic extract and its partitioned fractions. The crude hydro-alcoholic extract (1000 mg/kg) and its partitioned fractions viz., the aqueous, ethyl acetate and ethanol fractions (100 mg/kg) inhibited carrageenan-induced edema in male rats. Due to non-availability of sufficient quantity of aqueous, ethyl acetate and ethanol fractions, only hydro-alcoholic extract was assessed for anti-inflammatory activity using other experimental models. Hydro-alcoholic extract inhibited the cotton pellet granuloma and the increase of vascular permeability induced by histamine, 5-hydroxytryptamine and prostaglandin E$_2$. The hydro-alcoholic extract and ethanol fraction inhibited the appearance of gastric lesions induced by indomethacin, ethanol and pylorus ligature but not those induced by stress, thus, confirming its anti-ulcerogenic activity. The extract and the fraction were also evaluated for analgesic activity in the writhing test using acetic acid, but both did not exhibit any activity. Another Brazilian group of researchers (Gracioso et al., 2002) evaluated an aqueous fraction (AqF) of the aerial parts of *T. ulmifolia* for its antiulcerogenic activity on gastric and duodenal mucosa in mice and rats, respectively. The AqF significantly reduced the formation of lesions associated with HCl/ethanol administration as well as lesions induced by a combination of indomethacin and bethanechol at doses of 500 mg/kg and 1000 mg/kg. In stress-induced gastric ulcer, the inhibition by the AqF was 48%, 57%, and 58% at doses of 250, 500 and 1000 mg/kg, respectively. Okoli et al. (2003) also reported the use of *T. ulmifolia* in the treatment of inflammatory disorders in various *in vivo* and *in vitro* inflammatory models. The highest dose of the AqF significantly affected the gastric juice parameters by increasing the pH and decreasing the acid output in pyloric ligature experiment. Essential oil of *T. ulmifolia* has been reported to

### 1.4.5 Clinical studies

In a double blind placebo controlled study, Argin Max (a nutritional supplement for the enhancement of female sex function) containing Damiana as one of the constituents was given to 34 women for 4 weeks (Ito et al., 2001). It showed improvement in sexual desire, reduction of vaginal dryness, increase in sexual intercourse and orgasm, and improvement in clitoral sensation. A twin study investigating Argin Max effects on men indicated that over 80% had improved erection (Ito et al., 1998; Rowland and Tai, 2003). Argin Max did not exhibit estrogeneric activity with an *in vitro* estrogen bioassay, which was performed using a human endometrial adenocarcinoma cell line, Ishikawa, which contains an alkaline phosphatase enzyme sensitive to estrogen stimulation (Polan et al., 2004). A double-blind placebo-controlled study was performed on a herbal preparation ‘YGD’ containing leaves of *Ilex paraguayensis*, seeds of *Paulinia cupana* and leaves of *T. diffusa* to determine the effect on gastric emptying and weight loss over 10 days and 45 days (Andersen and Fogh, 2001). The preparation significantly delayed gastric emptying and induced significant weight loss over 45 days in overweight patients. Fifteen patients were administered an apetite suppressant composition, containing Damiana as one of the ingredients, twice a day (Mann, 1993). Significant weight loss was observed in all the patients at the end of the 42 days. In a separate set of double-blind randomized crossover study performed on 47 patients using Damiana containing herbal formulation, a significant decrease in body weight was observed after 45 days of treatment (Hessel and Lundsgaard, 1999).

### 1.4.6 Toxicology

Due to occurrence of cyanogenic constituents in *Turnera* species, their toxicity cannot be ruled out. Low levels of cyanide like compounds suggest the potential for adverse effects, with possible toxicity to the liver and kidneys.
(Rowland and Tai, 2003). There has been a report of a case of poisoning of individual who consumed 8 ounces of an extract of Damiana and who underwent tetanus like convulsions and paroxysms which resulted in symptoms like those of strychnine poisoning (Dominguez and Hinojosa, 1976). This may have occurred due to cyanide poisoning. The alcoholic extracts of the roots of *Turnera* species have been reported to exhibit oxytocic activity, and, hence, should be avoided during pregnancy (Vieira et al., 1968). The leaves have a slight laxative effect and may cause loosening of the stools at higher amounts (Mills, 1991).

### 1.5 Standardization of plant drugs

Although herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored (Christopher, 2004). There are estimated 2,50,000 species of plants on earth, and approximately 5000 plants, a very small fraction of the world’s plants, have been studied scientifically. Of the 121 most frequently used prescription drugs in different countries, 74% are derived from only 90 species of plants. There are about 7000 firms manufacturing traditional medicines with or without standardization (Dubey et al., 2004). It has been estimated that 800 companies producing standardized medicinal plant products with revenues in excess of $ 4.5 billion (Murch et al., 2004).

India has 16 agro climatic zones, 45,000 different plant species and 15,000 medicinal plants (India Herbs, 2002). Indian systems of medicine have identified 1500 medicinal plants, of which 500 species are mostly used in the preparation of drugs. Three of the ten most widely selling herbal medicines in the developed countries, namely preparation of *Allium sativum*, *Aloe barbadensis* and *Panax* spp. are available in India (Dubey et al., 2004). Despite a vast flora and fauna of medicinal plants, India’s share in global export of medicinal plants related trade is just 0.5%.

Global market of herbal medicines and herbal personal care products/herbal cosmetics was US $8 billion and US $22 billion respectively in year 1999 with annual growth rate of 20% (Herbal Sector, 2004; Wakdikar, 2004). The European market of herbal medicines is US $6.8 billion (Calixto, 2000; Expo Europe, 2004). The German market corresponds to about 42% of European
market. This market is followed by France 25%, Italy 9%, United Kingdom 8%, Poland 5%, Spain 3%, The Netherlands 3% and others 5%. The herbal medicine markets in Asia and Japan are US $2.3 and 2.1 billion respectively. The domestic market of Indian systems of medicine and Homoeopathy was of the order of Rs. 4,000 crores in year 2000 (India Herbs, 2002).

During the last twenty years, herbal medicines have enjoyed renaissance among consumers throughout the developed world (Hylands, 2002). For centuries, preparations based on traditional folk uses have served as the main form of medical treatment. In most western countries, plant-based preparations or medicines were discarded during the early part of the twentieth century with the advent of modern medical science. However, in many parts of the world, especially, Asia, traditional herbal medicines are still the integral part of primary health care. With the advent of twenty first century, plant based medicines and nutraceuticals have once again occupied the centre stage for treating many of today’s common illnesses and health problems as most of modern synthetic medicines are associated with severe adverse effects. While consumer acceptance of these products is high, there are concerns voiced mainly by regulatory agencies that current quality control and standardization procedures for botanical products are not sufficient to ensure their efficacy and safety. If plant-based medicines are to gain wider acceptance not only from the public, but from the regulatory authorities as well, then there must be new approaches to solve the problems of quality control and standardization.

Standardization of a drug guarantees its chemical consistency, therapeutic efficacy and reproducible biological activity (Hawkins, 2001). Standardization of plant derived drugs involves the collection of information and application of stringent quality control measures at every step of the process from the growing of a medicinal plant to the finished therapeutic substance (Bonati, 1991). It includes complete description of the starting drug, control and monitor on the factors viz., growing conditions, harvesting time, part of plant harvested, absence of toxic pesticides or other contaminants, drying methods, freshness and storage, extraction process, and analytical controls which are required for providing constancy of the quality of an extract. Nomenclature which includes the name of the drug, physical state, solvent for extraction and composition of the extract also form an important part of standardization. Another aspect of standardization is
that it guarantees the content of one or more active constituents or marker compounds. According to Willard (1999), “Standardization should involve the compilation of complete data on a herb such as the season in which the herb is picked, the ripeness, the taste, smell, appearance, drying, storage, processing and fingerprinting which needs much larger spectrum of constituents, usually five or more active or marker constituents”. Standardization is also considered a way to deal with the regulations, framed by regulatory authorities, that require drug measurability, and active ingredients to be stated on product labels (Linda, 2002).

Non-availability of pharmacopoeial standards for plant derived medicines is the major drawback which often frustrates a physician as well as a patient, and can over-shadow the time tested healing properties of plant medicines. This can only be countered by the process of standardization of plant medicines (Plotnikoff and George, 1999). By way of standardization, plant derived phyto-pharmaceuticals can be evaluated for their performances, limitations, optimal dosage, contraindications, and applications (Hawkins, 2001).

Inconsistent and variable biological effects of plant-derived medicines are, perhaps, the main discouraging issues for researchers in the field of natural products. Reproducible efficacy and safety of the phyto-pharmaceuticals are based on reproducible quality. Therefore, a phyto-pharmaceutical could only be considered as a rational drug if it is standardized and its pharmaceutical quality is approved. Also, in pharmacological, toxicological, and clinical studies with herbal drugs, their composition needs to be well documented in order to obtain reproducible results (Bauer, 1998; Food and Drug Administration, 1998). The World health Organization (WHO) has recognized this problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines (Bauer et al., 1994). Besides research, this concept must be followed in the commercial production and therapeutic application of phyto-pharmaceuticals. Usually this undesirable biological and phytochemical variability of the plant material is due to different growth, harvest, drying and storage conditions. Therefore, cultivation of plants under standardized conditions is desirable. The polarity of solvent, mode of extraction, instability of the constituents may influence the composition of extracts, and must, therefore, be kept constant (Bauer, 1998; Food and Drug Administration, 1998).
The WHO has issued the following guidelines for the assessment of herbal medicine (WHO, 1991):

1. **Quality assessment**: crude plant material, plant preparation and finished product.
2. **Stability**: shelf life.
3. **Safety assessment**: documentation of safety based on experience or toxicology studies.
4. **Assessment of efficacy**: documented evidence of traditional use and/or activity determination on animal or human models.

The steps in standardization and quality control include:

1. Determining the identity of the plants from classical texts and equating it to scientific botanical identity.
2. Ensuring that the correctly identified raw material is supplied and used.
3. Ensuring that the correct formulation and the correct quantities are used.
4. Ensuring that the correct procedures of preparation, storage and packaging are adopted.

Standardized herbal extracts are of two main types: an *active constituent extract* where there is a known and accepted active biochemical principle, and a *marker extract* where the active biochemical principle is not known and a characteristic compound is used as a marker (Tierra, 1998). An active constituent extract, is like a drug and may have undesirable side effects which are normally absent in the herb. In the marker extract, a unique constituent is selected as a marker and as no single active constituent is known, so the entire extract is treated as active. Table 2 shows examples of active constituent extract and marker extract.

**Table 2**: Examples of active constituent extract and marker extract.

<table>
<thead>
<tr>
<th>Standardized extract</th>
<th>Plant</th>
<th>Biochemical principle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active constituent extract</strong></td>
<td><em>Ginkgo biloba</em></td>
<td>24% flavoglycosides</td>
</tr>
<tr>
<td></td>
<td><em>Silybum marianum</em></td>
<td>80% silymarin</td>
</tr>
</tbody>
</table>
Standardization of Ayurvedic drugs is necessary seeing the growing popularity of medicine in western countries (Singh, 2002). The standardized medicinal herbs include:

1. *Adhatoda vasica* (0.5% vasicine)
2. *Allium sativum* (0.6% allicin)
3. *Andrographis paniculata* (10% andrographolide)
4. *Asparagus racemosus* (30% saponins)
5. *Azadirachta indica* (2% azadiractin)
6. *Bacopa monnieri* (20% bacoside)
7. *Boswellia seratata* (40 and 70% boswellic acid)
8. *Capricum frutescens* (0.62% capsaicinoids)
9. *Commiphora mukul* (5% guggal-sterones)
10. *Embelia ribes* (8% embelin)
11. *Gymnema sylvestre* (75% gymnemic acid)
12. *Momordica charantia* (3% bitters)
13. *Ocimum sanctum* (8% ursolic acid)
14. *Phyllanthus niruri* (2% bitters)
15. *Picrorrhiza kurroa* (10% kutkosides)
16. *Pueraria tuberosa* (7% diosgenin)
17. *Saraca indica* (8% tannins)
18. *Terminalia arjuna* (8% tannins)
19. *T. belerica* (40% tannins)
20. *T. chebula* (60% tannins)
21. *Trifolium pratense* (20 and 40% saponins)
22. *Trigonella foenum graecum* (10% saponins)
23. *Withania somnifera* (1.5% withanolides)
24. *Zingiber officinale* (5% gingrols)

Bonati (1991) has described the standardized extracts as the ones:

1. Having consistent levels of specified compounds.
2. Having recognized active constituents as well as a variety of other plant constituents.
3. Subjected to rigorous quality controls during all phases of growth, harvesting and manufacturing processes.

Standardized extracts have consistent activity which allows more accurate prescribing resulting in consistent clinical results. Further, extensive quality control ensures the quality and safety of standardized extracts.

One of the major problems in standardizing plants is that they contain many complex and different chemical constituents (Hylands, 2002). Oxford
Natural Products (ONP) has developed an authority which has specified stringent criteria for standardizing plant-based medicines. This helps manufacturers to standardize and validate plant products. Over the last two years, total quality profiling (TQP) has been developed which combines a number of technologies in a unique way to overcome problems associated with product definition and variability. Through the use of TQP manufacturing, one can achieve careful control on characterization of the plant materials and their preparation through an innovative combination of plant science, chemistry and biology applied in a uniquely complementary way. Phytotrat – a proprietary software system, contains the information about all key stages through which plant raw material passes from field to formulation such as, agronomic performance, soil conditions and environmental elements including rainfall, chemistry and microbiology of the crop.

The quality and purity required in a drug are achieved by standards given in the official work of reference. To establish the identity and quality of the drug several methods may be considered (Handa, 1995; Evans, 1996; Indian Pharmacopoeia, 1996; Kokate et al., 1999; Wallis, 1999). These are enumerated below:

a) **Morphology and Organoleptic evaluation.** In case of whole drug, the macroscopic and sensory characters are usually sufficient to enable the drug to be identified. These include colour, odour, taste, size, shape, fracture, etc.

b) **Histology and Microscopy.** These are valuable both for powders and unground drugs. Type of epidermal parenchyma, stomata, trichomes, fibres, vessels, calcium oxalate crystals, etc., help in the identification of drug.

c) **Quantitative Microscopy.** Microscopical determinations such as vein-islet number, veinlet termination number, palisade ratio, stomatal number, stomatal index, determination of size of fibres, vessels, etc., help in the identification of the plant, and in differentiating closely allied species.
d) **Solubilities**, especially exceptional behaviour towards solvents, are useful for the examination of many oils, oleo-resins, etc.

e) **Qualitative chemical tests.** Most of these tests are colour reactions which are specific for certain substances. These tests include the general tests for alkaloids, glycosides, tannins, flavonoids, etc.

f) **Quantitative chemical tests.** A number of quantitative chemical tests viz., acid value, iodine value, saponification value, ester value, unsaponifiable matter, acetyl value and volatile acidity are useful in the evaluation of fixed oils, resins, balsams, volatile oils and gums.

g) **Physical constants** such as specific gravity, optical rotation, viscosity and refractive index are especially valuable for the evaluation of oils and fats, oleo-resins, balsams, and similar substances.

h) **Ash values.** These are useful for detecting excess of sandy or earthy matters. The presence of ash is determined as total ash, acid insoluble ash, water soluble ash and sulphated ash.

i) **Extractive values.** The determination of extractable matter refers to the amount of constituents in a plant material extracted with specific solvents. It indicates the nature of constituents of the plant drug, and also helps in detecting low grade exhausted drugs.

j) **Moisture content.** Moisture content must be determined and controlled so as to prevent decomposition in the plant material. Methods which are commonly employed for the determination of moisture are loss on drying, toluene distillation, gas chromatographic method, Karl Fischer method and spectroscopic methods.

k) **Volatile oil.** Pharmaceutical significance of aromatic drugs is due to their odorous principles, i.e., volatile oils. These drugs are standardized on the basis of their volatile oil content.
l) **Crude fibre.** Determination of crude fibre is done to determine the presence of excessive woody material.

m) **Microbial contamination.** It is applied for crude drugs which are to be taken internally.

n) **Toxic residues.** These may arise due to pesticide application and fumigation. It can be reduced by use of infusions of the dried plant material.

o) **Chemical fingerprinting.** Various chromatographic procedures and spectroscopic methods are employed to develop the chemical fingerprint profile, thus allowing all components in the extract to be detected (Hylands, 2002).

p) **Biological profiling.** Biological profiling identifies biologically active plants allowing highly sophisticated standardization and quality control.

q) **Assays.** Crude drugs may be assayed for a particular group of constituents using chemical, spectrometric and radioimmunoassays.

1.5.1 **Challenges in the standardization of plant drugs**

1. **Biological variations:** Plants are rich source of chemicals and potential sources of effective medicines, but the chemical constituents of plants vary depending on the species, variety and part of the plant, conditions of growth (soil, water and temperature) and age of the plant (Therapeutics Letter, 1998). These complexities and variations of chemical content make standardization essential.

2. **Selection of markers:** The major drawback associated with the process of standardization is selection of markers (Tierra, 1998). Most of standardized herbal extracts are not consistently standardized to one
marker because it is not certain which of its constituents are responsible for its therapeutic actions. Nettle root is standardized by one company to 5% amino acids, by another to 8% sterols, and a third uses 35 ppm scopoline. *Echinacea* can be standardized to three different constituents, i.e., echinacosides, polysaccharides or polybutylides. Sometimes an active compound for any given herb may change in time such as the hyperforin of St. John’s wort recently understood to be more active than its previous marker, hypericin. Moreover, any medicinal herb does not exhibit single biological activity, but has long tradition of use in the treatment of various ailments and different constituents are responsible for different activities.

**Turmeric**

- **Analgesic** – Borneol, caffeic acid, curcumin, p-cymene, eugenol
- **Anti-dermatitic** – Guaiacol
- **Anti-edemic** – Borneol, caffeic acid, caryophyllene, curcuminoids, eugenol
- **Anti-inflammatory** – Azulene, borneol, caffeic acid, caryophyllene, cinnamic acid, curcumin, eugenol, protocatechuic acid, vanillic acid, β-sitosterol
- **Inhibit cyclooxygenase** – Curcumin, galangin
- **Inhibit lipoxygenase** – Borneol, caffeic acid, cinnamic acid
- **Inhibit 12-lipoxygenase** – Curcumin
- **Inhibit production of tumour necrosis factor** – Curcumin

Turmeric is generally standardized on the basis of curcumin (95%) but this standardized extract can not be relied upon to be effective for skin conditions (anti-dermatitic).

3. **Role of Federal Regulatory Authorities (FRA):** Lack of proper control by FRA results in marked variation in strength of marketed herbal formulations. Consumer reports found that 25% of ginseng products on health food store shelves did not contain any of the listed compounds, and the contents of products varied widely (Herbal Roulette, 1995). Another study commissioned by Los Angeles Times found that three of the ten different national brands of St. John’s wort over the counter remedies, contained less than half the active ingredients claimed on the label and
seven products contained between 75 to 135% of the active ingredients as labeled hypericin (Barret, 2000).

4. Safety issues: In the United States, botanical products are marketed as “dietary supplements” (Calixto, 2000). Other countries treat the herbal preparations as drugs, and to be registered these products to be tested to prove their safety and chemical efficacy. There is general conception that herbs are ‘natural’ so these are completely safe. This is not true; the dangers of nutraceuticals are well documented (Holt, 1998; AAPS News Magazine, 2001). In 1994, use of *Ephedra* led to 39 deaths and 695 cases of serious illness, ranging from insomnia, nervousness and arrhythmia to hypertension, heart attack, seizures and stroke. Liquorice is reported to cause oedema and hypertension if used for a long time (Dubey et al., 2004). Ginseng causes hypertension, gynaecomastia and vaginal bleeding.

Further, toxicities caused by herbal drugs have been shown in table 3 (Saxena, 1985). Table 4 shows herbal drug interactions with modern drugs and their consequences (Saxena, 1985; Cupp, 1999; Meschino, 2003).

**Table 3:** Toxicities caused by herbal drugs.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Use</th>
<th>Possible toxin</th>
<th>Toxic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aristolochia</em> spp.</td>
<td>Antidermatitis</td>
<td>Aristolohic acid</td>
<td>Dose dependent carcinogenesis</td>
</tr>
<tr>
<td></td>
<td>Antirheumatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antigout</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Panax ginseng</em></td>
<td>Adaptogen</td>
<td>Estrogen like substances</td>
<td>Gynaecomastia Ginseng abuse syndrome (excitation, arousal, nervousness, tension, hypertension)</td>
</tr>
<tr>
<td><em>Pennyroyal oil</em></td>
<td>Abortifacient</td>
<td>Pulegone</td>
<td>Severe hepatotoxicity</td>
</tr>
<tr>
<td>(Mentha pulegium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Hedeoma pulgioides)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sassafras</em></td>
<td>Antirheumatic</td>
<td>Safrole</td>
<td>Hepatotoxic Hepatocarcinogenic</td>
</tr>
<tr>
<td></td>
<td>Carminative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flavoring agent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 3:** Continued.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Effect(s)</th>
<th>Metabolite</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senecio crotalaria</td>
<td>Stimulant</td>
<td>Pyrrolizidine</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alkaloids</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Centrilobular necrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hepatic failure</td>
</tr>
<tr>
<td>Symphytum officinale</td>
<td>Stimulant</td>
<td>Pyrrolizidine</td>
<td>Hepatocellular damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alkaloids</td>
<td></td>
</tr>
<tr>
<td>Viscum album</td>
<td>Diuretic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antihypertensive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anticancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antispasmodic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkaloids</td>
<td>Cytoxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haemagglutination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mitogenic</td>
</tr>
</tbody>
</table>

**Table 4:** Herbal drug interactions with modern drugs and their consequences.

<table>
<thead>
<tr>
<th>Herbal drug</th>
<th>Modern drug</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aesculus hippocastanum</td>
<td>Coumarin</td>
<td>Synergism (potentiation of anticoagulant effect)</td>
</tr>
<tr>
<td>Convallaria majalis Nerium indicum</td>
<td>Digoxin</td>
<td>Synergism (potentiation of digitalis toxicity)</td>
</tr>
<tr>
<td>Echinacea</td>
<td>Immunosuppressive agents</td>
<td>Counteract effect of the drug</td>
</tr>
<tr>
<td>Ephedra sinica</td>
<td>Caffeine, Decongestant, Stimulant</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Antidepressant</td>
<td>Severe hypertension</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>Aspirin, Warfarin, Clopidogrel, Dipyridamole</td>
<td>Synergism (increases bleeding, hemorrhages)</td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
<td>Cortisone</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Antihypertensives</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>Antidepressant</td>
<td>Synergism (dizziness, confusion, allergic reactions, fatigue and gastrointestinal symptoms)</td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>Chlorpropamide</td>
<td>Synergism (upsets diabetic control)</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>Warfarin</td>
<td>Decreases warfarin effectiveness</td>
</tr>
<tr>
<td></td>
<td>Antidepressant</td>
<td>Euphoria and CNS stimulation</td>
</tr>
<tr>
<td>Panax ginseng saponins</td>
<td>Antihypertensives</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Piper methysticum</td>
<td>Sedatives, Antipsychotics, Ethanol</td>
<td>Synergism (lethargic)</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Warfarin</td>
<td>Synergism (increases bleeding)</td>
</tr>
<tr>
<td>Allium cepa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanacetum parthenium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbal drugs with diuretic actions</td>
<td>Antihypertensives</td>
<td>Potentiation of hypotension</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.5.2 Solutions for problems associated with plant drug standardization

1. Control and monitor on growing conditions, harvesting time, part of plant harvested, absence of toxic pesticides or other contaminants, drying methods, freshness and storage, processing, extract solvents, whole extraction process, and analytical controls.
2. Establishment of pharmacognostic standards as mentioned above.
3. Biological evaluation on the basis of traditional reports.
4. Toxicological studies (LD<sub>50</sub> studies).
5. Isolation of biologically active constituent(s) using bioactivity-guided fractionation.
6. Estimation of biological markers using HPTLC/HPLC.
7. Preparation of standardized formulation.
8. Dosage schedule.
9. Control on formulation and manufacture of dosage form, packaging, storage and stability.
10. Control on batch to batch variation.
11. Control by FRA on finished products.

1.6 Experimental models for assessing anti-anxiety activity

Various experimental models for evaluating anxiolytic activity are discussed under subheads based on anxiogenic stimulus involved to induce anxiety in animals.

1.6.1 Elevation

The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated maze. The manifestation of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by time spent by the animal in the open arms.

1.6.1.1 Elevated plus maze

The plus maze consists of two open arms, 50×10×40 cm and two closed arms, 50×10×40 cm with an open roof, arranged so that the open arms are opposite to each other (Vogel and Vogel, 1997). The maze is elevated to a height
of 50 cm. The rats are housed in pairs for 10 days prior to testing in the apparatus. During this time, the rats are handled by the investigator on alternate days to reduce stress.

Thirty minutes after administration of the test or the standard drug, the rat is placed in the centre of the maze, facing one of the closed arms. During a 5-minute test period, the following measures are recorded: the number of entries into open and closed arms; time spent in open and closed arms; the total number of entries to the open/closed arms. The whole procedure is conducted preferably in a sound attenuated room. The anxiolytic behaviour is evaluated by observing motor activity. Open arm exploratory time is registered.

1.6.1.2 Elevated zero maze

The maze comprises a black perspex annular platform (10.5 cm in diameter, 10 cm width) elevated to 65 cm above the ground level, divided equally into four quadrants (Kumar, et al., 2000b). The two opposite quadrants are enclosed by a black perspex wall (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite quadrants are surrounded by perspex lip (1 cm high) which serves as a tactile guide to animals on these open areas. Rats are placed on one of the enclosed quadrants for a 5-minute test period. The maze is cleaned with 5% ethanol/water solution and dried between test sessions. Time spent in open arms, number of head dips over the edges of platform, number of stretch postures attend from closed to open arms are recorded.

1.6.1.3 Elevated T maze

The T-maze consists of three arms of equal dimensions (50×12 cm) (Onusic, et al., 2003). One arm is enclosed by 40 cm high walls and disposed perpendicularly to the two opposing open arms. The entire apparatus is elevated 50 cm above the floor. To avoid falls, the open arms are surrounded by a plexiglas rim 1 cm high. The animal is placed at the distal end of the enclosed arm of the ETM, facing the intersection of the arms, 30 minutes after treatment. The time taken by the rat to leave this arm with four paws is recorded (baseline latency). The same measurement is repeated in two subsequent trials (avoidance 1 and 2) at 30 seconds intervals. Following avoidance training, rats are placed at the end of
the right open arm of the maze and the latency to leave this arm with the four paws is recorded for the three consecutive times (escape 1, 2 and 3), again with 30 seconds intervals.

1.6.2 Novel environment

Exposure to novel environment induces approach-avoidance behaviour in animals.

1.6.2.1 Open field behaviour

The apparatus is composed of a white circular plexiglas floor (40 cm in diameter) surrounded by a grey PVC wall (30 cm in height) (Roy et al., 2001). The floor is divided into one central and six peripheral parts of equal surface. A 100 W white bulb placed 70 cm above the open field provides a 500-lx illumination at floor level. At the beginning of the test, the mice are placed in a peripheral part of the open field and video recorded for 5 minutes. Neurobehavioural parameters recorded are as follows:

(a) Latency before leaving the initial part
(b) The total number of entries into peripheral and central parts
(c) Rearings and defecation
(d) Stretched attend postures

1.6.2.2 Hole board test

The apparatus consists of a grey perspex panel (40×40 cm, 2.2 cm thick) with 16 equidistant holes (3 cm diameter) in the floor (Costa-Campos et al., 2004; Kalueff and Tuohimaa, 2004). Photocells below the surface of the holes provide the measure of the number of head dip. The board is positioned 15 cm above the table and is divided with black water-resistant marker into 9 squares of 10×10 cm. Each animal is individually placed in the centre of the board (facing away from the observer) and following parameters are noted during 5-minute test period: (a) the latency of first head dip, (b) number and duration of head dips and number of rearings and (c) spontaneous moments (number of squares crossed with all four paws).
1.6.2.3 Social interaction in rats

In an unfamiliar and brightly lit environment, the normal social interaction of rats (sniffing, nipping, grooming) is suppressed (Vogel and Vogel, 1997). Anxiolytics counteract this suppression.

The apparatus used for the detection of changes in social behaviour and exploratory behaviour consists of a perspex open topped box (51×51×20 cm) with 17×17 cm marked areas on the floor. One hour prior to the test, two naive rats from separate housing cages are treated with the test compound orally. They are placed into the box (with 60 W bright illumination 17 cm above) and their behaviour is observed over a 10 minutes period by remote video recording. Social interaction between the animals is determined by noting the time period of sniffing of the partner, crawling under or climbing over the partner, genital investigation of the partner, and following the partner. Exploratory motion is measured as the number of crossing of the lines marked on the floor on the test box. Six pairs are used for each dose. The anxiolytic activity is determined by comparing the values of treated partners with the data from pairs of untreated animals using single factor analysis of variance.

1.6.2.4 Suppression of feeding by novelty

The apparatus consists of a wooden box (60×60×35 cm) with a solid floor (Bhattacharya et al., 1996). The floor is covered with a 2 cm wooden chip layer, and 15 laboratory chow pellets are evenly placed on the floor. A similar arrangement is made in home cage rats. The rats are food deprived for 24 hours prior to testing, but are provided with drinking water. The rats are placed individually in the test chamber and the latency to begin feeding is recorded. If the rats have not eaten within 300 seconds, the test is terminated and a latency score of 300 seconds is recorded.

1.6.2.5 Staircase test

The staircase test is used for evaluating anxiolytic activity by purporting step-climbing to reflect exploratory or locomotor activity, while rearing behavior is an index of anxiety state (Vogel and Vogel, 1997).

For experiments with mice, the staircase is composed of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is
constant along the whole length of the staircase. In this test, each animal is used only once. Twelve mice are used for the untreated control group, each drug group, and for the group receiving standard. The drug or the standard is administered orally 1 hour or 30 minutes subcutaneously prior to the test. The animal is placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears are counted over a 3 minute period. A step is considered to be climbed only if the mouse has placed all four paws on the step. In order to simplify the observation, the number of steps descended is not taken in account. After each test, the box has to be cleaned in order to eliminate any olfactory cues which might modify the behaviour of the next animal. In this experimental model, average number of steps and rearing of control group is taken as 100%, and the values of treated animals are expressed as percentage of the control.

1.6.3 Bright illumination

Exploration of mice or rats is inhibited by bright illumination, which is highly aversive for rodents.

1.6.3.1 Light/dark model

In light and dark model, animals are placed on the brightly lit side of a two compartment chamber and the number of crossings between the light and dark sites is recorded (Vogel and Vogel, 1997). Anxiolytics produces a dose-dependent increase in crossings. The apparatus consists of cage which is one third darkened with a cover and separated with a wall from the otherwise brightly illuminated area. A round hole (diameter 13 cm) allows the rat to pass from the illuminated to the darkened compartment. The cage is placed on an Animex®-activity counter. The animals are treated orally with the test compound 30 minutes before the session. A group of 6-8 animals is used for each dose. At the start of the test, the rat is placed in the middle of the illuminated part of the cage. The number of crossings is registered during 10 minutes. The anxiolytic activity is screened by comparing the average number of crossings in the treated groups with the saline treated control.
1.6.4 Mirror image

It has been observed that animal species exhibit approach-avoidance response upon placement of a mirror within their environment. The parameter, i.e., latency to enter and total time spent in the mirrored chamber can be used to evaluate anxiolytic drugs.

1.6.4.1 Mirrored chamber test

The apparatus consists of a mirrored cube (30 cm on a side) open on one side that is placed inside a square wooden box (40×40×30.5 cm) (Kulkarni and Reddy, 1996; Kulkarni, 2003). The mirrored cube is constructed of five pieces of mirrored glass. The mirrors used are mirrored on one surface only (back surface being painted dark brown). The three mirrored side panes, a top pane, and the floor pane face the interior of the cube. The mirrored cube is placed in the centre of the wooden container to form a 5 cm corridor that completely surrounded the mirror chamber. A mirror is also placed on the container wall so that it faces the single open side of the mirrored chamber. The other three walls of the container are painted dark brown.

Thirty minutes after the administration of the test drug or standard, the animals are placed individually in the chamber of mirrors at a fixed corner. During a 5-minute test period, the following parameters are noted: (a) latency to enter the chamber, i.e., the time spent in seconds for the first entry into the chamber of mirrors, (b) number of entries in mirrored chamber and (c) the time spent with each entry is calculated by dividing the total time spent with number of entries.

1.6.5 Physical discomfort

1.6.5.1 Four plate test in mice

The four plate test is used for evaluating anxiolytic activity by delivering the shocks to reflect locomotor activity (Vogel and Vogel, 1997).

The test box has the shape of a rectangle (25×10×16 cm). The floor is covered with four identical rectangular metal plates (8×11 cm) separated from one another by a gap of 4 mm. The plates are connected to a source of continuous current which applies to two adjacent plates a mild electrical shock of 0.35 mA for 0.5 sec. Adult male Swiss albino mice are randomly divided into different groups.
Thirty minutes before the test, the animals are administered the test drug or the vehicle.

At the beginning of the test, the mouse is gently dropped onto a plate and is allowed to explore the enclosure for 15 seconds. After this, every time the animal crosses from one plate to another the experimenter electrifies the whole floor for 0.5 second which evokes a clear flight reaction to the mouse. The number of times the apparatus is electrified is counted each minute for 10 minutes. The delivery of shocks decreases dramatically the motor activity. The number of shocks received during the first minute is taken as the parameter for evaluating anxiolytic activity.

1.6.5.2 Foot shock-induced freezing behaviour in rats

In this test, anxiolytic activity is determined by freezing behaviour (Vogel and Vogel, 1997). The animals receive a single test compound or the vehicle 30 minutes prior to being placed in a standard conditioning chamber (e.g. Coulborn Instrument) for a 6.5 minutes session. Two minutes after the start of the session, a scrambled foot-shock (0.5 mA, 0.5 second) is delivered through the grid floor of the chamber. Using an assembly of push buttons interfaced with a computer, an observer monitors the amount of time each animal spends engaged in the following mutually exclusive behaviours.

- Freezing: immobility with rigid body posture.
- Sedated posture: sitting or sleeping.
- Small exploratory movements: movements involving the torso or front paw only, vertical movements of the head or sniffing.
- Locomotion: activity involving hind paws, grooming or rearing.

Frequency of rearing is also counted. All behaviours are monitored for the entire 6.5 minutes session. Evaluation of the anti-anxiety effect can be done on the basis of duration of foot-shock induced freezing after administration of test compound and control.

1.6.5.3 Distress vocalization in rat pups

It has been observed when rat pups are held by tail, they emit ultrasound (Vogel and Vogel, 1997). This parameter, i.e., number of sounds produced by rat
pups can be used to evaluate anxiolytic drugs. The pups are tested at 9-12 days of age. In the morning, all pups are subjected to handling stress and the magnitude of their ultrasound emission is observed. The stress consists of holding the pup by the base of the tail between forefinger and thumb of the experimenter, and then suspending it 5 cm above the bench for 30 seconds. A control recording (30 sec) is taken when the pup is held gently, whereby the pups emit only a few ultrasounds. Responses when held by the tail are more than 10 times higher. The entire hand tail holding procedure is immediately repeated. Ultrasounds are recorded with suitable detectors with 42 KHz as the center of a 10 KHz recording range. The output of the detectors is fed into pen recorders. The total number of ultrasonic cries in the two sessions of hand holding and the two sessions of tail holding are calculated. These are used as the control activity of each pup. Any pup producing less than 50 ultrasounds when held by the tail is excluded from the drug study.

Three to four hours after the first test, the pups are randomly allocated to several equally sized groups, weighed, marked, treated with the vehicle or drug, and placed back in the home cage. Thirty minutes after treatment, each pup is subjected to the same handling stress as that used in the morning session, and the total number of sounds produced is calculated in the same way. The anti-anxiety effect can be evaluated by comparing the total number of sounds produced by the treated group and control.

1.6.5.4 Anticipatory anxiety in mice

It has been observed when group-housed mice are removed one by one from their home cage, the last mice removed have always higher rectal temperature than those removed first (Vogel and Vogel, 1997). This parameter, i.e., anticipatory fear for an aversive event (handling causes stress induced hyperthermia) can be used to screen anxiolytic activity.

In this experiment, mice are housed at constant room temperature and relative humidity for at least seven days in Makrolon cages to adapt to the environment. Test drugs or standard (diazepam) drug are administered orally in various doses to the group of 18 mice prior to the test. Thirty minutes later, first mouse is removed from the cage and the rectal temperature registered by inserting a silicone lubricated thermistor probe (2 mm diameter) for 2.5 cm into the rectum. The average temperature of 3 mice is taken as basal value. Mice number 4 through
15 are simply removed and again returned to the cage, and thereafter body temperature is determined in the remaining three animals. The difference of the mean value of these mice and the basal value is calculated as increase in the body temperature, i.e., anticipatory fear.

1.6.5.5 Unconditioned conflict procedure

1.6.5.5.1 Vogel’s conflict test

To check the anti-anxiety activity, simple and reliable conflict procedure is used (Vogel and Vogel, 1997). Thirty rats are administered shocks while licking water. The apparatus is a clear plexiglas box (38×38 cm) with a black plexiglas compartment (10×10.5 cm) attached to one wall and an opening from the large box to the small compartment. The entire apparatus has a stainless steel grid floor. A water bottle with a metal drinking tube is fitted to the outside of the small compartment so that the tube extended into the box at a height of 3 cm above the grid. Rats lick in bursts with a relatively constant rate of 7 licks per second. A drinkometer circuit is connected between the drinking tube and the grid floor of the apparatus, so that the rat completes the circuit whenever it licks the tube. Shock is administered to the feet of the animal by switching the connections to the drinking tube and grids from the drinkometer to a shocker which applies an unscrambled shock between the drinking tube and grid floor. The rat is placed in the apparatus and allowed to find the drinking tube and to complete 20 licks before shock (available at the tube for 2 seconds) is applied. The rat controls the shock duration by withdrawing from the tube. A 3 minutes timer is automatically started after the termination of the first shock. During 3-minute period, shocks are delivered following each twentieth lick. The number of shocks delivered during the 3 minutes session is recorded for each animal. The number of shocks received after treatment is compared with untreated animals.

1.6.5.5.2 Geller type conflict test

Animals are subjected to food deprivation in order to induce hunger (Umezu, 2000). They are trained under MULT FR20/FR20-punishment schedule of food reinforcement, using the apparatus for the Geller type conflict test (GT-8510, GT-8005 and GT-7715). The schedule consists of four pairs of an alternating safe period; the mouse’s lever pressing is reinforced by food pellets at
FR20 without electric shock. During the alarm period, which is indicated by a warning stimulus (tonic signal: 800 Hz, 90 dB), every 20th lever press is punished using an electric shock (50-90 v, ca 0.3mA, 50 Hz AC, duration 0.3s). The response rate of the animal is recorded during the safe as well as alarm period.

1.6.6 Social separation

Social separation stress results in vocalizations and nociceptive responses in animals, and is sensitive to the anxiolytic drugs.

1.6.6.1 Chick social separation-stress

In this procedure, a chick is placed into the observation chamber in isolation for a 3-minute test session (Sufka, et al., 2001). To index stress-induced analgesia, a 50 μl injection of 0.10% formalin was administered into the plantar region of the Chick’s foot immediately before placement into the chamber. The following observations are recorded:

1. (a) Foot lift frequency and foot lift duration in response to formalin
   (b) To index sedation
   (c) Ventral recumbent latency that resembles a sleep-like posture. A composite pain score (CPS) is derived from the following formula:
   \[ \text{CPS} = 4 \times (z\text{-score } \text{foot lift}) + [z\text{-score } (\text{duration/total number of lifts})]. \]

2. Number of vocalization to index separation-stress.

1.6.7 Exposure to predator/predator's odor

1.6.7.1 Cat odor exposure

This paradigm is based on defensive behaviours displayed by rodents when confronted to a predator but also to its odor (Johnston and File, 1988; Roy, et al., 2001).

Mice are divided into three groups, i.e., control (no odor), neutral odor (modeling clay) and predator odor (cat feces). Cat feces are obtained from a 2 year old male domestic cat, collected out doors quickly after defecation. The neutral odor is provided by a 2 cm diameter ball of blue modeling clay of the same volume as cat feces. The mice are individually brought to the testing room dimly
lit with a 40-w red bulb (15-lx on the testing location). After the removal of the grid, and depending on the group, an odorant stimulus is placed on the saw dust, at the opposite location of the food compartment. The same procedure is followed for the control group except that no stimulus is given. In order to avoid any disruption between odors, the mice from the control group are always tested first followed by those from the neutral odor group and the predator odor group. The grid is then replaced and the behaviour of each mouse is recorded from the side during 5 minutes. The parameters recorded are discussed below.

(a) The time spent at the opposite location of the odorant stimulus
(b) The number of entries into the two parts of the cage (i.e. under the food compartment or stimulus location)
(c) The number of contacts with the odorant stimulus
(d) The number of stretch attend posture
(e) The number of burrows in odorant stimulus

The cat odor can also be obtained by rubbing a damp cloth (20x20 cm) against the fur of male laboratory housed domestic cats for 5 minutes (Onusic, et al., 2003). Clothes with cat odor are kept in sealed plastic bags. Each cloth is used for four exposures only.

1.6.7.2 Mouse defense test battery

In this assay, mice are placed in a test alley and approached with an anesthetized rat, a natural predator of mice that triggers unconditioned aversive reactions (Tecott and Nestler, 2004). A series of typical behaviours is assessed including flight, risk assessment (repeated episodes of extending and withdrawing body in the direction of threat), and defensive threat and attack response.

1.6.8 Anxiogenic agents
1.6.8.1 Antagonism of discrimination stimuli produced by anxiogenic drugs

It has been seen that most of anxiolytics block the discriminative effect of PTZ, whereas anticonvulsants without anxiolytic effects do not (Barnett, 1985).

A sub-convulsive dose (20 mg/kg, i.p.) of PTZ is used as an anxiogenic stimulus in rats. The basic method involves training of rats to press an appropriate lever for food in the presence of an injection of PTZ. Responses on second lever
are also recorded. A rat is considered trained when it can reliably press the lever appropriate to a PTZ versus saline injection. Treatment with anxiolytic drug block this discriminative effect of PTZ.

1.6.8.2 \(\beta\)-carbolines-induced behavioural syndrome in monkeys

Ethyl ester of \(\beta\)-carboline-3-carboxylate (\(\beta\)-CCE) possesses a high affinity for BZD receptor binding site, thus, causes a behavioural syndrome, which is blocked by diazepam (Barnett, 1985).

The procedure involves chaired monkeys with indwelling intravenous catheters. \(\beta\)-CCE is administered i.v. and behaviours observed include marked head and body turning, and distress vocalization. Plasma samples show increases in cortisol, epinephrine, and norepinephrine. Drugs with anti-anxiety activity are considered to block these behavioural changes.

This model is time consuming procedure. Moreover, rhesus monkeys are expensive and difficult to obtain. These disadvantages associated with this model limit its use for preliminary screening of anxiolytic drugs. Therefore, this procedure fits best as a ‘tertiary’ evaluation, after there is sufficient evidence of anxiolytic potential from other preliminary measures.

Behavioural measures in experimental models of anxiety have been summarized in table 5.

**Table 5: Behavioural measures in experimental models of anxiety.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Anxiogenic stimulus</th>
<th>Test</th>
<th>Neurobehavioural parameters</th>
<th>Anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Elevation</td>
<td>Elevated plus maze</td>
<td>Open arm entries</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>Elevated zero maze</td>
<td>Time spent in the open arms</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Open arm entries (%)</td>
<td>–</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Time spent in the open arms (%)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Elevated T-maze</td>
<td>Latency to leave open arm</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Novel environment</td>
<td>Open field exploratory behaviour</td>
<td>Approach latency</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>The total number of entries in peripheral and central parts</td>
<td>–</td>
</tr>
<tr>
<td>Novel environment</td>
<td>Hole board test</td>
<td>Latency of first head dip</td>
<td>+</td>
<td></td>
</tr>
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<td>-------------------</td>
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<td>---------------------------</td>
<td>---</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Number and duration of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>head dips</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Number of squares crossed</td>
<td></td>
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<tr>
<td>Social interaction in rats</td>
<td>Sniffing of partner</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crawling under or climbing over the partner</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genital investigation of partner</td>
<td>–</td>
<td></td>
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<tr>
<td>Suppression of feeding by novelty</td>
<td>Latency to start eating food</td>
<td>+</td>
<td></td>
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<tr>
<td>Staircase test</td>
<td>Number of steps climbed</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of rearings</td>
<td>–</td>
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<td>3</td>
<td>Bright illumination</td>
<td>Number of light box entries</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>Time spent in light box</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mirror image</td>
<td>Mirrored chamber test</td>
<td>Latency to enter the chamber</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of entries</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total time spent in the chamber</td>
<td>–</td>
<td></td>
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<tr>
<td>5</td>
<td>Physical discomfort</td>
<td>Four plate test</td>
<td>Flight reaction</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>Foot shock induced freezing behaviour in rats (Antifighting test)</td>
<td>Freezing</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Sedated posture</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exploratory movements</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Distress vocalization in rat pups</td>
<td>Total number of sound produced</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anticipatory anxiety in mice</td>
<td>Stress induced hyperthermia</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unconditioned conflict procedure</td>
<td>Number of shocks delivered</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Vogel’s conflict</td>
<td>Number of punished lever presses</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geller’s conflict</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>Social separation</td>
<td>Chick social separation stress</td>
<td>Total number of vocalization</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Foot lift frequency/duration</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sedation</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Exposure to predator/predator’s odor</td>
<td>Cat odor exposure</td>
<td>Time spent at the opposite location of the odorant stimulus</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number of contacts with the odorant stimulus</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number of entries into stimulus compartment</td>
<td>–</td>
</tr>
</tbody>
</table>
1.7 Research envisaged

About 85 species of the genus *Turnera* have been reported in various floras. An exhaustive survey of the literature on this genus revealed that sporadic information is available only on 27 species. Amongst these 27 species, ethnopharmacological reports are available on, and little pharmacological studies have been carried out only on two species viz., *T. aphrodisiaca* and *T. ulmifolia*. Further, only 10 species of *Turnera* (Table 1, pp 30-31) have been subjected to phytochemical investigations.

A close scrutiny of the available literature on *Turnera* reveals that only *T. aphrodisiaca* is widely known for its medicinal properties. The plant has a long history of use, in various traditional as well as alternative and complementary systems of therapeutics, as an aphrodisiac, nerve tonic, and in various other ailments. *T. aphrodisiaca* has also been available commercially in the United States since 1874, and has been approved by the FDA as a food additive (Abby’s Herbal Newsletter Archives, 1998). Despite a strong ethnopharmacological record of its medicinal use, no systematic work has been carried out on *T. aphrodisiaca* as is evident from the availability of only three pharmacological reports on this plant, and that too of contradictory nature (Section 1.4.4, pp 32-34). Improper selection of the plant material, geographical or seasonal variation and wrong time of collection might be responsible for these controversial reports. Even the authors of the German Commission E monographs feel that the traditional use of *T. aphrodisiaca* has not been justified by modern research (Blumenthal et al., 1998).

Keeping in mind the traditional/alternative and complementary medicinal uses, sporadic phytochemical and pharmacological reports, *T. aphrodisiaca* seems