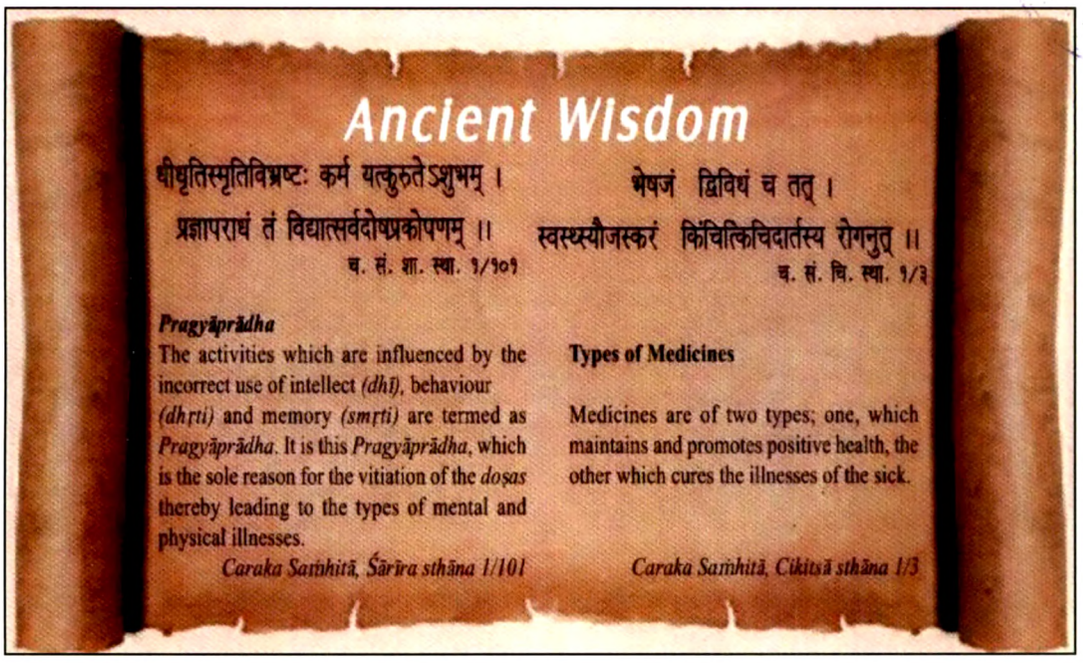
CHAPTER - 1

Introduction

&

Review of literature



Adaptogens

Ancient Ayurvedic literature has focused a great deal on the concept of Rasayana chikitsa. The aim of this specialized therapy is to preserve the health and ensure longevity by optimizing the cellular nutrition. This subject has been of great interest for scientists, as the concept, encompass a comprehensive health of human being. A number of herbs are described as Rasayana in Ayurveda. (Brahma SK et al., 2003)

Sushruta has mentioned that a person whose *Doshas*, *Dhatus,* and physiological functions like digestion, metabolism and excretion remain in the balanced form, his sense organs function properly, both the soul and mind remain cheerful, is regarded as healthy individual and any deviation from the above is regarded as diseased one. Human beings receive every moment a number of stimuli from the environment *(Agantuka)* and from their own systems *(Nija)* as well. These stimuli are expressed in excessive *(Atiyoga)* manner, inadequate *(Hina yoga)* manner or perverted *(Mithya yoga)* manner.

If a human being is not capable enough to cope these stimuli, then he is subjected to stress thus ultimately suffer from different kinds of stress related diseases. In Ayurveda three causative factors for diseases are described. These are *Pragyaparadha* (perverted actions against your knowledge and intellect), *Asatmendriyartha samyoga* (adverse interactions between soul, sense organs and senses) and *Kala parinama* (due to influence of time or nature or environmental factors) all these factors may be included in the group of stressors.

Ayurvedic concept of ***Pragyaparadha*** (errors of thought and behaviour) and their role in the causing disease can be critically correlated with the modern neuro-endocrine mechanisms involved with stress.(Tortora) These stressors vitiate the *doshas*, which ultimately vitiate the *dhatus* thus causing a number of stress related problems. The objective of the treatment here is to balance the body physiology by re establishing the most effective interactions between the *doshas* and *dhatus* already thrown into several imbalances. (Brahma et al., 2006)

Natural products, including plants, animals and minerals, have been the basis of treatment of human diseases since ages. History of medicine dates back practically to the existence of human civilization. The modern medicine or allopathy has gradually been developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics (Patwardhan et al., 1992).

Traditional medicines have a long history. These are based on sum total of knowledge, skills and practices derived from theories, beliefs and experiences indigenous to different cultures. Education, training and research in this area have not been accorded due attention and support. The quality and quantity of the safety and efficacy data on traditional medicines are far from sufficient to meet the criteria needed to support their use worldwide. The reason may be unavailability of adequate and scientifically accepted methodology for evaluating traditional medicines.

The contribution of plants to pharmacology has been estimated in billions of dollars over the past twenty years. In particular, the development of pathological disorders related to heightened stress sensitivity and dysregulation of stress-coping mechanisms is of great concern today. The neuroendocrine system is one of our major auto-regulatory systems.

1.1 Neuroendocrine system

The complex interplay of various components of the human body is in a constant state of flux, whereby the Hypothalamus–Pituitary–Adrenal Axis (HPAA) senses all within and around us. It is then able to prioritize and decipher how energy should be directed. The HPAA is central to our entire endocrine system. The endocrine system itself is a classic cybernetic system, with feedback mechanisms throughout at various levels. Very often, when one endocrine gland manifests a problem other glands are also suffering. For example, with hypothyroidism we can often see reproductive hormones out of balance and/or insulin signaling being affected. What is even more important and often relative is that underneath all of this is an imbalance, or lack of adaptability, centered in the HPAA. It is theorized that the central regulator – the hypothalamus – is responsible for the shifting of homeostasis (allostasis).

The disruption of the HPAA is often central to most health problems, syndromes, diseases, and even aging itself.

This comprehension of the human body is important to maximize the quality of life and longevity. This understanding is also valuable for the prevention of illness, the best possible vitality or improving mental and physical performance.

There are many common threads among the systems of the human body that control our ability to heal from injury, recover from infection and/or illness (acute and chronic), improve performance, and spare lean muscle mass, maintaining a positive nitrogen balance (anabolic state) and of course dealing with stress (the 'stress response').

The health of the HPAA is the root of our entire body; it is truly the key to the strength of our "Life-Force".

"Biological age", the term is frequently used to describe the functional status of the human body as opposed to chronological age. To measure this, investigators have used a variety of biomarkers including skin thickness, strength, stamina, body composition, reaction time, vision, hearing, blood and neurological tests. All of these tests however, have one common feature: they measure aspects of anabolic and catabolic activity. Biological age should include one's metabolic status as it relates to the level of anabolic activity **(repair, rebuild and rejuvenate).** It is the understanding that aging is fundamentally a shift from youthful anabolic metabolism to increasing levels of catabolic activity. Other models of aging, such as wear and tear, free radicals, enzyme depletion and even accumulated genetic error can all be placed under the umbrella of this metabolic shift. The "Metabolic Model" resolves this issue by pointing out that cell defenses, including the production of DNA repair

enzymes (eg; DNA polymerase) and endogenously produced antioxidant enzymes (eg; glutathione, SOD) are influenced by anabolic hormone levels.
Over time, the effects of prolonged stress cause hormone balance to be shifted towards a catabolic process of releasing more stress hormones in order to attempt to handle the stress better. This shift actually causes more oxidative damage and diminishes levels of anabolic hormones, thus negatively affecting the endocrine system (thyroid, pancreas, reproductive, thymus etc.) Whether or not an aging clock truly exists, the fact remains that anabolic metabolism determines a great deal more than one's muscle mass.
It influences immunity, protein synthesis, cell proliferation, bioenergetics, cell communication, endocrine function, and even mind, mood and behavior.

Hypothalamus–Pituitary–Adrenal Axis dysfunction has been shown to lead to some of the following conditions: (Donald R Yance)

- ❑ Dysregulation of catecholamines (fight or flight stress hormones)
- ❑ Dysregulation of glucocorticoids (stress hormones)
- ❑ Dysregulation of cytokines (disrupted immune system response)
- ❑ Receptor desensitization (disruptions in cellular communication)
- ❑ Atrophy of nerve cells in the amygdala and hippocampus of the brain
- ❑ Bone mineral loss
- ❑ High lipids
- ❑ Sarcopenia - loss of lean muscle mass (catabolic)
- ❑ Abdominal obesity
- ❑ Increased risk of cardiovascular disease
- ❑ Chronic fatigue
- ❑ Mild depression and anxiety
- ❑ Anorexia (cachexia)
- ❑ Poor sleep patterns
- ❑ Altered cognitive performance
- ❑ Decreased sexual behaviour

Modern medicine offers vitamins and tonics against most of ill-defined complaints like lethargy, fatigue, tiredness, decreased mental activity, forgetfulness, loss of appetite etc that are byproducts of stressed life style. Modern medicine is in need of novel remedies that can treat conditions resulting from dysfunctional stress response. An obscure pharmacological class of medicinal plants called *adaptogens* suggests novel strategies for treating stress-related disorders.

1.2 Stress

Stress is a common problem and a major health hazard of life, which is prevalent in almost all age groups. It is anything that threatens the body or has ill effects on its functioning.

Stress can be defined as "Pattern of physiological reactions that prepares an organism for action" (WHO, 1971). It varies from individual to individual. With rapid advancement of civilization, industrialization and over population, there has been proportional rise in the stressors of diverse nature. Modern man is constantly being exposed to stressors like atmospheric pollution, food adulteration, highly ambitious and competitive life style, synthetic drugs etc.

Biological stress syndrome (Tortora)

The work of Hans Selye provides the classic model for adaptation to stress. He observed that given any source of external biological stress, an organism would respond with a predictable biological pattern in an attempt to restore its internal homeostasis. He termed this the General Adaptation Syndrome or Biological Stress Syndrome, and divided the response into four categories:

BIOLOGICAL STRESS SYNDROME OF HANS SELYE	
Phase	Neuroendocrine effect
Alarm reaction	Activation of nervous system and adrenal glands
Resistance Phase	HPA axis activation
Tissue changes	Adrenal hypertrophy, gastrointestinal ulceration, Thymic and lymphoid atrophy
Exhaustion Phase	May culminate in death

1.3 Physiology of Stress

Within seconds after an acutely stressful event or danger, norepinephrine is released from nerve endings in preparation for a rapid response. Almost instantly, the adrenal glands release epinephrine and nor epinephrine into the bloodstream. The combination of the release of nor epinephrine and epinephrine results in the familiar "fight or flight" response. Within minutes of a stressful event (and possibly lasting for several hours), a much more elaborate interaction between the nervous and endocrine systems and other forms of internal communication occurs, resulting in a very complex adaptive response to deal with the stress. At this point adrenal glands release extra amounts of cortisol into the circulation. Several other endocrine glands are also critical to the stress response. The hypothalamus, responds to stress by releasing a hormone called corticotrophin-releasing factor (CRF). This hormone signals the pituitary gland to release adrenocorticotropic hormone (ACTH), which stimulates the adrenal glands to release cortisol. With the rise in stress hormones, a complex mechanism of feedback controls is set in motion, eventually signaling the hypothalamus to stop producing its messenger hormone. (Gregory S Kelly, 1999)

Cortisol causes a corresponding drop in our anti-aging and immune enhancing hormone dehydroepiandrosterone (DHEA). A tremendous body of research has shown that when cortisol goes up, DHEA drops and when DHEA is normal, cortisol also normalizes. Low DHEA levels are seen in those that are immune compromised, have arteriosclerosis (hardening of the arteries), diabetes, certain forms of cancer, and autoimmune diseases such as lupus. In aged animals, DHEA restores the cytokine secretion profiles and balance to those of much younger animals. In addition, DHEA increases IL-2 secretion, enhances antibody production and reduces IL-6 and TNF-α. DHEA thus appears to help reverse immune senescence.

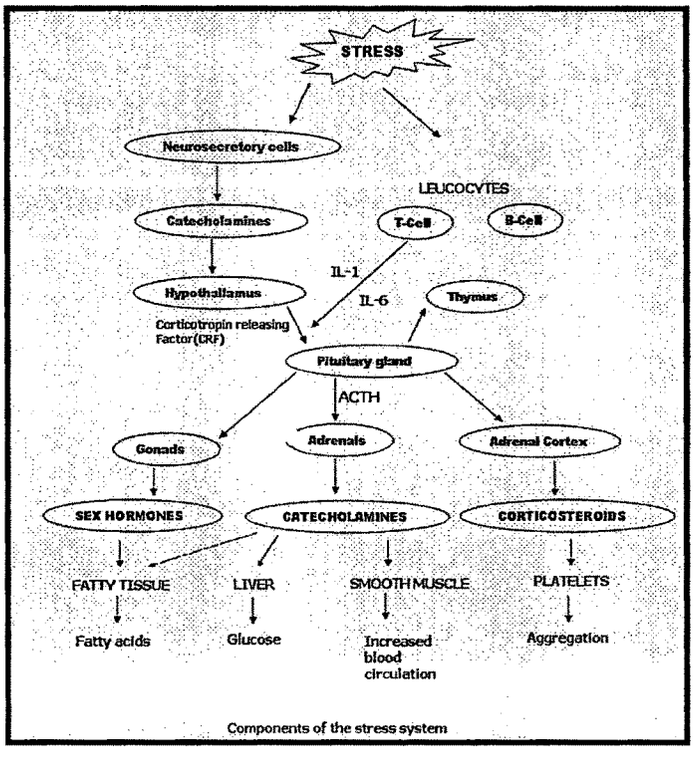


Fig. 1.1 Components of Stress system

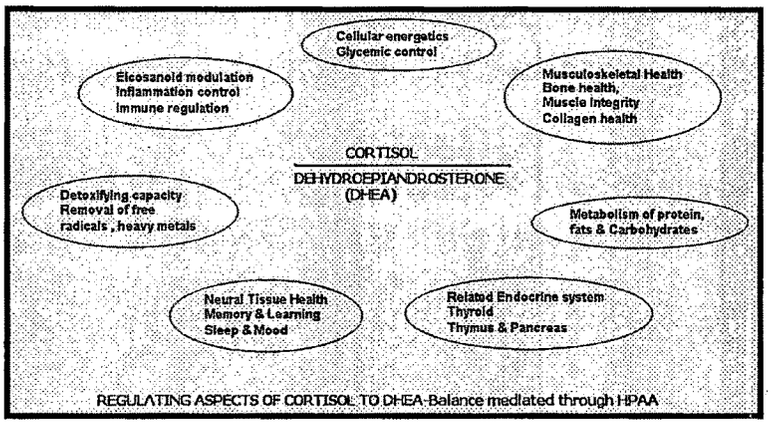


Fig.1.2 Regulating aspects of Cortisol to DHEA-balance mediated through HPAA

Stress itself is a defense response of an organism to external factors (strain), which stimulates formation of endogenous activating messengers, such as catecholamines, prostaglandins (PG), cytokines, nitric oxide (NO) etc. ("switch on"-system), which in turn activates energetic and metabolic resources of the organism.

Corticosteroids, CRF and PGE2 are endogenous mediators of cellular communications, which protect cells and the whole organism from overreacting to the activating messengers ("switch off" system). The balance between the activities of the "switch on" and "switch off" systems – "reactivity" – reflects the sensitivity of the organism to stressors and the level of protection against their damaging effects.

An overall restoration of anabolic metabolism can result in significant upregulation of immunity, demonstrated by greater monocyte, NK cell and T-cell numbers and activity. Since these enhancements do in fact lead to more competent anti-bacterial, anti-cancer and anti-viral defense, one principal anti-aging goal is achieved: the restoration of immune profiles to those seen in youth. In the Indigenous system of medicine there are large number of herbal

drugs and formulations recommended to withstand stress and strain of life without altering physiological functions of the body. These agents induce resistance against aversive stimuli and impart immunity to protect individuals against disease and also postpone ageing, improve vigor, vitality and longevity. This type of activity is known as adaptogenic activity and the drugs are known as adaptogens.

Adaptogens constitute a new class of metabolic regulators (of natural origin) which increase the ability of the organism to adapt to environmental distress and to avoid damage from such factors. (Panossian A, 2003)

Adaptogens are the new concepts in the health conscious world, which are popularly being used to stay fit and prolong and improve the quality of life.

Adaptogens combat the negative effects of stress, improve health and well-being, and enhance athletic performance capacity. They increase the body's vitality and reserve, a key component to any comprehensive longevity program. They are revered because they enhance the 'Life-Force', encourage natural harmony, enhance one's adaptability, and as a result generate "radiant health."

Humans, like all living things, are in a constant flux, adapting so as to harmonize within and around its environment. We are constantly adjusting to a multitude of slight or very large changes, some of which are obvious while others go unnoticed. Good health can be measured by our ability to adapt. A healthy, adaptive person will survive and/or maintain good health where an unhealthy person, less adaptive, will fall, become ill, or even perish. Adaptability is probably the most distinctive characteristic of life. Our over or under adjustment will lead to disharmony. An adaptogen, possesses anabolic activity, increases resistance to a wide range of stressors in a non-specific manner and normalizes hypo- and hyper-conditions.

Adaptogens have been used and recommended suggests that the active principles of these "universal remedies" are directed towards those regulatory systems which are common for all the tissues involved in the regulation of homeostasis (immune or/and hormonal, CNS). (Panossian A, 1997)

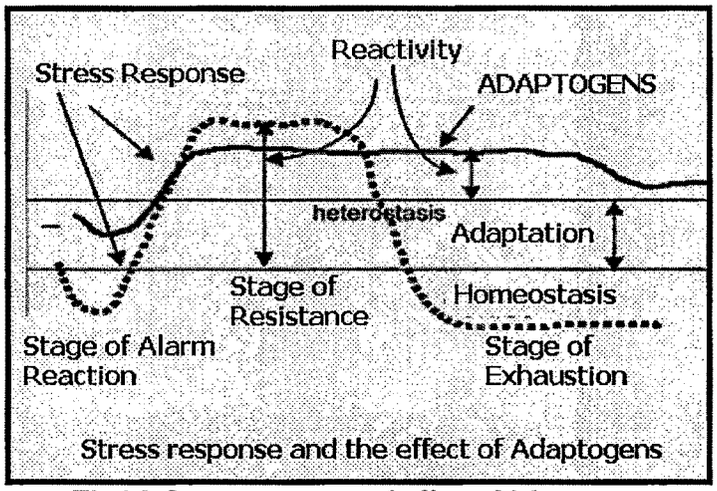


Fig 1.3 Stress response and effect of Adaptogens

1.4 Drugs Known for Adaptogenic Effects

Because the concept of the "adaptogenic effect" is of relatively recent occurrence, one cannot find it in old drug lists. The assignment into the group of adaptogens happens, therefore retrospectively, based on experiential medical data and in a few cases, on data gained from /in vitro/ and /in vivo/ tests. Adaptogenic drugs are found in the most diverse families they are distinctly different in the pattern of their constituents.

The most widely used adaptogenic plant drugs are

Panax ginseng, Elutherococcus senticosus, Withania somnifera, Centella asiatica, Bacopa monera, Asparagus racemosus, Tinospora cordifolia, Ocimum sanctum, Emblica officinalis, Rhodiola rosea, Bryonia alba, , Pfaffia paniculata, Albizzia junibrissin, Mucuna pruriens, etc. (Charles O. Essemone et al.,)

Withania somnifera (Ashwagandha) (INDIAN GINSENG)

The leaves of this plant are used in India as a folk medicine for a local treatment for skin tumors. The root drug is considered a tonic and roborant. It is said to "protect the organism from illness through maintaining the healthy balance of the physical energies (Bhattacharya et. al., 1987)

The root contains the steroid lactone withaferin A and related withanolides, beside various alkaloids. The sitoindosides IX and X isolated by Ghosal et al. represent C-27-glycowithanolides, the sitoindosides VII and VIII, acylesterylglucosides (Ghosal et al., 1989).

The early reports on some plant extracts containing glycosides showed an increase in resistance of animals and human to various kinds of stresses. (Brekman and Dardymov , 1969). These reports paved ways for screening of Ashwagandha as a possible adaptogen. Its efficacy in typical experimental models of stress have shown to improve swimming performance of rats in swimming endurance test (Gupta et al., 1977) The investigations have shown that Ashwagandha enhances survival during stress and induces a state of non-specifically induced resistance during physical and chemical stress in mice and rats (Singh et al., 1982) Vaswani et al., (1983) have reported Ashwagandha as an adaptogen by exhibiting its property that increases WBC and RBC counts as well as haemoglobin levels in mice. Sheshadri and Sudareshan (1986) have reported that the milk fortified with Ashwagandha increases the body weight, total protein and haemoglobin and hence is useful as an adaptogen. The extracts of Ashwagandha are reported to exhibit immunomodulatory activity (Dahanukar et al., 1986).

Panax Ginseng Ginseng (KOREAN GINSENG)

Ginseng has been the most popular adaptogenic herb.The tonifying effect of the ginseng root has been described in a Chinese text as early as the 1st century after Christ. According to current understanding, the adaptogenic effect of the drug is ascribed to the ginsenosides or panaxosides. Additional constituents previously cited include essential oil, the sesquiterpene beta-elemene, polyacetylenes (12,13), salicylic- and vanillic-acid (14), polysaccharides, as well as ubiquitously-occurring amino acids, fatty acids, sterines and sugar. Fransworth and Bederka in 1973 reported ginseng as a general tonic with reference to its adaptogenic effects. Saito et al., (1977) found that the saponins of Ginseng protect stress induced ulcer. Molokovsky et al., (1989) proved ginseng extract as adaptogenic agent in alloxan induced diabetes in rats and mice. Ginseng extracts were found to exhibit endurance promoting properties by Ramachandra et al (1990) using a new experimental model for evaluation of adaptogenic activity. Takahashi et al (1992) have proved the anti-stress effect of ginseng by demonstrating the inhibition of development of morphine tolerance in stressed mice.

Eleutherococcus senticosus Taiga root (SIBERIAN GINSENG)

In search of a drug which could replace the expensive ginseng root, one came across the Taiga root, originating from Siberia. Its phytochemical and pharmacological processing goes back to Russian works, in particular, that of Brekhman and his circle. The chief constituents are considerably different than those of the ginseng root . They may be arranged in the following groups (Marina Davydov et al., 2000).

1. Phenyl propane compounds: syringin = eleutheroside B, sinapin alcohol, coniferyl aldehyde, chlorogenic acid, caffeic acid derivatives.

2. Lignanes: syringaresinol-4-4'-0-beta-D-diglucoside = Eleutheroside E (D), syringa-resinol monoglucoside, syringaresinol, sesamin.

3. Cumarins: e.g. Isofraxidin-7-0-glucoside and its aglycon, isofraxidin.

4. Polysaccharides.

5. Additional constituents, such as sterins, oleanolic acid, essential oil, sugar.
The anti-stress effect of Eleutherococcus extracts have been demonstrated in animal experiments, through a raised protection from the typical organic changes during the alarm phase, as described Selye (Brekhman 1969).
Endocrine effects of /Eleutherococcus/ can be read from an increase in the weight of the adrenal cortex, while the simultaneous decrease in the content of cholesterol and ascorbic acid indicates an increased formation of corticosteroids. In recently performed examinations, a rise in corticosterone-serum values after the application of intra-peritoneal application of Eleutherococcus extracts on rats has been proven.

Ocimum sanctum Tulsi, Holy Basil

Ocimum sanctum is a plant that is known in India as Tulsi, and "Holy Basil." It has gained a solid place as a tonic in traditional Indian medicine. *Ocimum sanctum* leaves contain an essential oil of varying compositions. The chief components are eugenol, methylchavicol, alpha- and beta-bisabolen (Laakso et al., 1990). Additional constituents are the flavonaglyca luteolin and apigenin and their 7-0-glucuronides as well as the C-glycosides orientin and molludistin and the triterpene acid ursolic acid (Nair et al., 1982).
Singh et al., (1981) reported the anti-stress property of Tulsi and other plants described to be tonics in Ayurveda. This was further investigated in rats and

mice by Bhargava et al (1985) and found to possess adaptogenic properties. Methanolic extract and aqueous suspension of Tulsi leaves were investigated for immunoregulatory profile. The results of the study indicates immunostimulant capability which may be contributory in explaining the adaptogenic action of the plant by Lawrence et al., (1972) and Godhwani et al., (1988).

Asparagus racemosus Shatavari

Shatavari is a popular general tonic. Root extracts of shatavari were reported to possess adaptogenic property by Thakur et al., (1995). Jacob et al., (1988) proved Shatavari as an adaptogen by evaluating its protective property against stress induced gastric and duodenal ulcers. Dahanukar et al., (1989) have shown immunotherapeutic modification of experimental infections by shatavari. Gupta et al., (1991) have proved shatavari as an adaptogen by evaluating its immunomodulatory properties.

Tinospora cordifolia Guduchi

Guduchi is a valuable tonic in many debilitating diseases. It is prescribed along with Ashwagandha and Shatavari in Ayurveda to strengthen host defence. Guduchi has been reported to possess immunomodulatory property by Thatte et al., (1989) , Guduchi is reported to be an adaptogen by Khosa et al., (1993)

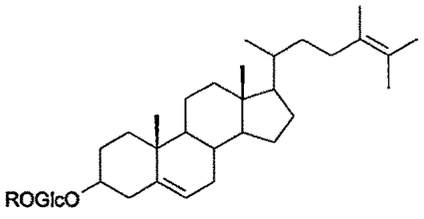
There is plenty of evidence indicating that single administration of an adaptogen activates ACTH and corticosteroid formation, and that subchronic pretreatment with adaptogens normalizes stress hormone levels (Wagner et al., 1994; Panossian et al., 1997).

For example Cucurbitacin R diglucoside, [DCR,16α,20-dihydroxy-2α,25-di(1-O-αD-glucopyranosyloxy)-cucurbiten-5-trion-3,11,22], one of the active principles of *Bryonia alba* L. roots,

The active principles of plant adaptogens as far as investigated can be divided into two main chemical groups

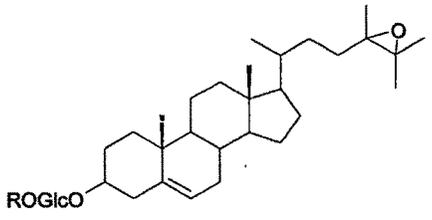
Steroids with a tetracyclic skeleton like cortisol:

Sitoindosides *(Withania somnifera)* (Bhattacharya et al., 1987; Ghosal et al., 1989)



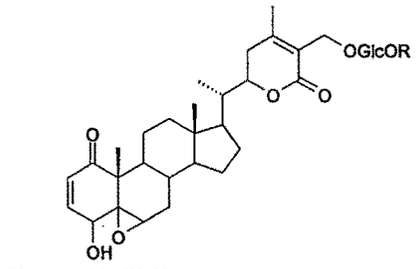
Sitoindoside VII

R = Parmitoyl



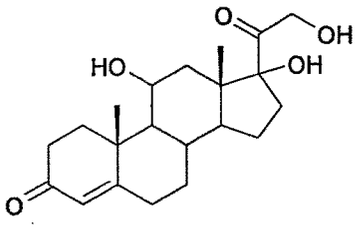
Sitoindoside VIII

R = Parmitoyl



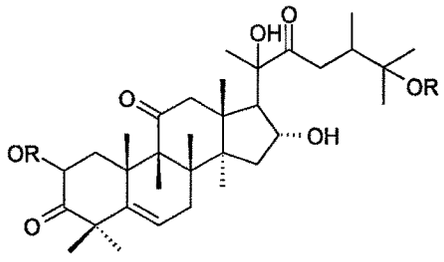
Sitoindoside IX, X

R = H, Palmitoyl



Cortosol

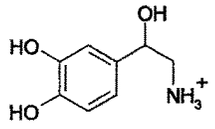
Cucurbitacin R glucoside *(Bryonia alba)* (Panossian et al., 1997)



Cucurbitacin R diglugoside

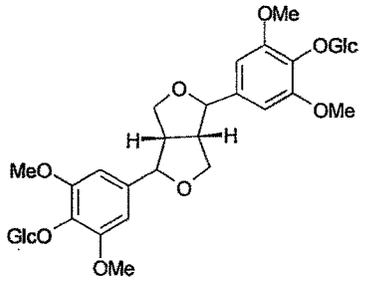
R = Glc

Aromatic compounds, structurally similar to catecholamines

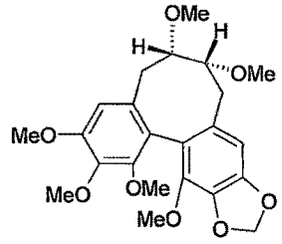


Norepinephrine

Lignans: Eleuteroside E *(Eleuterococcus senticosus)* Schizandrin B *(Schizandra chinensis)* (Kochetkov et al., 1962)



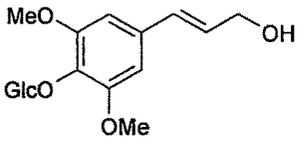
Eleuteroside E



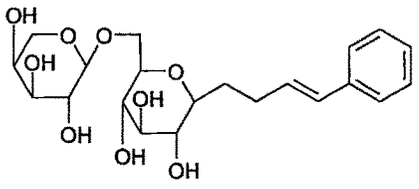
Schizandrin B

Phenylpropane derivatives:

Syringin *(Eleuterococcus senticosus)* (Wagner et al., 1982) Rosavin *(Rhodiola rosea)* (Kurkin and Zapesochnaya, 1986)

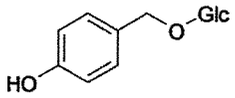


Syringin



Rosavin

Phenylethane deivatives: Salidroside *(Rhodiola rosea)* (Ssaratikov et al., 1968)



Salidroside

Adaptogens of other origin

Fungi.

Medicinal fungi have a long history of use in China. These fungi are cultivated and marketed for their adaptogenic properties. Some of the more well-known species are shiitake (*Lentinula edodes*), reishi (*Ganoderma lucidum*), maitake (*Grifola frondosa*), chaga (*Inonotus obliquus*), turkey tail (*Trametes versicolor*), and caterpillar fungi (*Cordyceps sinensis*) (Hobbs, 1995). The main compounds thought to be active as adaptogens are the polysaccharides and triterpenes.

Shilajit. Known also as mumie, researchers first began to investigate the bioactivity and chemical composition of shilajit in the early 1970s. It is believed that this substance is the result of humification of a combination of resinous plant material, lichen, and fungi. Chemical analysis shows that shilajit is primarily composed of humus, a mixture of decomposed organic matter and

soil compounds such as fulvic acid and humic acid. These latter substances contain compounds such as uronic acids, phenolic glycosides, triterpenes, phenolic lipids, and amino acids (Schepetkin et al., 2003). Indigenous peoples have collected the blackish-brown substance for centuries from the high mountains of Nepal, Tibet, Bhutan, China, Pakistan, Afghanistan, the Urals, and Norway. It is found on steep rock faces at altitudes between 1000 and 5000 meters. In Indian folk medicine, shilajit is a legendary rejuvenator and immunomodulator, termed *rasayana.* It is primarily used for longevity and to arrest the process of aging. Combined with other herbs and substances, shilajit is included in various formulas for debility, convalescence and wasting disease, to enhance the libido and to treat stress and immune system deficiency (Schliebs et al., 1997;Bhattacharya et al., 2000; Puri, 2003).

Deer Antler.

Deer horn (cervi cornu parvum), deer antler velvet (cervus lu rong), mature deer antler (*cervus lu jiao jiao*), and deer antler gelatin (*cervi colla cornus*) have been in use in traditional Chinese medicine for centuries (Bensky and Gamble, 1986). These substances are used primarily for neuroendocrine deficiency (*qi* deficiency) and believed to be adaptogenic and a metabolic restorative (Holmes, 1996). Deer antler contains many amino acids, polyamines, androgens, estrogens, sphingomyelin, cholesterol, ectosaponins, calcium phosphate/carbonate, magnesium, phosphous, sphingmyelin, ganglioside, chondroitin and choline analogues (Holmes, 1996).

Realizing the importance and common use of adaptogens it was thought worthwhile to investigate upon some plants, which are constituents of many formulations available in traditional system of medicine for adaptogenic activity.

Many medicinal plants classified, as Rasayana in Ayurveda are believed to be useful in strengthening the immune system, to postpone ageing improve vigor, vitality and longevity.

A literature search on some such plants reported for rejuvenating property revealed Kapikachchhu (*Mucuna pruriens* Linn.) to be one of the popular and important medicinal plants of India is a constituent of more than 200 indigenous drug formulations. The plant is called by common names like Cowitch, Velvet bean and Cowhage in different part of the country. All parts of *Mucuna pruriens* possess valuable medicinal properties.

Mucuna finds traditional use in number of diseases. Root is bitter, thermogenic, emolient, stimulant, purgative, aphrodisiac, diuretic, emmenagogue, anthelmintic, febrifuge and tonic. The Ayurvedic usage of roots still extends for constipation, nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, fever and delirium. Leaves are Aphrodisiac, anthelmintic, tonic, and are useful in ulcers, inflammation, helminthiasis, cephalalgia and general debility. Seeds have a long history of use in Indian Ayurvedic medicine, where it is used for worms, dysentery, diarrhea, snakebite, sexual debility, cough, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhea, sterility, gout, delirium, dysmenorrhea, diabetes, and cancer.

An exhaustive scientific data on phytochemical, pharmacological and clinical aspects of the seeds of *Mucuna pruriens* is available but according to the available literature, all parts of *Mucuna pruriens* possess valuable medicinal properties and very little or no information regarding the scientific studies on roots of *Mucuna pruriens* is available

Tagara, another plant mentioned as drug, in the Ayurvedic classics, forms an important ingredient of several preparations used in the treatment of diseases, such as anemia, jaundice, bleeding, hemorrhoids, tuberculosis, mental disorders, epilepsy, fever, cough, asthma and brain tonic and general tonic. The botanical source of Tagara is roots of *Valeriana jatamansi* Jones, and *V. hardwickii* Wall. belonging to family Valerianaceae. Both these species occur only in the Himalayas. Tagara is the substitute for *Valeriana wallichi DC.*. The literature search revealed that in the state of Karnataka in India, and also in other centers of south India, roots of another plant known as Granthika Tagara (Kannada) is used as substitute for Tagara in the markets whereas these belong to the roots of *Nymphoides macrospermum* family Menyanthaceae.

Literature survey on *Nymphoides macrospermum* revealed that no scientific information is available on phytochemical and biological aspects of this plant. However, it forms a substitution for Valerian in southern parts of the country and sometimes replaced by the vendors.

Therefore, roots of *Mucuna pruriens* as well as roots of *Nymphoides macrospermum* were selected for systematic investigations in order to evolve certain parameters for their standardization and also to assess their biological activities,thereby to justify their traditional claims.

1.5. MUCUNA AT A GLANCE

Mucuna is a genus of 33 accepted species of climbing vines and shrubs of the family Fabaceae, found worldwide. The leaves are 3-palmate, alternate or spiraled, and the flowers are pea-like but larger, with distinctive curved petals, and occurring in racemes. *Mucuna* species are generally bat-pollinated and produce seeds that are said to look like the eyes of a bull or deer. Like other legumes, *Mucuna* plants bear pods The pods of some species are covered in coarse hairs that cause itchy blisters when they come in contact with skin. Other parts of the plant have medicinal properties.

1.5.1 *Mucuna pruriens*

Mucuna pruriens (syn. *Mucuna prurita, Carpopogon pruriens, Dolichos pruriens*) is a tropical legume known by a multitude of common names,

India *Atmagupta/Cowage/Kapikachchu/Kavanch/Kawanch/Kowez/Baidhok/Alkusi*, Japan-*Velvet bean*, Thailand-*Sijeh/Mijeh/Horseeye bean*, Srilanka *Wanduru-me*, Nepal-*Kauso/Kausva/Kaocho/Cowhage* Medagascar-*Taingilotra*, Pakisthan-*Konchkari/Kawanch/Alkushi/Goncha*, (Indian Medicinal Plants)

Taxonomically *Mucuna pruriens* belongs to the

Kingdom –*Plants*, Phylum –*Angiosperms*, Class-*Magnoliatae*, Sub-class- *Rosidae* Order- *Rosales*, Family -*Leguminosae-Papilionoidae*

Table 1.1 Uses of different species of *Mucuna* found in India. (Wealth of India)

Species	Uses
Mucuna cochinchinensis .Cheval Syn: *Mucuna. nivea* (Roxb)	Useful for fodder, cover crop or green manure. Fleshy and tender fruits of the plant after removal of velvet skin are used as vegetable.
Mucuna deeringiana (Bort) Syn: *Stizolobium deeringianum* Bort.	Useful for fodder, cover crop or green manure. Seeds from unripe pods are used as vegetable. Dry pods and seeds are used as a good source of proteins for fattening of cattle and sheep.
Mucuna monosperma DC *Carpopogon monospermum*	Possess expectorant property and is used in Coughs, asthma and affections of tongue. Trichomes are irritant to skin and cause dermatitis. Seeds are bitter, sweet, refrigerant, tonic, cardiotonic and Aphrodisiac.
Mucuna gigantean DC *Carpopogon gigenteum*	Powdered seeds are said to be used as purgative, bark is used in rheumatic complaints. It is pulverized mixed with dry ginger and rubbed over the parts. Hairs on pods produce intense irritation and dermatitis.
Mucuna nigricans Steud Syn: *Mucuna imbricate* DC	Watery sap from the stem is used for coughs and fevers in Philippines, hairs on pods produce dermatitis.
Mucuna bracteata	Pods and trichomes from pods are reported to possess medicinal properties similar to those of *M.prurita*
Mucuna capitata	Seeds are considered tonic and also used as animal feed after detoxification.
Mucuna utilis Wall	Resembles *M.prurita* and *M. deeringiana* to some extent. Seeds are more toxic compared to *M. prurita*
Mucuna atropurpurea	Bristles of the pods are irritant and cause dermatitis.
Mucuna hirsuta Wight & Arn *Mucuna macrocarpa* Wall	Bristles on pods cause dermatitis
Mucuna pruriens	Described below

Some more species of Mucuna described in the texts are

Mucuna argyrophylla, Mucuna birdwoodiana, Mucuna elliptica, Mucuna fawcettii, Mucuna glabrialata, Mucuna holtonii, Mucuna killipiana, Mucuna melanocarpa, Mucuna mutisiana, Mucuna novo-guineensis, Mucuna pacifica, Mucuna pallida, Mucuna platyphylla, Mucuna poggei, Mucuna reptans ,Mucuna reptans, Mucuna urens, Mucuna rostrata, Mucuna mollis, Mucuna sempervirens, Mucuna sloanei, Mucuna stans, Mucuna warburgii,Mucuna membranacea,

BOTANICAL ASPECTS

Mucuna pruriens is a large half-woody twinner, with long slender cylindrical branches, at first covered with short reflexed hairs afterwards nearly smooth.

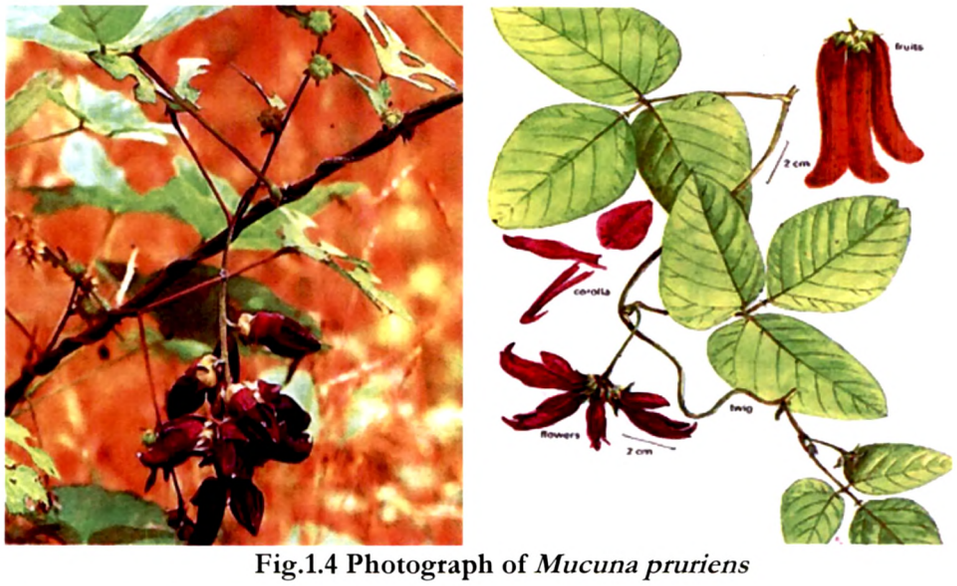


Fig.1.4 Photograph of *Mucuna pruriens*

Leaves alternate, pinnately trifoliate, on hairy petioles 6-12 inches long stipules small, lanceolate, leaflets on short, thick ,hairy stalks, with setaceous stipellae at their base, 6-8 inches long, the terminal one the smallest, rhomboid-ovate, and lateral ones broadly ovate, very unequal at the base, the lower side being much expanded, all acute or acuminate, entire, membranous, green on both surfaces, nearly smooth above, covered below with adpressed white hairs, especially abundant on the prominent veins.

Flowers: large, shortly stalked, in cluster of two or three together, in a pendulous, long-stalked, axillary raceme a foot in length, rachis and pedicels pilose, bracts ½ an inch long, lanceolate, densely hairy, falling before the flowering period. Calyx cup-shaped, silky externally, deeply cleft in a somewhat two-hipped manner, the two upper segments being perfectly united to form a single triangular one, and the lower three lanceolate, subulate, the middle one the longest. Corolla papilionaceous, standard broadly ovate, acute, about ¾ inch long, with a short claw, pale purplish, wings nearly 1 ½ inch long, narrow, oblong, blunt, slightly falcate, dull dark purple tinged with pale yellowish green. Keel petals narrow, a little longer than the wings, nearly straight, except at the end, where they become hard and cartilaginous, and curve upwards, forming a prominent stiff, greenish beak. Stamens 10, 9 combined by their filaments, the upper one distinct, fore part of the filaments somewhat dilated, anthers small, soon falling, oblong. Ovary surrounded at the base by a small crenulate disk, shortly stalked, hairy, tapering into the longer slender style, stigma small terminal Legume nearly sessile, about 3 inches long by more than ½ inch broad, falcately curved at each end, somewhat compressed, slightly contracted between the seeds, dark brown, very densely covered with a thick felt of stiff, short, sharp pale reddish hairs, which pint backwards and are readily detached.

When young the pods have a strongly marked rib down each valve, which is concealed by the hairs.

Seeds 4 or 5 Ovoid, slightly compressed, with a persistent oblong , funicular hilum, dark brown with spots, usually 1.2-1.8 cm long, 0.8-1.2 cm wide , hard, smooth to touch, not easily breakable, Odour, not distinct: taste, sweetish-bitter (Wealth of India)

Ethnomedicinal uses of *Mucuna pruriens*

Seed- Used to cure night dreams and impotency and to promote fertility,as an aphrodisiac to increase seminal fluid and manly vigour, as a nervine, as an emmenagogue, for diarrhea, as an antivenin, or diabetes, for scorpion stings and snakebite, to treat male impotence and sterility, for abortion, for sexual debility and spermatorrhea,for persistent coughs, for pulmonary tuberculosis, as an aphrodisiac, and for seminal weakness and impotence,for rheumatic disorders and muscular pain,For worm infestation, gonorrhea, sterility and general debility.

Root-Used as an emmenagogue, for rheumatism and gout, for delirium, as a blood purifier and diuretic, for kidney stones, to relieve dysmenorrhea, dropsy and catarrh, for cholera, and general debility.

Leaf- used as a uterine stimulant, in dysentery, as a nerve tonic, an aphrodisiac and diuretic, for scorpion stings. (Indian Medicinal Plants)

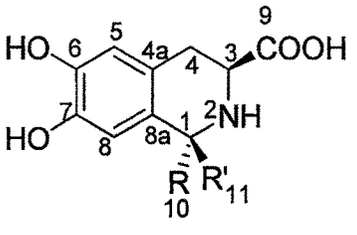
PHYTOCHEMICAL ASPECTS

Mucuna pruriens contains many diverse Phytochemicals like alkaloids, L-dopa, alkylamines, phenolics, saponins, proteins, minerals, fatty acids, sterols , serotonin, carbohydrates, fiber, etc.

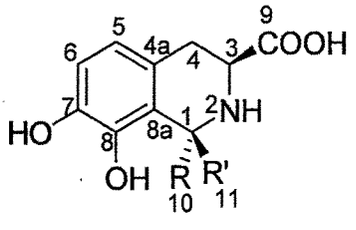
Alkaloids

Studies of Rashit & Mazumdar (1956) and Ghosal et al (1971), revealed the presence of 0.53% total alkaloids like Prurienine ($C_8H_{16}O_2N_2$, mp 213-14), Prurienidine ($C_6H_{15}O_3N_3$, m.p 287-88), five other bases designated as base P ($C_{17}H_{26}O_6N$ b.p 118-19), base Q (b.p 220-210), base R ($C_{23}H_{35}O_4N$ bp 320^0), base S (b.p above 320^0) and base X ($C_{11}H_{25}O_3N$ m.p 94-95). Recently Mishra et al have isolated 4-tetrahydroisoquinoline alkaloids from Mucuna priuriens.L-3-Carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, (-)-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline,(-)3-Carboxy-1,1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and (-)3-Carboxy-1,1-dimethyl-7,8-dihydroxy-1,2,3,4 tetrahydroisoquinoline from Mucuna seeds.

1, 2, 3, 4-tetrahydroisoquinoline -3-Carboxylic acids are very potent and selective towards opoid receptors for peptide hormones and neurotransmitters.



1, R = R' = H
2, R= H, R' = Me
3, R = R' = Me



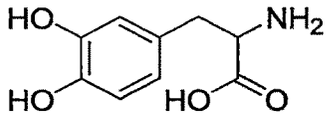
4, R = R' = Me

L-Dopa

l-3, 4 –DihydroxyPhenylalanine (L-Dopa) was first isolated from *Vicia faba* in 1913.(T. Torquati, M. Guggenheim Z) and later from the seeds of Georgia velvet bean *Stizolobium deeringianum* by Miller 1920. Although L-Dopa occurs in other plant families and legume genera, in a survey of 511 species representing 7 families and 246 genera, Daxenbichler et al found major concentration of L-dopa in the seeds of only 7 species all of which belonged to the genus Mucuna Accumulation of high concentration of L-dopa in seeds may be a peculiarity of Mucuna. Concentration exceeding 5% of the dry tissue wt have only been reported in the seed embryos of six species of Mucuna. Damodaran et al isolated 30g of the pure amino acid L-Dopa from 2kg of Mucuna seeds. Bell et al 1971 have reported L-Dopa to be the major free amino acid by detecting by paper chromatography and high voltage electrophorosis. Cultures derived from *Mucuna pruriens* have shown to accumulate high levels of L-Dopa, concentration of 2,4 D influences its accumulation. Bruins N et al have developed methods for biotransformation of p-Hydroxyphenyl aceticacid to 3,4 dihydroxyphenoxyacetic acid from calcium alginate immobilized cell suspension cultures of *Mucuna pruriens* and isolation followed by HPLC with electrochem and UV detection confirming its identity and purity by lc/ms.(Bruins AP et al.,1984) A rapid Reversed phase HPLC method has been developed by siddhuraju et. al for the extraction and quantitative determination of L-Dopa , L-3-Carboxy-6,7 dihydroxy-1,2,3,4-tetrahydroisoquinoline and 1-methyl -3-Carboxy-6,7 –Dihydroxy-1,2,3,4-tetrahydroisoquinoline in *Mucuna pruriens* seeds.

The L-DOPA could be obtained in good yield on extraction with EtOH-H_2O mixture (1:1) using ascorbic acid as protector

Misra et al have developed methods for extraction of L-DOPA/other active components from the seeds using different solvents, TLC and HPLC fingerprinting of the extracts for amino acid components

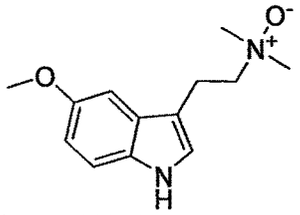


3,4-Dihydroxyphenyl alanine

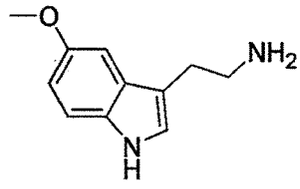
Alkylamines

Ghosal et. al revealed the presence of 4-Indole-3-alkylamines, 5-methoxy tryptamine, 5-methoxy-N-N-dimethyl tryptamine-N-oxide, β-Carboline and choline in seeds, pods, leaves and roots and 0.015% Serotonin (5-Hydroxytryptamine) in pod trichomes and also have suggested the possible routes of Serotonin metabolism.

Itching caused by the trichomes of pod is due to presence of histamine liberating principle Mucunain a proteinase which causes local itching which lasts for 3-5 min.



5-methoxy-N,N-dimethyltryptamine-N-oxide



5-methoxy tryptamine

Total phenolics and Tannins.

The total phenolic content was found to be very high(7.75%), and Gurumoorthi et al have reported a high tannin content (0.24%) which are found to be located in the seed coat with only traces in the cotyledons

Amino acids and Minerals

Vadivel et al have analysed the proximate composition, mineral profile and amino acid profile of total seed protein reporting minerals like Sodium, Potassium, Calcium, Magnesium, Phosphorus, Iron, Copper, Zinc and Manganese, amino acids like Alanine, Arginine, Aspartic-acid, Cystine, , Glutamic-acid, Glycine, Histidine, L-DOPA, Leucine, isoleucine, Lysine, Methionine, Proline, Phenyalanine Threonine, Tryptamine, Tyrosine, Valine. Studies of Adebowale et al shows that the seeds have highest total Essential Amino acids , its comparision with FAO/WHO requirement pattern indicated higher values than that recommended for preschool children.

Fatty acids

Seeds contain an oil (4.5%) containing fatty acids like arachidic acid, linoleic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid and stearic acid. Kapoor et al

Studies of Hassan et al, Smith et al ,Panikkar et al and Prakash et al. have documented the presence of Vernolic acid (4%), Bufotenine, lecithins and Trypsin

PHARMACOLOGICAL ASPECTS

Traditionally the seed have been used indigenously throughout the world for snakebite and several *in vivo* studies validated this traditional use.

Anticoagulant & Procoagulant activity

Aqueous extract of the leaves showed a dose related ability to prolong the time taken to clot for blood treated with a standardized dose of Echis carinatus venom. (Houghton et al., 1994)

Echis carinatus venom contains a mixture of proteins that affect the coagulative cascade, causing severe bleeding and haemorrhage. An increase in procoagulant activity was found in prothrombin activation by Echis carinatus venom invitro by clotting and chromogenic assay of the seed extract (Guerranti et al., 2001)

Myotoxic, cytotoxic and coagulation activities

Rats pretreated with a single dose(21mg/kg)and multiple doses (21mg/kg) of seed aqueous extract 24 h and 3wk maintained the normal LDH, SGPT, CK enzyme levels and significantly inhibited the lethal Echis carinatus venom (0.5mg/kg i.p) induced myotoxic, cytotoxic and coagulation effects .(Guerranti et al., 2001)

Antivenin activity

Guerranti, R. et al (2002) assessed the antivenin property in mice. The serum of mice treated with water extract of seeds was tested for its immunological properties. Two proteins of Echis carinatus venom with apparent molecular masses of 25 and 16kDa were detected by Western blot analysis carried out

using IgG of mice immunized with extract or its partially purified protein fraction. By enzymatic in-gel digestion and electro spray ionization –mass spectrometry/mass spectrometry analysis of immunoreactive venom proteins, phospholipase A (2) the most toxic enzyme of snake venom, was identified, these results demonstrate that the observed antivenin activity has an immune mechanism. Antibodies of mice treated with non-lethal doses of venom reacted against some proteins of M pruriens extract. Proteins of E carinatus venom and M pruriens extract have at least one epitope in common as confirmed by immnodiffusion assay (Aguiyi et al., 1999)

Mucuna pruriens derived serum immunoglobulins produced a high and sustained immune response with high survival rates against immunization with a minimum lethal dose of purified cobra venom after 4, 24, 72 and 168 hr in mice. (Guerranti, et al., 2004) A glycoprotein was purified from the protein extract of M pruriens seeds using Concanavalin A affinity chromatography. The multiform glycoprotein (gpMuc) (whose immunogenic properties mainly reside in its glycan-chains) MPE immunogen generates antibodies that cross-reacts with the venom proteins. The gpMuc contains both N-and O-glycans suggesting the probability of O-glycans involved in the antigenicity.
Fattepur S. et al have reported an Appreciable protective effect against the most toxic sea snake *Enhydrina schistosa* venom with alcoholic extract of *Mucuna pruriens* in both in-vitro and in-vivo studies.

Antidiabetic effect

Several *in vivo* studies have been reported on the blood-sugar-lowering effect of Velvet bean. These studies all validate the traditional use of the plant for diabetes.

Treatment with powdered seeds 0.5, 1 and 2g/kg in alloxan –diabetic rabbits significantly reduced the blood glucose levels at a dose of 1 and 2g/kg body weight. (Akhtar et al., 1999) *Mucuna pruriens* extract (200mg/kg) was administered for 50 days in STZ induced diabetic mice, the plasma glucose concentration was reduced by 7.45%. However extract did not exert any favorable change in Tail flick latency and gastric transit percentage. (Grover JK et al., 2002) Treatment with *Mucuna pruriens* (200mg/kg) extract for 40 days on Streptozotocin-diabetic rats reduced plasma glucose levels by 9.07%.with a significant reduction in urine volume and urinary albumin levels, whereas Mucuna failed to modify renal hypertrophy. (Grover JK et al., 2001) Alcoholic extract of *Mucuna pruriens* at 100,200 and 400mg/kg/day when assessed for antihyperglycemic effect on varying degrees of hyperglycaemia and diabetic complications. in a pilot study (Plasma glucose >180mg/dL,21days), a chronic study in alloxanized rats (Plasma glucose >280mg/dL 120days) and streptozotocin mice (Plasma glucose >400mg/dL 60days) a maximum antihyperglycemic effect occurred at week 6 at a dose of 200mg/kg/day. In chronic alloxanized rats, at 1, 2, 3 and 4 months MP showed a decrease in plasma glucose level by 40.71%, 45.63%, 50.33% and 51.01% respectively. Whereas in chronic STZ diabetic mice, MP had no significant effect. (Rathi et al., 2002) D-chiro-inositol and its two galacto-derivatives have been identified by chromatographic and NMR techniques, which explains the well established antiglycaemic effect of *Mucuna pruriens* seeds.(Donati et al., 2005)

Antiparkinson activity

The beneficial impact of Mucuna in Parkinson's disease has been documented in a number of studies. In a Comparative study of CNS and cardiovascular effects of levodopa (L-dopa) with seed powder in rats, mice, and dogs, seed

powder and levodopa showed equivalent hypothermic and antiparkinsonian activity. The seed powder had a faster onset of action and was more active than levodopa and also antagonized apomorphine induced hypermotility. (Rajendran et al., 1996) Alcoholic extract of *Mucuna pruriens* seed (MPE) (200 mg/kg, oral) exhibited antagonism of Catalepsy induced by haloperidol (0.1-0.5 mg/kg, i.p.) after day-2 onwards and persisted even after Haloperidol challenge on day-8. A combined treatment of L-DOPA with MPE showed significant antagonism of catalepsy from day-2 onwards which reached to maximum on day-3 and persisted till day-6 and sustained even after Haloperidol challenge on day-8. (Urmila Aswar et al., 2006)

In a comparative neurorestorative study, with levodopa in 6-hydroxydopamine (6OHDA) lesioned rat model of Parkinson's disease. Mucuna cotyledon powder treatment significantly restored the endogenous levodopa dopamine, norepinephrine and serotonin content in the substantia nigra and significantly increased the brain mitochondrial complex-I activity but did not affect the total monoamine oxidase activity. Neurorestorative benefit by Mucuna cotyledon powder on the degenerating dopaminergic neurons in the substantia nigra may be due to increased complex –I activity and the presence of NADH and coenzyme Q-10. (Manyam et al., 2004)

Spraque-Dawley rats fed with Mucuna endocarp in the form of HP-200 at a dose of 2.5, 5.0,or 10g/kg/day (mixed with rat chow and fed daily ad lib) for 52 weeks were analyzed for neurotransmitters in the cortex, hippocampus, substantia nigra and striatum. It produced a significant effect on nor epinephrine or dopamine, serotonin and their metabolites-HVA, DOPAC ad 5-HIAA in the nigrostriatal tract. (Manyam et al., 2004)

Sexual behaviour

Mucuna pruriens seed powder (3g) containing 100 mg levodopa (L-dopa) was assessed for sexual behaviour in female rats. Where the females showed rejections and exhibited decreased lordosis when males attempted to mount. The rejections were reflected as a significant reduction in the lordosis quotient and an increase in the rejection quotient. While both levodopa and *M. pruriens* inhibited sexual behaviour in females, the inhibitory effect of *M. pruriens* was greater than that of levodopa, indicating the presence of other constituents in *M. pruriens* contributing to this effect. (Rajendran et al., 1997)

Learning and Memory studies

Mucuna seeds (1g/kg bw) was administered to evaluate for heuristic processes in rats, significant effect (93±3.3% memory retrieval)of drug on learning skills and memory retrieval processes was observed in Group receiving drug during both training and memory retrieval sessions. (Salma Khanam et al., 2005)

Androgenic activity

Increased spermatogenesis, body weight, weight of testes, seminal vesicles and prostate were observed in rats treated with total alkaloids from the seeds. Seed Powder when screened for aphrodisiac activity produced a significant increase in mounting frequency, Intromission frequency and Ejaculatory latency indicating an increase in libido and sexual performance.(Saksena et al., 1987)

Spasmolytic effect

In a chemical and pharmacological study on whole plant material of Indian *Mucuna pruriens* the pod trichomes contained only serotonin, the pods themselves, seeds, leaves, and roots yielded four indole-3 alkylamines. Extracts

and individual alkaloids produced spasmolytic effect on smooth frog muscle and hypertension in dogs, frogs, and rats. (Ghosal et al., 1971)

Anti-oxidant activity

Alcoholic extract of the seeds exhibited antilipid peroxidation property, in iron-induced lipid peroxidation, and with hydroxyl and super oxide radicals in vivo and invitro studies on rat liver homogenate by significantly inhibiting $FeSo_4$ induced lipid peroxidation, and specific chemical reactions induced by superoxides and hydroxyl radicals through direct chemical interaction. The methanol extract of *Mucuna pruriens* seeds showed a strong antioxidant activity by inhibiting DPPH & hydroxyl radicals, Nitric oxide and Superoxide scavenging and reducing power activities when compared with Curcumin, Quercetin, alpha –Tocopherol and L-Ascorbic acid. (Yerra Rajeshwar 2005)

Analgesic, antipyretic, and anti-inflammatory effects

Alcoholic extracts of the leaves and fruits of *Mucuna pruriens* exhibited an increased pain threshold, decreased body temperature, and anti-inflammatory activity when administered in mice and rats at a dose of 3.0g/kg and 1.0g/kg respectively. (Iauk et al., 1993)

Hypotensive and Hypocholesterolaemic activity

Mucuna seed extract produced a significant hypotensive and hypocholesterolaemic activity in normal rats. (Pant ML et al., 1968)

Anthelmintic property

The oil sample of *Mucuna pruriens* showed significant *In vitro* anthelmintic effect (against *Pheritima posthuma*) at 10 mg/ml and 50 mg/ml as compared to standard (10 mg/ml) Piperazine citrate. (Jalalpure SS et al., 2007)

CLINICAL ASPECTS

Antiparkinsonian Activity

Several clinical studies have proved that the seeds of *M. pruriens* possess the anti Parkinson activity

Neurologic diseases (Vata rogas, Sanskrit) and the pharmacological treatment of them were described in the ancient Indian Medical system-Ayurveda. paralysis agitans, is described under the name Kampavata. *Mucuna pruriens* (Atmagupta,) which contains levodopa, was used in the treatment of Kampavata.(Manyam BV 1990) A Significant improvement in daily living activities (ADL), better response in tremor, bradykinesia, stiffness and cramps (on motor examination as per UPDRS rating) was observed in a study with an Ayurveda, therapy (a concoction in cow's milk of powdered Mucuna and Hyoscyamus reticulates seeds and Withania somnifera and sida cordifolia roots and minerals) in 18 clinically diagnosed Parkinsonian patients .(Nagashayana et al., 2001)

The efficacy and tolerability of HP-200, derived from Mucuna in patients with Parkinson's disease were examined in sixty patients (46 male and 14 female) with a mean (±SD) age of 59± 9 years treated in an open study for 12 weeks. Statistically significant reduction in Hoehn and Yahr stage and unified Parkinson's disease Rating scale UPDRS scores were seen from baseline to the end of the 12–week treatment ($p < 0.0001$, t- test) Adverse effects were mild

and were mainly gastrointestinal in nature. No Adverse effects were seen in clinical laboratory reports.(Manyam BV 1995)

In an L-dopa pharmacokinetics comparative study, Parkinson's disease patients were challenged with single doses of 200/50mg Levodopa/Carbidopa, and 15 and 30g of Mucuna preparation in randomized order at weekly intervals. 30g Mucuna preparation led to a considerably faster onset of effect (34.6v68.5min p=0.012), reflected in shorter latencies to peak L-dopa plasma concentrations. Mean on time was 21.9% (37min) longer with 30g Mucuna than with LD/CD (p=0,021). Peak L-dopa plasma concentrations were 110% higher and the area under the plasma concentration v time curve (area under curve) was 165.3% larger (p=0.012). Mucuna seed powder formulation suggests that this natural source of L-dopa might possess advantages over conventional L-dopa preparations. (Katzenschlager et al., 2004)

Penis erectile stimulant

Mucuna seed extract taken orally for 4 weeks by human adults showed improvement in erection, duration of coitus and post coital satisfaction in a case study with 56 patients. (Sankaran et al., 1984)

1.6. TAGARA AT A GLANCE

Tagara an Ayurvedic drug mentioned in the Ayurvedic classics forms an important ingredient of several Ayurvedic preparations used in the treatment of diseases, such as anemia, jaundice, bleeding, hemorrhoids, tuberculosis, mental disorders, epilepsy, fever, cough, asthma and brain tonic and general tonic.

Valeriana wallichii DC, commonly known as 'Tagara,' is a wild herb common in the temperate Himalaya and has been used since long in Ayurvedic and Unani systems of medicine. The ancient Indian Charak Samhita has described Tagara as cure for obesity, skin disease, insanity, epilepsy snake poisoning and restorative remedies for the nervous system has been discussed among the bitter tasting and fragrant group of plants. All parts of *V wallichii* are used for nervous debility and failing reflexes, as a hypnotic and in the treatment of spastic disorders as constituent of Ayurvedic medicine. The roots are highly aromatic but the leaves when fresh have sharp odor though much less as compared to the roots. Valerian commonly used in Northern European medicine, derived from the underground parts of *Valeriana officinalis* Linn. is among the most popular herbal remedies because of its effectiveness as sedative and hypnotic properties. Related species are used in traditional medicine in many other parts of the world. (Yoganarasimhan et al)

Chemical constituents (Peter J Houghton)

Valerian contains several compounds with demonstrable pharmacological activity. These include the essential oil and its sesquiterpenoids (valerenic acid), epoxy iridoid esters (valepotriates) and their decomposition products such as baldrinal and homobaldrinal, amino acids (arginine, GABA, glutamine, tyrosine), and alkaloids. Valerian also possesses small amounts of phenolic

acids and flavonoids, valerosidatum, chlorogenic acid, caffeic acid, choline, β-sitosterol, fatty acids, and various minerals.

Essential Oil (0.1% to 0.6%)

The essential oil content and composition of valerian varies significantly. The essential oil contains mono- and sesquiterpene hydrocarbons with the main constituents being bornyl acetate, valerianol, valeranone, cryptofauronol, and valerenal, The sesquiterpenes have three types of structures based on kessane, valeranone, and valerenic acid skeletons. Valerenic acid and its derivatives (acetoxyvalerenic acid and hydroxyvalerenic acid) have been reported to be characteristic of Valerian and its subspecies. More than 150 compounds have been reported in the essential oil, including acyclic, monocyclic, and bicyclic hydrocarbons as well as oxygen-containing derivatives such as alcohols, aldehydes, ketones, phenols, oxides, and esters. Bornyl acetate and isovalerate have been reported to be the primary components of the essential oil of Valerian. Other primary compounds include valerianol, valeranone, cryptofauronol, or valerenal. Other secondary compounds include isoborneol, borneol, isobornyl acetate, isobornyl isovalerate, isoeugenyl-isovalerate, valeranone (ca. 0.005% to 40%), terpinolene, α-pinene (6.76%), camphene (ca. 16%), β-pinene (ca. 6.5%), β-caryopyllene, limonene (1% to 2%), carveyl acetate (ca. 5%), and dihydrocarveyl acetate (1% to 2%)

Epoxy Iridoid Esters (Valepotriates)

Valepotriates are triesters of a terpenoid, trihydric alcohol. This alcohol has the structure of an iridoid cyclopenta-(c)-pyran with an attached epoxide ring. Numerous acid residues are found. Valtrate makes up approximately 80% to 90% of total valepotriates with the remainder consisting of acevaltrate,

didrovaltrate, and isovaleroxyhydroxydidrovaltrate (IVHD valtrate) Valepotriates degrade rapidly, especially in acidic solutions.

A literature search revealed that in Karnataka and other centres in south India a drug under the name Granthika Tagara (Kannada) is available in the market in place of the ayurvedic drug Tagara which is been identified as *Nymphoides macrospermum.*

1.6.1 GranthikaTagara (*Nymphoides macrospermum)*

Nymphoides is a genus of aquatic flowering plants in the family Menyanthaceae. The genus name refers to their resemblance to the water lily *Nymphaea. Nymphoides* are aquatic plants with submerged roots and floating leaves that hold the small flowers above the water surface. Flowers are sympetalous, most often divided into five lobes (petals). The petals are either yellow or white, and may be adorned with lateral wings or covered in small hairs. The inflorescence consists of either an umbellate cluster of flowers or a lax raceme, with internodes occurring between generally paired flowers.

Taxonomically *Nymphoides macrospermum* belongs to.

Kingdom- *Plantae*, Division-*Magnoliophyta*, Class-*Magnoliopsida* Order-*Asterales*
Family-*Menyanthaceae*, Genus- *Nymphoides*

Numerous species of *Nymphoides* are found in Australia, and others exist in Southeast Asia, Africa and South America.

There are approximately 50 species of *Nymphoides*, including following are found in different part of world. (http://medicinalplantindia.com)

- *Nymphoides aquatica* (J.F.Gmel.) Kuntze - [S.E. North America]
- *N. aurantiaca* (Dalzell) Kuntze - [S.E. Asia and Australia]

- *N. beaglensis* Aston - [Australia]
- *N.ymphoides bosseri* A.Raynal - [Africa]
- *N. brevipedicellata* (Vatke) A.Raynal - [Africa]
- *N. cordata* (Elliott) Fernald - [N.E. North America]
- *N. coreana* (Léveille) Hara - [Asia]
- *N. crenata* (F.Muell.) Kuntze - [Australia]
- *N. disperma* Aston - [Australia]
- *N. elegans* A.Raynal - [Africa]
- *N. elliptica* Aston - [Australia]
- *N. exigua* (F.Muell.) Kuntze - [Australia]
- *N. exiliflora* (F.Muell.) Kuntze - [Australia]
- *N. ezannoi* Berhaut - [Africa]
- *N. fallax* Ornduff - [Central America]
- *N. flaccida* L.Sm. - [South America]
- *N. forbesiana* (Griseb.) Kuntze - [Africa]
- *N. furculifolia* Specht - [Australia]
- *N. geminata* (R.Br.) Kuntze - [Australia]
- *N. grayana* (Griseb.) Kuntze - [Central America]
- *N. guineensis* A.Raynal - [Africa]
- *N. hastata* (Dop) Kerr - [Asia]
- *N. herzogii* A.Galàn-Mera & G.Navarro - [South America]
- *N. humboldtiana* (Kunth) Kuntze - [Central and South America]
- *N. humilis* A.Raynal - [Africa]
- *N. hydrocharioides* (F.Muell.) Kuntze - [Australia]
- *N. hydrophylla* (Lour.) Kuntze - [India]
- *N. indica* (L.) Kuntze - [pan-tropical]
- *N. krishnakesara* K.T.Joseph & Sivar. - [India]
- *N. lungtanensis* S.P.Li, T.H.Hsieh & C.C.Lin - [Asia]
- *N. macrosperma* Vasudevan - [India]
- *N. microphylla* (A.St.-Hil.) Kuntze - [South America]
- *N. milnei* A.Raynal - [Africa]
- *N. minima* (F.Muell.) Kuntze - [Australia]
- *N. montana* Aston - [Australia]
- *N. moratiana* A.Raynal - [Africa]
- *N. parvifolia* (Wall.) Kuntze - [Asia and Australia]
- *N. peltata* (S.G.Gmel.) Kuntze - [Europe and Asia]
- *N. planosperma* Aston - [Australia]
- *N. quadriloba* Aston - [Australia]
- *N. rautaneni* (N.E.Br.) A.Raynal - [Africa]
- *N. siamensis* (Ostenf.) Kerr - [Asia]
- *N. simulans* Aston - [Australia]

- *N. sivarajanii* K.T.Joseph - [India]
- *N. spinulosperma* Aston - [Australia]
- *N. spongiosa* Aston - [Australia]
- *N. stygia* (J.M.Black) H.Eichler - [Australia]
- *N. subacuta* Aston - [Australia]
- *N. tenuissima* A.Raynal - [Africa]
- *N. thunbergiana* (Griseb.) Kuntze - [South Africa]
- *N. tonkinense* (Dop) P.H.Ho - [Asia]
- *N. triangularis* Aston - [Australia]
- *N. verrucosa* (R.E.Fries)A.Galàn-Mera & G.Navarro - [South America]

Botonical Aspects

Nymphoides macrospermum is annual or perennial herb, usually found in deep water in lagoons and slowly flowing streams at sea level , also sometimes seen in temporary water in ditches and rice fields. Dwarf plants are sometimes found flowering and fruiting on wet mud after the water has receded. It is dioecious but male plants are more abundant than females.

Rhizome up to 2 cm thick, developing elongate stolons. Leaf blades up to ±20 cm in diameter, green and gland-dotted below, rather thick and leathery. Fertile shoots dimorphic, primary shoots petiole-like, up to 2.5 cm long each bearing a single long leaf, secondary shoots arising from the primary ones, each terminating in a leaf and cluster of flowers. Flowers unisexual, clustered at proximal and distal end of the stem. Pedicels 2-6cm long, sepal lobes oblong, 1.5-2mm long, 3-4 mm broad, acute. Petal lobes oblong, ±6mm long, ±2mm broad, pure white, basal part of upper surface and the margins fimbriate, with glands at base. Stamens inserted at the petal tbe throat, filaments up to 2 mm long, anthers ±1mm long, pale purple , rarely pale yellow before anthesis. Pistillode in male flowers prominent, ±3 mm long, resembling a gynoecium.

Staminodes in female flowers reduced to short, white filaments, stigmas with 1 series of long, radiating hairs. Capsule irregularly subglobose, reddish, ±6 mm

long, ±8mm in diameter, usually 6 seeded, dehiscing irregularly. Seeds 2-6, obovate-lenticular, ± 3.5 mm long, ±4.5 mm in diameter, slightly rough. (Christopher DK Cook.,)

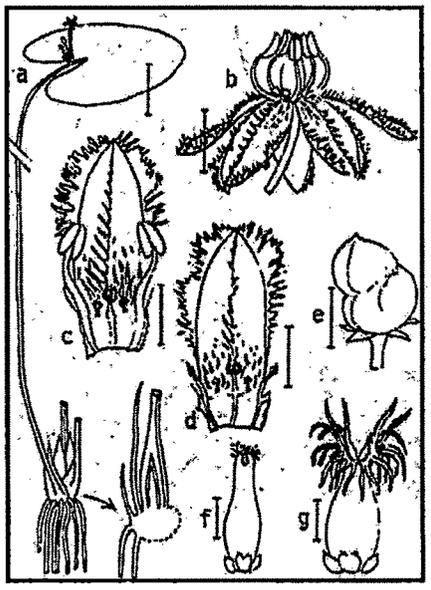


Fig. 1.5 *Nymphoides macrospermum. a-Flowering plant(2cm) with axillary shoot shortened and a longitudinal section of the base of an axillary branch. b- male flower(3mm); c-petal of male flower with two stamens and three glands (2mm); d-female petal with two staminodes and three glands (2mm);e-fruit(4mm); f-Pistallode (1mm); g- Carpel (1mm).*

Literature survey on *Nymphoides macrospermum* revealed no scientific information on its phytochemical and biological aspects except a report from Anita et al regarding the anticonvulsant and sedative activity where Alcohol extract of NM showed significant reduction in severity and increase in latency of convulsions induced by pentylenetetrazole (PTZ), and locomotor activity

Fig.1.6 *Nymphoides macrospermum* *Valeriana wallichii*

Research envisaged

The present studies were proposed with the purpose, first to develop the methods for standardization of selected plant material and thereby to provide a means for true identification and secondly to evaluate biological activity in order to justify their role in therapeutics. The detailed steps to undertake studies were planned as follows:

- Collection and identification of the plant material.
- Determination of macroscopic and microscopic characters, physicochemical constants, of selected plant material.
- Determination of content of in-organic elements including heavy metals in the selected plant materials as per who guidelines.
- Preparation of the extracts and preliminary phytochemical screening.
- Toxicity studies of the extracts and their fractions of the selected plant materials.
- Evaluation of extracts and their fractions for anti-stress and immunomodulatory activities using suitable pharmacological models.
- HPTLC fingerprint profiles of different extracts of selected plant materials.
- Isolation and characterization of the important phytoconstituents from the extracts and bioactive fractions.
- Development of methods for identification and quantification of suitable markers and their assessment in the selected plant extracts using HPTLC.