Evaluation of locomotor activity & exploratory behavioral activity of *Thysanolaena maxima* by open field method

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EAST WEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF PHARMACY.

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Dedication

DEDICATED TO

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Abstract

The present study was carried out to Evaluate of locomotortivity & exploratory behavioral activity of methanol extract of *Thysanolaena maxima* on the central nervous system of rodent mice. The plant *Thysanolaena maxima* has been used for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases especially as an antitubercular drug in the hilly areas of this subcontinent.The evaluation of locomotor activity & exploratory behavioral activity of methanol extract of *Thysanolaena maxima* was carried out by using 'open field' method for the dose of 200mg/kg and 400 mg/kg. A group of mice was kept as positive control and was given diazepam as standard drug.Methanol extract of *Thysanolaena maxima* 200 mg/kg the central locomotion was started from 2.75± 0.25 at 0 minute and ended in 2.25± 0.75 at 120 minutes. Methanol extract of *Thysanolaena maxima* 400 mg/kg the central locomotion was started from 2.00± 0.00 at 0 minute and ended in 1.00± 1.00 at 120 minutes. We can see the central movement has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.Methanol extract of *Thysanolaena maxima* 200 mg/kg the peripheral locomotion was started from 11.00±0.00 at 0 minute and ended in at 120 minutes. Methanol extract of *Thysanolaena maxima* 400 mg/kg the peripheral locomotion was started from 22.75± 11.30 at 0 minute and ended in 21.75±14.46 at 120 minutes. We can see the peripheral movement has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.Methanol extract of *Thysanolaena maxima* 200 mg/kg the leaning count was started from 24.5±12.14 at 0 minute and ended in 1.50± 0.86 at 120 minutes. Methanol extracts of *Thysanolaena maxima* 400 mg/kg the leaning count was started from 10.25± 0.25 at 0 minute and ended in 1.25± 0.47 at 120 minutes. We can see the leaning count has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.Methanol extract of *Thysanolaena maxima* 200 mg/kg the grooming count was started from 17.50±1.19 at 0 minute and ended in 29.00 ± 3.42 at 120 minutes. Methanol extracts of *Thysanolaena maxima* 400 mg/kg the grooming count was started from 19.00±5.12 at 0 minute and ended in 29.75±0.62 at 120 minutes. We can see the grooming count has increased for both the dose 200mg/kg and 400 mg/kg as the time proceeds. Methanol extract of *Thysanolaena maxima* 200 mg/kg the defecation count was started from 1.50± 0.28 at 0 minute and ended in 0.75± 0.25 at 120 minutes. Methanol extracts of

Thysanolaena maxima 400 mg/kg the defecation count was started from 1.00± 0.81 at 0 minute and ended in 20.25± 0.28 at 120 minutes. We can see the defecation count has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds. The result indicated that the extract decreased locomotor activity which indicates it has CNS depressant activity. However, further studies are needed in order to isolate the active principles responsible for the observed activity.

Some Abbreviations

ADEM - Acute Disseminated encephalomyelitis

CAE - Childhood-Onset Absence Epilepsy

CPT-2 - Carnitine-Palmitoyl Transferase Type II

EEG - Electroencephalogram

GTCS - Generalized Tonic–Clonic Seizures

HMSN - Hereditary Motor and Sensory Neuropathy

JAE - Juvenile Absence Epilepsy

JME - Juvenile Myoclonic Epilepsy

MRI - Magnetic Resonance Imaging

PKAN - Panthotenate Kinase-Associated Neurodegeneration

CHAPTER 1

INTRODUCTION

1.1 Human Central Nervous System

The central nervous system is a part of nervous system, which consists of the brain and spinal cord, integrates and processes information sent by nerves. The central nervous system (Figure-1) is the structural and functional centre for the entire nervous system. The site of neural integration and processing, the central nervous system receives information from the senses, evaluates this information, and initiates outgoing responses to the body. Damage to the central nervous system can therefore affect temperament, motor control, and homeostasis Neuron is the basic functional unit of central nervous system. (Marieb, 1995)

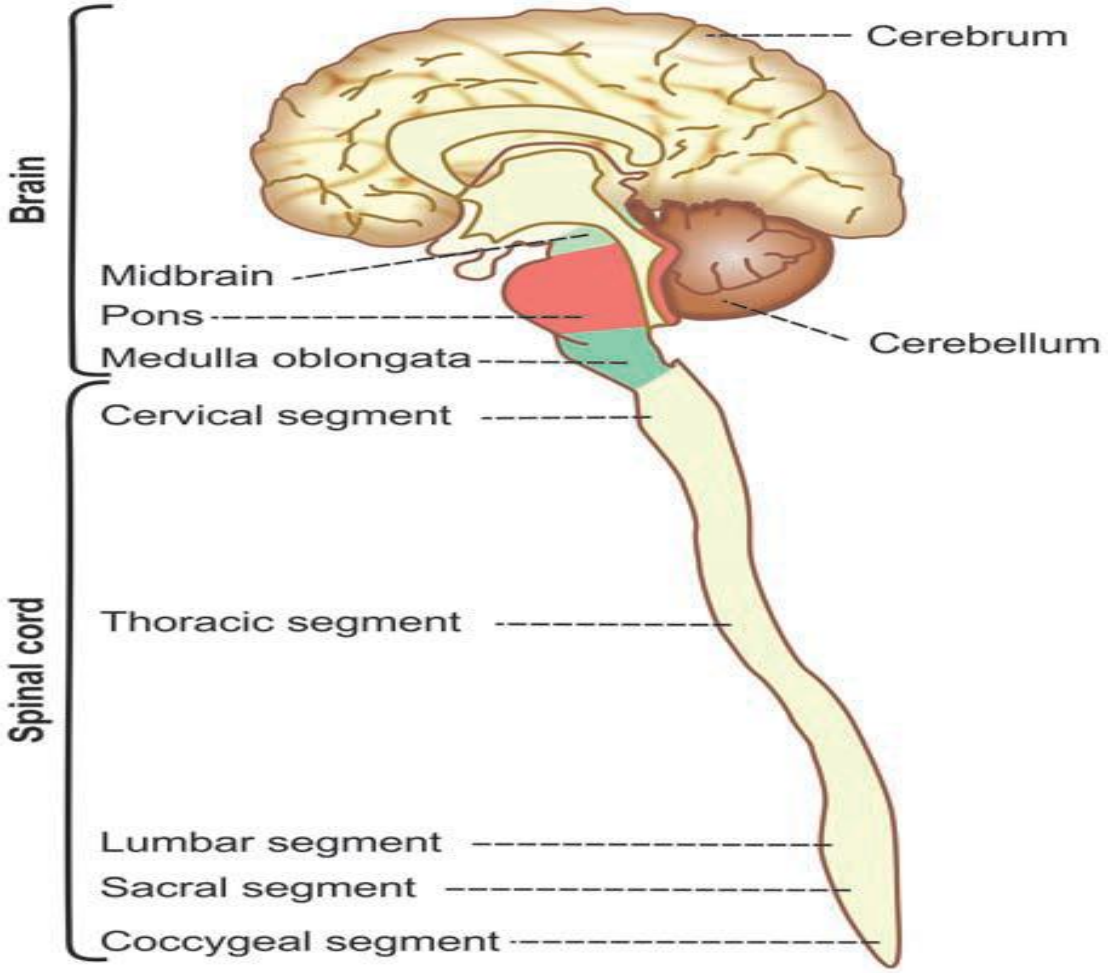


Figure-1.1: Central Nervous System

1.1.1 Composition of central nervous system

The central nervous system is composed of two types of nervous tissue: grey matter and white matter. **Grey matter** is grey because it contains mostly cell bodies, dendrites, and short, unmyelinated white matter consists of myelinated nerve fibers. The butterfly-shaped core is made up of grey matter, which contains unmyelinated neurons as well as the cell bodies and dendrites of many spinal neurons. The delicate tissues of the spinal cord are protected by cerebrospinal fluid, soft tissue layers, and the spinal column, a series of backbones (vertebrae). Injury to the spinal column can also damage the spinal cord, resulting in paralysis. (Marieb, 1995)

1.1.2 Neuron- The basic functional unit of central nervous system

Neurons have many of the same features as other body cells. In addition, neurons have specialized cell structures that enable them to transmit nerve impulses. Different types of neurons are different shapes and sizes. In general, however, they share four common features: dendrites, a cell body (soma), an axon, and branching ends called dendrites

Dendrites are short, branching terminals that receive nerve impulses from other neurons or sensory receptors, and relay the impulse to the cell body. The dendrites are numerous and highly branched, which increases the surface area available to receive information.

The cell body contains the nucleus and is the site of the cell's metabolic reactions. The cell body also processes input from the dendrites. If the input received is large enough, the cell body relays it to the axon, where an impulse is initiated. A neuron typically has one axon, which conducts impulses away from the cell body. Axons range in length from 1 mm to 1 m, depending on the neuron's location in the body. For example, the sciatic nerve in the leg contains neuronal axons that extend from the spinal cord all the way to the muscles in the foot, a distance of over 1 m. The terminal end of an axon branches into many fibers. To communicate with adjacent neurons glands, or muscles, the axon terminal releases chemical signals into the space between it and the receptors or dendrites of neighboring cells.

The axons of some neurons are enclosed in a fatty, insulating layer called the myelin sheath, which gives the axons a glistening white appearance. These axons are said to be myelinated. Axons without a myelin sheath are said to be unmyelinated. The myelin sheath protects

myelinated neurons and speeds the rate of nerve impulse transmission. Schwann cells, a type of glial cell, form myelin by wrapping themselves around the axon. (Marieb, 1995)

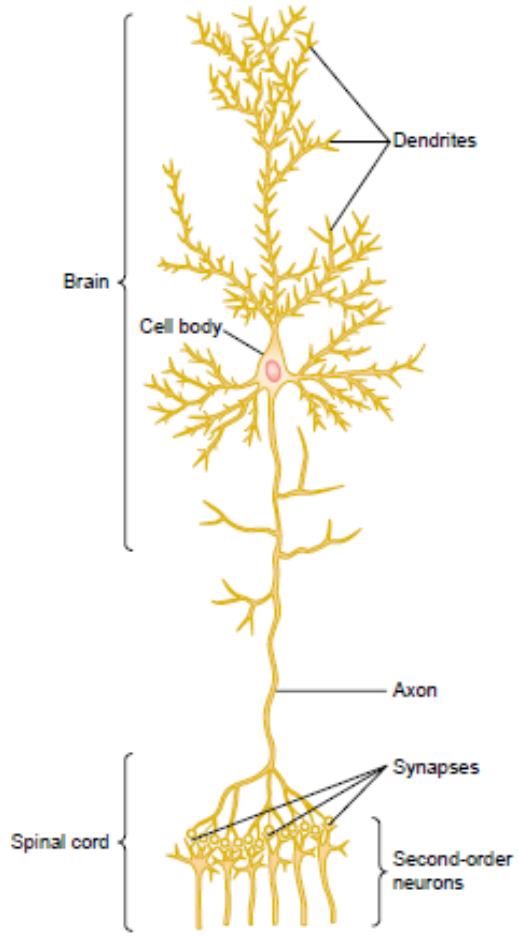


Figure -1.2: A neuron of central nervous system

1.1.3 Central Nervous System Synapses

All the information is transmitted in the central nervous system mainly in the form of nerve action potentials, called simply "nerve impulses," through a succession of neurons, one after another. However, in addition, each impulse

(1) Can be blocked in its transmission from one neuron to the next,

(2) Can be changed from a single impulse into repetitive impulses, or

(3) Can be integrated with impulses from other neurons to cause highly intricate patterns of impulses in successive neurons. All these functions can be classified as *synaptic functions of neurons.* (Guyton and Hall, 2000)

1.1.4 Types of Synapses—Chemical and Electrical

There are two major types of synapses:

(1) The chemical synapse and

(2) The electrical synapse.

Almost all the synapses used for signal transmission in the central nervous system of the human being are chemical synapses. In these, the first neuron secretes at its nerve ending synapse a chemical substance called a neurotransmitter (or often called simply transmitter substance), and this transmitter in turn acts on receptor proteins in the membrane of the next neuron to excite the neuron, inhibit it, or modify its sensitivity in some other way. More than 40 important transmitter substances have been discovered thus far. Some of the best known are acetylcholine, norepinephrine, epinephrine, histamine, gamma-aminobutyric acid (GABA), glycine, serotonin, and glutamate. Electrical synapses, in contrast, are characterized by direct open fluid channels that conduct electricity from one cell to the next. Most of these consist of small protein tubular structures called gap junctions that allow free movement of ions from the interior of one cell to the interior of the next. Such only a few examples of gap junctions have been found in the central nervous system. However, it is by way of gap junctions and other similar junctions that action potentials are transmitted from one smooth muscle fiber to the next in visceral smooth muscle and from one cardiac muscle cell to the next in cardiac muscle. (Guyton and Hall, 2000)

1.1.5 'One-Way' conduction at chemical synapses.

Chemical synapses have one exceedingly important characteristic that makes them highly desirable for transmitting most nervous system signals: they always transmit the signals in one direction: that is, from the neuron that secretes the transmitter substance, called the pre-synaptic neuron, to the neuron on which the transmitter acts, called the postsynaptic neuron. This is the principle of one-way conduction at chemical synapses, and it is quite different from conduction through electrical synapses, which often transmit signals in either direction.

The importance of the one-way conduction mechanism is it allows signals to be directed toward specific goals. Indeed, it is this specific transmission of signals to discrete and highly focused areas both within the nervous system and at the terminals of the peripheral nerves that allows the nervous system to perform its myriad functions of sensation, motor control, memory, and many others. (Guyton and Hall, 2000)

1.1.6 Signal transmission across a synapse

The simplest neural pathways have at least two neurons and one connection between the neurons. Other neural pathways can involve thousands of neurons and their connections as an impulse travels from the origin of the stimulus, through the sensory neurons to the brain, and back through motor neurons to the muscles or glands. The connection between two neurons, or a neuron and an effector, is called a *synapse*. A neuromuscular junction is a synapse between a motor neuron and a muscle cell. An impulse travels the length of the axon until it reaches the far end, called the synaptic terminal. Most neurons are not directly connected, but have a gap between them called the synaptic cleft. These neurons are not close enough for the impulse to jump from one to the other.

Chemical messengers called neurotransmitters carry the neural signal from one neuron to another. Neurotransmitters can also carry the neural signal from a neuron to an effector, such as a gland or muscle fiber. When an action potential arrives at the end of a pre-synaptic neuron, the impulse causes sacs that contain neurotransmitters to fuse with the membrane of the axon. These sacs, called synaptic vesicles, release their contents into the synaptic cleft by exocytosis. The neurotransmitters then diffuse across the synapse, taking about 0.5 to 1 ms to reach the dendrites of the postsynaptic neuron, or cell membrane of the effector. Upon reaching the postsynaptic membrane, the neurotransmitters bind to specific receptor proteins in this membrane. The receptor proteins trigger ion-specific channels to open. This depolarizes the postsynaptic membrane and, if the threshold potential is reached, initiates an action potential. The impulse will travel along the postsynaptic axon to its terminal and to the next neuron or an effector. Neurotransmitters have either excitatory or inhibitory effects on the postsynaptic membrane. If the effect is excitatory, the receptor proteins will trigger ion channels that open to allow positive

ions, such as sodium, to flow into the postsynaptic neuron. As a result, the membrane becomes slightly depolarized. The membrane of the neuron cannot experience an action potential but the slight depolarization spreads throughout the nerve cell, lowering its threshold level.

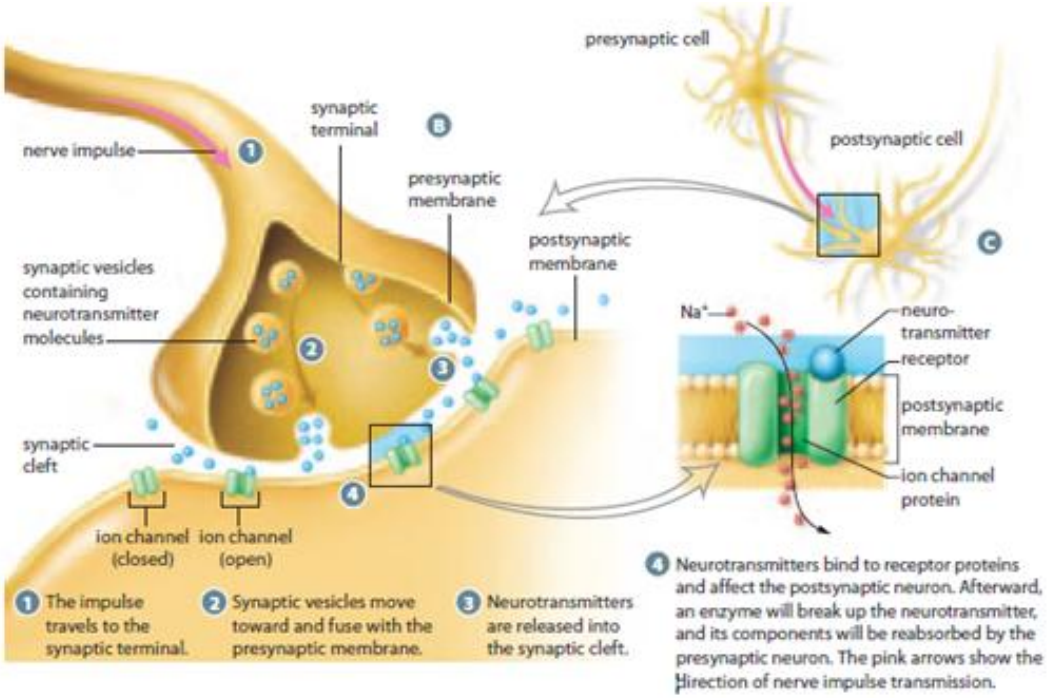


Figure -1.3: Signal transmission across a synapse

If the neurotransmitter is inhibitory, the receptor will trigger potassium channels to open, allowing potassium ions to flow out. This results in a more negative transmembrane potential, resulting in hyperpolarization. A single cell body may be receiving signals from many pre-synaptic neurons at the same time. Some can be excitatory and others can be inhibitory. One of the functions of the cell body is to integrate all of the incoming signals. The combined effect of all of the stimuli spreads across the cell body. If the excitatory stimuli are strong enough, the depolarization will reach the point at which the axon is connected to the cell body and an impulse will be generated. The postsynaptic neuron will then return to resting potential. After the neurotransmitter has had its effect, enzymes break it down and inactivate it so that its components can be reabsorbed by the pre-synaptic cell. (Marieb, 1995)

1.1.7 Normal neurodevelopment in adolescence

Adolescents show significant neuropsychological progress in the years leading up to adulthood, although this may lack the dramatic effect of a toddlers taking their first steps or uttering their first words. Structurally, the brain continues to increase in total volume until the age of approximately 14 years. A longitudinal magnetic resonance imaging (MRI) study showed that the total white matter volume continues to increase into the early 20s, frontal and parietal grey matter volume peaks at approximately 14 years of age before declining, and the grey matter in the occipital and temporal lobes continues to increase until 20 years of age. The decrease in frontal grey matter volume is probably due to massive synaptic loss during this period; data from primate models have estimated that up to 30 000 synapses may be lost per second over the entire cortex, particularly from the frontal regions. Although the precise reason for this is unknown, it is speculated that the brain is developing on the basis of experience and pertinent environmental needs—the "use it or lose it" theory. Finally, there seems to be much more focal activation of the brain in adolescence compared with early childhood, with a marked increase in the degree to which each hemisphere can process information independently.

1.1.8 Common central nervous system disorders

1.1.8.1 Neurological Disorders

1.1.8.1.1 Epilepsy

Epilepsy is the most common neurological disorder of adolescence. Epilepsy may have an onset at this time or pre-existing epilepsy may continue to remit or deteriorate. Accurate history taking is crucial to the diagnosis. As teenagers usually attend clinic with parents who have not witnessed the paroxysmal events, it may be necessary to talk to their friends and schoolteachers to obtain useful, and even diagnostic, information. Investigations are used to classify epilepsy syndromes in order to guide treatment and inform on prognosis and to identify any underlying cause. There are some important epilepsy syndromes commonly present in adolescence. (Macleod and Appleton, 2006)

1.1.8.2 Neurodegenerative disorders

Neurodegenerative disorders, although individually rare, are collectively an important group of disorders to consider at this age. Regression, whether cognitive, motor or a mixture of both, poses a specific diagnostic challenge. The first question that must be dealt with in the adolescent presenting with an apparent loss of skills is whether this reflects a genuine neurodegenerative disorder or a pseudoregression due to some other aetiology. The first symptoms of a neurodegenerative disorder may be a change in personality or a declining school performance, or often a combination of both. The change from a primary school, single classroom environment to the secondary school with large varied classrooms as well as the increasing academic and organizational demands can often unmask pre-existing static difficulties.

In a child with pre-existing neurological difficulties it can be difficult to differentiate between a plateauing of skills, a pseudoregression (important as the cause may be reversible) and real onset of a neurodegenerative condition. In these situations, it is vital to consider the validity of the original diagnosis. The progressive myoclonic epilepsies are an excellent example of this problem, as discussed above. Other examples of this particular problem include the child with erroneously diagnosed "diplegic cerebral palsy" who has in fact a genetic disorder such as dopa-responsive dystonia or idiopathic torsion dystonia, hereditary spastic paraplegia or pantothenate kinase-associated neurodegeneration (PKAN). Causes of pseudoregression include depression, which is becoming increasingly recognized (and often overlooked) in teenagers, and other non-neurological conditions including acquired hypothyroidism and substance misuse. Poorly controlled and subtle epileptic seizures or frequent spike and wave activity on the EEG represent other potentially treatable causes of pseudoregression.

1.1.8.2.1 Subacute sclerosing panencephalitis (SSPE)

In the UK, SSPE is rare (four patients (aged 11–15 years) have been diagnosed in the past 15 years at Alder Hey), but this condition is likely to increase in the next 10–15 years, with the reduced uptake of measles immunization in the second year of life. The onset is usually insidious, often with a decline in school performance (poor concentration and short-term memory) and behavioral disturbances, which may be initially ascribed to psychological problems

or depression. Rare presentations include visual failure and frequent atypical absences, occasionally with myoclonic seizures. Progression varies from child to child, but characteristically includes clumsiness, myoclonic and tonic–clonic seizures. Over the ensuing weeks, months or years, dysphagia, dysarthria, involuntary movements and cortical visual impairment develop, eventually leading to coma and death. The EEG is characteristic (often suggesting the diagnosis) and shows very high-amplitude and periodic triphasic slow wave complexes; diagnosis is confirmed by finding an increased anti-measles antibody titre in the cerebrospinal fluid.

1.1.8.2.2 Variant Creutzfeldt–Jakob disease

Variant Creutzfeldt–Jakob disease is important, although fortunately its incidence would not seem to be as high as predicted when first identified a decade ago. The vast majorities of paediatric cases have either had an onset around, or have presented after, 12 years of age. The most common presentation is with psychiatric symptoms (depression, anxiety or social withdrawal); cognitive involvement occurs early, but may be masked and initially overlooked because of the psychiatric symptoms. Sensory symptoms (paraesthesiae/painful dysaesthesiae) usually develop within 6 months of the onset, followed by ataxia and involuntary movements, typically dystonia, chorea and myoclonus. The EEG does not show the characteristic periodic paroxysms seen in sporadic Creutzfeldt–Jakob disease, but brain MRI usually shows symmetrical high signal in the posterior thalamic (pulvinar) regions. Death occurs usually at a median of 14–18 months from disease onset.

1.1.8.2.3 Wilson's disease

In children with Wilson's disease (hepatolenticular degeneration), presentation after 10 or 12 years of age is typically neurological although this may initially be subtle and limited to a single symptom (eg, dysarthria or gait disturbance). Psychiatric features ranging from behavioral disturbance to a paranoid psychosis may precede any neurological manifestations in up to 20% of patients. Neurological deterioration occurs in the late teenage years with worsening dysarthria, dystonia, a fixed pseudosmile, tremor, postural abnormalities and rigidity; dementia is a later complication and epilepsy is uncommon. The Kayser–Fleisher ring, an orange–brown

discoloration at the limbus of the cornea, will be seen in most teenagers with neurological symptoms and, although usually visible with an ophthalmoscope, are far better visualized on slit-lamp examination. Diagnosis can be confirmed by low serum caeruloplasmin and copper levels and high 24-h urinary copper excretion; the defective gene is on chromosome 13q. The diagnosis of Wilson's disease is important as it is one of the very few treatable neurodegenerative disorders.

1.1.8.2.4 Friedreich's ataxia

The most common progressive disorder affecting primarily motor function in adolescence is Friedrich's ataxia, an autosomal recessive disorder. It has a mean age at onset of 11–12 years (range 4–16 years) and presentation is usually with clumsiness, ataxia and dysarthria; these children may initially be diagnosed as having dyspraxia. Presentation in the teenage years may also be with pes cavus, or less commonly with scoliosis or cardiomyopathy. The ataxia is relentlessly progressive, but the rate of progression varies between (and occasionally within) families. Ambulation is lost between 6 and 10 years after onset. A hypertrophic cardiomyopathy is very common and may be shown early in the course of the disease by echocardiography in asymptomatic individuals. Muscle stretch/deep tendon reflexes are absent and plantar responses are extensor. Diagnosis is confirmed by finding the mutation of the frataxin gene on chromosome 9q, which is present in approximately 90% of affected individuals. Vitamin E-responsive ataxia may present with a very similar clinical phenotype although the cardiomyopathy is rare; the Friedrich's ataxia mutation is negative and the ataxia improve with high-dose vitamin E supplementation.

1.1.8.2.5 Pantothenate kinase–associated neurodegeneration

PKAN (previously known as Hallervorden–Spatz disease) is a rare autosomal recessive disorder that usually presents after 10–12 years of age with extra-pyramidal dysfunction as manifest by rigidity (not spasticity), dystonia and, subsequently, choreoathetosis and dementia. A pigmentary retinopathy is commonly found. Initial diagnoses in these young teenagers include cerebral palsy, dyspraxia and suspicion of substance abuse. The reported pathognomic brain MRI feature (the eye of the tiger sign) is not always seen in affected individuals, even with progressive

neurological symptoms and signs, and may also disappear during the course of the disease.Identification of the mutation encoding the enzyme pantothenate kinase 2 in approximately 60% of patients has allowed a more accurate diagnosis, but its absence does not preclude a diagnosis of PKAN.

1.1.8.2.6 Idiopathic torsion dystonia

Children with idiopathic torsion dystonia usually present between 7 and 12 years of age, but occasionally later. In most cases (80%), the disorder is inherited in an autosomal dominant pattern (but with incomplete penetrance) and present with bilateral (although occasionally asymmetric) lower limb dystonia and an abnormal, frequently bizarre gait that usually becomes more generalized to involve the upper limbs, neck and bulbar muscles. Initial diagnoses in these children may include cerebral palsy and a functional disorder. Generalization is less probable if the initial presentation is in the upper limbs or trunk. The generalized form tends to progress relatively slowly over 5–10 (or more) years. Neuroimaging is normal and DNA analysis may show the presence of the DYT1 gene on chromosome 9q34 in about 60% of individual.

1.1.8.3 Neuromascular disorders

Adolescents with recent-onset neuromuscular disorders tend to have specific problems, usually related to self-help skills and activities of daily living This contrasts with younger children who more typically present with gross motor developmental delay, stumbling or an inability to keep up with their peer group. Although most neuromuscular disorders in adolescence present with weakness or muscle cramps or both, a number of rarer, and predominantly metabolic muscle disorders may present with fatigue.

Most of the neuromuscular disorders involving the muscle or peripheral nerve are inherited (often in an autosomal dominant pattern), and show clinical variation within a family. It is therefore important to always examine the child's biological parents and siblings in detail (including, where appropriate, undressed), as this may be important in identifying a specific neuromuscular disorder. In the authors' experience, this is particularly true for myotonic dystrophy, facio-scapulo-humeral dystrophy, hereditary motor and sensory neuropathy (HMSN)

type IA, and central core disease—all of which are typically inherited in an autosomal dominant pattern. Diagnosis is confirmed by neurophysiological investigations (nerve conduction studies, electromyography), muscle biopsy, DNA and other analyses (e.g., positive anti-acetylcholine receptor antibodies in juvenile auto-immune myasthenia), depending on the specific disorder.

In general, progressive proximal muscle weakness in childhood is usually caused by a myopathy. Included in this group are the dystrophies and the inflammatory, endocrine and metabolic myopathies.

1.1.8.3.1 Becker muscular dystrophy

Patients with Duchenne muscular dystrophy always present in the first decade of life, whereas in Becker muscular dystrophy the onset is usually after 5 years of age, but also at any time in childhood or even in early adult life. Boys with Becker muscular dystrophy usually have a history of poor sporting activities and quite severe muscle pains after even moderate exercise, often leading to an initial referral to an orthopedic surgeon or rheumatologist—with a consequent delay in diagnosis. Marked calf pseudo-hypertrophy is common, and, as with the muscle pains, tends to be more marked than in Duchenne muscular dystrophy. The creatine phosphokinase level is always raised (>10–20 times normal) and echocardiography may show cardiac involvement in the absence of cardiac symptoms.

1.1.8.3.2 Facio-scapulo-humeral dystrophy

Facio-scapulo-humeral dystrophy is an autosomal dominant dystrophy presenting in late childhood or early adolescence with progressive facial weakness, scapular winging and weakness of muscles in the shoulder girdle leading to difficulties in raising the arms. Disease progression is variable, with periods of apparent arrest. Many patients do not become disabled and their life expectancy is normal, whereas others become wheelchair dependent in adult life.

1.1.8.3.3 Myasthenia gravis

Myasthenia gravis in adolescents (predominantly in females) is usually an autoimmune disease that presents in a similar fashion as the adult disease. Ptosis and diplopia are usually the initial

presenting features, but weakness may become more generalised. Affected individuals may only have increasing fatigue as the day progresses. A considerable minority may present acutely over hours or days (often precipitated by an intercurrent illness or infection) in a myasthenic crisis with severe bulbar and respiratory difficulties, which is a medical emergency. The course tends to be slowly progressive.

1.1.8.3.4 Myotonic dystrophy

The onset of symptoms in myotonic dystrophy is usually in adolescence or early adult life, although symptomatic myotonia (abnormal muscle relaxation after contraction) may be seen in childhood. Presenting features include muscle weakness, particularly of the face and distal limb muscles, and the teenager may already have been diagnosed with diabetes mellitus. Often, a background of mild to moderate learning difficulties is observed, which together with the presence of motor difficulties may lead to an initial diagnosis of dyspraxia. The affected parent in childhood/teenager-onset myotonic dystrophy is typically the father, in contrast with the congenital form, where the affected parent is the mother. The course is slowly progressive, with severe weakness in the hands and feet in adult life.

1.1.8.3.5 Peripheral neuropathies

Most peripheral neuropathies in childhood and adolescence are hereditary; the most important exception is acute inflammatory demyelinating polyradiculoneuropathy (Guillain–Barré syndrome), which commonly presents between 11 and 15 years of age. Of the inherited neuropathies, HMSN type IA and type II tend to be the most common and usually have a peak incidence in the second decade of life. HMSN type IA is commonly inherited in an autosomal dominant manner. Weakness begins in the anterior tibial compartment and causes foot drop, pes cavus deformities and, eventually, clawing of the toes. Sensory disturbance may be found on formal examination, but is rarely clinically significant. Muscle stretch reflexes are typically absent at the ankles, but may be reduced or absent elsewhere, depending on the person's age. Symptom progression is slow and significant disability does not develop until middle adult life. Acquired neuropathies are most commonly seen in hospital, particularly in oncology patients

receiving antineoplastic drugs and undergoing intensive care (critical illness neuropathy or myopathy).

1.1.8.3.6 Metabolic myopathies

A number of rare metabolic muscle disorders may also present in adolescence. Carnitine-palmitoyl transferase type II (CPT-2) has an onset in late childhood/adolescence and usually presents with myalgia, fatigability during or after exercise, and myoglobinuria after sustained aerobic exercise. Respiratory muscles may be involved and fatal rhabdomyolysis has been reported rarely. There is no residual weakness after individual episodes, but repeated episodes over years may lead to a permanent, although mild, myopathy. The CPT-2 concentration can be measured to confirm the diagnosis. Patients with CPT-2 are at risk of malignant hyperthermia syndrome and must be counseled accordingly. Very-long-chain acyl coenzyme A dehydrogenase deficiency also usually presents after the age of 10 years with severe pain and myoglobinuria on exercise. The other important genetic metabolic myopathy is that seen in the mitochondrial cytopathies, a multi-organ group of conditions that can present at any age (commonly in childhood and early adult life) and with any symptoms, depending on the predominant organs involved. A predominant myopathic or neuropathic presentation is seen in myoclonic epilepsy and ragged red fibres (MERRF) (on muscle biopsy), and neurogenic atrophy ataxia and retinitis pigmentosa (NARP), respectively. Diagnosis of both myoclonic epilepsy and ragged red fibres and neurogenic atrophy, ataxia and retinitis pigmentosa may be made by finding the specific mutation on blood DNA analysis, although these mutations may be negative and diagnosis will subsequently be made on muscle biopsy (through histological analysis or finding the specific mutation on muscle DNA). (Macleod and Appleton, 2006)

1.1.8.4 Inflammatory disorders of the central nervous system in adolescence

1.1.8.4.1 Multiple sclerosis

Multiple sclerosis occurring in the early to late teenage years is reported to represent between 3.6% and 4.4% of all cases of multiple sclerosis, although the figure may be higher with improved diagnostic techniques, including MRI scanners of greater magnetic strength, and an increased awareness that multiple sclerosis can occur in childhood. It is more common in

females, particularly after puberty, and follows a relapsing and remitting course in approximately 60% of patients as in adults. The symptoms and signs of childhood multiple sclerosis are similar to those in adults, with visual involvement (optic neuritis or an internuclear ophthalmoplegia) or sensorimotor disturbances representing the most common manifestations.

1.1.8.5 Miscellaneous

Isolated location-specific episodes of demyelination are relatively common in adolescents and include optic neuritis, transverse myelitis and rarely neuromyelitis optica (Devic disease). Acute disseminated encephalomyelitis (ADEM), usually a post-infectious or, rarely, postvaccination disorder, can also present in adolescence and tends to be a monophasic illness; it is probably the most common demyelinating disorder in children. The presentation of ADEM is more often with an encephalopathic illness than with multiple sclerosis. The MRI changes in multiple sclerosis and ADEM may be similar, although characteristically are more widespread and dramatic in ADEM. However, relapses may occur although the risk is unclear; one recent case series suggested a risk of 57%.Debate continues as to whether such relapses should be labeled as polyphasic ADEM or simply multiple sclerosis

The diagnosis of both multiple sclerosis and ADEM is made on the basis of the child's presenting symptoms and signs and MRI findings. Importantly, multiple sclerosis should never be diagnosed after a single episode of demyelination. Cerebrospinal fluid analysis is important in both confirming the diagnosis, particularly in multiple sclerosis, with the finding of oligoclonal bands (which may also be present in ADEM and some disorders which involve the breakdown of the blood-cerebrospinal fluid barrier), and excluding other causes including infections and some malignancies. (Macleod and Appleton, 2006)

1.2 Medicinal Plants

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste. (Hassan, 2012)

Most species are used only in folk medicine, traditional systems of formal medicine using relatively few (e.g. 500-600 commonly in Traditionally Chinese Medicine). Around 100 plant species have contributed significantly to modern drugs. The use of medicinal plants is increasing worldwide, related to the persistence and sometimes expansion of traditional medicine and a growing interest in herbal treatment. (Plantlife.org.uk,2010)

At present the term "Alternative Medicine" became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. (Hassan, 2012). The medicinal uses of plants grade into their uses for other purposes, as for food, cleaning, personal care and perfumery. Plants are used in medicine to maintain and augment health - physically, mentally and spiritually - as well as to treat specific conditions and ailments. (Petrovska, 2012). Like in case of laxatives, blood thinners, antibiotics and anti-malaria medications, contain ingredients from plants. Moreover the active ingredients of taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively. (Hassan, 2012)

1.2.1 Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- **Synergic medicine-** The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
- **Support of official medicine**- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

- **Preventive medicine**- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. (Hassan, 2012)

1.2.2 Historical Overview of Medicinal Plants

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Contemporary science has acknowledged their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life.

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants' use were instinctive, as is the case with animals. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis. Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again. (Petrovska, 2012)

1.2.3 Historical sources relevant for study of medicinal plants' use

The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.

The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: *Rhei rhisoma*, camphor, *Theae folium, Podophyllum*, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra.

The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc.

The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc.

According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense.

In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to. Some of them were given the names after mythological characters from these epics; for instance, Elecampane (*Inula helenium* L. Asteraceae) was named in honor of Elena, who was the centre of the Trojan War. As regards the plants from the genus *Artemisia*, which were believed to restore strength and protect health, their name was derived from the Greek word *Artemis*, meaning "healthy."Herodotus (500 BC) referred to castor oil plant, Orpheus to the fragrant hellebore and garlic, and Pythagoras to the sea onion (*Scilla maritima*), mustard, and cabbage. The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (*Centaurium umbellatum Gilib*) were applied against fever;

garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents.

Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium"—Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time. Among others; he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth. In the description of the plant toxic action, Theophrast underscored the important feature for humans to become accustomed to them by a gradual increase of the doses. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants.

In his work "*De re medica*" the renowned medical writer Celsus (25 BC–50 AD) quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc.

In ancient history, the most prominent writer on plant drugs was Dioscorides, "the father of pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army. Circa 77 AD he wrote the work "De Materia Medica." This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic *materia medica* until the late middle Ages and the Renaissance. Of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic effect. In addition to the plant description, the names in other languages coupled with the localities where they occur or are grown are provided. The plants having mild effect are dominant, but there are also references to those containing alkaloid or other matter with strong effect (fragrant hellebore, false hellebore, poppy, buttercup, jimson weed, henbane, deadly nightshade). Dioscorides' most appreciated domestic plants are as follows: willow, chamomile, garlic, onion, marsh mallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore). Camomile (*Matricaria recucita* L.), known

under the name Chamaemelon, is used as an antiphlogistic to cure wounds, stings, burns, and ulcers, then for cleansing and rinsing the eyes, ears, nose, and mouth. Owing to its mild carminative action, it is particularly appropriate for usage with children. Dioscorides deemed that it had abortive action, on which he wrote, "The flower, root, and the entire plant accelerate menstruation, the release of the embryo, and the discharge of urine and stone, provided that they are used in the form of an infusion and baths." This untrue belief was later embraced by both the Romans and the Arabs; hence the Latin name *Matricaria*, derived from two words: *mater* denoting "mother," i.e. matrix, denoting 'uterus'. Dioscorides differentiated between a number of species from the genus *Mentha*, which were grown and used to relieve headache and stomach ache. The bulbs of sea onion and parsley were utilized as diuretics, oak bark was used for gynaecological purposes, while white willow was used as an antipyretic. As maintained by Dioscorides, *Scillae bulbus* was also applied as an expectorant, cardiac stimulant, and antihydrotic.It is worth underscoring that Dioscorides pointed to the possibility of forgery of drugs, both the domestic ones such as opium forged by a yellow poppy (*Glaucium flavum*) milk sap and poppy, and the more expensive oriental drugs, transported by the Arab merchants from the Far East, such as iris, calamus, caradmomum, incense, etc.

Pliny the Elder (23 AD-79), who was a contemporary of Dioscorides, travelled throughout Germany and Spain, wrote about approximately 1000 medicinal plants in his book "Historia naturalis." Pliny's and Dioscorides' works incorporated all knowledge of medicinal plants at the time.

The most distinguished Roman physician (concurrently a pharmacist), Galen (131 AD–200), compiled the first list of drugs with similar or identical action (parallel drugs), which are interchangeable—"De succedanus." From today's point of view, some of the proposed substitutes do not correspond in a pharmacological context and are absolutely unacceptable. Galen also introduced several new plant drugs in therapy that Dioscorides had not described, for instance, *Uvae ursi folium*, used as an uroantiseptic and a mild diuretic even in this day and age.

In the seventh century AD the Slavic people used *Rosmarinus officinalis, Ocimum basilicum, Iris germanica,* and *Mentha viridis* in cosmetics, *Alium sativum* as a remedy and *Veratrum album,*

Cucumis sativus, Urtica dioica, Achilea millefolium, Artemisia maritime L., *Lavandula officinalis, Sambuci flos* against several injurios insects, i.e. louses, fleas, moths, mosquitos, and spiders and *Aconitum napellus* as a poison in hunting.

In the middle Ages, the skills of healing, cultivation of medicinal plants, and preparation of drugs moved to monasteries. Therapy was based on 16 medicinal plants, which the physicians-monks commonly grew within the monasteries as follows: sage, anise, mint, Greek seed, savory, tansy, etc.

Charles the Great (742 AD–814), the founder of the reputed medical school in Salerno, in his "Capitularies" ordered which medicinal plants were to be grown on the state-owned lands. Around 100 different plants were quoted, which have been used till present days such as sage, sea onion, iris, mint, common centaury, poppy, marsh mallow, etc. The great emperor especially appreciated the sage (*Salvia officinalis* L.). The Latin name of sage originates from the old Latins, who called it a salvation plant (*salvare* meaning "save, cure"). Even today sage is a mandatory plant in all Catholic monasteries.

The Arabs introduced numerous new plants in pharmacotherapy, mostly from India, a country they used to have trade relations with, whereas the majority of the plants were with real medicinal value, and they have persisted in all pharmacopoeias in the world till today. The Arabs used aloe, deadly nightshade, henbane, coffee, ginger, strychnos, saffron, curcuma, pepper, cinnamon, rheum, senna, and so forth. Certain drugs with strong action were replaced by drugs with mild action, for instance, *Sennae folium* was used as a mild laxative, compared to the purgatives *Heleborus odorus* and *Euphorbium* used until then.

Throughout the Middle Ages European physicians consulted the Arab works "De Re Medica" by John Mesue (850 AD), "Canon Medicinae" by Avicenna (980-1037), and "Liber Magnae Collectionis Simplicum Alimentorum ET Medicamentorum" by Ibn Baitar (1197-1248), in which over 1000 medicinal plants were described.

For Macedonia, St Clement and St Naum of Ohrid's work are of particular significance. They referred to the Nikeian pharmacological codex dating from year 850, and transferred his extensive knowledge on medicinal plants to his disciples and via them to the masses.

Marco Polo's journeys (1254-1324) in tropical Asia, China, and Persia, the discovery of America (1492), and Vasco De Gama's journeys to India (1498), resulted in many medicinal plants being brought into Europe. Botanical gardens emerged all over Europe, and attempts were made for cultivation of domestic medicinal plants and of the ones imported from the old and the new world. With the discovery of America, materia medica was enriched with a large number of new medicinal plants: *Cinchona, Ipecacuanha, Cacao, Ratanhia, Lobelia, Jalapa, Podophylum, Senega, Vanilla, Mate,* tobacco, red pepper, etc. In 17th century, *Cortex Chinae,* yielded from quinine bark *Cinchona succirubra* , under the name countess' powder, since the Countess of Chinchon was the first one who used it, was introduced to European medicine. Quinine bark rapidly overwhelmed England, France, and Germany despite the fact that there was many an opponent to its use among distinguished physicians—members of a range of academies.

Paracelsus (1493-1541) was one of the proponents of chemically prepared drugs out of raw plants and mineral substances; nonetheless, he was a firm believer that the collection of those substances ought to be astrologically determined. He continuously emphasized his belief in observation, and simultaneously supported the "Signatura doctrinae"—the signature doctrine. According to this belief, God designated his own sign on the healing substances, which indicated their application for certain diseases. For example, the haselwort is reminiscent of the liver; thus, it must be beneficial for liver diseases; St John's wort *Hypericum perforatum* L. would be beneficial for treatment of wounds and stings given that the plant leaves appear as if they had been stung.

While the old peoples used medicinal plants primarily as simple pharmaceutical forms—infusions, decoctions and macerations—in the Middle Ages, and in particular between 16th and 18th centuries, the demand for compound drugs was increasing. The compound drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug the theriac was

produced from a number of medicinal plants, rare animals, and minerals, it was highly valued and sold expensively. (Petrovska, 2012)

In the 18th century knowledge about plant derived drugs expanded, but attempts to indentify the active ingredients from plants were unsuccessful. (Clardy and Walsh, 2004). In his work *Species Plantarium* (1753), Linnaeus (1707-1788) provided a brief description and classification of the species described until then. The species were described and named without taking into consideration whether some of them had previously been described somewhere. For the naming, a polynomial system was employed where the first word denoted the genus while the remaining polynomial phrase explained other features of the plant (e.g. the willow Clusius was named *Salix pumila angustifolia antera*). Linnaeus altered the naming system into a binominal one. The name of each species consisted of the genus name, with an initial capital letter, and the species name, with an initial small letter. (Petrovska, 2012)

Early 19th century was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipecacuanha (1817), strychnos (1817), quinine (1820), pomegranate (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy. With the upgrading of the chemical methods, other active substances from medicinal plants were also discovered such as tannins, saponosides, etheric oils, vitamins, hormones, etc.

In the early 19th century, the term ‘pharmacognosy' was coined by Johann Adam Schmid (1759-1809), but the main shift came when it became clear that the pharmaceutical properties of plants are due to specific molecules that can be isolated and characterized. Another achievement in the field of medicinal plants was the development of methods to study the pharmacological effect of natural products and vegetable extracts. Claude Bernard (1813-1878), who conducted detailed studies on the pharmacological effects of curare (a drug and arrow poison used by the American Indians of the Amazon), is considered one of the first scientists in this field. (Clardy and Walsh, 2004)

In late 19th and early 20th centuries, there was a great danger of elimination of medicinal plants from therapy. Many authors wrote that drugs obtained from them had many shortcomings due to

the destructive action of enzymes, which cause fundamental changes during the process of medicinal plants drying, i.e. medicinal plants' healing action depends on the mode of drying. In 19th century, therapeutics, alkaloids, and glycosides isolated in pure form were increasingly supplanting the drugs from which they had been isolated. Nevertheless, it was soon ascertained that although the action of pure alkaloids was faster, the action of alkaloid drugs was full and long-lasting. In early 20th century, stabilization methods for fresh medicinal plants were proposed, especially the ones with labile medicinal components. Besides, much effort was invested in study of the conditions of manufacturing and cultivation of medicinal plants.

On account of chemical, physiological, and clinical studies, numerous forgotten plants and drugs obtained thereof were restored to pharmacy: *Aconitum, Punica granatum, Hyosciamus, Stramonium, Secale cornutum, Filix mas, Opium, Styrax, Colchicum, Ricinus*, and so forth. The active components of medicinal plants are a product of the natural, most seamless laboratory. The human organism accepts the drug obtained from them best in view of the fact that man is an integral part of nature. There are scores of examples of this kind; perhaps they will instigate serious research into the old manuscripts on medicinal plants, which would not be observed out of curiosity about history but as potential sources of contemporary pharmacotherapy. (Petrovska, 2012)

1.2.4 Medicinal Plant in present times

In present days, almost all pharmacopoeias in the world—Ph Eur 6, USP XXXI, BP 2007—proscribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany) that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine). For the sake of adequate and successfully applied therapy, knowledge of the precise diagnosis of the illness as well as of medicinal plants, i.e. the pharmacological effect of their components is essential. Plant drugs and

phytopreparations, most commonly with defined active components, verified action and, sometimes, therapeutic efficiency, are applied as therapeutic means. In the major European producer and consumer of herbal preparations—Germany, rational phytotherapy is employed, based on applications of preparations whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs.

With the new Law on Drugs and Medical Devices dated September 200 and enacted in the Republic of Macedonia, dry or sometimes fresh parts of medicinal plants (herbal substances) may be used for preparation of herbal drugs, herbal processed products, and traditional herbal drugs. Herbal substances may also be utilized for manufacture of homeopathic drugs, which are stipulated in the current law, too. In the Republic of Macedonia herbal preparations are dispensed without a medical prescription, as "over the counter" (OTC) preparations. (Petrovska, 2012)

1.2.5 Medicinal Plant in traditional medicine

By definition, 'traditional' use of herbal medicines implies substantial historical use and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. An example is the use of ephedra (= Ma huang) for weight loss or athletic performance enhancement. (Shaw, 1998). While in some countries, herbal medicines are subject to rigorous manufacturing standards, this is not so everywhere. In Germany, for example, where herbal products are sold as 'phytomedicines', they are subject to the same criteria for efficacy, safety and quality as are other drug products. In the USA, by contrast, most herbal products in the marketplace are marketed and regulated as

dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria. (Schulz *et al.*, 2001)

1.2.6 The role of herbal medicines in traditional healing

The pharmacological treatment of disease began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose. (Schulz *et al.*, 2001)

1.2.6.1 Traditional Chinese medicine

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12 000 items used by traditional healers, about 500 are in common use (Li, 2000). Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy. Traditional Chinese medicine is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market (Li, 2000).

1.2.6.2 Japanese traditional medicine

Many herbal remedies found their way from China into the Japanese systems of traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century (Saito, 2000)

1.2.6.3 Indian traditional medicine

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

1.2.6.4 Introduction of traditional herbal medicines into Europe, the USA and other developed countries

The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines particularly in Europe and North America, where herbal products have been incorporated into so-called 'alternative',' complementary', 'holistic' or 'integrative' medical systems. (Tyler, 2000)

During the latter part of the twentieth century, increasing interest in self-care resulted in an enormous growth in popularity of traditional healing modalities, including the use of herbal remedies; this has been particularly true in the USA. Consumers have reported positive attitudes towards these products, in large part because they believe them to be of 'natural' rather than 'synthetic' origin, they believe that such products are more likely to be safe than are drugs, they are considered part of a healthy lifestyle, and they can help to avoid unnecessary contact with conventional 'western' medicine.

While centuries of use in traditional settings can be used as testimony that a particular herbal ingredient is effective or safe, several problems must be addressed as these ingredients are incorporated into modern practice.

One problem is that ingredients once used for symptomatic management in traditional healing are now used in developed countries as part of health promotion or disease prevention strategies; thus, acute treatment has been replaced by chronic exposure (e.g., herbal products used for weight loss). (Allison *et al*., 2001). This means that a statement about 'thousands of years of evidence that a product is safe' may not be valid for the way the product is now being used. This does not expressly mean that an ingredient is unsafe; it does mean that safety in the modern context cannot be assumed.

A second problem is that efficacy and effectiveness have rarely been demonstrated using modern scientific investigations. An evidence-based approach to this issue has only recently been implemented, and the results reveal that for most herbal products, considerable gaps in knowledge need to be remedied before one can be convinced about their efficacy.

1.2.7 Medicinal plants & modern medicine

1.2.7.1 Importance of research on medicinal plants

Plants have been used as medicine for millennia. Out of estimated 250 000 to 350 000 plant species identified so far, about 35 000 are used worldwide for medicinal purposes. It has been confirmed by WHO that herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries. Meanwhile, consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives. The recent resurgence of plant remedies results from several factors:

1) The effectiveness of plant medicines;

2) The side effect of most modern drugs; and

3) The development of science and technology.

It has been estimated that in the mid-1990s over 200 companies and research organizations worldwide are screening plant and animal compounds for medicinal properties. Actually, several important drugs used in modern medicine have come from medicinal plant studies, e.g., taxol/paclitaxel, vinblastine, vincristine, topotecan, irinotecan, etoposide, teniposide, etc. As for drugs derived from orchids, some novel discoveries, both in phytochemical and pharmacological properties, were reported by some universities. However, studies on plants are very limited. Only about a third of the million or so species of higher plants have been identified and named by scientists. Of those named, only a tiny fraction has been studied. Nowadays the linking of the indigenous knowledge of medicinal plants to modern research activities provides a new approach, which makes the rate of discovery of drugs much more effective than with random collection. (Jin-Ming *et al*., 2003)

1.2.7.2 Recent Advancements

The 20th century saw the integration of ethnobotanical, pharmacological and phytochemical studies, a process that had taken many and many years, but which allowed the development of a new approach to the study and the pharmaceutical use of plants. Ultimately, herbal remedies and natural products became transformed into chemically defined drugs. These offer large structural diversity (Clardy and Walsh, 2004), and modern techniques for separation, structure elucidation, screening and combinatorial synthesis (Ganesan, 2003); (Steinbeck, 2004), have lead to revitalization of plant products as a source of new drugs. This has opened up new opportunity and avenues for drug development.

Plant-based systems have continued to play an essential role in health care of many cultures and the World Health Organization (WHO) has estimated that approximately 65% of the world's population relies mainly on plant-derived traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining population, mainly residing in "developed" countries. Of 122 compounds identified in a survey of plant derived pure compounds used as drugs in countries hosting WHO-Traditional Medicine Centers, 80% were found to be used for the same or related ethnomedical purposes, and were derived from only 94 plant species. Relevant examples are given by Fabricant and Farnsworth. Probably the best example of ethnomedicine's role in guiding drug discovery and development is that of the antimalarial drugs, particularly, quinine and artemisinin. The isolation of quinine was reported in 1820 by the French pharmacists, Caventou and Pelletier from the bark of Cinchona species (e.g., *Cinchona officinalis).* The bark, long used by indigenous groups in the Amazon region for the treatment of fevers, was introduced into Europe in the early 1600s for the treatment of malaria, and quinine formed the basis for the synthesis of the commonly used antimalarial drugs, chloroquine and mefloquine, which largely replaced quinine in the mid-twentieth century. As resistance to both these drugs developed in many tropical regions, another plant having a long history of use in traditional Chinese medicine (TCM) for the treatment of fevers, Artemisia annua (Qinghaosu), gained prominence , and the discovery of artemisinin by Chinese scientists in 1971 provided an exciting new natural product lead compound. Artemisinin analogs, such as artesunate, are now used for the treatment of malaria in many countries, and

many other analogs of artemisinin have been prepared in attempts to improve its activity and utility. These include totally synthetic molecules with the trioxane moiety included, such as arterolane tosylate which is in Phase II trials under Ranbaxy, artemisinin dimers and the amino-artemisinin, artemisone.

Resistance to artemisinin-based drugs is now being observed. In order to counter this development, variations on the basic structure have been launched in combination with other antimalarials (usually variations on the chloroquine structure) such as dihydroartemisinin and piperaquine phosphate (Artekin); artemether and lumefantrine (Coartem); artesunate and mefloquine (Artequin); and artesunate, sulfamethoxypyrazine, and pyrimethamine (Co-Arinate). Currently there is one other fixed dose combination with an artemisinin derivative in Phase III clinical trials, pyronaridine/artesunate (Pyramax).While artemisinin and more soluble derivatives have altered the treatment of resistant malaria, the costs of collection of sufficient quantities of the source plants is high, and the overall cost of the drugs may exceed what can be afforded by the countries where the drug is required for general treatment. In an attempt to avoid dependence on wild or even cultivated plant harvesting and thereby reduce costs, the Keasling group, in conjunction with the Gates Foundation and Amyris Pharmaceuticals, has transferred the genes from the producing plant into Escherichia coli and also *Saccharomyces cerevisiae*. They have successfully expressed the base terpene (amorpha-4, 11-diene) and followed up with modification of the base structure both chemically, and to some extent, biochemically via P450 enzymes .Titers exceeding 25 g/L of amorpha-4, 11-diene have been produced by fermentation and are followed by chemical conversion to artemisinin, thereby allowing for the development of a potentially viable process to provide an alternative source of artemisinin .Other significant drugs developed from traditional medicinal plants include: the antihypertensive agent, reserpine, isolated from *Rauwolfia serpentina* used in Ayurvedic medicine for the treatment of snakebite and other ailments; ephedrine, from Ephedra sinica (Ma Huang), a plant long used in traditional Chinese medicine, and the basis for the synthesis of the antiasthma agents (beta agonists), salbutamol and salmetrol; and the muscle relaxant, tubocurarine, isolated from *Chondrodendron* and *Curarea* species used by indigenous groups in the Amazon as the basis for the arrow poison, curare. Although plants have a long history of use in the treatment of cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine, and

consequently many of the claims for the efficacy of such treatment should be viewed with some skepticism. Among the plant derived anticancer drugs in clinical use, some of the best known are the so-called vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus*; etoposide and teniposide which are semisynthetic derivatives of the natural product epipodophyllotoxin; paclitaxel (Taxol), which occurs along with several key precursors (the baccatins) in the leaves of various Taxus species, and the semisynthetic analog, docetaxel (Taxotere); and topotecan (hycamptamine), irinotecan (CPT-11), 9-amino- and 9-nitro-camptothecin, all semisynthetically derived from camptothecin, isolated from the Chinese ornamental tree, *Camptotheca acuminata*. These agents together with other plant-derived anticancer agents have been reviewed. (Cragg *et al*., 2012)

1.2.8 Distribution of medicinal plants as well as herbal medicine in developed countries

Plants and their metabolites constituents have a long history of use in modern "western" medicine and in certain systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, dioxin, morphine, quinine. Use of herbal medicines in developed countries has expanded sharply in the latter half of the twentieth century. In recent years, the use of traditional medicine information on plant research has again received considerable interest. While the western use of such information has also come under increasing scrutiny and the national and indigenous rights on these resources has become acknowledged by most academic and industrial researchers. Meanwhile, the need for basic scientific investigations on medicinal plants using indigenous medical systems becomes imminent. The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines, particularly in Europe and North America, where herbal products have been incorporated into so-called alternative, "complementary", "holistic" or "integrative" medical systems. Monographs on selected herbs are available from a number of sources, including the European Scientific Cooperative on Phytotherapy, German Commission E and the World Health Organization. The WHO monographs, for example, describe the herb itself by a number of criteria (including synonyms and vernacular names) and the herb part commonly used, its geographical distribution, tests used to identify and characterize the herb (including macroscopic and microscopic

examination and purity testing), the active principles, dosage forms and dosing, medicinal uses, pharmacology, contra-indications and adverse reactions. During the latter part of the twentieth century, increasing interest in self-care resulted in an enormous growth in popularity of traditional healing modalities, including the use of herbal remedies; this has been particularly true in the USA. In the European market there are a lot of products derived from natural plants, which are recognized to possess different biological properties, such as antioxidant, antiseptic, diuretic, stimulating the central nervous system, sedative, expectorant, digestive, etc. Some of these plants have been used in traditional medicine since ancient times and are available on market as infusions, tablets and/or extracts. Consumers have reported positive attitudes towards these products, in large part because they believe them to be of "natural" rather than "synthetic" origin, they believe that such products are more likely to be safe than are drugs, they are considered part of a healthy lifestyle, and they can help to avoid un necessary contact with conventional "western" medicine. (Hosseinzadeh *et al*., 2015)

1.2.9 Medicinal Plants in Bangladesh

Bangladesh contains about 5,700 species of higher plants being situated in a larger part of South-Asian center of plant genetic diversity. Of which 260 species are cultivated and the rest of the species are virtually left on growing in natural vegetation in forests and in village thickets. Chittagong Hill Tracts posses the largest tropical rain forest of Bangladesh, which includes a vast amount of plant resources. Majority of the tribal communities of the country live in this area and depend on the plant re-sources for their food, fuel, fruit, vegetables and medicine. The use of wild plants forms part of their traditional or indigenous systems of knowledge and practice that have accumulated and developed over generations. The widely used medicinal plant species are - Neemada (*Buddleja asiatica*), Mondessa (*Campanumoea celebica*), Kanphutki (*Cardiospermum halicacabum*) Pahari bichuti (*Cnesmone javanica*), Pidaghi (*Cratoxylum sum-stranum*), Madanmasta (*Dehaasia kurzii*), Chotra-pata (*Laportea crenulata*), Mughal *manigach* (*Nelsonia campestris*), Kulla (*Desmos longiflorus*) etc. Also, a vast majority of forests of Bangladesh lies in Sylhet division in the Northeast Bangladesh Ecological Zone located on a series of low hills on the southern and southeastern parts of Habiganj and Moulavibazar districts. Sylhet forests alone have 790 species of flowering plants distributed in 95 families. The most significant fact is

that 25 of 95 families in this region are each represented only by a single taxon. Plant species which are becoming rare in Sylhet Region are: Kumbhi (Carea arbo-rea), Kalija (Cordia dichotoma), Panihijal (Salix tetrasperma), Kurta (Plaquium polyanthum) etc. Among these wild and semi-wild plant species, medicinal and aromatic plants have been used over the millennia for human welfare in the promotion of health and as drugs and fragrance materials. In Bangladesh, the importance of medicinal plants needs no mention. Even today, use of medicinal plants in primary health care systems is very important, especially in remote rural communities and poorly accessible areas. Wild collections of herbs mainly by the poor are a livelihood activity and often a major source of cash income for these groups.Ayurvedic and Unani companies (phytopharmaceuticals) use large number of medicinal plants species as traditional medicines since ancient times. Most important medicinal plants are:

Terminalia arjuna (Arjun*), T. chebula* (Hartaki), *T. bellirica* (Bohera), *Aegle marmelos* (Bael), *Withania somnifera* (Aswagandha), *Cassia angustifolia* (Sonapata), *Saraca asoca* (Ashok). (Rahman and Fakir, 2015)

1.2.10 Some medicinal plants and their uses

Table-1.1: Information of some medicinal plants

Local name	Scientific name	Source of drug	Uses
Nayantara	*Catharanthus roseus*	leaves	Cancer, insomnia, blood pressure, diabetes
Sarpagandha	*Rauvolfia serpentian benth*	Roots	Insomnia, blood pressure, brain disorder, dysentery
Shatamuli	*Asparagus racemosus*	Roots.	Cancer, bacteria and fungal disease, tonic, appetizer, diabetes, jaundice
Dadmardan	*Cassia alata*	Leaves, roots	Insects bites, ring worm, eczema,

			bronchitis, stomach disorder
Ghritkumari	*Aloe indica*	Leaves	Constipation, fistula, anthelemintic, leucorrhoea, piles, burns, jaundice
Lajjabati	*Mimosa pudica*	Whole plants	Blood purification, toothache, convulsion,fistula, piles
Bohera	*Terminalia belerica*	Fruits, barks	Constipation, diarrhea, dysentery, piles, rheumatisms, leprosy
Neem	*Azadirachta indica*	Leaves	Anathematic, dermatitis, fever, stomach disorder, jaundice, nausea, ruminates.
Haritaki	*Terminalia chebula*	Fruits, barks	Indigestions, jaundice, piles, skin disease, ulceration of gums

Tulsi	*Ocimum sanctum*	Leaves, flower, seeds,whole plants	Stomach disorder, stimulant, cough, fever, malaria, common cold, hypertension
Thankuni	*Cliotoria ternatea*	Whole plants.	Weakness, dermatitis, jaundice, stomach disorder
Mehedi	*Lawsonia inermis*	Leaves, flowers, fruits, seeds	Skin disease, pox, burns,dandruff, insomnia
Elache	*Elettaria cardamomum*	Fruits, backs.	Asthma, constipation, skindisease, rheumatistic
Basak	*Adhatoda vasica*	Roots, leaves,flowers	Cough, asthma, arthritis, dysentery, malaria.

(Scribd, n.d.)

1.3 Plant review

Plant name*: Thysanolaena maxima*

Thysanolaena maxima is a long-lived clumping grass that is becoming common in cultivation as an ornamental garden plant. This species is native to the Indian sub-continent, China, Japan and south-eastern Asia. However, it is starting to spread from cultivation in Queensland and is now thought to be a potential environmental weed. (Technigro, 2011)

Figure-1.4: Large plants of *Thysanolaena maxima* persisting in an abandoned garden.

Figure-1.5: (a)Dense clump of bamboo-like stems., & (b) Large and relatively broad leaves.

Figure-1.6: Young seed-head.

1.3.1 Description

Tiger grass is a long-lived plant that usually grows 2-3 m tall and eventually forms a large and dense clump. It produces numerous upright or arching stems (up to 10 mm thick) that are unbranched and have joints at regular intervals. These stems are bamboo-like in appearance and bear large alternatively arranged leaves. The leaves consist of a sheath at the base, which encloses the stem, and a spreading leaf blade. The very large leaf blades are relatively broad (25-60 cm long and 3-7 cm wide) with pointed tips and entire margins. The large seed-heads (30-60 cm long) are usually open in appearance with numerous very slender spreading branches. These branches can be up to 30 cm long and each bears numerous tiny flower spikelets (1.5-2 mm long). The flower spikelets at first appear hairless, but as they mature the small hairs become more obvious, giving the seed-head a slightly feathery appearance. Each flower spikelet contains a single tiny seed about 0.5 mm long. (Technigro, 2011)

1.3.2 Vernacular Names

Jhadughas (Hindi), Amliso (Nepali), Taza(Nishi), Kamgang (Adi), Eppane -Nani(Apatani), Phool Jhadu (Assamese), Bouquet grass, Tiger grass (English), Boom grass (English) (BISHT and AHLAWAT, n.d.)

1.3.3 Distribution

This species has recently been reported growing away from cultivation in south-eastern and northern Queensland. A single plant was noticed growing on the edge of a waterway in Moorooka in the southern suburbs of Brisbane in 2009. More recently, in July 2011, a larger population of numerous large clumping plants was found scattered along the steep banks of a waterway in the southern suburbs of Cairns. (Technigro, 2011)

1.3.4 Morphology

Huge tufted grass, up to 3 m tall, culms solid, leaf-sheaths at least the upper ones, tight, glabrous, terete, smooth, the nodes glabrous, margins with some short stiff hairs towards the throat ; blades lanceolate-acuminate, abruptly contracted to a short petiole for a subcordate base, acuminate to a fine point, glabrous, the margins scaberulous, upto 50 cm long and 7 cm wide ; ligule a shallow membrane 1-2 mm deep, backed by short stiff hairs ; Inflorescence a huge and drooping panicle 60 - 90 cm long or more wide at anthesis, the axis and branches at first rounded, ultimately, capillary, not sharply angled ; spikelets numerous, often in pairs on a common peduncle, each pedicel distinct ; lower glume clasping, ovate-acute, obscurely 1 nerved, upto 6.5 mm long ; upper glume more transparent ; lower- lemma lanceolate-acuminate, sub-hyaline, with 1 or 2 long setose hairs near the margin ; upper lemma lanceolate-acuminate, 3 nerved, green between the nerves, hyaline thence to the margin, with stiff setose hairs along the hyaline portion on both sides ; palea a narrow, 2 nerved, hyaline scale ; stamens 2 (3) ; stigmata 2, plumose; reddish brown, the rachilla continues as a flattened process with an expanded tip, beyond and behind the upper lemma. The aspect of the spikelets changes with the onset of anthesis when the upper lemma emerges and its setose hairs gradually adopt a stance at right anglesto the lemma's surface. (Bisht and Ahlawat, n.d.)

1.3.5 Classification

Kingdom Plantae

Subkingdom Trachebionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Liliopsida

Subclass Commelinidae

Order Cyperales

Family Poaceae

Genus Thysanolaena

Species *Thysanolaena maxima* (Roxb.) Kuntze

(Plants.usda.gov, n.d.)

1.3.6 Natural regeneration

It regenerates through seeds under natural condition. The seeds mature during February to March and disseminate by wind to long distances due to their lightweight. Seed dispersal is also affected by water in some areas. The seed germinates in the beginning of the rainy season. The seedling establishment and the growth is good on loose and exposed areas such as landslides and freshly disturbed soil specially near road construction sites where light availability is good. (Bisht and Ahlawat, n.d.)

1.3.7 Artificial regeneration

Cultivation of broom-grass is comparatively easy and requires less financial inputs. It can be grown on marginal lands, wastelands and jhum fallow. It grows well on a wide range of soils varying from sandy loam to clay loam. The planting can be done by seeds or rhizomes. Some people also collect and transplant the wild seedlings for propagation but it are always better to get quality seedlings from reputed nurseries. (Bisht and Ahlawat, n.d.)

1.3.8 Medicinal use of Tiger grass

1. It can be used as a tonic for drink. (Chiramongkolgarn and Paisooksantivatana, n.d.)
2. To reduce vomiting tendency, and stomach trouble crushed flowers can be taken with water. (Rahmatullah et al., 2013)
3. Roots are used in treatment of flatulence. (Maity *et al*., 2004)
4. A paste of flower along with country liquor and honey acts as traditional contraceptive for women. (Maity *et al.*, 2004)
5. Decoction of roots along with salt is a remedy for mouth sore. (Maity *et al*.,2004)

CHAPTER 2

LITERATURE REVIEW

2.1 Mechanical and dielectric Properties of *Thysanolaena Maxima* (broom grass) long fiber reinforced polyester composites

A new natural fiber i.e. broom grass botanically called "*Thysanolaena maxima*" is introduced in the present work with a view to understand its properties initially. Then the untreated and chemically treated fiber is reinforced into the polyester matrix and the composites are fabricated to test their mechanical and dielectric properties strictly as per ASTM procedures. The highest tensile strength of 82.39 MPa, modulus of 1.05 GPa is obtained for broom grass CT – 1, CT – 2 fibres respectively. With CT – 2, 3 broom grass FRP composites achieved highest tensile strength, modulus respectively at maximum fiber volume fraction. Broom grass CT – 2 FRP composites have shown good flexural strength, modulus of 78.51 MPa, 5.31 GPa respectively than the other composites investigated in the present work. Impact strength of 91.11 kJ/m2 is achieved for broom grass FRP composites at 39.35% fiber volume fraction. The insulating light weight material according to required dielectric strength is selected from the composites reinforced with broom grass at different volume fractions of fiber. Fiber morphology is understood from the scanning electron micrographs of fiber. (Srinivasababu *et al*., 2014)

2.2 Antimicrobial Activity of Some Medicinal Plants from East and Central Part of Nepal

Seven indigenous medicinal plants, *Mallotus philippensis, Pogostemon cablin, Colebrookea oppositifolia, Mussaenda macrophylla, Celosia argentea, Pilea symmeria and Thysanolaena maxima*, have been investigated for their antimicrobial activity and antioxidant activity. The ethanol extract of these medicinal plants were subjected to evaluate their antibacterial properties and their antioxidant potential. The antibacterial screening against four bacteria, *Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris and Escherichia coli*, was done by disc diffusion method and Zone of Inhibition (ZOI) was observed. The ZOI obtained ranges from 7 to 16 mm. The Antioxidant activity of the extract was tested using scavenging activity of DPPH (1, 1-Diphenyl-2- Picrylhydrazyl) radical method. Ascorbic acid was taken as standard. IC50 of four extracts were obtained < 100 μg/ml whereas three had > 100 μg/ml. The overall result shows that almost all the plant extracts have interesting antibacterial activity and among seven, four had remarkable radical scavenging potential to be used as an antioxidant. (Basnet and Subba, 2014)

2.3 Existing marketing system and economic analysis of Broom grass (*Thysanolaena maxima* Roxb: Poaceae)

A comprehensive field survey was made throughout Chittagong, Cox's Bazar, Bandarban, Rangamati and Khagrachari hill districts during 2007-2010 to study the species diversity, processing, existing marketing system, export potentialities, economic implication of article processed from broom grass (*Thysanolaena maxima* Roxb: Poaceae). Another field experiment was also conducted to know the Benefit-Cost (B/C), IRR ratio for economic analysis of this species. Seven different types of broom grass were identified in the study areas. The local name of these broom grass were Moishaphool, Jathiphool, Shadajathiphool, Khairijathiphool, Lalchejathiphool, Harinaphool and Biniphool. Those broom grass had 4 different panicle colors i.e. black, red, green and golden. Among them Jathiphool, Harinaphool and Biniphool were found exportable. After harvesting these were dried, binded and then transported to different places for selling through a certain channel. Geometric increase of price of broom grass in each selling stage was also observed. From harvesters to wholesale market it become double, after processing it increased 10th times, and in rural and urban area it increased around 15th and 18th times, respectively. The price increased 20th times from the harvesters' price when these were exported. The revenue collection from broom grass in Rangamati hill tracts was Tk 406300.00 in the year 2006-2007. January to July was found broom grass released period in the year, where February was the pick-period and highest revenue (Tk 274600.00) was earned in the month of February. The field experiment findings revealed that B/C ratio at 10, 15 and 20% AIR was 1.61, 1.54 and 1.48, respectively and IRR 69% at 0.50 ha experimental plantation of T. maxima. (Alam *et al*., 2013)

2.4 A viable model for broom grass cultivation and management in Tripura

Realizing severe decrease in broom grass production in wild and ever increasing market potential, it has become the need of hour to domesticate it in farmer's fields. This can be achieved through sustainable cultivation under agro forestry. Introducing the plantation in a new site has many implications such as suitability of land, willingness of farmers for sparing their own fields, choice of perennial and seasonal but profitable and suitable intercrops and market

linkages. The participatory approach adopted at every stage, established a pathway to reach the objectives taking care of all the implications. Extension strategies by using SWOT exercise have been significantly effectual to understand the problems and prospects. The analysis helps to motivate farmers for participatory planning and execution of the On-farm research and demonstration. The observations and experiences from the study site reveal that the practice is much viable (Benefit-Cost Ratio of 6.21) and sustainable. It has a potential scope for extension in Tripura and north-east hill regions of India as well. (Kaushik and D, 2014)

2.5 Effect of plant density on growth and yield of *Thysanolaena maxima*: important non-timber forest product of Meghalaya

Thysanolaena maxima is a wild grass cultivated by the farmers of Meghalaya. When the demand for broom increased, many erstwhile shifting cultivators got motivated to take up cultivation of this plant.In this paper; they report the findings of field experiments conducted to investigate the effect of plant density on growth and yield of T.maxima. The experiments were laid in Mynska village of Meghalaya and the study was conducted between July 2012 and February 2014 using Randomized Complete Block Design with four replicates and five spacing treatment. The study revealed that the growth and yield parameters are not impacted by plant density during the first year of its growth. During the second year, the effect of density on growth and yield became pronounced and 1.5x2.0 m spacing gave optimum number of tiller, tiller diameter, internodal length, leaf number, panicles number, harvest index and height and diameter of tussock. The yield of panicles was however maximum in the treatment 1.0x1.0 m spacing. The study concludes that up 1.5x2.0 m spacing may be adopted if farmers are interested for green biomass (fodder). However, foroptimum production of broom grass panicles (broom), 1.0x1.0 m spacing is most appropriate. (Lapasam and B.K, 2015)

2.6 Fungi on the grasses, *Thysanolaena latifolia* and *Saccharum spontaneum*, in northern Thailand

Fungi associated with dead leaves and stems of *Thysanolaena latifolia* and *Saccharum spontaneum* were collected and identified at two sites. T. latifolia yielded 67 taxa, comprising 24 ascomycetes ,33 hyphomycetes, 9 coelomycetes and 1 myxomycete. The most common genera

were Leptosphaeria, Niptera, Periconia, Septoria, Stachybotrys, Tetraploa, and Verticillium. S. spontaneum yielded 79 taxa comprising 32 ascomycetes, 37 hyphomycetes, and 10 coelomycetes. The most common genera were Cladosporium, Massarina, Periconia and Tetraploa. The highest species diversity index was recorded on S. spontaneum (H = 6.5), while T. latifolia was lower (H = 5.5).The mycota at the two sites differed significantly in species composition. Percentage similarity for *T. latifolia* between the two sites was 50.5% while for *S. spontaneum* it was 52.3 %. (W et al., 2010)

2.7 Medicinal Plants in Tao Dam Forest, Wangkrajae Village, Sai Yok District, Kanchanaburi Province

Ethnobotanical survey of medicinal plants in Tao Dam Forest, Wangkrajae village, Sai Yok district, Kanchanaburi province, was conducted at 2 months interval during June 1999 to June 2000, in order to reveal the forest potential as medicinal plant resources. Both dried and living specimens were deposited at the herbarium, Department of Horticulture, Kasetsart University, Kamphaengsaen Campus. Three hundred and fifteen species were identified. Thirty-seven species were used as medicines.There are two groups of uses: internal uses (31 spp.) and external uses (6 spp.). Six species were also used as vegetables. In addition, five species were new recorded of medicinal uses. (Chiramongkolgarn and Paisooksantivatana, n.d.)

2.8 Medicinal formulations of a Kanda tribal healer – a tribe on the verge of disappearance in Bangladesh

The Kanda tribe is one of the lesser known small tribes of Bangladesh with an estimated population of about 1700 people (according to them), and on the verge of extinction as a separate entity. To some extent, they have assimilated with the surrounding mainstream Bengali-speaking population, but they still maintain their cultural practices including traditional medicinal practices, for which they have their own tribal healers. Nothing at all has been documented thus far about their traditional medicinal practices and formulations, which are on the verge of disappearance. The Kanda tribe can be found only in scattered tea gardens of Sreemangal in Sylhet district of Bangladesh; dispersion of the tribe into small separated communities is also contributing to the fast losing of traditional medicinal practices. The objective of the present

study was to conduct an ethnomedicinal survey among the traditional healers of the Kanda tribe (in fact, only one such healer was found after extensive searches). Information was collected from the healer with the help of a semi-structured questionnaire and the guided field-walk method. A total of 24 formulations were obtained from the healer containing 34 plants including two plants, which could not be identified. Besides medicinal plants, the Kanda healer also used the body hairs of the Asiatic black bear (Ursus thibetanus) and bats (Pteropus giganteus giganteus) in one of his formulation for treatment of fever with shivering. The ailments treated by the Kanda healer were fairly common ailments like cuts and wounds, skin diseases, helminthiasis, fever, respiratory problems (coughs, asthma), gastrointestinal disorders (stomach pain, constipation, diarrhea), burning sensations during urination, various types of pain (headache, body ache, toothache, ear ache), conjunctivitis, poisonous snake, insect or reptile bites, jaundice, and bone fractures. A number of important drugs in allopathic medicine like quinine, artemisinin, and morphine (to name only a few) have been discovered from observing indigenous medicinal practices. From that view point, the formulations used by the Kanda healer merit scientific studies for their potential in the discovery of cheap and effective new drugs. Scientific validation of the medicinal formulations of the Kanda healer can also be effective for treatment of ailments among this tribe, which does not have or does not want to have any contact with modern medicine. (Rahmatullah *et al*., 2013)

2.9 Phytostabilization Potential of Pb Mine Tailings by Two Grass Species, *Thysanolaena maxima* and *Vetiveria zizanioides*

Pot and field experiments were conducted to elucidate the phytostabilization potential of two grassspecies *(Thysanolaena maxima* and *Vetiveria zizanioides)* with respect to lead (Pb) tailing soil. Three fertilizers (Osmocote® fertilizer, cow manure, and organic fertilizer) were used to improve the physicochemical properties of tailing soil. *V. zizanioides* treated with organic fertilizer and cow manure showed the highest biomass (14.0±2.6 and 10.5±2.6 g per plant, respectively) and the highest Pb uptake in the organic fertilizer treatment (*T. maxima*, 413.3 μg per plant; *V. zizanioides*, 519.5 μg per plant) in the pot study, whereas in field trials, *T. maxima* attained, the best performances of dry biomass production (217.0±57.9 g per plant) and Pb uptake (32.1mg per plant) in the Osmocote® treatment. In addition, both grasses showed low

translocation factor (<1) values and bioconcentration coefficients for root (>1). During a 1-year field trial, *T.maxima* also produced the longest shoot (103.9±29.7 cm), followed by *V. zizanioides* (70.6±16.8 cm), in Osmocote® treatment. Both grass species showed potential as excluder plants suitable for phytostabilization applications in Pb contaminated areas. (Meeinkuirt *et al*., 2013)

2.10 Folk uses of some medicinal plant from North Sikkim

The local inhabitants in the North Sikkim area have inherited rich traditional knowledge of the use of many plants or plant parts for the treatment of their common diseases. They often have the information on how to use the plants and to take or to apply the medicine for different diseases and health care. Information on medicinal uses of 15 types of tubers, rhizomes or roots used by the inhabitants of North Sikkim, viz. Lepchas, Nepalese and Bhutias is presented here. (Maity, Pradhan and Chauhan, 2004)

2.11 Sustainable management of degraded jhum fallow through plantation of *Thysanolaena maxima* (roxb.) o. kutze (broom grass) in different spacing trial

Shifting cultivation (Jhum) is a traditional and cultural integrated form of agricultural system now considered as a major cause of environmental degradation having a disastrous impact on the ecology. It is blamed as the causal factor of deforestation, loss of biodiversity, soil erosion, lowering productivity, depletion of soil fertility and finally deepening impoverishment of jhum dependent communities. Rehabilitation of such degraded shifting cultivation land through plantation of non timber forest produce like broom grass is a means of sustainable land management. This grass is considered as multipurpose, non-perishable cash crop that can withstand a wide range of agro-climatic conditions. Present study revealed better performance of growth in cultivation of broom grass in 2.5m spacing trial while highest yield was recorded in 2m spacing. From the study conducted in two successive years it was observed that the production of brooms was significantly high in selected individuals than the locally available plants. Cultivation of selected varieties of broom grass for two consecutive years contributes a profit of Rs. 8200/- per hectare. (Barua *et al*., 2011)

2.12 The chemopreventive effects of *Thysanolaena latifolia* against carbon tetrachloride (CCl_4)-induced oxidative stress in rats.

Present study was undertaken to evaluate the chemopreventive effects of *Thysanolaena latifolia* against carbon tetrachloride (CCl_4)-induced oxidative stress in rats. (Gnanaraj *et al*., 2012)

2.13 Paper from *Thysanolaena maxima*

Thysanolaena maxima (Roxb) (tiger or broom grass), is a tall reed-like perennial grass, grown on shady slopes in forests in India and the Nicobar Islands. Culms are solid, smooth and rounded and up to a height of 4 m. The leaves and the tips are used as fodder; the bushy, fox-tail-like panicles (30–90 cm) are used for making brooms. When the panicles are cut, the stem portion (3–4 m) is left out in the field and is burnt. Fibers, of average 1.25 mm in length at 45% yield (unbleached), could be obtained from this grass. The laboratory handmade paper sheets exhibited good properties, with a burst factor of 30, a breaking length of 3555 m and a tear factor of 106. Hence, it can be suggested that T. maxima may become a potential source of raw material for pulp- and paper-making either alone or in combination with the conventional pulp- and paper-making raw materials. This material could help to meet the future demand for pulp- and paper-making raw material, if properly exploited. (Saikiz *et al.*, 1992)

2.14 Growth pattern, production, and marketing of *Thysanolaena maxima* (Roxb.) Kuntze : An important non-timber forest product of Meghalaya, India.

Broomgrass is an important semi-domesticated non-timber forest produce of Meghalaya. The distribution, growth pattern, biomass production, and productivity of broomgrass processing and the cost-benefit analysis of cultivation, along with marketing linkages of the product, have been studied using standard methods employed in ecological and socio-economic research. The study revealed that broomgrass grows in all parts of Meghalaya below 1,600 m.a.s.l., on a wide range of soils. An analysis of growth parameters, namely leaf number, leaf area index, basal area, canopy cover, and tussock height, revealed that broomgrass is a fast growing perennial grass that

attains maximum growth in four years. The cost-benefit ratio analysis of broomgrass cultivation was found to be 3.46, 3.32, and 3.19 at 10, 15, and 20% annual interest rates, respectively. The study concludes that broomgrass cultivation could be an effective instrument for generation of cash income in rural Meghalaya, as its cultivation needs minimum input of labour and generates a very attractive economic return. (Tiwari et al., 2012)

CHAPTER 3

MATERIALS

&

METHODS

3.1 Preparation of Plant Extract for Experiments

3.1.1 Materials:

Reagent:

1. Methanol

Equipments:

1. Beaker
2. Funnel
3. Glass rod
4. Grinding machine
5. Filter paper
6. Cotton
7. Separating funnel

3.1.2 Collection & Preparation of Plant Material

Thysanolaena Maxima plant was collected from Chittagong Hill tracts. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24hours at considerably low temperature for better grinding. The dried leaves was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.1.3 Washing and Drying of *Thysanolaena Maxima* Plant

At first the leaves were thoroughly washed with tap water to remove dust, soil, bird's droppings etc. within them. The leaves were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 500C for 2 hours.

3.1.4 Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of

previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 700 g and was measured using electronic balance.

3.1.5 Extraction of the Dried Powdered Sample

The fine powder of *Thysanolaena Maxima* leaves was dissolved in 8000 ml methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.1.6 Filtration of the Extract

After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper was prepared for rotary evaporation.

3.1.7 Solvent Evaporation

The filtrate was kept in rotary evaporator for complete evaporation of the solvent. The solution was also kept in the hot plate and stirred frequently for solvent evaporation. After running this procedure, a gummy extraction was obtained which was preserved in refrigerator.

3.2 Principle of a Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (e.g., "the sample was evaporated under reduced pressure").Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts.

Figure-3.1: Rotary Evaporator

A simple rotary evaporator system was invented by Lyman C. Craig. It was first commercialized by the Swiss company Büchi in 1957. Other common evaporator brands are Heidolph, LabTech, Stuart, Hydrion Scientific, SENCO, IKA and EYELA. In research the most common form is the 1L bench-top unit, whereas large scale (e.g., 20L-50L) versions are used in pilot plants in commercial chemical operations.

3.3 Test for CNS activity

3.3.1 Experimental design

Twenty four experimental animals were randomly selected and divided into four groups denoted as experimental group *Thysanolaena Maxima* methanol part (200mg, 400mg) , negative control group and positive control group . Each group of mouse was weighed properly & dose of the test sample & control materials was adjusted accordingly.

3.3.2 Drugs and chemicals

1. Carboxy methyl cellulose (CMC)

2. Water for injection

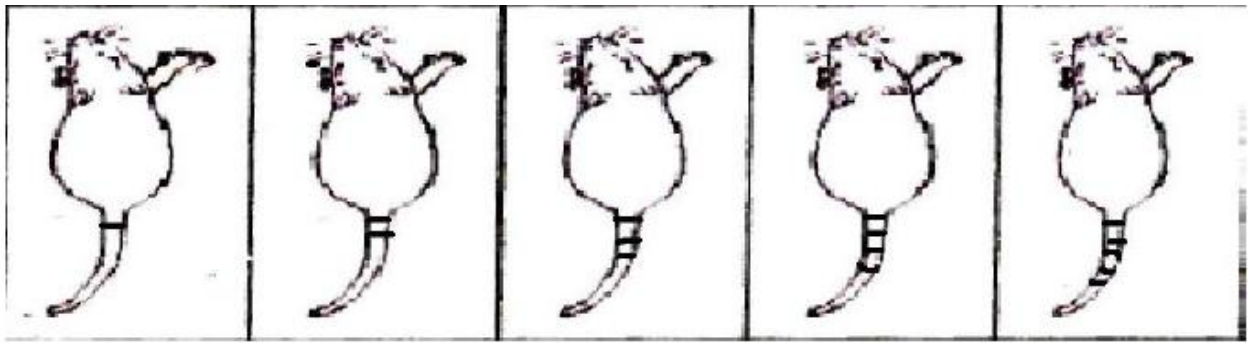
3. Diazepam

4. *Thysanolaena Maxima* (methanol part)

3.3.3 Method of identification of animals

Each group consists of five animals. It was difficult to observe the biological response of six mice at a time receiving same treatment. It is quite necessary to identify individual animal of groups during treatment. The animals were individualized in the following way i.e. marked as

M_1=mice 1, M_2=mice 2, M_3=mice3, M_4=mice 4, M_5=mice 5 & M_6=mice 6.



3.3.4. Animal

For the experiment male Swiss albino mice of 3 - 4 weeks of age, weighing between 20 - 25 gm, were collected from the laboratory of Jahangirnagar University. Animals were kept in standard environmental conditions and had free access to feed and water.

Figure-3.2: Image of mice

3.3.5. Preparation of test material

In order to administer the crude extract of Methanol at dose 200 & 400 mg/kg body weight of mice. The extract was collect by calculating of mice weight & was triturated in unidirectional way by the addition of 3 ml of distilled water. For proper mixing, small amount of suspending agent CMC was slowly added. The final volume of the suspension was made 1.2 ml. The final volume of the suspension was made 1.2 ml. To stabilize the suspension it was stirred well. For the preparation of positive control group (5 mg/kg) Diazepam is taken & a suspension of 1.2 ml is made.

3.3.6 Open field test

This experiment was carried out as described by Gupta (Gupta et al., 1971). The animals were divided into control and test groups containing 6 mice each. The test group received Geodorumdensiflorum extract at the doses of 200 and 400 mg/kg body weight orally whereas the negative control group received vehicle (CMC in water) & positive control group received Diazepam (1mg/kg).The floor of an open field of half square meter was divided into a series of squares each alternatively coloured black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 & 120 min after oral administration of the test drugs. Then measure the behaviors' Line crossing, Center square, Peripheral crossing, Rearing,Leaning, Grooming, Defecation.

Figure-3.3: Open Field Method

CHAPTER 4

RESULT

&

DISCUSSION

4.1 Result

Open Field Method

Table-4.1: Number of central movement

Treatment	0 min	30 min	60 min	90 min	120 min
Standard	7.00± 3.36	2.50± 0.28	2.25± 0.25	2.00± 0.00	2.50±0.28
Control	2.00± 0.00	2.50± 0.50	4.75± 1.70	3.50± 0.50	2.25±0.25
Methanol 200mg	2.75± 0.25	2.75± 0.25	2.50± .50	2.50± 0.50	2.25± 0.75
Methanol 400mg	2.00± 0.00	2.00± 0.00	1.75± 0.25	2.00± 0.00	1.00± 1.00

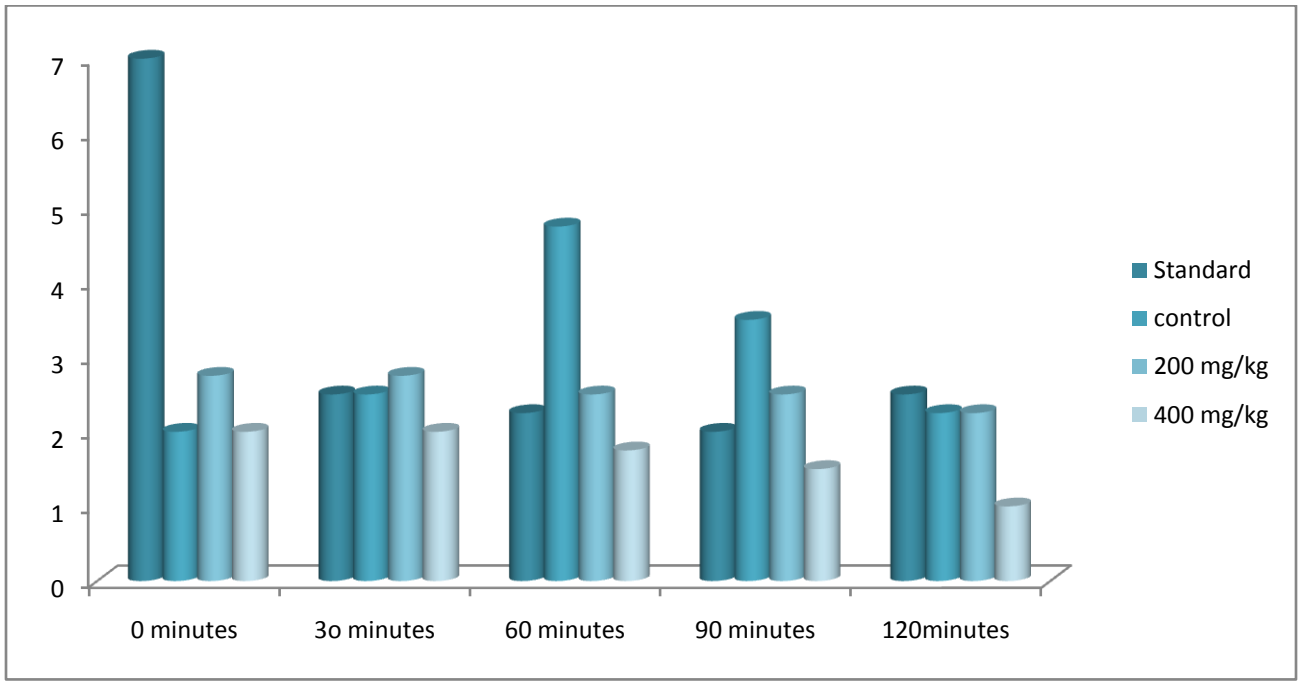


Figure-4.1: Graphical representation of number of central movement

From Figure-4.1 we can see the central movement has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

Table-4.2: Number of peripheral movement

Treatment	0 min	30 min	60 min	90 min	120 min
Standard	158.25± 17.54	72.00±2.12	69.00± 15.74	71.00±25.43	92.50± 8.41
Control	93.50± 17.62	83.75±7.04	73.50± 11.92	44.25 ± 16.94	52.00± 15.01
Methanol 200mg	24.50± 12.14	55.75±13.03	42.50± 14.20	24.00±1.68	35.00± 11.00
Methanol 400mg	22.75± 11.30	47.98±23.99	44.75± 11.18	32.25± 20.88	21.75± 14.46

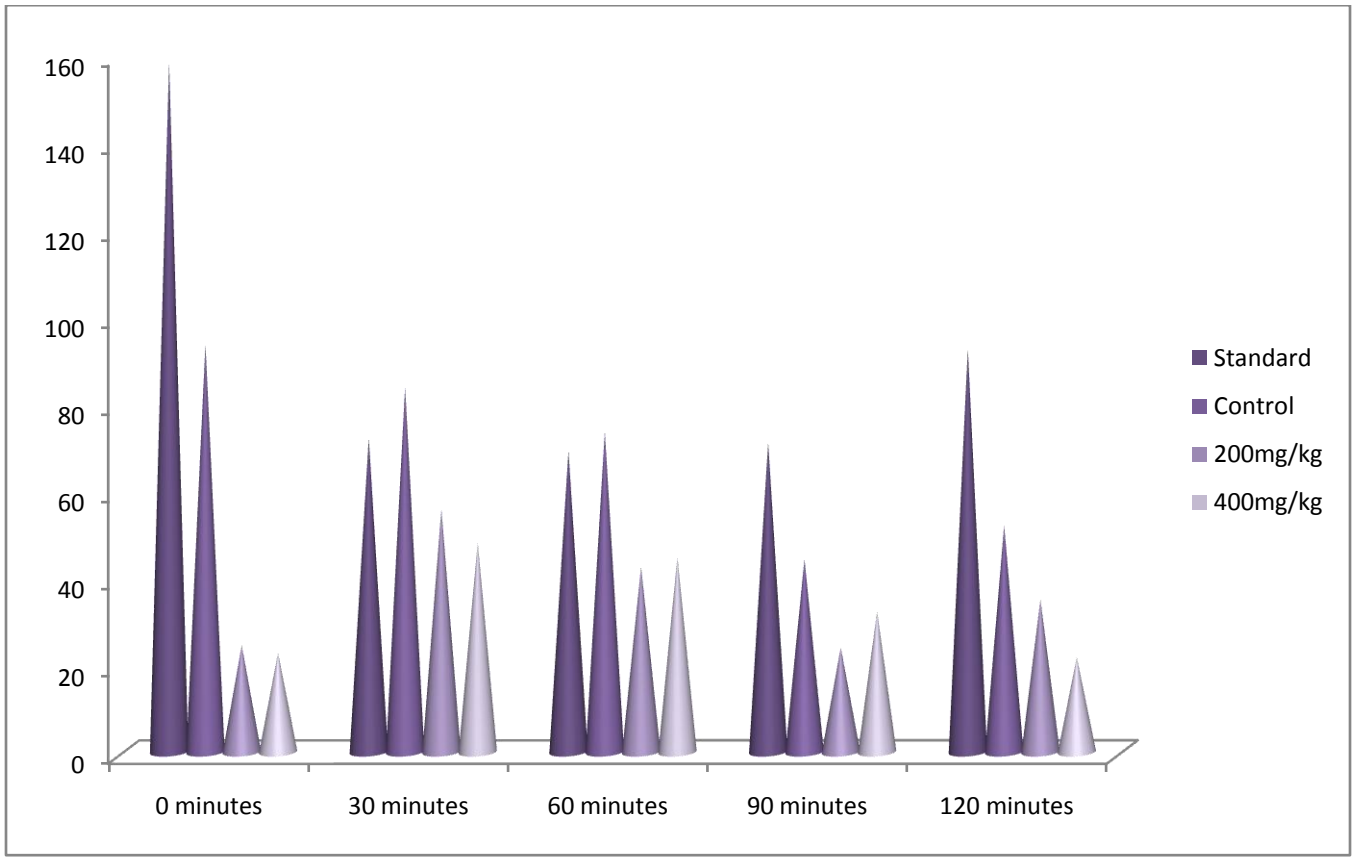


Figure-4.2: Graphical representation of number of peripheral movement

From Figure-4.2 we can see the peripheral movement has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

Table-4.3: Number of leaning

Treatment	0 min	30 min	60 min	90 min	120 min
Standard	7.75±1.79	3.25± 0.62	1.75± 0.85	1.50± 1.50	1.25± 1.19
Control	12.50±3.09	16.25± 3.40	13.50± 1.25	11.25± 2.17	10.75± 3.62
Methanol 200mg	11.00±0.00	9.50± 1.25	9.75± 3.42	5.50± 2.50	1.50± 0.86
Methanol 400mg	10.25± 0.25	6.50± 2.10	6.50±1.84	1.50± 1.50	1.25± 0.47

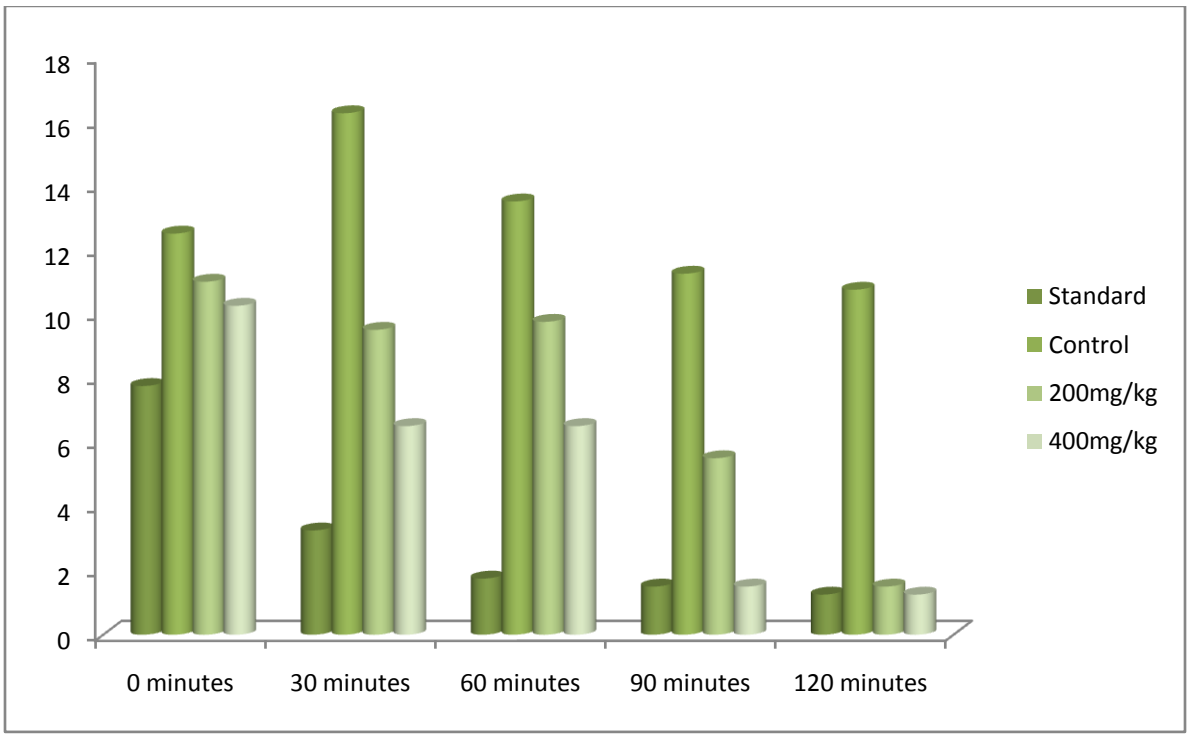


Figure-4.3: Graphical representation of leaning count

From Figure-4.3 we can see the leaning count has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

Table-4.4: Number of grooming

Treatment	0 min	30 min	60 min	90 min	120 min
Standard	1.75± 0.75	3.25± 0.85	4.50± 0.28	6.75± 0.75	7.00± 0.00
Control	2.00±1.08	5.75± 1.49	6.25±1.10	8.50±1.58	13.25± 0.47
Methanol 200mg	17.50±1.19	19.00± 4.32	22.75±1.93	27.00± 0.91	29.00±3.42
Methanol 400mg	19.00±5.12	20.00± 1.04	23.50±0.64	25.75±0.75	29.75±0.62

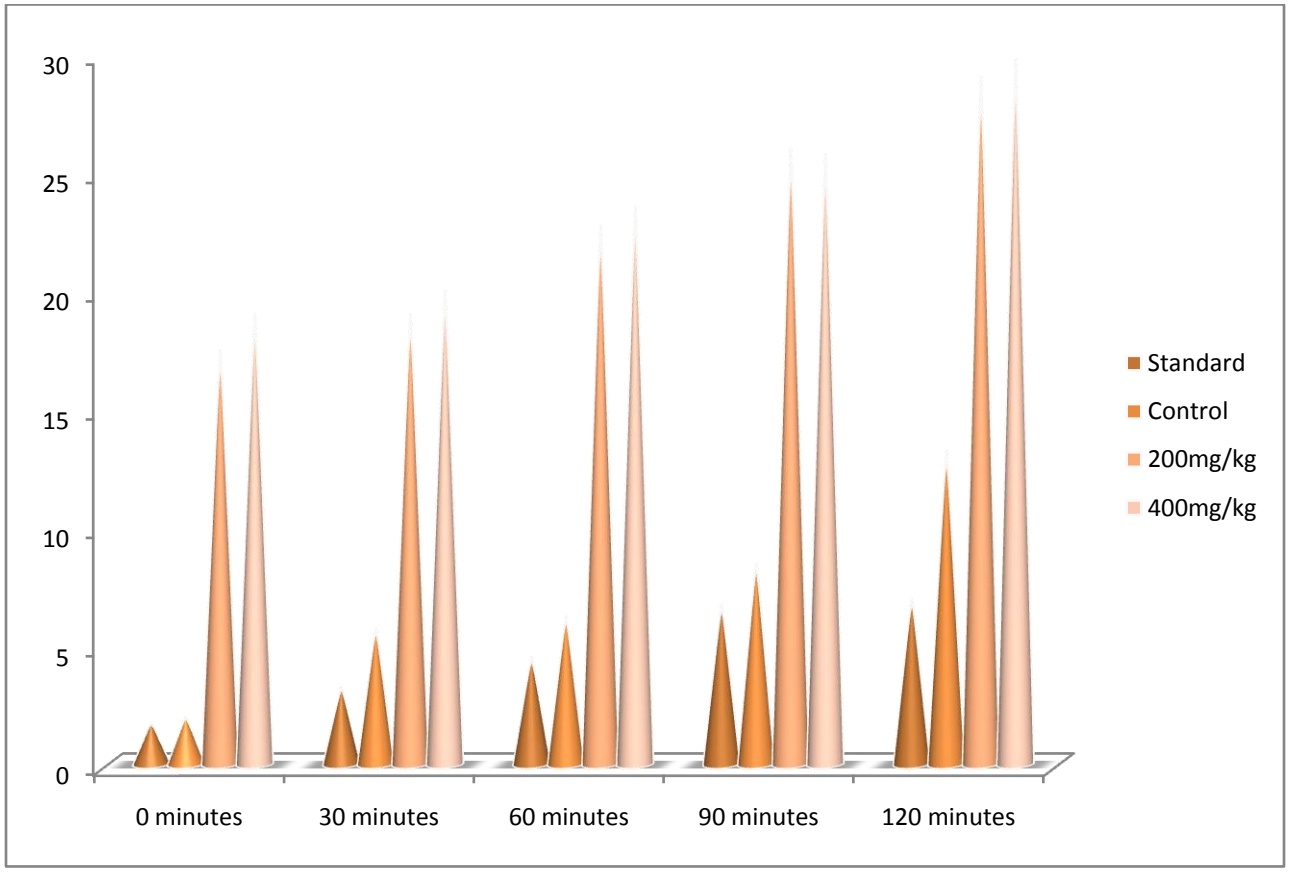


Figure-4.4: Graphical representation of grooming count

From Figure-4.4 we can see the grooming count has increased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

Table-4.5: Number of defecation

Treatment	0 min	30 min	60 min	90 min	120 min
Standard	1.50±0.64	1.00± 0.57	0.00± 0.00	0.50± 0.28	.50± 0.40
Control	1.75±0.47	1.50± 0.64	1.50± 0.40	1.75± 0.75	1.25± 0.86
Methanol 200mg	1.50± 0.28	0.00± 0.00	1.25± 0.25	1.25 ± 0.25	0.75± 0.25
Methanol 400mg	1.00± 0.81	0.75± 0.64	0.50± 1.25	0.25 ± 0.25	0.25± 0.28

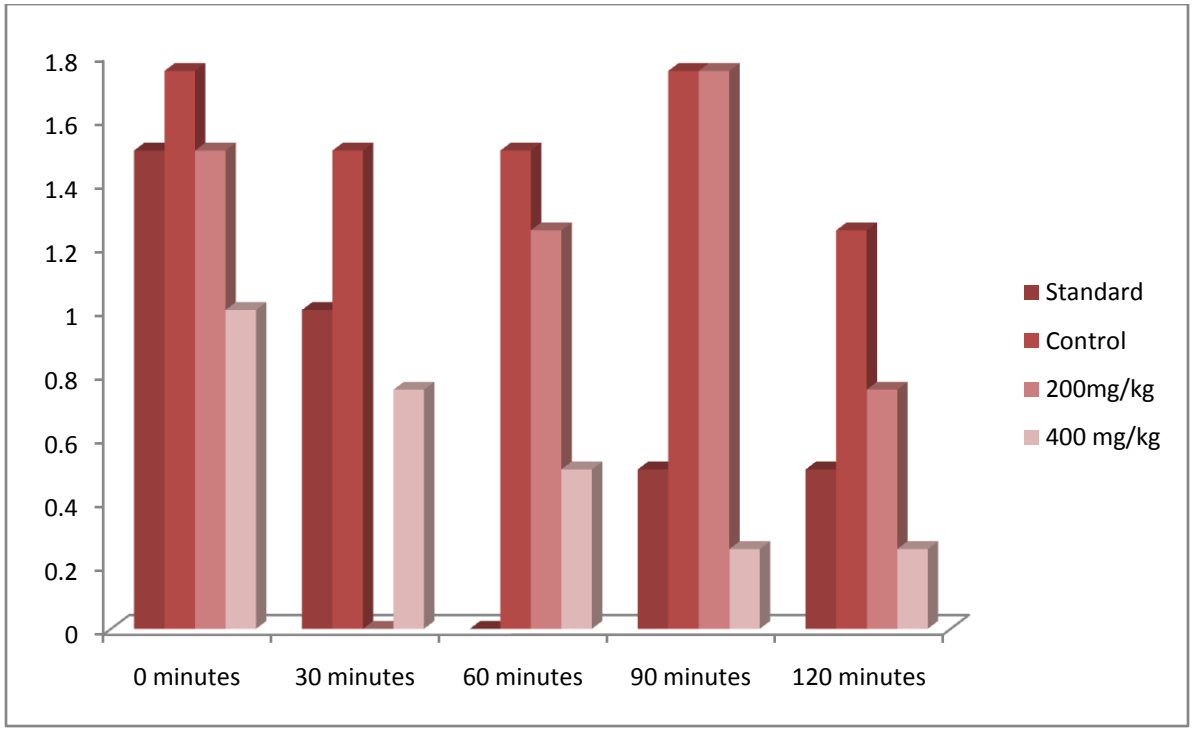


Figure-4.5: Graphical representation of defecation count

From Figure-4.5 we can see the grooming count has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

4.2 Discussion

According to a previous study, CNS depressant activity of Ethanol extract of *Cymbidium aloifolium (L.)* was evaluated by open field test, which has clearly verified the CNS depressant activity evidenced by decreased fall off time. A most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. Inhibitory effects on spontaneous motor activity of the plant extract indicated depressant activity. The movement is a measure of the level of excitability of the CNS and its decrease may be intimately related to sedation resulting from depression of the CNS.(Howlader and Alam,2011)

In the present study, the effect of methanol extract of *Thysanolaena maxima* on CNS has been evaluated for the doses of 200mg/kg and 400 mg/kg by using 'open field method' to two groups of mice. A positive control group was given diazepam with the dose of 1mg/kg and a negative control group was given 1% carboxy methyl cellulose solution.

From table 4.1 it is found that, methanol extract of *Thysanolaena maxima* 200 mg/kg the central locomotion was started from 2.75± 0.25 at 0 minute and ended in 2.25± 0.75 at 120 minutes. Methanol extract of *Thysanolaena maxima* 400 mg/kg the central locomotion was started from 2.00± 0.00 at 0 minute and ended in 1.00± 1.00 at 120 minutes. we can see the central movement has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

From table 4.2 it is found that, methanol extract of *Thysanolaena maxima* 200 mg/kg the peripheral locomotion was started from 11.00±0.00 at 0 minute and ended in at 120 minutes. Methanol extract of *Thysanolaena maxima* 400 mg/kg the peripheral locomotion was started from 22.75± 11.30 at 0 minute and ended in 21.75±14.46 at 120 minutes. We can see the peripheral movement has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

From table 4.3 it is found that, methanol extract of *Thysanolaena maxima* 200 mg/kg the leaning count was started from 24.5±12.14 at 0 minute and ended in 1.50± 0.86 at 120 minutes. Methanol extracts of *Thysanolaena maxima* 400 mg/kg the leaning count was started from 10.25± 0.25 at 0 minute and ended in 1.25± 0.47 at 120 minutes. We can see the leaning count has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

From table 4.4 it is found that, methanol extract of *Thysanolaena maxima* 200 mg/kg the grooming count was started from 17.50±1.19 at 0 minute and ended in 29.00 ± 3.42 at 120 minutes. Methanol extracts of *Thysanolaena maxima* 400 mg/kg the grooming count was started from 19.00±5.12 at 0 minute and ended in 29.75±0.62 at 120 minutes. We can see the grooming count has increased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

From table 4.5 it is found that, methanol extract of *Thysanolaena maxima* 200 mg/kg the defecation count was started from 1.50± 0.28 at 0 minute and ended in 0.75± 0.25 at 120 minutes. Methanol extracts of *Thysanolaena maxima* 400 mg/kg the defecation count was started from 1.00± 0.81 at 0 minute and ended in 20.25± 0.28 at 120 minutes. We can see the defecation count has decreased for both the dose 200mg/kg and 400 mg/kg as the time proceeds.

The result indicated that the extract decreased locomotor activity which indicates it has CNS depressant activity. Locomotor activity refers to an increase in alertness and decrease in locomotor activity considered as sedative effect.

Conclusion

In conclusion, *Thysanolaena maximum is* a plant which is mostly use with a view to ornamentation and rarely used traditionally. This research was the first attempt to investigate its effect on central nervous system. It could be suggested that the crude methanol extract of *Thysanolaena maxima* surely possess central nervous system depressant activities. However, further research is needed to be carried out to determine the precise mechanisms involved as well as the chemical constituents responsible for the pharmacological activities.

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