CNS DEPRESSANT ACTIVITY OF METHANOL EXTRACT OF *Thysanolaena maxima* BY HOLE CROSS 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.

Submitted By

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DECLARATION BY THE RESEARCH CANDIDATE

I, Fahmida Alam Ria, ID: 2012-1-70-010, hereby declare that the dissertation entitled **"CNS Depressant Activity of Methanol Extract of *Thysanolaena maxima* by Hole Cross Method''** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine & authentic research work carried out by me. The contents of this dissertation, in full or in parts, have not been submitted to any other institute or University for the award of any degree or Diploma of Fellowship.

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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled **"CNS Depressant Activity of Methanol Extract of *Thysanolaena maxima* by Hole Cross Method''** is a bona fide research work done by Fahmida Alam Ria (ID: 2012-1-70-010), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy under my supervision.

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This is to certify that the dissertation, entitled **"CNS Depressant Activity of Methanol Extract of *Thysanolaena maxima* by Hole Cross Method''** is a bona fide research work done by Fahmida Alam Ria (ID: 2011-1-70-010), under the guidance of **Nazia Hoque**, Senior Lecturer, Department of Pharmacy, in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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DEDICATION

This research paper is dedicated to my beloved parents and my friend Mohsin Ibna Amin for their unconditional support.

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ALS	Amyotrophic Lateral Sclerosis
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DMT	Dimethyltryptamine
GABA	Gamma-Aminobutyric Acid
GC	Gas Chromatography
GHB	Gamma Hydroxybutyrate
HPLC	High Performance of Liquid Chromatography
LAI	Leaf Area Index
LSD	Lysergic Acid Diethylamide
METM	Methanol Extract of *Thysanolaena Maxima*
nAChR	Nicotinic Acetycholine Receptor
PET	Positron Emission Tomography
SEM	Standard Error of the Mean
SPSS	Statistical Package for Social Sciences
TIA	Transient Ischemic Attack
TLC	Thin Layer Chromatography
TM	*Thysanolaena maxima*
ZSS	*Zizyphi spinosi semen*

Abstract

Thysanolaena maxima are a perennial grass plant found in hilly regions of Nepal, northern and eastern parts of India, Bhutan, and Philippines. The flowers of this plant are used as cleaning tool or broom, which is known as *kuchcho* in Nepali. It also is called "broom grass" in areas where its flowers are used as a cleaning tool. 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. *Thysanolaena maxima* (TM) have been studied for antioxidant, antimicrobial and chemo preventive effects. However, scientific information on TM regarding the neuropharmacological effect is limited. This study was designed to evaluate the central nervous system (CNS) depressant activity of the methanolic extract of *Thysanolaena maxima* (METM). For this purpose, the whole plants of *T. maxima* were extracted with methanol. Diazepam at the dose of 1 mg/kg was used as a reference drug in the experiment. Swiss male albino mice of 20–25 g were used in the study. The animals were kept in standard laboratory and were provided with standard diet and clean water. CNS depressant study of the extract (200mg/kg and 400 mg/kg body weight) was done by hole-cross test. A significant decrease in locomotor activity was observed at all doses in the hole-cross test. The study suggests that METM may possess moderate to high CNS depressant activity. In conclusion, further investigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported CNS depressant properties of *Thysanolaena maxima.*

Key words: *Thysanolaena maxima, CNS depressant, Diazepam*

CHAPTER ONE
INTRODUCTION

1.1 Plants and Human

Plants and man are inseparable. Plants existed on the earth in the geological past form the early history of the earth. The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. From the early stages of human civilization, plants, especially medicinal plants have played a pioneering role for the welfare of human beings. Recently, dramatic changes have taken place in the primary health care system of world population through the development of science, technology and medical science, but till to day 400 cores of people of the world are totally dependent on herbal medicine. It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources and herbal medicines are used by about 75-80% of the world's population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects (Sofowora, 1982).

1.2 Historical Overview on Medicinal Plant

Plants have been used from ancient times to attempt cures for diseases and to relive physical suffering. Ancient peoples all had acquired some knowledge of medicinal plants. Oftentimes these primitive attempts at medicine were based on superstition and speculation. Evil spirits in the body were thought to be the cause of medical problems. They could be driven out of the body through the use of poisonous or disagreeable plant substances that rendered the body a disagreeable habitat. Medicine men or women of a tribe were usually charged with knowledge of such plants. The progress of medicine has often been guided by the earlier observations and beliefs.

Drug plants were always of especial interest. As early as 5,000 B.C. many drugs were in use in China. Sanskrit writings testify to methods of gathering and preparing drugs in these early times. The Babylonians, ancient Hebrews and Assyrians were all familiar with medicinal plants. From Egypt there are records dating to 1,600 B.C. naming many of the medicinal plants used by physicians of that period, among which myrrh, opium, cannabis, aloes, cassia and hemlock are prominent. The Greeks were familiar with many of the drugs of today, evidenced by the works of Hippocrates, Theophrastus, Aristotle and Pythagoras. However, the supernatural element continued to remain prominent in their culture.

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 center 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, camomile, garlic, onion, marshmallow, 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 'uteri. 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.

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.

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 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).

1.3 Herbalism: Overview

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts.

Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phytotherapy. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Pharmacognosy is the study of medicines derived from natural sources.

Traditional use of medicines is recognized as a way to learn about potential future medicines.

In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from "ethnomedical" plant sources; 80% of these compounds were used in the same or related manner as the traditional ethnomedical use.

Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases. Such secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives.

At least 12,000 have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on the human body by binding to receptor molecules present in the body; such processes are identical to those already well understood for conventional drugs and as such herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be in principle just as effective as conventional medicines but also gives them the same potential to cause harmful side effects. Many of the herbs and spices used by humans to season food yield useful medicinal compounds.

Similarly, to prescription drugs, a number of herbs are thought to be likely to cause adverse effects. Furthermore, “adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal” (Crystalinks, n.d.).

1.3.1 Definition of Medicinal Plants

The term of medicinal plants includes a various types of plants used in herbalism and some of these plants have a medicinal activity. 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.

The medicinal plants in particular have received the attention of scientists form chemical, pharmacological and clinical angles from all over the world. Studies on medicinal plants through surveys are also gaining importance. A vast knowledge on plants exists as traditional medicine and a large amount of potent medicinal herbs are found growing wild. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. When a plant is designated as "medicinal", it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants are important therapeutic aid for various ailments.

Accordingly, World Health Organization (WHO) has formulated a definition of medicinal plants in the following way: "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs". Today there is widespread interest in drugs deriving from medicinal plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have toxic side effects. Clinically useful drugs which have recently been isolated from plants including the anticancer agent including the paclitaxel from the yew tree, antimalarial agent artimisinin from a Chinese herb *Artemsia annua* (wormwood). Moreover, many of antibiotics, vitamins and hormones are used today resulted from purification of such extracts and isolation and identification of active principles. *Thysanolaena maxima* as a medicinal herb shows sedative effect as well as further research also show its anticancer activities.

1.3.2 Herbal Medicinal Products

According to Council Directive 65/65/EEC, which has been implemented in national law in all Member States, medicinal products require prior marketing approval before gaining access to the market. In almost all Member States, herbal medicinal products are considered as medicinal products, and are, in principle, subject to the general regulations for medicines as laid down in the various national medicine laws. In many cases, a specific definition of herbal medicinal products is available, which is in line with the EU Guideline ‘Quality of Herbal Medicinal Products’. This includes plants, parts of plants and their preparations, mostly presented with therapeutic or prophylactic claims. Different categories of medicinal products containing plant preparations exist or are in the process of being created. For instance, draft legislation in Spain includes the definitions ‘Herbal Medicinal Products’ and ‘Phytotraditional Products’. The latter are not considered as ‘pharmaceutical specialties’ and are therefore not classified as herbal medicinal products.

1.3.2.1 Classification of Herbal Products

Generally, herbal products are classified as medicinal products if they claim therapeutic or prophylactic indication, and are not considered as medicinal products when they do not make these claims. Products not classified as medicinal in most cases belong to the food or cosmetic areas, although they sometimes contain plants which have pharmacological properties. For example, senna pods (from *Cassia* plants, used as laxatives) can be marketed as food in Belgium. Specific categories of non-medicinal products exist in some Member States, such as the so-called ‘therapeutic supplement products’ in Austria. In Ireland, Spain and the United Kingdom, there is exist preparations defined as medicinal products, which are under specific conditions exempt from licensing requirements.

1.3.2.2 Combination Products

Herbal ingredients used in combination are widely used in Europe, and their assessment is often performed according to specific guidelines. Combinations of herbal and homeopathic ingredients exist in a few countries. Their assessment follows rather strict criteria, usually those of a ‘full’ application procedure. Combinations of herbal ingredients and vitamins are available in many countries (IARC, 2002).

1.4 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.

1.4.1 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 12000 items used by traditional healers, about 500 are in common use. 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.

1.4.2 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.

1.4.3 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 (IARC, 2002).

1.5 The Role of Herbal Medicines in Modern Society

The use of herbs to treat disease is almost universal among non-industrialized societies. A number of traditions came to dominate the practice of herbal medicine at the end of the twentieth century:

- The "classical" herbal medicine system, based on Greek and Roman sources
- The Siddha and Ayurveda medicine systems from various South Asian Countries
- Chinese herbal medicine
- Traditional African medicine
- Unani-Tibb medicine
- Shamanic herbalism: a catch-all phrase for information mostly supplied from South America and the Himalayas
- Native American medicine.

Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the world's population presently uses herbal medicine for some aspect of primary health care. Pharmaceuticals are prohibitively expensive for most of the world's population, half of which lives on less than $2 U.S. per day. In comparison, herbal medicines can be grown from seed or gathered from nature for little or no cost.

In addition to the use in the developing world, herbal medicine is used in industrialized nations by alternative medicine practitioners such as naturopaths.

A 1998 survey of herbalists in the UK found that many of the herbs recommended by them were used traditionally but had not been evaluated in clinical trials.

In Australia, a 2007 survey found that these Western herbalists tend to prescribe liquid herbal combinations of herbs rather than tablets of single herbs.

The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants (Crystalinks, n.d.).

1.6 Alternative Medicine

These days the term "Alternative Medicine" became very common in western culture, it focuses 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. Like in case of laxatives, blood thinners, antibiotics and antimalarial medications, contain ingredients from plants. Moreover, the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively.

A plant's medicinal value is due to the presence in its tissues of some chemical substance or substances that produce a physiological action on the body. Most important are the alkaloids, compounds of carbon, hydrogen, oxygen and nitrogen. Glucosides, essential oils, fatty oils, resins, mucilages, tannins and gums are all utilized. Some of these are powerful poisons so that the preparation and administering of them should be entirely supervised by physicians (Rasool, 2012).

1.7 Difference of Herbal and Conventional Drugs

Although superficially similar, herbal medicine and Conventional pharmacotherapy have three important Differences: Use of whole plants- herbalists generally use unpurified Plant extracts containing several different constituents. It is claimed that these can work together synergistically so that the effect of the whole herb is greater than the summed effects of its components. It is also claimed that toxicity is reduced when whole herbs are used instead of isolated active ingredients (“buffering”). Although two samples of a Particular herbal drug may contain constituent compounds in different proportions, practitioners claim that this does not generally cause clinical problems. There is some Experimental evidence for synergy and buffering in certain Whole plant preparations, but how far this is applicable to all herbal products is not known. Herb combining- often several different herbs are used Together. Practitioners say that the principles of synergy and Buffering apply to combinations of plants and claim that combining herbs improves efficacy and reduces adverse Effect. This contrasts with conventional practice, where Polypharmacy is generally avoided whenever possible. Diagnosis- herbal practitioners use different diagnostic Principles from conventional practitioners. For example, when treating arthritis, they might observe, “Under Functioning of a patient’s symptoms of elimination” and decide that the arthritis results from “An accumulation of metabolic waste products”. A diuretic, cholerectic or laxative Combination of herbs might then be prescribed alongside Herbs with anti-inflammatory properties (Pal and Shukla, 2002).

1.8 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.

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. 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.

1.9 Present Status of Herbal Medicine

The wide spread use of herbal medicine is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and German regularly prescribe herbal medicine. The number of patients seeking herbal approaches for therapy is also growing exponentially. With the US Food & Drug Administration (FDA) relaxing guidelines for the sale of herbal supplement, the market is booming with herbal products (Brevoort, 1998). As per the available records, the herbal medicine market in 1991 in the countries of the European Union was about $6 billion (may be over $20 billion now), with Germany account for $3 billion, France $1.6 billion and Italy $0.6 billion. In 1996, the US herbal medicine market was about $4 billion, which have doubled by now. The Indian herbal drug market is about $ one billion and the export of herbal crude extract is about $80 million.

In the last few decades, a curious thing has happened to botanical medicine. Instead of being killed off by medical science and pharmaceutical chemistry, it has made come back. Herbal medicine has benefited from the objective analysis of the medical science, while fanciful and emotional claims for herbal cures have been thrown out, herbal treatments and plant medicine that works have been acknowledge. And herbal medicine has been found to have some impressive credentials. Developed empirically by trial and error, many herbal treatments were nevertheless remarkably effective. In a recent survey estimated that 39% of all 520 new approved drugs in 1983-1994 were natural products or derived from natural products and 60-80% of antibacterial and anticancer drugs were derived from natural products (Harvey, 1999).

The penicillin that replaced mercury in the treatment of syphilis and put an end to so many of the deadly epidemics comes from plant mold. *Belladona* still provides the chemical used in ophthalmological preparations and in antiseptics used to treat gastrointestinal disorders. *Rauvolfia serpentina* (The Indian snake root) which has active ingredient, reserpine, was the basic constituent of a variety of tranquilizer first used in the 1950’s to treat certain types of emotional and mental problems. Though reserpine is seldom used today for this purpose, its discovery was a breakthrough in the treatment of mental illness. It is also the principal ingredient in a number of modern pharmaceutical preparations for treating hypertension. But reserpine can have a serious side effect-severe depression. On the other hand, tea made of *R. serpentina* has been used in India as a sedative for thousands of years. Examination of the history of medicine and pharmacy reveals a definite pattern. Humankind first utilized materials found in the environment on an empirical basis to cure various ailments. These plant, animal parts and even microorganisms were initially employed in unmodified form, then as concentrated extract to improve their intensity and uniformity of action. Subsequently, pure chemical compounds as prototypes synthetic chemical entities were developed that possessed even greater. In fact, plant substance remains the basis for a very large proportion of the medications used today for treating heart diseases, hypertension, depression, pain, cancer, asthma, neurological disorders, irritable bowel syndrome, liver diseases and other ailments (Vickers and Zollman, 1999; Alschuler *et al.*, 1997; Carter, 1999; Bensoussan *et al.*, 1998; Schuppan *et al.*, 1999). By 1994, pharmacologist Norman Farnswoth had identified over 119 plant-derived substances that are used globally as drug. Many of the prescription drugs sold in United States are molecules derived from or modeled after naturally occurring molecules in plant. Interest in natural product research has been rekindled by discoveries of novel molecules from marine organisms (such as bryostatin) and potent new chemotherapeutic agents from plants (such as Taxol). Research has been facilitated by new rapid–through put bioassays in which robotic arms and computer controlled cameras test exceedingly small quantities of plant samples for the presence of the compounds active against a multiplicity of disease targets. It is possible to accomplish in a few minutes that once took months to analyze in laboratory. Even with new technology, it appears that one of the best sources for finding plant species to test is still the healer's pouch, because such plants have often been tested by generations of indigenous people. Yet at this crescendo of enthusiasm for herbal medicine, an increasing number of aged healers are dying with their knowledge left unrecorded. Too often, though forests disappear without any notice. Currently 12.5 percent of all plant species are threatened with immediate extinction. Most botanists regard this estimate by the International Union for the Conservation of Nature (IUCN) as conservative, because it considers only species known to science; numerous undiscovered species pass from the world unrecorded and unmourned (Cox, 2000).

1.10 Potential of Medicinal Plants in Economy

Global estimates suggest that over three-fourths of the total world population cannot afford the products of allopathic medicine and thus have to rely upon the use of traditional medicines, which are largely derived from plants. Through scientific studies, only about 1% of the total known medicinal plant species (folk, ethno and traditional medicine) is acknowledged to have therapeutic value for human beings. The World Health Organization is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health-care programs. Besides, the demand for herbal drugs has reached a new high in recent years in the West as well. About 1400 herbal preparations are used widely, according to a recent survey in Member States of European Union. Such popularity in health-care, plant-derived products reflects their increasing acceptance among the elite as well.

India is one of the major raw material-producing nations of South Asia and within the country; the Himalaya supports a large number of such species. Export statistics available indicate that between 1992 and 1995 the country exported about 32,600 tonnes of crude drugs valued at $US 46 million. Conservation, propagation and utilization of medicinal plants have, over the years, become a major thrust area of research activity throughout the country (Kala, 2005).

1.11 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 characterizes 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).

1.12 Significances of Medicinal Plants to Human Being

Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.

Many food crops have medicinal effects, for example garlic. Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species.

Plant metabolites include: primary metabolites and secondary Metabolites *Phytotherapy* is the use of plants or plant extracts for medicinal purposes (especially plants that are not part of the normal diet). *Phytochemistry* is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants.

•Thin layer chromatography (TLC)
•Gel (column) chromatography)
•High performance of liquid chromatography (HPLC)
•Gas chromatography (GC)
•Mass spectrometry
•Nuclear magnetic resonance (Singh, 2015).

1.13 Some Established Drugs of Natural Origin

1.13.1 Antimalarial Agents

Quinine is considered to be the drug of choice for severe chloroquine-resistant malaria due to *P. falciparum*. In the United States, the related alkaloid quinidine is recommended for this purpose Because of its wide availability, therein its use as an antiarrhythmic agent.

1.13.2 Cardiovascular Drugs

The cardiac glycosides, which include Digoxin, Digitoxin and Deslanoside, exert a powerful and selective positive inotropic action on the cardiac muscles.

1.13.3 CNS Drugs

One of the most cited examples of important natural product-derived drugs is the neuromuscular blocker, d-tubocurarine, which recently helped recognition of the possibility that a number of vastly different CNS and peripheral nervous system diseases may be therapeutically controlled by selective nicotinic acetycholine receptor (nAChR) agonists and has opened a new area of drugs design based on the Nicotine molecule.

1.13.4 Antibiotics

Today, new important anti-infective are being discovered from microbial, plant and animal sources. For example, the antimalarial agent, Artemisinin, was isolated from the Chinese medicinal plant *Artemesia annua*.

1.13.5 Antineoplastic Agents

The major anticancer drugs are natural products from plants ormicroorganisms. Examples of such important anticancer drugs are Bleomycin, Doxorubicin, Daunorubicin, Vincristine,

1.13.6 Cholesterol-Lowering Agents (Hypolipidimics)

These drug acts by inhibition of 3-hydroxy–3– methylgutaryl coenzyme- a reductase (HMGCoA reductase), an enzyme critical in the biosynthesis of cholesterol. The first of the HMG-CoA reductase inhibitors were isolated from *Penicillium sp.*

1.13.7 Immunomodulators

The immunomodulator Cyclosporin was originally isolated from a soil fungus, *Trichoderma polysporum*. This compound was a major breakthrough for organ transplantation.

1.13.8 Antihyperglycemics

The major antihyperglycemic drugs are natural products derived from popular plant such as *Momordica charantia* (Karela); *Tinospora cordifolia* (Guduchi); *Gymnema sylevestre* (Gurmar); *Azadirachta indica* (Neem); *Ficus benghalensis* (Indian banyan tree); *Aegle marmelos* (bel or bilva); *Aloe vera*; *Allium sativum* – Garlic etc.

1.14 Medicinal Plants in Bangladesh

In Bangladesh there are about 297 Unani, 204 Ayurvedic and 77 Homeopatheic drug manufacturing industries where the medicinal plants are extensively used in both raw and semi– processed forms of medicine in various pharmaceutical dose formulations. These plants also serve as important raw materials for many modern medicinal preparations. The market value of drugs produced by these industries from medicinal plants is about Tk. 300 crores. Besides, village Kobiraj, street Vendors and Tribal people also use a large number of medicinal plants for the treatment of various diseases. There is no actual figure how many medicinal plants are used in Bangladesh. Chowdhury at SAARC workshop (held on 16-18 June, 2002) gave a brief idea about the amount of medicinal plants used annually in Bangladesh. A few of them are mentioned here: Ashwagondha (Withania somnifera) - 56,000 kg, Anantamul (Hemidesmus indicus) - 50,000 kg, Kurchi (Holarrhena antidysenterica)-1, 00,000 kg, Gulancha (Tinospora cordifolia) - 127,000 kg. According to Hamdard Laboratories (WAQF), in Bangladesh the annual demand for a few medicinal plants are- Satomuli (Asparagas racemosus)– 800 tons, Sarpagondha (Rauvolfia serpentina)– 1,000 tons, Ghritokumari (Aloe vera)– 24,000 tons, Kalomegh (Andrographis paniculata)– 1,000 tons (Hassan, 2003). Every year Bangladesh imports a large quantity raw materials belonging to of medicinal plants mostly under the banner of spices and spends more than 64 crores Taka annually for this purpose. Ironically, 70% of these imported raw materials can be met from the indigenous sources from Bangladesh (Begum, 2003).

Table 1.1: Medicinal Plant Species Listed by WHO Which Can Be Grown in Bangladesh Commercially

Scientific Name	Bengali Name	English Name	Used Parts	Used As Patent Drugs
Winthania somnifera Dunal	Ashwagandha	Winter Cherry	Root, Leaf, Fruit, Seed, Whole Plant	Syrup Masturin, Arq Ashwaganda. Magun Sohag Soonth
Aloe vera Tour. ex Linn.	Ghritokumari	Aloe	Leaf	Tablet Suranjan, Tablet Mudir, Syrup Belgiri
Andrographis panniculata Wall.ex Nees.	Kalomegh	Creat	Leaf, Stem, Whole Plant	Syrup Safi, Syrup Kurchi
Asparagus racemosus Willd.	Satomuli	Aspargus	Tuberous Root, Leaf, Flower, Fruit	Tablet Abiaj, Khisandha, Ka-4, Sufoof Gigian
Plumbago zeylanica Linn.	Chita		Root	Majoon Falasefa, Syrup Kurchi
Adhatoda zeylanica Nees. (Syn. name- A. vasica Linn.)	Vasak	Vasaka	Leaf, Stem, Bark, Root, Flower	Syrup Saduri, Chawan Prash, Tablet Sualin, Syrup Ajaj
Rauvolfia serpentine (Linn.) Benth.	Swarpagandha	Snake root	Root	Syrup Mangurin

Glycyrrhiza glabra Linn.	Jastimodhu	Liqourice root	Root, Stem	Tablet Sualin, Mauol Hiat, Syrup Badian, Tablet Kafur

(Islam, 2003)

1.15 Rationale of Herbal Drug Research: Special Reference to Bangladesh

Most of the people of our country have no or little access to allopathic medicine due to their uncompromisable low income in respect of high cost of allopathic medicine. A survey conducted in 1990 in different villages of Bangladesh shows that on average of 14% if people suffering illness approach qualified allopathic doctors, 29% contact unqualified village doctors, 10% contact mollahs, 29% contact quack and 19% contact homeopaths. The survey indicates an extensive use of medicinal plants, most of which are served in a crude and substandard form, by our people. The use of such crude and substandard herbal drug is dangerous and may threaten public health. Thus the analysis of plants for exploring the bounty of chemical entities and their biological screening is the current need for standardization of herbal medication (Ghani, 1998).

Since Bangladesh is a country of low economic growth, a proper health care system can be established by supplying low cost medicines to its population. This may be only possible by utilizing our natural resources of medicinal plants and their constituents. So, scientific exploration and standardization of these potential crude drugs is an urgent need to revolutionize our drug sector. Besides, Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to produce drugs and medicines. During the last five years Bangladesh has spent more than 1500 crore Taka for importing chemicals, raw materials and semi-processed drugs of plant origin from neighboring and other countries and this trend is growing upwards day by day. This huge foreign exchange can be saved if the indigenous medicinal plants or its semi processed products are utilized by the manufacturer to satisfy their need (Ghani, 1998).

1.16 Central Nervous System

The central nervous system (CNS) is the processing center for the nervous system. It receives information from and sends information to the peripheral nervous system. The two main organs of the CNS are the brain and spinal cord. The brain processes and interprets sensory information sent from the spinal cord. Both the brain and spinal cord are protected by three layers of connective tissue called the meninges.

Within the central nervous system is a system of hollow cavities called ventricles. The network of linked cavities in the brain (cerebral ventricles) is continuous with the central canal of the spinal cord. The ventricles are filled with cerebrospinal fluid which is produced by specialized epithelium located within the ventricles called the choroid plexus. Cerebrospinal fluid surrounds, cushions, and protects the brain and spinal cord from trauma. It also assists in the circulation of nutrients to the brain (Bailey, 2016).

1.16.1 Central Nervous System Disorders

A **central nervous system** (**CNS**) **disease** can affect either the brain or the spinal cord, resulting in neurological or psychiatric **disorders**. Causes of **CNS** diseases are trauma, infections, degeneration, autoimmune **disorders**, structural defects, tumors, and stroke.

Disorders of the nervous system may involve the following:

- **Vascular disorders**, such as stroke, transient ischemic attack (TIA), subarachnoid hemorrhage, subdural hemorrhage and hematoma, and extradural hemorrhage
- **Infections**, such as meningitis, encephalitis, polio, and epidural abscess
- **Structural disorders**, such as brain or spinal cord injury, Bell's palsy, cervical spondylosis, carpal tunnel syndrome, brain or spinal cord tumors, peripheral neuropathy, and Guillain-Barré syndrome
- **Functional disorders**, such as headache, epilepsy, dizziness, and neuralgia
- **Degeneration**, such as Parkinson disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), Huntington chorea, and Alzheimer disease (Johns Hopkins Medicine Health Library, n.d.).

1.16.2 Chemical Classifications of CNS Drugs

Each of the regulated drugs that act on the central nervous system or alter your feelings and perceptions can be classified according to their physical and psychological effects. The different drug types include the following:

- **Depressants:** Drugs that suppress or slow the activity of the brain and nerves, acting directly on the central nervous system to create a calming or sedating effect. This category includes barbiturates (phenobarbital, thiopental, and butalbital), benzodiazepines (alprazolam, diazepam, clonazepam, lorazepam, and midazolam), alcohol, and gamma hydroxybutyrate (GHB). Depressants are taken to relieve anxiety, promote sleep and manage seizure activity.
- **Stimulants:** Drugs that accelerate the activity of the central nervous system. Stimulants can make you feel energetic, focused, and alert. This class of drugs can also make you feel edgy, angry, or paranoid. Stimulants include drugs such as cocaine, crack cocaine, amphetamine, and methamphetamine. According to the recent World published by the United Nations Office on Drugs and Crime, amphetamine-derived stimulants like ecstasy and methamphetamine are the most commonly abused drugs around the world after marijuana.
- **Hallucinogens**: Also known as psychedelics, these drugs act on the central nervous system to alter your perception of reality, time, and space. Hallucinogens may cause you to hear or see things that don't exist or imagine situations that aren't real. Hallucinogenic drugs include psilocybin (found in magic mushrooms), lysergic acid diethylamide (LSD), peyote, and dimethyltryptamine (DMT).
- **Opioids:** These are the drugs that act through the opioid receptors. Opioids are one of the most commonly prescribed medicines worldwide and are commonly used to treat pain and cough. These include drugs such asheroin, codeine, morphine, fentanyl, hydrocodone, oxycodone, buprenorphine, and methadone.
- **Inhalants:** These are a broad class of drugs with the shared trait of being primarily consumed through inhalation. Most of the substances in this class can exist in vapor form at room temperature. As many of these substances can be found as household items, inhalants are frequently abused by children and adolescents. These

include substances such as paint, glue, paint thinners, gasoline, marker or pen ink, and others. Though ultimately all of these substances cross through the lungs into the bloodstream, their precise method of abuse may vary but can include sniffing, spraying, huffing, bagging, and inhaling, among other delivery routes.

- **Cannabis**: Cannabis is a plant-derived drug that is the most commonly used illicit drug worldwide. It acts through the cannabinoid receptors in the brain. Cannabis is abused in various forms including bhang, ganja, charas, and hashish oil.
- **New psychoactive substances (NPS):** These are drugs designed to evade the existing drug laws. Drugs such as synthetic cannabinoids, synthetic cathinones, ketamine, piperazines, and some plant-based drugs such as khatand kratom are examples of NPS (Balhara, n.d.).

1.16.3 CNS Depressants

A drug that reversibly depresses the activity of the central nervous system usually called CNS depressant, used chiefly to induce sleep and to allay anxiety. Barbiturates, benzodiazepines, and other sedative-hypnotics have diverse chemical and pharmacological properties that share the ability to depress the activity of all excitable tissue, especially the arousal center in the brainstem. Sedative-hypnotics are used in the treatment of insomnia, acute convulsive conditions, and anxiety states and in facilitation of the induction of anesthesia. Although sedative-hypnotics have a soporific effect, they may interfere with rapid eye movement sleep associated with dreaming and, when administered to patients with fever, may act paradoxically and cause excitement rather than relaxation. Sedative-hypnotics may interfere with temperature regulation, depress oxygen consumption in various tissues, and produce nausea and skin rashes. In elderly patients they may cause dizziness, confusion, and ataxia. Drugs in this group have a high potential for abuse that often results in physical and psychological dependence. Treatment of dependence involves gradual reduction of the dosage because abrupt withdrawal frequently causes serious disorders, including convulsions. Acute reactions to an overdose of a sedative-hypnotic may be treated with an emetic, activated charcoal, gastric lavage, and measures to maintain airway patency.

1.16.3.1 Pathophysiology

All the sedative-hypnotics are general CNS depressants. Most stimulate the activity of GABA, the principal inhibitory neurotransmitter in the CNS. Benzodiazepines, which are one of the most frequently prescribed medications in the world, enhance the effect of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABAA receptor.

GHB is a sedative-hypnotic that is banned for sale to the public because of frequent abuse and serious toxic adverse effects. GHB is a neuroinhibitory neurotransmitter or neuromodulator in the CNS. It also appears to increase GABA B receptor activity and dopamine levels in the CNS (Cooper, 2015).

1.16.3.2 Sleep Disorder: Insomnia

Insomnia, defined as persistent difficulty in falling or staying asleep that affects daytime function, can induce significant psychological and physical disorders. Most patients engage in long-term use of benzodiazepines analogs to treat insomnia. But these drugs have limited benefits with obvious side-effects, such as impaired cognitive function, memory and general daytime performance. In addition, long-term administration results in tolerance and dependence.

Numerous herbal medicines are recognized as active in the central nervous system (CNS) and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches or epilepsy, that do not respond well to conventional treatments.

People from different regions of the world have used herbal medicines to alleviate affective disorders for many years and as a consequence, the search for novel pharmacotherapy from medicinal plants has progressed significantly in the past decade. An increasing number of herbal products have been introduced into psychiatric practice

1.16.3.3 Theories of Hypnotic Action

An approach to understanding sedative/hypnotics has been to hypothesize that sleep results from drug-induced reduction in energy metabolism; barbiturates, for instance, decrease cerebral glucose metabolic rate in human positron emission tomography (PET) scan studies. On the other hand, the results of animal studies have been more variable, such that barbiturates may and benzodiazepines may not decrease cerebral metabolic rate of oxygen (CMRO2). A more cogent argument against the notion that hypnotics induce sleep by lowering metabolic rate is that it stems from a view of sleep as being a very passive process, which seems to contradict the more contemporary understanding of sleep as a multifaceted, actively regulated process. Indeed, at doses that induce sleep (and prior to achieving anesthetic doses), patients receiving most hypnotic medications demonstrate the alternating ultradian rhythm of NREM and REM sleep, which indicates an active regulatory mechanism. It seems more parsimonious, then, to hypothesize that sedative/hypnotics act at specific sites involved in sleep regulation, rather than producing a nonspecific ‘‘slowing’’ of the nervous system.

1.16.3.3.1 The Molecular Level: The Central GABAA–Benzodiazepine Receptor Complex

A major insight into the action of hypnotics began in the 1970s with the discovery of high affinity, stereospecific receptors for benzodiazepines in the central nervous system. GABAA-benzodiazepine receptors are the most abundant inhibitory receptor system in the central nervous system (CNS). They can be viewed as representatives of a large family of hetero-oligomeric ligand-gated ion channels (e.g., nicotinic acetylcholine, glycine, and serotonin-3 receptors).Functionally, they contain three distinct but interacting moieties: recognition sites for GABA and benzodiazepines and a chloride ionophore. Binding of a benzodiazepine agonist to its recognition site results in increased chloride ion flux, which in turn hyperpolarizes the postsynaptic membrane at a level below that at which spike generation is possible. Molecular cloning data indicate that the GABAA–benzodiazepine receptor complex is comprised of at least five subunits; these in turn may have various isoforms. Each subunit is comprised of four membrane-spanning regions. The intracellular loops of some subunits contain phosphorylation sites, which have been hypothesized to be a locus of receptor modulation. It is possible, for instance, that the receptor system might respond to GABA differently in the phosphorylated versus dephosphorylated state. Alpha and beta Subunits need to be present in order to be responsive to benzodiazepines, and a complete system of alpha, beta, and gamma needed for a fully responsive receptor. Recent data using murine gene targeting techniques indicate that the alpha-1 isoform is not required for the anxiolytic actions of benzodiazepines, but may mediate sedation and ataxic effects; in principle, this might make it possible to develop anxiolytics with a more benign side effect profile than those currently available. The gamma-1 subunit, which in some senses may be viewed as a marker of the subgroup of GABA receptors representing the GABAA–benzodiazepine receptor complex, is found in 60% to 90% of GABA receptors. GABAA-benzodiazepine receptors have also been divided into Type I and II, differing on the particular alpha subunit isoform; whether this distinction has pharmacologic significance in terms of effects of drugs that selectively bind to the Type I receptor is still under investigation. There are peripheral and ‘‘Valium insensitive’’ receptors that appear not to be involved in sleep-related processes in addition to the central receptors. (Mirshafa, Azadbakht and Ahangar, 2013).

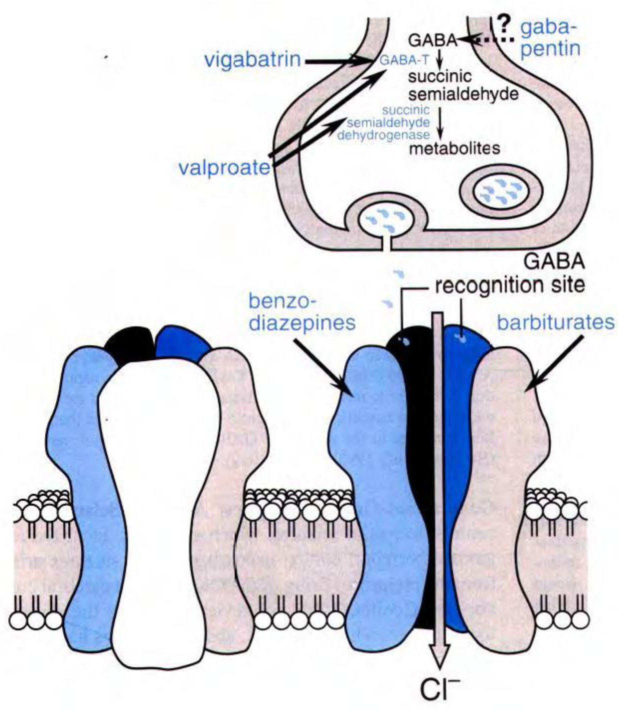

Fig. 1.1: Modulation Mode of the Central Inhibitory Transmitter Gaba and The Action Sites of Drugs

1.16.3.4 Sedative Effects Obtained by Some Phytochemicals

Traditional health practitioners treat insomnia by making herbal preparations from leaves, stems, barks and roots of medicinal plants. Tea made from these plants and taken has been shown to be quite effective. Researchers have taken a step further to isolate and determine those bioactive principles in these plant parts; and there modes of action. A number of modern therapeutic agents have been derived from tropical forest species. Most pharmaceutical products currently in use are plant derived and some 75% of these were discovered by examining the use of these plants in traditional medicine. The isolated compounds are categorized into the following classes of phytochemicals (terpenoids, flavonoids, alkaloids, saponins, etc.). Also considered are the sedative properties of different extracts and other compounds which are not referred to as phytochemicals.

1.16.3.4.1 Terpenoids

Many essential oils and monoterpenes are used therapeutically as relaxing drugs and traquilizers. The monoterpenes are present in volatile oils of many plant species such as *Mentha piperita, Zanthoxyum schinifolium and Mentha X villosa*. Recently, much attention has been focused on the sedative, relaxant activities and behavioural effect of medicinal plants in aromatherapy. It is believed that the inhalation of fragrance is a powerful means of relaxation. Some of the odors are used to treat depression, anxiety and tension. The inhalation of the crude extract of *Kaempferia galanga* L showed sedative effects at doses of 1.5 mg. The essential oils Linalool. 1, 8-cineole and α- terpineol were shown to possess sedative properties.

1.16.3.4.2 Flavonoids

Albizzia julibrissin Durazz flowers are used as sedatives in oriental traditional medicine. The phytochemical study of this plant led to the isolation of two flavonol glycosides, quercetrin and isoquercetrin. These compounds were observed to increase pentobarbital-induced sleeping time in a dose-dependent manner in mice. It was reported that the flavonoid 2S (-)-hesperidin isolated from *Valeriana officinalis* has sedative and sleep enhancing properties whereas 6-methylapigenin also isolated from *Veleriana officinalis* exhibited ability to increase the sleep enhancing properties of hesperidin.

1.16.3.4.3 Alkaloids

The alkaloids in sanjoin were found to exist in certain ratios and some drug interactions such as additivity, synergistic or counteracting interaction was postulated. In order to determine if there was any additivity, synergistic or counteracting interaction between the alkaloids isolated from the plant; the potent alkaloids were co- administered with the non-potent alkaloids. Coclaurine and sanjoinine-A were co-administered with nuciferine and sanjoinine-A with coclaurine. It was observed that there is additivity between sanjoinine-A and nuciferine, while coclaurine did not enhance the sedative activity of sanjoinine-A. The sedative activity of the alkaloid was monitored by measuring the hexibarbital induced sleeping time. It was also postulated that since the major constituent of the butanol fraction obtained was zizyphusine, a quartinary aporphine alkaloid which did not exhibit any sedative activity; the sedative activity of the butanol fraction was attributed to the presence of flavonoids or the minor components such as aporphine alkaloids.

1.16.3.4.4 Steroids

Two bufadienolides known as diagremontianin and bersaldegenin-1, 3, 5-orthoacetate isolated from *Kalanchoe diagremontiana* were shown to have a pronounced sedative effect. β-sitisterol isolated from *Tillia americana* var, Mexicana exhibit sedative activity at a dose of 30 mg/Kg in mice. A dose response curve of β-sitisterol in the range of 1-30 mg/kg doses indicated that this compound produces an anxiolytic-like action from 1-10 mg/kg and a sedative response when the dose was increased to 30 mg/kg. These effects were observed to resemble those produced by diazepam.

1.16.3.4.5 Saponins

The saponins jujuboside A and B isolated from the seeds of *Zizyphus vulgaris* Lamark var. spinosus Bunge (Rhamnaceae) was thought to be partly responsible for the sedative property of the plant observed that jujuboside A had no inhibitory activity but exerts a synergism with phenylalanine on the central nervous system function, therefore concluded that jujuboside A is not a sedative agent. But it was showed that saponins extracted from the species *Ziziphus jujuba* Semen that grows in China exhibited significant effect on walking time and coordinated movement, and prolonged the suprathreshold barbiturate induced sleeping time.

1.16.3.4.6 Quinoids

Ternstroemia pringlei is used in Mexico for the treatment of insomnia. Bioactivity guided fractionation of the methanolic extract led to the isolation of the sedative compound jacaranone which is a quinoid.

1.16.3.5 Sedative Effects of Some Non-Phytochemicals

Table 1.2: List of Some Non-Phytochemicals and Their Plant Sources

Non-Phytochemicals	Plant sources
Lactons	Two lactone compounds protoanemonin and anemonin isolated from the flowering aerial parts of *Pusatilla alpine Subsp*. Apiifolia were shown to exhibit sedative activity
Cannabinoid	Pickens (1981) showed the sedative activity of cannabidiol which was isolated from *Cannabis sativa.* It was observed to be the major constituent of the plant, representing about 40% in its extract.
Cinnamates	Ethyl trans-p-methoxycinnamate and ethyl cinnamate obtained from the n-hexane extract of *Kaempferia galanga* was shown to possess sedative effects at 0.0014 g and 0.0012 mg respectively. They showed significant reduction of locomotor activity
Nitrite	*Ixora pavetta* Vahl. (Rubiaceae) is a small tree whose flowers are used for the treatment of insomnia. The amyl nitrite isolated from the flowers exhibited sedative effects

Valepotriates and Iridoids	In Bulgaria *Valeriana officinalis* L. (Valerianaceae) roots are used to treat insomnia. Its powerful sedative action was observed to be due to the presence of valepotriates and epoxy-iridoid esters

(Kuponiyi, 2013)

1.16.3.6 Some Medicinal Plants Giving Sedative-Hypnotic Effect

1.15.3.6.1 *Piper methysticum*

Piper methysticum originates in the Pacific Polynesian Islands, and its extract, kava-kava, has been traditionally used as an herbal medicine to treat insomnia and anxiety in the South Pacific since the 18thcentury. There are eighteen kava lactones isolated from kava-kava root extract, four chiral and two achiral enantiomers of them perform most of the activities including yangonin, desmethoxyyangonin (5,6-dehydrokawain), methysticin, 7,8-dihydromethysticin, kawain, and 7,8-dihydrokawain. It also have been reported that administration of the lactones alone did not exert the same pharmacologic activity as whole kava-kava extract, some researchers suggested that this may due to the modulation of transport and/or metabolism

1.16.3.6.2 *Zizyphus jujuba*

Zizyphi spinosi semen (ZSS), the maturate seed of *Zizyphus jujuba* Mill var. *spinosa*, is a historic classic herbal medication widely used in treating insomnia in Asian countries. There are three main types of ingredients in ZSS that also display lipid oxidation resistance properties: saponins, alkaloids, and flavonoids. The underlying mechanism of ZSS remains unclear although numerous studies have been conducted over the past years. One of the major alkaloid compounds, sanjoinine A, enhances pentobarbital-induced sleeping behaviors, which are more commonly referred to as hypnotic effects.

1.16.3.6.3 California Poppy (E*schscholzia californica*)

This is a great herb for insomnia due to restlessness and anxiety (and it's safe and gentle enough for children). This herb not only helps to fall asleep, it improves the quality of sleep as well. It can be coupled with valerian, if anyone need a stronger sedative. A tincture is the most powerful way to take this herb.

1.16.3.6.4 Passion Flower (*Passiflora incarnata*)

Passion flower is wonderful for those who tend to wake frequently throughout the night. This, too, is safe for children and those with compromised health. It can be taken in large doses (I usually keep taking it until I feel calm and tired) and for long periods of time.

1.16.3.6.5 Hops (*Humulus lupulus*)

Hops is a fast-acting nervine and sedative, good for anxiety, hysteria, digestion, and stress-related illness. This herb is completely safe for most of the population (although due to the natural steroids found in hops, pregnant women and children under two years of age should avoid it) (Jirsa, 2012).

1.16.3.6.6 Valerian (*Valeriana officinalis*)

In the United States, herbalists use valerian extensively for its sedative action against insomnia, nervousness, and restlessness. It is recommended for those types of people who have a hard time falling asleep, because it shortens sleep latency. It also reduces nighttime waking. Valerian is an excellent herbal sedative that has none of the negative side effects of Valium and other synthetic sedatives. It works well in combination with other sedative herbs, such as California poppy, skullcap, hops, and passion flower.

1.16.3.6.7 Wild Lettuce (*Lactuca virosa*)

Wild lettuce is a mild sedative and nervine used for restlessness and insomnia. It may be found in a variety of formulas for the treatment of acute and chronic insomnia. It is used homeopathically for restlessness and insomnia (Boericke, 1927). Because of its safety of use and calming effects, wild lettuce is a good children’s remedy.

1.16.3.6.8 Lavender (*Lavandula officinalis*)

Lavender is a gentle strengthening tonic for the nervous system. A few drops of lavender oil added to a bath before bedtime is recommended for persons with sleep disorders. Additionally, the oil may be used as compressor massage oil or simply inhaled to alleviate insomnia.

1.17 Plant Review

Scientific Name: *Thysanolaena maxima*

Thysanolaena maxima (Roxb.) Kuntze (family—Poaceae), popularly known as broomgrass, is an important NTFP and grows in almost all parts of South and Southeast Asia up to an elevation of 1,600m and climatic conditions ranging from tropical to subtropical. The inflorescence of T. *maxima* makes broom popular in Asia. The flowers of this plant are used as cleaning tool or broom. "Tiger Grass" is a common name for this plant throughout the tropics where it is grown as an ornamental. It may be used to create the effect of bamboo, which it resembles, but to which it is not related. It also is called "broom grass" in areas where its flowers are used as a cleaning tool (ZipcodeZoo, 2016). It grows wild in the hills of the northeastern region of India and in Darjeeling and Sikkim Himalayas. The contribution of this NTFP toward enhancing the livelihood of the people of the region has been studied by Bisht and Ahlawat 1998, Shankar *et al.* 2001, and Tiwari 2001. In Meghalaya, broomgrass was first introduced by the state forest department about three decades ago under a silvi-pastoral system in social forestry plantations for generating income during gestation periods, that is, periods between plantation and harvest of timber. Subsequently, the plant has been domesticated and cultivated on large scale by upland farmers. This plant, therefore, provides a typical case for understanding the role of domesticated NTFPs in enhancing the livelihood of the rural poor. Being a recently domesticated plant, little is known about its growth features, productivity, and plantation management. The broom made out of the inflorescence of this plant is sold across South and Southeast Asia and the market is expanding.

Fig. 1.2: *Thysanolaena maxima*

1.17.1 Distribution

Broomgrass grows in almost all parts of Meghalaya, where it covers an estimated 127 sq. km (Tiwari *et al.* 1995). Broomgrass grows below 1,600 m.a.s.l. on a wide range of soils. It naturally colonizes areas with newly exposed soils due to land slip, road sides, abandoned quarries, abandoned jhum (shifting cultivation) areas, and waste lands. Large areas of abandoned jhum fields have also been converted to broomgrass plantations in the last two decades, due to an increase in demand for brooms from various parts of the country. The Ri Bhoi and East Khasi Hills districts account for more than 70% of the total production of brooms in Meghalaya (Tiwari *et al.*, 2012).

1.17.2 Growing Conditions and Uses

Thysanolaena Maxima is a fast growing clumping grass that will quickly form a clump of 1m or more across.

Growing to 2.5m to 3m, *Thysanolaena Maxima* makes a great screening or background plant. In some Asian countries the broad leaves are used to wrap food.

It is easy to care for, can be grown a green filler plant for containers and grows well in part shade to full sun.

The only real problems with Tiger Grass is frost and cold, so in the cooler climates of Victoria and Tasmania it can struggle a little, although can grow well in Melbourne in sheltered courtyards. It is fast growing so need space and should consider a root barrier to contain it.

- ✓ Only its panicle is used for making brooms. Farmers use its stem as a building material or as fuel and fodder, for mulching or staking crops or sell it to the paper pulp industry. The leaves and tender shoots are used as fodder in times of scarcity.
- ✓ Cultivation of broomgrass on marginal lands unsuitable for food production can generate additional household income.
- ✓ It is tolerant of harsh environmental conditions such as shallow soil, drought and heavy rainfall. It grows easily on shady slopes, damp and steep river banks, degraded areas and gravelly soil on weathered rock surfaces.
- ✓ Broomgrass can grow on degraded, marginal or steep land unsuitable for food production. It can be grown as a hedge in an alley cropping system and is recommended as part of a shifting cultivation system.
- ✓ Typical successful adopters are subsistence farmers cultivating steep, fragile or degraded hillsides.

- ✓ Broomgrass is produced in a five-year cycle with the lowest yield in the first and fifth years, and highest in the second and third years.
- ✓ Al though the first year of production is labor-intensive, requiring planting and weeding, the crop does not require much attention after that.

1.17.3 Vernacular Names of *Thysanolaena maxima*

Table 1.3: Vernacular Names of *Thysanolaena maxima*

English	**Asian Broom Grass, Bamboo grass, bouquet grass, broom grass, tiger grass**
India	bushnia, chir, chiten, deobahari, garajono, hmunphiah, jharu, jurna, karsar, konda, phuljharu, pirlu, saper
Indonesia	awis, lantebung, menjalinwuwu
Laos	dokkhein, kheemkhoong
Malaysia	bulohteberau, rumputbuloh
Nepal	Amriso
Philippine	buybuyeagadu, lasa, tagadeu, tagisa
Thailand	khoeilaa, khoei la, laolaeng, toing kong, tong kongyakapphaiyai
Tibet	khrergod
Vietnam	cay le, dong trung ha thao, omganh, say

1.17.4 Taxonomic Hierarchy of the Investigated Plant

Table 1.4: Taxonomic Hierarchy of the Investigated Plant

Rank	Scientific name & common name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Subclass	Liliopsida – Monocotyledons
Class	Commelinidae
Order	Cyperales
Family	Poaceae – Grass family
Genus	*Thysanolaena* Nees – tiger grass
Species	*Thysanolaena maxima* (Roxb.) Kuntze [excluded]

1.17.5 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 ; lowerlemma 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; 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 angles to the lemma's surface.

1.17.6 Growth Pattern

Broomgrass forms tussocks. The culms arise centrifugally during the peak growth period (June–July) and bear inflorescence at the end of vegetative growth. The appearance and growth of culms in a tussock depict a characteristic order that probably controls the extent of culm growth, as well as the size, number, and length of the leaves and the overall shape of the crown. Broomgrass is usually planted during April and May, and peak vegetative growth takes place during June and July. The productive period starts with the flowering of the plant in the months of October to March. The inflorescence becomes ready for harvest by December and January and the harvest continues until March. The maximum height of a tussock is attained in three years, while basal girth and culms numbers continue to increase

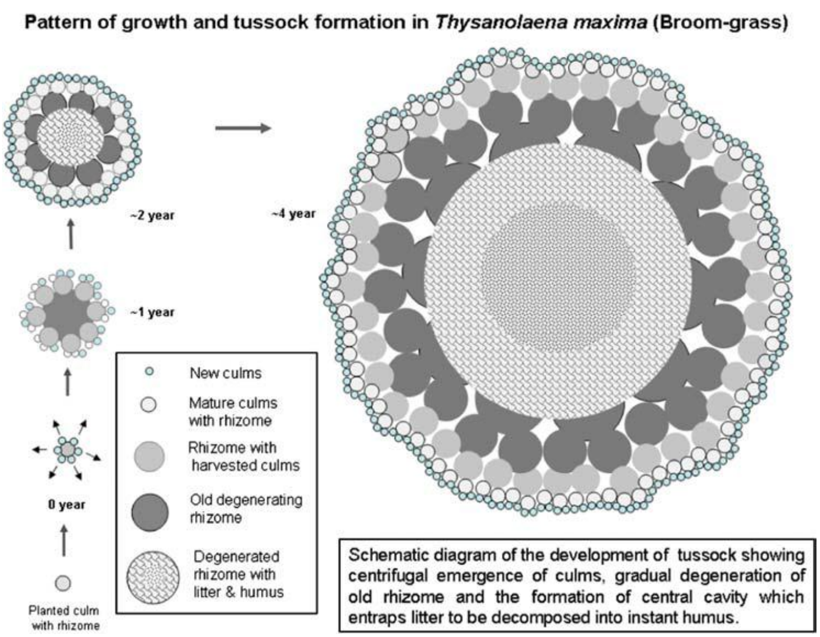

Fig. 1.3: Centrifugal Emergence and Growth of Tussock Year-Wise

1.17.7 Crown Structure

As the number of culms per tussock and basal area of the culms increased with age of the plantation, the growth became much more vigorous, resulting in longer internode length and larger leaf size. It caused a steady increase in the cumulative leaf area of the tussock up to year 4. The number of leaves per culm, however, remained almost unchanged due to seasonal leaf-fall. The canopy cover of the tussock was measured as the area of shade cast by its canopy when the sun was overhead. The canopy cover increased at a fast rate and reached its maximum at the end of year 4. Likewise, the Leaf Area Index (LAI) also increased fast, up to the third year of growth, but lowered down subsequently. In tussocks of four-year-old plantations, the culms laden with leaves and brooms tended to droop down, resulting in maximum canopy cover and moderate LAI. The vigour was fairly sustained during the fourth year with a slight decrease, but it significantly decreased beyond the fifth year. The culms grow only toward the periphery in centrifugal order. The resulting central cavity of the tussock, therefore, remains devoid of any culm. The growth of the tussocks attained its maximum toward years 3 and 4, after which the tussocks showed signs of withering from the inner side, and the area of central cavity or empty space increased significantly. The culms emerged so closely at the periphery of the cavity that most dead leaf and another biomass remains were locked within the cavity. This consequence of the species growth pattern helps conserving nutrients in the close vicinity of the newly arising culms, which is particularly important in degraded sites prone to soil erosion.

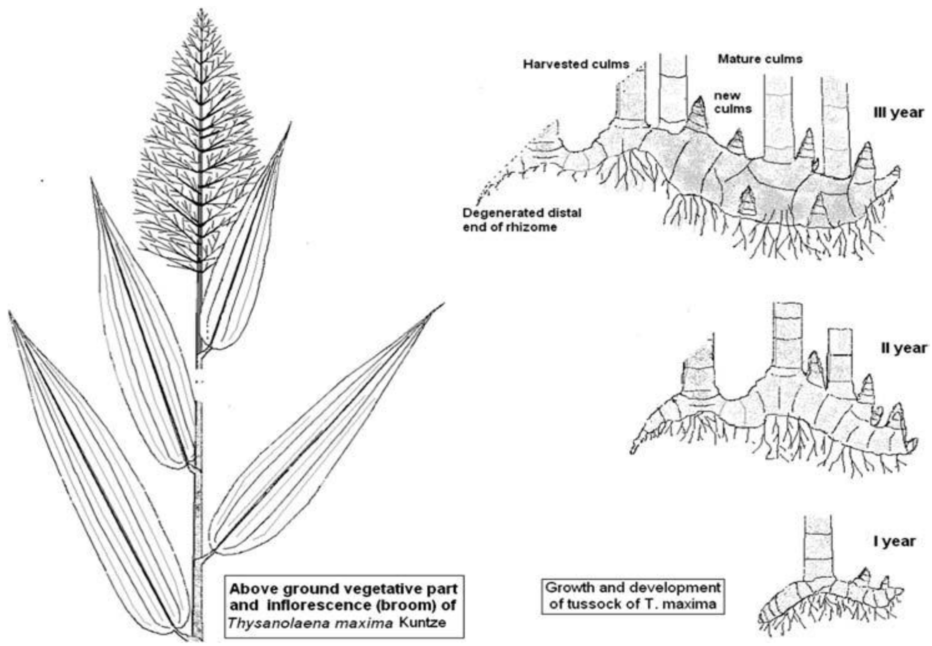

Fig. 1.4: Part of the Culm with Inflorescence (Broom) and Year-Wise Growth Pattern of Rhizome

1.17.8 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 especially near road construction sites where light availability is good.

1.17.9 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 seedling for propagation but it is always better to get quality seedlings from reputed nurseries.

1.17.10 Production and Processing

The quality of broom depends upon the time of harvesting. Shorter inflorescences, generally collected in the early stages of inflorescence development, are considered the best quality. The product is classified under three categories:

(a) Class-(I) or best quality: those types in which the flower has not yet opened and is collected in the months of January and February.

(b) Class-(II) or medium quality: those types that are cut immediately after flowering and are collected in the months of (late) February and March.

(c) Class-(III) or inferior quality: those types that have remained in the culms for longer periods and are collected in the months of April and May. After harvesting, the product is transported to homestead for processing, which is usually a simple process. A frame-like structure in the form of trays made of bamboo is used for drying the inflorescence. Sometimes, the inflorescences are tied in small bundles and hanged over fixed bamboo poles. The drying operation is done over three to four days for hardening the stems in order to prevent rotting. The product is then packed in large bundles and transported to the market or stocked in one place in the villages to sell to middlemen or traders. The majority of the product enters the market and is transported to other places at this level of processing only. Value addition, that is, the making of broom, is done manually by very few households and for a small quantity of the total harvest. Meghalaya has now emerged as one of the largest producers and exporters of broomgrass in the country. Ninety percent of the brooms produced are exported outside the state. The production of brooms in Meghalaya from the years 2004 to 2009 is given in. There is a trend of an increase in production, price, and growers' income. This may be attributed to the expanding market for the product. The steady increase in the price shows that the price was regulated by external demand. The drop in price during 2005 and 2006 may be attributed to the doubling in the production within a year, possibly causing a glut in the market. However, during the subsequent years, when production either increased moderately or plateaued, the price continued to increase. (Tiwari *et al.*, 2012)

1.17.11 Additional Plant Information

1.17.11.1 Planting

It prefers a warm, shaded position, mimicking its tropical habitat however, perhaps surprisingly, it will handle light frosts. Direct mid-summer sun can burn the leaves, particularly the tips. If multi planting, plant at 1m-1.5m intervals.

1.17.11.2 Watering

Water in well when first planted and water regularly until established. After that, moderate levels of rainfall should be sufficient to keep the plants happy and healthy.

1.17.11.3 Fertilizer

Feed with a nitrogen-rich liquid fertilizer a few times a year to keep the plant looking lush and healthy.

1.17.11.4 Pruning

To keep the clump looking fresh, it is best to prune some of the oldest canes back to the ground each year. Start this process after 3 years. Remove tired old stems by cutting back to the stem’s base. Trim the plant to shape if required. The plant produces broom-like flowers on mature plants, so you can remove spent flower heads as well.

1.17.12 Landscaping Uses

It is excellent in a Balinese or Japanese style garden. Useful as a screening plant and can be used as a feature in containers. Suited for use as a screen or hedge in narrow spaces where other hedging plants are not suited. Best to be planted away from swimming pools as the flowers will drop litter as they fade.

Tiger Grass will also look great growing in a pot on a balcony or patio. If it is decided to grow in a pot, keep in mind that they will need plenty of water over summer.

Table 1.5: Different uses of *Thysanolaena maxima*

USES FOR THIS BAMBOO SPECIES	ENVIRONMENTS & AREAS SUITABLE FOR THIS BAMBOO SPECIES
Screening	Full Sun – Full Shade
Hedging	Partial Sun – Partial Shade
Dust Suppression	Hot Environments
Dust Capture	Cold Environments
Ornamental	Cities – Suburban Areas
Shading	Rural Areas – Swamps
Live Fence	Beach Front Areas

1.17.13 Chemical Composition and Nutritive Value of *Thysanolaena maxima*

Thysanolaena maxima grass contained 15.3% crude protein, 3.82% ether extract, 23.58% crude fibre, 47.69% nitrogen-free extract, 9.60% total acids, 0.61% calcium and 0.21% phosphorus in DM (Bhuyan, 2016).

1.17.14 Medicinal Values

The extract from the root suckers is used to check boils. It has been observed that in some cases, boils were healed overnight. In case of retention of placenta in cows, the ‘amliso’ plant is fed for easy and immediate release. When the navel cord of a newly-born child has to be cut, the ‘amliso’ leaf is used as a knife without any infection. The root extract is also used as a mouth wash. Dried stem and bark powder is used as tonic, antiperiodic, aphrodisiac and also used in malaria (Vardhana, 2008).

1.17.15 Environmental Sustainability

The planting of broom grass has a direct impact on preventing surface soil erosion on steep hillsides. Broom grass grows in clumps and has many tangled up roots that grow to about one meter below the ground. This makes it highly effective in preventing soil erosion on hillsides as the grass is less likely to fall compared to other plants and trees that would have been planted there. The roots and leaves of the plant slow down water drops and the flow of water after heavy rain by absorbing the water in the soil. Growing broom grass on degraded land has been proven to help rehabilitate it as it helps retain ground moisture and promote fertility. There is no irrigation required to grow the grass and it does not produce any wastewater. No external inputs or energy is needed to grow the plant as it only requires human labor, which can be extensive in the first year of growing. Broom grass farming is highly recommended in new shifting cultivation systems on marginal lands to repair the damage from previous slash and burn methods.

1.17.16 Impact on Promoting Local Biodiversity

The start of growing broom grass has increased the local biodiversity in the communities. Now that the farmers have to tie up their livestock since they feed on the broom grass, other plant species in the area can successfully regrow and multiply. Broom grass that have been planted in areas where slash and burn cultivation took place has caused tree stumps to grow branches and other vegetation to grow back. Endangered animals such as the Barking Deer and Monkey are now reappearing in the infertile slash and burn areas where they once lived, as the broom grass used to rehabilitate the soil helps promote the growth of other vegetation the animals use for food. Broom grass does not compete for land with cereal crops so they can be grown simultaneously (Vardhana, 2008).

CHAPTER TWO
LITERATURE REIVIEW

2.1 Growth and Lead Accumulation by *Thysanolaena maxima* in Lead Contaminated Soil Amended with Pig Manure and Fertilizer: A Glasshouse Study.

Bo Ngam lead mine soils contain high concentrations of lead (up 1% total Pb) and low amounts of organic matter and major nutrients (N, P, K). A glasshouse study was conducted to compare growth performance, metal tolerance and metal uptake by two grasses, *Thysanolaena maxima* (Roxb.) O. Kuntze and four ecotypes of *Vetiveria zizanioides (L.) Nash, syn. Chrysopogon zizanioides (L.)* Roberty (three from Thailand: Surat Thani, Songkhla and Kamphaeng Phet, and one from Sri Lanka) and to study the effects of pig manure (20% and 40% w/w) and inorganic fertilizer (75 and 150 mg kg (-1)) amendments to this lead mine soil. The results showed that both *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla) could tolerate high Pb concentrations in soil (10750 mg kg (-1)) and had very good growth performance. Application of pig manure increased electrical conductivity (EC) and reduced DTPA-extractable Pb concentration in the soils. Pig manure application improved the growth of vetiver, especially at 20%, application dosage. *V. zizanioides* had the highest biomass. *T. maxima* could not tolerate high EC values. The uptake by roots and transport of Pb to shoots of both species was reduced when soils were amended with pig manure. Application of inorganic fertilizer did not improve growth of *vetiver* but did improve that of *T. maxima*. Fertilizer application did not have any great influence on the Pb uptake in *vetiver* while *T. maxima* took up more Pb as a result of the fertilizer enhancing its biomass yield. Both species transported low Pb concentrations to shoots (8.3-179 mg kg (-1)) and accumulated higher concentrations in roots (107-911 mg kg (-1)). In summary, both species may be species well suited for phytostabilization in tropical lead mine areas (Rotkittikhun *et al*., 2007).

2.2 The Chemo preventive Effects of *Thysanolaena Latifolia* against Carbon Tetrachloride (CCl_4).

Present study was undertaken to evaluate the chemopreventive effects of *Thysanolaena latifolia* against carbon tetrachloride (CCl_4)-induced oxidative stress in rats. Methods: Rats were pre-treated with *T.latifolia* prior to the administration of CCl_4. Hepatic damage and toxicity were evaluated by measuring the levels of serum transaminases, malondialdehyde content, reduced glutathione, antioxidant enzymatic molecules and histopathological changes in rats. Free radical scavenging activity of *T.latifolia* was evaluated by in vitro 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Results: Administration of CCl_4 to rats (1.2 ml/kg, p.o.) showed a significant elevation of lipid peroxidation and decreased enzymatic and nonenzymatic antioxidants. Consistent with these changes, CCl_4 treatment enhances hepatic damage as evidenced by sharp increases in serum transaminases. In addition, the impairment of liver function corresponded with histolopathological changes. Pretreatment of animals with *T.latifolia* (150 and 300 mg/kg, p.o.) showed significant and dose-dependent protection against CCl_4 -mediated oxidative liver damage and toxicity in rats. Furthermore, histopathology of *T.latifolia* treated animals showed tendency toward normalization of cytoarchitecture of liver. *T.latifolia* was found to possess 20.3 ± 0.72 mg/g total phenolic content expressed as gallic acid equivalent and to scavenge DPPH radical significantly (Gnanaraj, Haque and Iqbal, 2012).

2.3 Antimicrobial and Antioxidant Activity of Some Indigenous Plants of Nepal.

This study was investigated the Antimicrobial and Antioxidant Activity of *Thysanolaena maxima* (Roxb.) .The ethanol extract *of Thysanolaena maxima* was subjected to evaluate its 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 by *T.maxima* was 8 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. *Thysanolaena maxima* have IC_{50} of 250µg/ml. The overall result shows that the plant has interesting antibacterial and antioxidant activity (Basnet and Subba, 2014).

2.4 Ethnomedicinal Study and Antibacterial Activities of Selected Plants of Palpa District, Nepal.

This study was investigated the common medicinal plants of palpa district for their Ethno medicinal uses and screened for their antibacterial activity. The disk diffusion method was used to test the antibacterial activity. Four strains of bacteria employed in test were two-gram positive *Bacillus subtilis* and *Staphylococus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa.* Result shows that, root juice *of T.maxima* has very good Anthelmentic effect. (Mahato and Chaudhary, 2014)

2.5 Herbal Remedies among the Khasi Traditional Healers and Village Folks in Meghalaya.

The study investigated the use of medicinal plant among the Khasi traditional healers. They use the Inflorescence paste of *Thysanolaena maxima* (Roxb.), mixed with a pinch of a slaked lime and applied locally for treatment of boils and cancer. Young stem juice is applied on the eye when eyes become red and dirty. (Hynniewta and Kumar, 2008)

2.6 Antioxidant, Antibacterial and Cytotoxic Activities of Various Extracts of *Thysanolaena maxima* (roxb) *kuntze* Available in Chittagong Hill Tracts of Bangladesh.

Phytochemical screening was conducted using the specific standard procedure. Antioxidant activity of the extracts was evaluated using DPPH radical scavenging assay and reducing power assay. Determination of total phenolic and flavonoid contents was also carried out. Antibacterial and cytotoxic activities were investigated using disc diffusion method and brine shrimp lethality bioassay, respectively.

The methanol extract showed highest DPPH radical scavenging activity as well as possessed highest phenolic content (IC_{50} value for DPPH is 36.94±0.62 µg/ml and total phenolic content is 74.39±2.87 in mg/g, GAE) compared to the petroleum ether and chloroform extracts. On the other hand, chloroform extract possessed maximum flavonoid content (81±7.542 in mg/g, QE) and highest reducing power compare to other extracts. All the extracts showed mild to moderate *in vitro* antibacterial activity with a zone of inhibition ranging from 7 mm to 16 mm. In brine shrimp lethality bioassay, the LC_{50}values for petroleum ether, chloroform and methanol extracts were found to be 579.05±78.08 µg/ml, 386.92±80.47 µg/ml and 494.29±104.82 µg/ml, respectively which revealed weak cytotoxic potentials of the extracts compared to the positive control

The results indicated that *T. maxima* could be a very potent source of natural radical scavenger. Isolation of active compounds from this plant responsible for producing such bioactivities is underway (Hoque *et al*., 2016).

2.7 Medicinal Plants in Tao Dam Forest, Wangkrajae Village, Saiyok District, Kanchanaburi Province.

This study was investigated for potential medicinal plant resources in Tao Dam Forest. *Thysanolaena maxima* (Roxb.) boiled with *Hyptis capitata* Jacq. And lin-ma (unknown species) for drink/ tonic (Chiramongkolgarn and Paisooksantivatana, 2014).

2.8 Growth Pattern, Production, And Marketing *of Thysanolaena maxima* (Roxb.) Kuntze: An Important Nontimber Forest Product of Meghalaya, India.

The dry weight of stems or leaves per culm did not increase significantly with the age of the tussock and the overall ratio of the leaf to stem weight per tussock during the first and second year of growth was around 1. The ratio, however, increased slightly during the third year and significantly during the fourth year. On the basis of measurement of reproductive allocation, it was found that the species, on average, allocated around 16% of total aboveground biomass toward its reproductive structure, that is, inflorescence that forms broom, the commercial forest produce. Total biomass per tussock increased from about half a kg at the end of the first year of growth to about 12 kg at the end of year 4. The average productivity increased up to the third year and decreased drastically beyond the fourth year onward. Farmers, however, still harvest the crops as new culms, arising during the fourth and fifth year of growth, providing some brooms even during the sixth year. The observations were not extended beyond the fourth year due to disarrayed patterns in vigor loss (Tiwari *et al*., 2012).

2.9 Exserohilum Leaf Spot on Tiger Grass.

Thysanolaena maxima (Roxb.) Kuntze (tiger grass) is a commercial containerized and landscape ornamental grass from the family Poaceae (subfamily: Centothecoideae, tribe: *Thysanolaeneae*) and similar in appearance to bamboo. In the summer of 2006, leaf spot on Tiger grass was first noticed in a South Florida nursery, and subsequently in several nurseries and landscapes throughout Miami-Dade Co. and the Florida Keys in Monroe Co. In addition, the same leaf spot symptoms were observed on young transplants from a production greenhouse at Apopka, FL.

Symptoms start as minute tan colored flecks often turning chlorotic to necrotic. These initial lesions elongate elliptically between the leaf veins, sometimes with a yellow halo, eventually turning necrotic. Lesions on young, naturally infected leaves vary from pinpoint size up to 0.2 cm wide (between the veins) and 1.2 cm long. Individual lesions gradually coalesce into large necrotic elliptical spots to blotches, sometimes interspersed with chlorosis (Fig. 2.1). Infected leaf tips may turn light brown to brown, curl and turn yellow below the necrotic leaf tip. The purpose of this study was to identify and characterize the causal agent of the disease.

Fig. 2.1: Tiger Grass Showing Tan-Colored Lesions That Are Typical of Leaf Spot Symptoms on Day of Receipt from the Supplier

Tiger grass leaf pieces each containing part of a lesion were excised with a small amount of surrounding asymptomatic tissue, surface sterilized for 45 sec in 50 ml 10% commercial NaOCl solution (Clorox, Clorox Co., Oakland, CA) with a drop of detergent (Tween 20), and rinsed three times in sterile deionized water. The leaf pieces were picked up with sterile tweezers, surface water was removed by brief placement on filter paper (Whatman #1), and placed in the center of V8-juice agar plates. V8-juice agar was prepared by mixing 1 can (330 mL) V8-juice (Campbell Soup Co., Camden, NJ), 670 mL deionized water, 3 mg calcium carbonate, and 15-gram agar, and autoclaving (121°C, 16 psi, 15 min). Single-spore isolates were obtained by transferring individual germinating conidia to fresh plates of V8-juice agar media. Cultures were maintained in an incubator at 21.1 to 26.3°C under fluorescent lighting at 970 lux for 12 h light and 12 h dark.

A dematiaceous fungus was consistently isolated from naturally occurring lesions on tiger grass and identified as Exserohilum rostratum based on conidial morphology according to Sivanesan's key (Fig 2.2).

Fig. 2.2: Conidia of *Exserohilum Rostratum* with the Protruding Hilum (Arrow), Which Distinguishes the Genus *Exserohilum* from Bipolaris

Fig. 2.3: Bipolar Conidial Germination of *Exserohilum rostratum*

(Brunings *et al*., 2009)

2.10 Enzymatic Hydrolysis of *Thysanolaena maxima*

Tiger grass (*Thysanolaena maxima*) is considered an important perennial energy crop in Southeast Asia with a high productivity and a low requirement for fertilizer. The monomeric sugar yield from T. maxima by two-stage microwave/chemical pretreatment and enzymatic hydrolysis is evaluated. The optimal conditions of the pretreatment were investigated by varying reaction times, reaction temperatures and chemical concentrations to maximize the amount of obtained monomeric sugar. The T. maxima was treated with microwave-assisted NaOH pretreatment using 15:1 liquid-to-solid ratio (LSR), 1% (w/v) NaOH at 140 °C for 15 min, followed by microwave-assisted H2SO4 pretreatment using 15:1 LSR, 0.5% (w/v) H2SO4 at 200 °C for 5 min. The maximum monomeric sugar released was 30.2 g/100 g of NaOH-pretreated solids. The enzymatic hydrolysis of the microwave-/chemical-pretreated T. maxima at pH 4.8, 45 °C for 120 h using enzyme amount of 160 µl/g pretreated solids produced an impressive maximum sugar yield of 110.4 g/100 g of NaOH-pretreated solids (Komolwanich *et al*., 2016).

CHAPTER THREE
MATERIALS & METHODS

3.1 Objective

The overall purpose and objective of the study is to investigate CNS activities of *Thysanolaena maxima.*

3.2 Study Area

The research was carried out in the Pharmacognosy Lab, Microbiology Lab, Chemistry Lab and Pharmacology Lab of Department of Pharmacy, East West University, Dhaka.

3.3 Data Collection

All the relevant data has been collected from two types of sources:

- Primary sources: direct personal contact and observations of the experiments carried out in the laboratory.
- Secondary sources: various publications like journals, papers, documents and websites.

3.4 Research Protocol

- Selection, identification, collection, drying and grinding of plants
- Extraction of the powders with methanol and collection of extract
- CNS depressant activity determination
- Studying and comparing the results obtained

3.5 Information Processing and Analysis

The data and the results collected were reviewed, compared, processed and organized. Some tests were repeated to be sure of the results. Some data were analyzed into flow charts and statistical tables where possible.

3.6 Preparation of Plant Extraction for Experiment

Extraction, the first step in the value addition of Medicinal plants bio resources is the production of herbal drug preparations (i.e. extracts), using a variety of methods from simple traditional technologies to advanced extraction techniques. Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

3.6.1 Materials

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

3.6.2 Collection and Identification

The whole plant was collected from Chittagong hill tracts region.

3.6.3 Drying of the Parts

The plant was thoroughly washed with water. Leaves were taken and cut into small pieces and spread in thin layers in trays. The leaves were then sun dried for seven days. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 500^{o}C for 2 hours.

3.6.4 Grinding and Storage of the Dried Samples

The dried leaves were ground to coarse powder with a mechanical grinder (Grinding Mill). This process breaks the dried leaves to smaller pieces thus exposing internal tissues and cells to solvents thus 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 weight of the total dry powder was 700 g and was measured using electronic balance.

3.6.5 Extraction of the Dried Powdered Sample

The dried leaves of *Thysanolaena maxima* were coarsely powdered by a milling machine and soaked in 8000 ml of methanol. The container with its contents was sealed and kept for 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a course of filtration by a piece of clean, white cotton material.

3.6.6 Condensation of the Leaf Extracts

The extracts were transferred to the round bottle flask of rotary evaporator. Then excess amount of solvents in the extracts were removed by rotary evaporator, with reduced pressure which was done by using a vacuum pump. The temperature of the rotary evaporator was set 50ºC. It run for 1h 10min and the RPM was set 120 for evaporation process. After evaporation extract was transferred in a beaker. Rest of the extract was removed from the round bottle flask by using dichloromethane. Then extract was kept in hot air oven to get more dried extract. All beakers were covered with aluminum foil. The extract was then collected and stored in a cool (4˚C) dry place for further assay.

3.7 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.

Fig. 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.

3.8 Test for CNS Depressant Activity

3.8.1 Experimental Animals

Swiss male Albino Mice of 20–25 g were collected from laboratory of Jahangir Nagar University, Bangladesh. The animals were kept in standard laboratory conditions (relative humidity 55–60%; room temperature 25°C; 12 h light/dark cycle) and were provided with standard diet and clean water.

Fig. 3.2: Albino Mice

3.8.2 Experimental Design

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

3.8.3 Drugs and Chemicals

1. Carboxy methyl cellulose (CMC)

2. Water for injection

3. Diazepam

4. *Thysanolaena maxima* (methanol extract)

3.8.4 Method of Identification of Animals

Each group consists of six 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= Mice 3, M_4= Mice 4, M_5= Mice 5 & M_6= Mice 6.

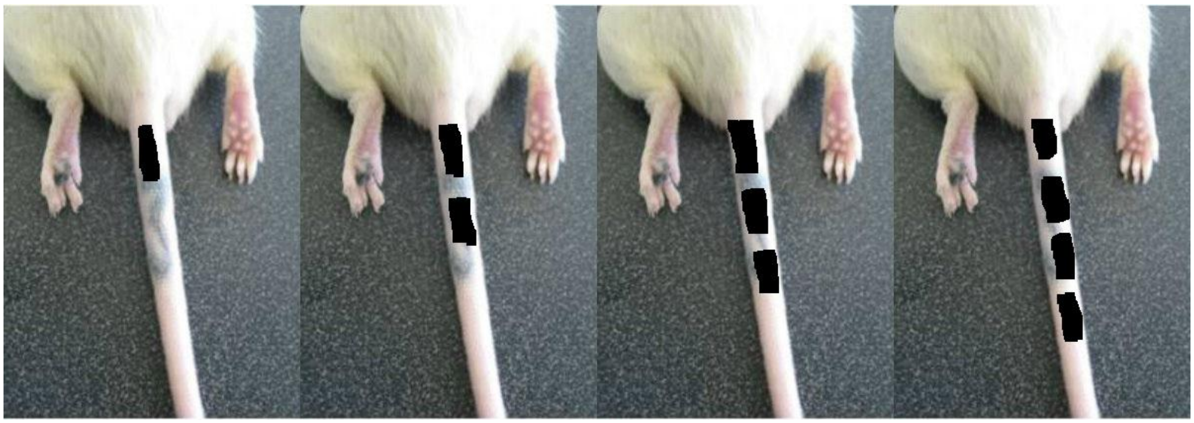

Fig. 3.3: Method of Identification of Animals

3.8.5 Preparation of Test Material

In order to administer the crude extract of Methanol at dose 200mg/kg & 400 mg/kg body weight of mice. The extract was collected 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. To stabilize the suspension, it was stirred well. For the preparation of positive control group (1mg/kg) Diazepam was taken & a suspension of 1.2 ml was made.

3.8.6 Hole Cross Test

The most reliable behavioral change is a hyperemotional response to novel environmental. The method was adopted as described by Takagi *et al*. The aim of this study was to characterize the emotional behavior of rodents using the hole-cross test.

For this experiment:

- A cage was used having a size of 30 × 20 × 14 cm with a fixed partition in the middle having a hole of 3 cm diameter.
- The animals were divided into control and test groups containing 6 mice each.
- The test group received *T.maxima* extract at the doses of 200mg/kg and 400 mg/kg body weight orally whereas the negative control group received vehicle (CMC in water) & positive control group received Diazepam (1mg/kg)
- Then they were allowed to cross the hole from one chamber to another.
- Mice were observer for 3 min and the number of passages was recorded at 0 and 30-minute interval following the treatments.

Fig 3.4: Mice in Hole-Cross Box

CHAPTER FOUR

RESULT

4.1 CNS Depressant Activity

4.1.1 Hole-Cross Test

Mice treated with *T.maxima* plant extract at two doses (200 mg/kg & 400mg/kg) showed dose dependent reduction in the locomotor activity which was comparable with standard drug diazepam. In case of control group, negligible variation in number of holes crossed from one chamber to another by mice was observed from 0 to 30 minute. Whereas groups treated with plant extract at the above mentioned doses showed significant decrease of movement from their primary value at 0 to 30 minute.

Table 4.1: CNS Depressant Activity of Methanol Extract of *T.maxima* on Hole-Cross Test in Mice

Treatment	Dose	No of Movements	
		0 minute	30 minute
Negative Control 1% CMC	1gm/100ml	12.8±1.16	13±1.41
Positive Control Diazepam	1mg/kg	13.80±0.86	5.40±0.68
Methanol extract of *T.maxima*	200mg/kg	7.25±2.096	1.25±.75
Methanol extract of *T.maxima*	400mg/kg	7.00±1.08	.50±.50

*All the values are expressed as mean ± SEM

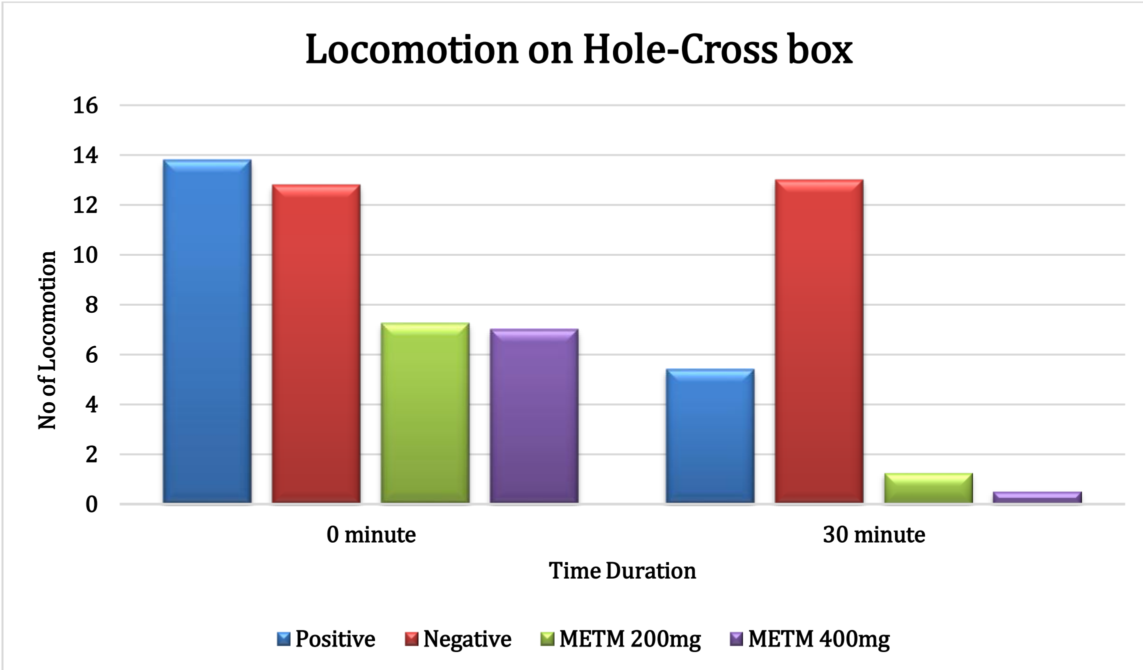

Fig 4.1: Locomotion Count on Hole-Cross Box

CHAPTER FIVE
DISCUSSION

Discussion

The current research was done to investigate the CNS depressant activity of methanolic extract of plant *T.maxima* in mice. To acquire evocative results regarding the effect of methanolic extract of *T.maxima* on CNS in mice, hole-cross method was implemented.

Anxiety and hypnosedation are principally mediated in the CNS by the GABAA receptor complex, which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, Parkinson syndrome, and Alzheimer's disease. Diverse drugs that are used in various psychological and neurological disorders might modify the GABA system at the level of the synthesis of GABA, induce anxiolysis or hypnosis in animals by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors, and thirdly by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents like barbiturates.

The extract showed significant CNS depressant effect in Hole-cross test in comparison to Standard drug Diazepam in dose dependent manner. For the study, 200mg/kg and 400mg/kg doses were administered in the mice orally. In comparison to the control group, a significant decrease in locomotor activity was observed to the test group. Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated the sedative effect. Extracts of *T.maxima* decreased locomotor activity indicates its CNS depressant activity. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABAA.

Different types of flavonoids and neuroactive steroids were found to be ligands for the GABAA receptors in the central nervous system; which led to assume that they can act as benzodiazepine like molecules. The sedation may be due to an interaction with benzodiazepines like compounds. Literature review of the plant revealed the presence of terpenoids, carbohydrates, tannins, flavonoids, saponins and glycosides in all extracts of *T. maxima* in varying amount. So might be this phytoconstituents are responsible for its

CNS depressant activity. The plant extracts may act by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization which diminish in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts.

CHAPTER SIX

CONCLUSION

Conclusion

Traditionally *Thysanolena maxima* are used as an antituberculosis drug in the hilly areas of this subcontinent. As the literature review suggests, the presence of several phytochemical compounds in *Thysanolaena maxima* makes the plant pharmacologically active. From the result of my study, it can be concluded that using in vitro experiments established that methanol extract of *Thysanolaena maxima* has moderate to high CNS depressant activity. However, further research is needed in order to find out the precise mechanisms and responsible chemical constituents for the CNS depression, analgesic, anti-inflammatory, antidiarrheal, anti-diabetic and other pharmacological activities. In the near future we will conduct experiments with purified fractions of the above extracts for further pharmacological and toxicological characterization.

CHAPTER SEVEN
REFERENCES

Alschuler L., Benjamin S.A. and Duke J.A. (1997). Herbal Medicine -What Works, What Is Safe. *Patient Care*, 10(3) pp.48-103.

Basnet, P. and Subba, B. (2014). Antimicrobial Activity of Some Medicinal Plants from East and Central Part of Nepal. *International Journal of Applied Sciences and Biotechnology*, 2(1), pp.36-42.

Bensoussan A., Talley N.J. and Hing M. (1998). Treatment of Irritable Bowel Syndrome with Chinese Herbal Medicine: Arandomized Controlled Trial. *Journal of the American Medical Association*, 280, 1585-1589

Bhuyan, K. (2016). *Chemical Composition and Nutritive value of Kuchi (Thysanolaena maxima Roxb) grass*. [online] Eurekamag. Available at: http://eurekamag.com/research/001/775/001775375.php#close [Accessed 25 May 2016].

Brevoort, P. (1998). The Booming US Botanical Market. A New Overview. *Herbal Gram*, 44 (1), pp.33-44.

Brunings, A., Datnoff, L., Palmateer, A., Locke, J. and Krause, C. (2009). Exserohilum Leaf Spot on Tiger Grass. *Plant Health Progress*, 2(1), pp.22-25.

Carter, A. (1999). Dwale: An Anesthetic from Old England. *British Medical Journal*, 19(2), pp.1623-1626.

Cooper, J. (2015). *Sedative-Hypnotic Toxicity: Background, Pathophysiology, Epidemiology*. [online] Medscape. Available at: http://emedicine.medscape.com/article/818430-overview#a6 [Accessed 24 May 2016].

Cox, P.A. (2000). Will Tribal Knowledge Survive the Millennium? *Science*, 287, pp.44-45.

Ghani, A. (1998), Dhaka: Asiatic Society, *Medicinal Plants of Bangladesh*, Dhaka. pp. 11-41.

Harvey, A. (1999). Medicines from Nature: Are Natural Product Still Relevant to Drug Discovery? *Trends in Pharmacological Sciences*, 20, pp. 196-198.

Hobbs, C. (n.d.). *Herbs and Natural Remedies for Insomnia*. [online] Christopherhobbs.com. Available at: http://www.christopherhobbs.com/library/articles-on-herbs-and-health/herbs-and-natural-remedies-for-insomnia/ [Accessed 24 May 2016].

Jirsa, A. (2012). *Herbs for Insomnia*. [online] Mindbodygreen. Available at: http://www.mindbodygreen.com/0-5104/Herbs-for-Insomnia.html [Accessed 20 Apr. 2016].

Kala, C. (2005). Current Status of Medicinal Plants Used by Traditional Vaidyas in Uttaranchal State of India. *Ethnobotany Research and Applications*, 3(2), p.267.

Komolwanich, T., Prasertwasu, S., Khumsupan, D., Tatijarern, P., Chaisuwan, T., Luengnaruemitchai, A. and Wongkasemjit, S. (2016). Evaluation of Highly Efficient Monomeric Sugar Yield from Thai Tiger Grass (*Thysanolaena maxima*). *Materials Research Innovations*, 20(4), pp.259-267.

Mirshafa, S., Azadbakht, M. and Ahangar, N. (2013). Study of Antidepressant and Sedative-Hypnotic Activity of Hydroalcoholic Extract of *Asperugo procumbens L*. Aerial Parts in Mice. *Iranian Journal of Pharmaceutical Researc*, 12(3), pp.529-535.

Pal, S. and Shukla, Y. (2002). Herbal Medicine: Current Status and the Future. *Asian Pacific Journal of Cancer Prevention*, 4, pp.281-288.

Petrovska, B. (2012). Historical Review of Medicinal Plants′ Usage. *Pharmacognosy Reviews*, 6(11), pp.1-5.

Rai, S. (1997). *Kuntz, A Multipurpose Plant*. [online] Agricultures Network. Available at: http://www.agriculturesnetwork.org/magazines/global/forging-partnerships/kuntz-a-multipurpose-plant [Accessed 26 Jun. 2016].

Rasool, B. (2012). Medicinal Plants (Importance and Uses). *Pharmaceutica Analytica Acta*, 3(10), pp.22-30.

Rath, S., Jagadev, P. and Patnaik, A. (1994). Genetic Variability and Character Association in Broomgrass (*Thysanolaena maxima Roxb*.). *The Indian Journal of Genetics and Plant Breeding*, 54(3), pp.300-303.

Rotkittikhun, P., Chaiyarat, R., Kruatrachue, M., Pokethitiyook, P. and Baker, A. (2007). Growth and Lead Accumulation by the Grasses *Vetiveria zizanioides* and *Thysanolaena maxima* in Lead-contaminated Soil Amended with Pig Manure and Fertilizer: A Glasshouse Study. *Chemosphere*, 66(1), pp.45-53.

Singh, R. (2015). Medicinal Plants: A Review. *Journal of Plant Sciences*, 3(1), pp.50-55.

Sofowora, A. 1982. Medicinal Plants and Traditional Medicine in Africa. John Wiley & Sons Limited, New York, pp.356-362.

Tiwari, B., Shukla, R., Lynser, M. and Tynsong, H. (2012). Growth Pattern, Production, and Marketing of *Thysanolaena maxima (Roxb.) Kuntze*: An Important Non-Timber Forest Product of Meghalaya, India. *Forests, Trees and Livelihoods*, 21(3), pp.176-187.

Vardhana, R. (2008). *Direct uses of medicinal plants and their identification*. New Delhi: Sarup & Sons, pp.225-290.

Vickers, A. and Zollman, C. (1999). ABC of Complementary Medicine: Herbal Medicine. *British Medical Journal*, 319, pp.1050 -1053.