# Evaluation of antimicrobial & antioxidant activities of methanol extract of *Thysanolaena maxima.*

**A Dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.**


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I hereby declare that this dissertation, entitled **"Evaluation of antimicrobial & antioxidant activities of methanol extract of *Thysanolaena maxima*"** is an authentic and genuine research work carried out by me under the guidance of **Nazia Hoque,** Senior lecturer, Department of Pharmacy, East West University, Dhaka.

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Mohammad Raju Khan

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*Dedication*

*This Research Paper is dedicated to*

*My beloved parents,*

*Who are my biggest inspirations...*

# ABSTRACT

The plant *Thysanolaena maxima* has been used by the natives in Tao Dam Forest, Bangkok and *Khashi* traditional healers and village folks in Meghalaya. It is used as a traditional medicine for the treatment of cancer, In case of Red eye and Dirty, in treatment of Dysentery, to facilitate Delivery, in veterinary medicine and as mouth wash in fever.

The aim of the present study was to evaluate the antimicrobial activity and antioxidant activity of methanol extract of *Thysanolaena maxima.*

The antimicrobial activities of methanol solvent extract of *Thysanolaena maxima* plant were tested against the gram-positive and gram-negative bacterial strains by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The crude methanolic extract of *Thysanolaena maxima* leaves extract showed very low antimicrobial activity against the microorganisms at concentrations of 600 μg/disc.

The antioxidant effect of methanolic extract of *Thysanolaena maxima* was very potent and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe3+ ions, which had a lesser reductive activity than the standard of Ascorbic acid.

In conclusion, further investigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported antimicrobial and antioxidant properties of *Thysanolaena maxima*.

**Key Words:** *Thysanolaena maxima*, Antimicrobial, Antioxidant.

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# Chapter: 1

# INTRODUCTION

## 1.1 Overview on Medicinal Plants

Fossil Records has revealed the use of medicinal plants by human beings around 60,000 years ago during Middle Paleolithic Age, (Fabricant & Farnsworth, 2001). These Fossil records suggest that even Neanderthal were not an exception who did not make use of medicinal plants, (Das and Choudhury, 2012).

Example of such medicinal plants is *Gingko biloba* which has been used medicinally for thousands of years. It is used for the treatment of numerous conditions such, many of which are under scientific investigation. The species has an evolutionary lineage that dates back to the Lower Jurassic, about 190 million years ago. Although this genus has undergone much change over this length of time, fossilized leaf material from the Tertiary species Ginkgo adiantoides is considered similar or even identical to that produced by modern *Ginkgo biloba* trees, (Jalalpour, et al 2012).

**Figure 1: A Fossilized *Gingko adiantoides* Leaf similar to its modern day predecessor *Gingko biloba***

The Gingko plant is wide used in Alzheimer's disease, Cerebrovascular Insufficiency, Cognitive Enhancement, Depression, Diabetes, Intermittent Claudication, Macular Depression, PMS, Sexual Dysfunction, Tinnitus, (Pelton, 2000).

The Plant kingdom consists of many different plant species containing different substances of medicinal importance. Some of these have already been explored for biological activity while some are not, (Rahman, *et al.* 2008).

As a source of medicine plant materials are important components of health care system. There are about 250,000 higher plant species (both Angiosperms and Gymnosperms) with a lower limit of 215,000 and upper limit of 500,000. Among these only 6% have been screened for biological activity and 15% have been evaluated phytochemically, (Fabricant & Farnsworth, 2001). Only just in South East Asia and its surrounding parts, there exist about 50,000 plant species among which 3,000 plants have been documented for potential medicinal properties and around 6,000 plants are used by traditional practitioners, (Shariff, *et al.* 2006).

So, Plants have been the traditional source of raw materials for medicine. It is known through the scholastic works of Atharva Veda and the writings of Charaka and Sushruta which gave huge knowledge of preventive and curative medicinal to the scientific community, (Chowdhury, *et al.* 2008).

Now, nearly 95% of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates, (Maridass *et al.* 2008).

It has been estimated that about 13000 plant species around the world are used as drugs. Since, the inclination of using natural product has increased; the exploration of active plant extracts has become frequent for new drug discovery, (Ferdous, *et al.* 2010).

Over 50% of all advanced clinical drugs are made of natural products that play an important role in drug development programs of the pharmaceutical industry. There are hundreds of medicinal plants which have a long history of curative properties against various diseases. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the higher plant, (Razvy, *et al.* 2011).

So, for being cheap, relatively safe and easily available, medicinal plants and herbs embody the foundation of traditional medicinal practice all over the world. Representing an untapped and

huge reservoir of drugs either known or novel in origin, the medicinal plants are center of research to find out novel lead compounds, (Ambikar, *et al*. 2010).

## 1.2 Medicinal Plants: An Evergreen Part of Medical Science

Death is authentic but unavoidable. Nobody can desire to lose his short but sweet life. Man is therefore, being continued his struggle to achieve mastery over the forces of nature- Diseases Decay and Death. Human struggle against the misery of three D"s-Disease, Decay and Death is eternal. From the very inception of civilization, the inherent concern for getting as well as staying healthy has been instigating human venture for cure from his surroundings. Illness, physical discomforts, injuries, wounds & fear of death had forced prehistoric man to use any natural substances that he/she could lay his/her hands on- "the green friends" PLANTs (Ogdan, 1981).

## 1.3 Humans and Plants

Humans need plants. All animals do. Humanity's relationship with plants has actually made it possible for us to have a civilization. Before we had cities, humans went around in little packs and were hunter-gatherers. We ate rats, birds, berries, and whatever food we could find. It wasn't very efficient. One day someone had the bright idea to plant the plants we like to eat. When humans did that, they were able to stay in one place full time. Then came the cities and a huge system of agriculture to support millions of people.

Everything we eat comes directly or indirectly from plants. Throughout human history, approximately 7,000 different plant species have been used as food by people. Plants regulate the water cycle: they help distribute and purify the planet's water. They also help move water from the soil to the atmosphere through a process called transpiration. Oxygen is brought to you by plants, as a byproduct of photosynthesis. Plants store carbon, and have helped keep much of the carbon dioxide produced from the burning of fossil fuels out of the atmosphere. Of course, aside from humans' myriad uses, plants make up the backbone of all habitats. Other species of fish and wildlife also depend on plants for food and shelter. (Bgci.org, 2014)

## 1.4 Traditional Medicine

Traditional medicine (also known as indigenous or folk medicine) comprises knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness

In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called alternative medicine. Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional Chinese medicine, traditional Korean medicine, acupuncture, Muti, Ifá, and traditional African medicine. Core disciplines which study traditional medicine include herbalism, ethnomedicine, ethnobotany, and medical anthropology

The WHO notes however that "inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems. The line between alternative medicine and quackery is a contentious subject.

Traditional medicine may include formalized aspects of folk medicine, that is to say longstanding remedies passed on and practiced by lay people. Folk medicine consists of the healing practices and ideas of body physiology and health preservation known to some in a culture, transmitted informally as general knowledge, and practiced or applied by anyone in the culture having prior experience. Folk medicine may also be referred to as traditional medicine, alternative medicine, indigenous medicine, or natural medicine. These terms are often considered interchangeable, even though some authors may prefer one or the other because of certain overtones they may be willing to highlight. In fact, out of these terms perhaps only indigenous medicine and traditional medicine have the same meaning folk medicine, while the others should be understood rather in a modern or modernized context. (Acharya, *et al.* 2008)

Similarly, a home remedy is a treatment to cure a disease or ailment that employs certain spices, vegetables, or other common items. Home remedies may or may not have medicinal properties that treat or cure the disease or ailment in question, as they are typically passed along by laypersons (which have been facilitated in recent years by the Internet).

### 1.4.1 Ayurveda

Ayurveda or Ayurvedic medicine is a system of Hindu traditional medicine native to the Indian subcontinent. Practices derived from Ayurvedic traditions are a type of alternative medicine. Ayurveda is a discipline of the upaveda or "auxiliary knowledge" in Vedic tradition. The origins of Ayurveda are also found in the Atharvaveda, which contains 114 hymns and incantations described as magical cures for disease. There are also various legendary accounts of the origin of Ayurveda, e.g., that it was received by Dhanvantari (or Divodasa) from Brahma. Ayurvedic practices include the use of herbal medicines, mineral or metal supplementation (rasa shastra), surgical techniques, opium, and application of oil by massages. (Wells& John C, 2009)

Originated in prehistoric times, some of the concepts of Ayurveda have been discovered since the times of Indus Valley Civilization and earlier. Ayurveda significantly developed during the Vedic period and later some of the non-Vedic systems such as Buddhism and Jainism also incorporated in the system. Balance is emphasized, and suppressing natural urges is considered unhealthy and claimed to lead to illness. Ayurveda names three elemental substances, the doshas (called Vata, Pitta and Kapha), and states that a balance of the doshas results in health, while imbalance results in disease. Ayurveda has eight canonical components, which are derived from classical Sanskrit literature. Some of the oldest known Ayurvedic texts include the SuśruthaSaṃhitā and CharakaSaṃhitā, which are written in Sanskrit. Ayurvedic practitioners had developed various medicinal preparations and surgical procedures by the medieval period. (Manohar, *et al* 2009)

Although laboratory experiments suggest it is possible that some substances in Ayurveda might be developed into effective treatments, there is no evidence that any are effective in themselves. Modern ayurvedic medicine is considered pseudoscientific. Other researchers consider it a proto-science, an unscientific, or trans-science system instead. Concerns were raised when 20% of

Ayurvedic U.S. and Indian-manufactured patent medicines sold through the Internet were found to contain toxic levels of heavy metals such as lead, mercury, and arsenic. (Quack, *et al*. 2011)

**Table 1: List of some Plants Used in Ayurveda**

| Species | Name | Uses in Ayurveda |
|---|---|---|
| *Achillea millefolium* | Biranjashipa | • Carminative<br>• Tonic |
| *Argyreia speciosa* | Elephant creeper | • Geriatric Tonic<br>• Mild Aphrodisiac |
| *Asparagus recemosus* | Wild Asparagus | • Blood Purifier<br>• Rejuvanative |
| *Capparis spinosa* | Capers | Hepatic Stimulant |
| *Cicorium intybus* | Wild Chicory | • Hepatic Stimulant |
| *Commiphora mukul* | Guggul | • Immunomodulator |
| *Crocus sativus* | Saffron | • Antioxidant |
| *Cyperus scariosus* | Umbrella‟s Edge | • Hepatoprotective |
| *Didymocarpus pedicellata* | Shilapushpa | • Diuretic |
| *Garcinia camboia* | Garcinia | • Cardiotonic |

| | | |
|---|---|---|
| *Glycyrrhiza glabra* | Licorice | • Antioxidan<br>• Laxative |
| *Gymnema sylvestre* | Gurmara | • Anti – diabetic |

### 1.4.2 Unani

Unani-tibb or Unani Medicine also spelled Yunani Medicine is a form of traditional medicine practiced in countries of the Middle East and South Asia. It refers to a tradition of Graeco-Arabic medicine, which is based on the teachings of Greek physicians Hippocrates and Galen, and developed into an elaborate medical system in the Middle Ages by Arabian and Persian physicians, such as Rhazes (al-Razi), Avicenna (IbnSena), Al-Zahrawi, and IbnNafis.

Unani medicine is based on the concept of the four humours: Phlegm (Balgham), Blood (Dam), Yellow bile (Ṣafrā') and Black bile (Saudā'). The time of origin is thus dated at circa 1025 AD, when Avicenna wrote The Canon of Medicine in Persia. While he was primarily influenced by Greek and Islamic medicine, he was also influenced by the Indian medical teachings of Sushruta and Charaka.

Unani medicine first arrived in India around 12th or 13th century with establishment of Delhi Sultanate (1206–1527) and Islamic rule over North India and subsequently flourished under Mughal Empire. AlauddinKhilji had several eminent Unani physicians (Hakims) in his royal courts. In the coming years this royal patronage meant development of Unani practice in India, but also of Unani literature with the aid of Indian Ayurvedic physicians. (Philosophy and Culture, New Delhi, 2001).

### 1.4.3 Traditional Chinese Medicine (TCM)

Traditional Chinese medicine is a broad range of medicine practices sharing common concepts which have been developed in China and are based on a tradition of more than 2,000 years, including various forms of herbal medicine, acupuncture, massage (Tuina), exercise (qigong), and dietary therapy. It is primarily used as a complementary alternative medicine approach. TCM is widely used in China and it is also used in the West. (Singh, *et al*. 2008)

TCM "holds that the body's vital energy circulates through channels, called meridians that have branches connected to bodily organs and functions." Concepts of the body and of disease used in TCM have notions of a superstitious pre-scientific culture, similar to European humoral theory. Scientific investigation has not found any histological or physiological evidence for traditional Chinese concepts such as qi, meridians, and acupuncture points. The TCM theory and practice are not based upon scientific knowledge, and its own practitioners disagree widely on what diagnosis and treatments should be used for any given patient. The effectiveness of Chinese herbal medicine remains poorly researched and documented. There are concerns over a number of potentially toxic plants, animal parts, and mineral Chinese medicinal. There is a lack of existing cost-effectiveness research for TCM. Pharmaceutical research has explored the potential for creating new drugs from traditional remedies, but few successful results have been found. A Nature editorial described TCM as "fraught with pseudoscience", and said that the most obvious reason why it hasn't delivered many cures is that the majority of its treatments have no logical mechanism of action, yet proponents argue that it is because research has missed key features of the art of TCM, such as the interactions between different ingredients. (Shang, *et al*. 2007)

The doctrines of Chinese medicine are rooted in books such as the Yellow Emperor's Inner Canon and the Treatise on Cold Damage, as well as in cosmological notions such as yin-yang and the five phases. Starting in the 1950s, these precepts were standardized in the People's Republic of China, including attempts to integrate them with modern notions of anatomy and pathology. In the 1950s, the Chinese government promoted a systematized form of TCM.

TCM's view of the body places little emphasis on anatomical structures, but is mainly concerned with the identification of functional entities (which regulate digestion, breathing, aging etc.). While health is perceived as harmonious interaction of these entities and the outside world, disease is interpreted as a disharmony in interaction. TCM diagnosis aims to trace symptoms to patterns of an underlying disharmony, by measuring the pulse, inspecting the tongue, skin, and eyes, and looking at the eating and sleeping habits of the person as well as many other things. (Steven Novella, 2012).

## 1.5 Goals of Using Medicinal Plants as Therapeutic Agents

The goals of using plants as sources of therapeutic agents are – a) To isolate bioactive compounds for direct use as drugs, (E.g. Digoxin, Digitoxin, Morphine, Reserpine, Taxol, Vinblastine, Vincristine); b) To produce bioactive compounds of novel or known origin as lead compounds for semi synthesis to produce molecules of higher activity and / or lower toxicity, (E.g. Metformin, Nabilone, Oxycodone and other narcotic analgesics, Taxotere, Teniposide, Verapamil, and Amiodarone, which are based on Galegine, Δ9 – tetrahydrocannabinol, Morphine, Taxol, Podophyllotoxin, Khellin respectively); c) To use agents as pharmacologic tools (E.g. LSD, Mescaline, Yohimbine); and d ) To use the whole plant or part of it as a herbal remedy, (E.g. Cranberry, Echinacea, Feverfew, Garlic, *Ginkgo biloba*). (Fabricant, *et al.* 2001).

## 1.6 Importance of Medicinal Plants as Drugs

According to WHO, 80% people of the developing countries chiefly rely on traditional medicines involving the use of plant extracts or their active constituents. Only a portion of the plants of the world have been screened thoroughly for their medicinal value in order to find out newer plant derived drugs. (Farnsworth, *et al.* 1991).

Plants have provided much life – saving pharmaceutical agents so far. And, there is an intense ongoing documentation of ethnomedical data and scientific research on medicinal plants by many developing countries. 14 of 35 in every 2000 drugs are either natural products or their derivatives. The plants that are not studied phytochemically can thus provide potential new leads for newer drug development. For example, Galegine from the herb *Galegaofficinalis* was the lead compound for the development of Metformin used in the treatment of type 2 diabetes. (Ahmed 2011)

**Table 2: Therapeutic Agents obtained from Flowering Plants**

| Plant Species | Therapeutic Agents |
|---|---|
| ***Atropa belladonna*** | Atropine |
| ***Camptotheca acuminate*** | Camptothecin |
| ***Catharanthus roseus*** | Vinblastine, Vincristin |
| ***Chondrodendron tomentosum*** | Tubocurarine |
| ***Digitalis lanata*** | Digitoxigenin, Gitoxigenin, Digoxigenin |
| ***Ephedra sinica*** | Ephedrine |

## 1.7 Medicinal Plants in Bangladesh

In Bangladesh 5,000 species of angiosperm are reported to occur. The number of medicinal plants included in the *Materia Medica of Traditional Medicine* in this subcontinent at present stands at about 2,000. More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. Dhaka, Rajshahi, Sylhet and Chittagong division is rich in medicinal plants where the cultivation of medicinal plants especially *Aloe vera* (Ghritakumari), *Asparagus racemosus* (Sotomuli), *Bombax ceiba* (Shimul), *Kaempferia angustifolia* (Misridana), *Ecolobium species* (Rajkantha) and*Ecolobium viride* (Nilkantha) are becoming popular, (Sharmin, 2004).

**Table 3: List of some Medicinal Plants used for Medicinal Purpose in Bangladesh**

| Scientific Name | Local Name | Traditional uses | Part(s) used |
|---|---|---|---|
| ***Bryonopsis laciniosa*** | Shivalingani | Skin Diseases, Dyspepsia, Jaundice | Whole Plant |
| ***Amorphophallus campanulatus*** | OlKachu | Piles, Tumors, Enlarged Spleen, Asthma, Rheumatism | Tuberous Roots |
| ***Hopea schaphula*** | Boilsur | Astringent, CNS depressant, Hypotensive | Stem Bark |
| ***Arachis hypogea*** | Cheenabadam | Emollient (Seeds), Bowel Astringent (Seeds Oil), Hemostatic Agent (Fruit Skin Extract) | Aerial Parts |
| ***Samanea saman*** | Fulkoroi | Diarrhea, Intestinal Diseases, Stomach Ache, Colds and Headache, Sore Throat | Bark |
| ***Michelia champaca*** | Champa | Fever, Colic, Leprosy, Post-Partum Protection, Eye Disorder | Seed and Flower |
| ***Aloe indica*** | Ghritakumari | Arthritis, hypertension Diabetes mellitus | Skin of Leaves |
| ***Swietenia mahagony*** | Mahagony | Diabetes, Malaria, Fever, Hypertension, | Seeds |

| | | | |
|---|---|---|---|
| | | Tuberculosis | |
| ***Caesalpinia nuga*** | Krung – khai | Analgesic, Anti Amyloidogenic, Antidiabetic / Hypoglycemic, Antifilarial, Anti-Inflammatory, Antimalarial, Antioxidant, Antitumor, Anxiolytic, Immunomodulatory. | Seed |
| ***Adansonia digitata*** | Baobab, Gadhagachh | Anti – malarial, Anti – pyretic, Anti – ulcerant, Health tonic | Leaf, Root, Flower |
| ***Jatropha gossypifolia*** | Karachuni, Bellyache Tree | Analgesic in toothache, Anti – diarrhoeal, Anti – malarial, | Leaf |
| ***Rauwolfia serpentina*** | Sharpagandha | Anti – hypertensive, Anti – malarial, Anti – psychotic | Root |
| ***Hodgsonia macrocarpa*** | Makal | Anti – malarial, Anti – pyretic | Fruit |

### 1.7.1 Use of Medicinal Plant in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (IUCN, 2003). The number of medicinal plants included in “Materiamedica” of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants,

majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (KritikarandBasu, 1980).

Bioactive compounds deposited in medicinal plants can serve as important raw materials for pharmaceutical manufacturing. Therefore, well-judged and scientific investigation of this wealth can significantly contribute to the public health. Again, it was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries. Thus, being available commodity of commerce, a country can also earn a good amount of foreign currency by exporting this natural wealth to other countries (Chopra, *et al*. 1982).

## 1.8 Economic Value

Medicinal plants are good repository of bioactive compounds. They serve as important therapeutic agents as well as essential raw materials for the manufacture of traditional and modern medicines. They, therefore, play a vital role to constitute a precious natural wealth of a country and contribute a great deal to its health care program. A huge amount of foreign exchange can be earned by exporting medicinal plants to other countries. India and Thailand are two examples of such countries which earn a lot of foreign exchange by exporting medicinal plants and their semi-processed products to other countries including Bangladesh. In this way indigenous medicinal plants take part significantly to build up a healthy economy of a country.

## 1.9 Research on Herbal Drug

Herbal drug may be defined as the plants, plant parts and plant products of all description, particularly those with medicinal properties. Herbal drugs are generally manufactured by the combination of two or more natural substances. The utility of these combinations are:

- To increase efficacy of the drug.

- To remove toxic effects.
- To reduce side-effects.
- To maintain stability.
- To keep pleasant taste, color and odor.

### 1.9.1 Scientific Basis of Herbal Drug

Herbal drug is often criticized as non-scientific, inactive and erroneous medicine. But phytochemical and biological investigation proves its medicinal value and therapeutic utility.

Traditional medicines that are used topically to treat skin disease contain tannin. Tannin is chemical having antiseptic and astringent property. When it is used topically it reacts with the proteins on infected area to produce a thin but strong barrier. This layer protects the infected area from micro-organism. Besides, tannin has antibiotic property. So it is said that there is no basic difference between herbal drug and allopathic medicine.

### 1.9.2 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).

## 1.10 Natural Sources: A Model for Synthetic Drugs

Natural sources are contributing to the development of modern synthetic drugs and medicines in a number of ways as stated below (Ghani, 1998):

- Novel structures of biological active chemical compounds, isolated from plant sources, often prompt the chemist to synthesize similar or better semi-synthetic compounds.
- Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant-derived compounds with known biological activity.
- Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by chemists for use as potent drugs.

Though most of the modern medicines are gift of synthetic chemistry, there are still some synthetic drugs where plant constituents act as "lead" (precursor) molecule. Procaine, a synthetic compound, displaces cocaine, isolated from coca leaves, due to its lacking of addiction property. Due to relatively low therapeutic index of procaine, search of new synthetic products lead to synthesis of Lidocaine, tetracaine and dibucaine. The discovery of diosgenin from Mexican Yams (Dioscoria) as a starting material for the synthesis of progesterone decreases the cost of progesterone from 80 U.S. $ per gm to 1.7 U.S. $ per gm. Also lifesaving antibiotic penicillin is synthesized from a natural product 6-aminopenicillanic acid derived from *Penicillium notatum* (Goldstein, *et al*., 1974).

### 1.10.1 Necessity of Drug Development from Plant Sources

The traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Since plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. On the contrary, a plant extract may have a chemical component in such a low concentration that it may not elicit the therapeutic action of interest. Besides, the crude extract may contain a number of ingredients performing the same therapeutic role.

Ingestion of such an extract may cause serious side-effects due to synergistic action of the constituents. So the application of herbal drug in crude form may be ineffective or may cause a toxic reaction. Vincristine, a prominent anticancer drug, was developed from periwinkle plant (*Vincarosea*) which was formerly prescribed for treating diabetes. The efficient hypotensive drug, reserpine, was developed from *Rauwolfia serpentine* which was previously provided as an antidote to snake-bites and in the treatment of lunatic patients (Chopra RN *et al*., 1982). Khelin, a coronary vasodilator drug prescribed as an effective remedy for angina pectoris, was developed from *Ammi visnaga* which was formerly used as a diuretic and antispasmodic in renal colic. Thus drug development from medicinal plants gives effective result (Ghani, 1998).

### 1.10.2 Procedure for Development

Sine drug development is an expensive practice, careful phytochemical analysis and pharmacological screening and if promising clinical tests are required. The way of developing drugs from plants involves several stages (Ghani, 1998), which include:

- Selection and correct identification of the proper medicinal plant.
- 2. Extraction with suitable solvent(s).
- Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.
- Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.
- Repeated fractionation of active fractions to isolate pure compound(s).
- Elucidation of chemical structure of pure compound(s) using spectroscopic methods.
- Evaluation of biological activity of pure compound(s)
- Toxicological tests with pure compound(s).
- Production of drug in appropriate dosage forms.

### 1.10.3 Bioactivity Guided Research of Medicinal Plants

However, natural products are currently undergoing a phase of reduced attention in drug discovery because of the enormous effort which is necessary to isolate the active principles and to elucidate their structures (Grabley, *et al*. 1999). Success in natural products research is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, information from traditional medicine, field observations or even random collection. One main strategy in the isolation of new leads consists of the so-called Bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds. Bioactivity guided phytochemical approach, has three phases of investigation.

First, biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones.

Second, the crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, until pure compounds are obtained. Third, the chemical structures of pure compounds are determined.
Only the bioactive extracts or fractions would be of connotation for next phytochemical and pharmacological analysis. So in medicinal plants research, bioactivity guided phytochemical approach might be a rational approach.

## 1.11 Plant Review

**Scientific name: *Thysanolaena maxima***

*Thysanolaena maxima* are a perennial grass plant found in hilly regions of Nepal, northern and eastern parts of India, and Bhutan. The flowers of this plant are used as cleaning tool or broom, which is known as “Amriso” in Nepali."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. (Quattrocchi, 2014)

*Thysanolaena maxima* or Tiger Grass has been under-used as a landscape plant, until recent years. It is easily mistaken for a Bamboo as they share a lot of the same traits. Tiger Grass is

made up of numerous long slender canes which are topped with drooping, green, bi-lobed leaves. The leaves only grow out of the very top of the canes which gives the plant a mushroom like appearance. When mature, Tiger Grass will start to produce purple flowers which resemble the tassels on corn. The canes don't produce side shoots, so plants maintain a neat and tidy appearance, unlike some Bamboo species. Another great feature of *Thysanolaena* is that it does not produce runners, which means your Neighbor'swon't find any popping up in their back yard.


**Figure 2: *Thysanolaena maxima***

**Table 4: 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 |

### 1.11.1 Taxonomic hierarchy of the investigated Plant

| Rank | Scientific Name and Common Name |
|---|---|
| Kingdom | Plantae – Plants |
| Subkingdom | Tracheobionta – Vascular plants |
| Superdivision | Spermatophyta – Seed plants |
| Division | Magnoliophyta – Flowering plants |
| Class | Liliopsida – Monocotyledons |
| Subclass | Commelinidae |
| Order | Cyperales |
| Family | Poaceae / Gramineae – Grass family |
| Genus | *Thysanolaena* Nees – tiger grass |
| Species | *Thysanolaena maxima* (Roxb.) Kuntze [excluded] |

(Plants.usda.gov, 2014)

### 1.11.2 Distribution

Broom grass grows in almost all parts of Meghalaya, where it covers an estimated 127 sq. km (Tiwari*et al.* 1995). Broom grass grows below 1,600 m.a.s.l. on a wide range of soils. It naturally colonies 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 broom grass plantations in the last two decades, due to an increase in demand for brooms from various parts of the country. The RiBhoi and East Khasi Hills districts account for more than 70% of the total production of brooms in Meghalaya.

### 1.11.3 Growth Pattern

Broom grass 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 (Figures 2a and 2b). Broom grass 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. (Tiwari, *et al*. 2000).

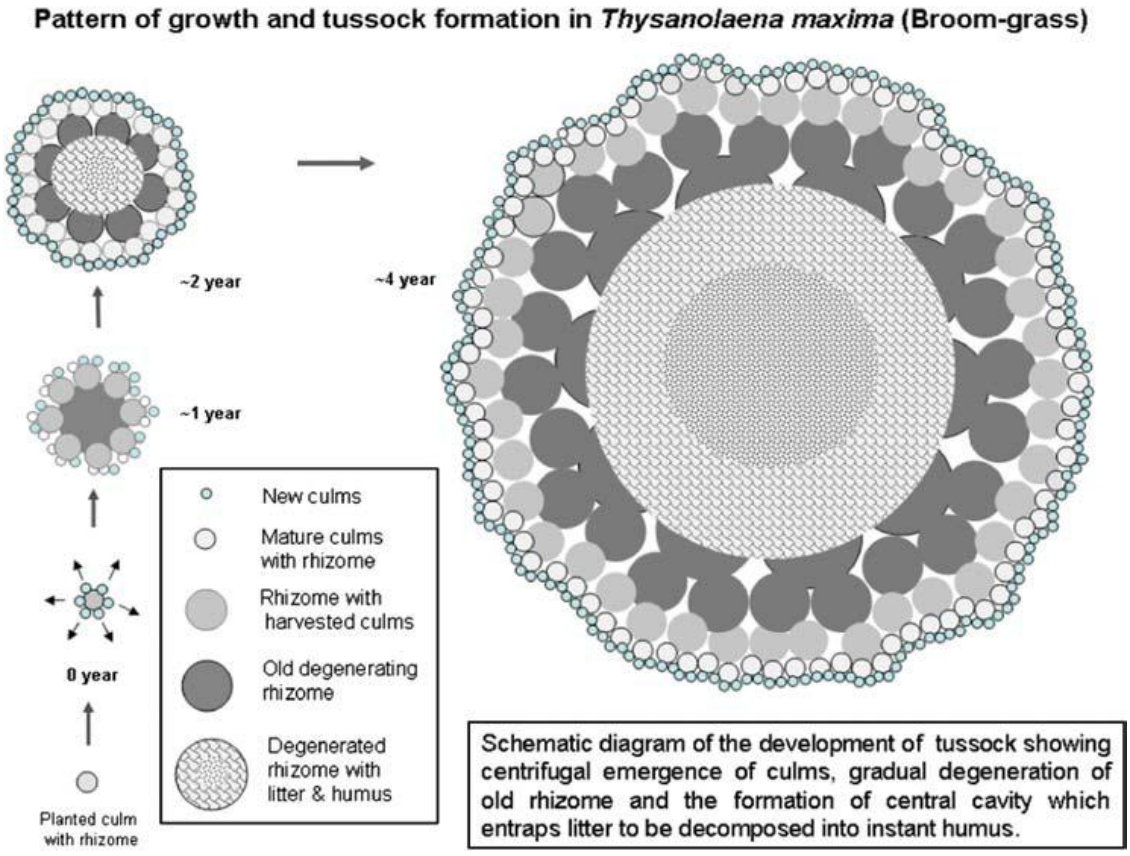


**Figure 3.Centrifugal emergence and growth of tussock year-wise.**

### 1.11.4 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 flowers have not yet opened and are 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 broom grass in the country. Ninety percent of the brooms produced are exported outside the state. 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.

## 1.11.5 Objectives

In order to achieve these aims, the following research objectives have been identified:

Table 5: Plant: *Thysanolaena maxima*. (Methanol Extract)

| SL No. | Experiment |
|---|---|
| **1.** | Pharmacological Activity Test |
| **a.** | **Antimicrobial Activity Study** |
| **b.** | **Antioxidant Activity Study** |

The overall purpose and objective of the study is to analyze phytochemical substances present in the plant and evaluate the biological activities of *Thysanolaena maxima*

## 1.11.6 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.

## 1.11.7 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.

## 1.11.8 Research protocol

- ✓ Selection, identification, collection, drying and grinding of plants.
- ✓ Extraction of the powders with methanol and collection of extract.
- ✓ Antioxidant activity determination.
- ✓ Anti-Microbial activity determination.

- ✓ Studying and comparing the results obtained.

### 1.11.9 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.

### 1.11.10 Plant material

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many

plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40°C for 72 h. In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties. (Das.K, *et al.* 2010).

### 1.11.11 Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted.

The various solvents that are used in the extraction procedures are:

1. **Water:** Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanin's) have no antimicrobial significance and water soluble phenolic only important as antioxidant compound. (Das K *et al.* 2010).
2. **Acetone:** Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. Both acetone and methanol were found to extract saponins which have antimicrobial activity. (Eloff JN, 1998)
3. **Alcohol**: The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. More

useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results. (Lapornik B*et al.* 2005).

4. **Chloroform:** Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents.(Cowan MM, 1999)
5. **Ether:** Ether is commonly used selectively for the extraction of coumarins and fatty acids.(Cowan MM, 1999)
6. **Dichloromethanol:** It is another solvent used for carrying out the extraction procedures. It is specially used for the selective extraction of only terpenoids. (Cowan MM, 1999)

## Table 6: Structural features and activities of various phytochemicals from plants

| Phytochemicals | Structural features | Example(s) | Activities |
|---|---|---|---|
| Phenols and Polyphenols | C3 side chain, - OH groups, phenol ring | Catechol, Epicatechin, Cinnamic acid | Antimicrobial, Anthelmintic, Antidiarrhoeal |
| Quinones | Aromatic rings, two ketone substitutions | Hypericin | Antimicrobial |

| | | | |
|---|---|---|---|
| Flavonoids | Phenolic structure, one carbonyl group Hydroxylated phenols, C6-C3 unit linked to an aromatic ring Flavones + 3-hydroxyl group | Chrysin, Quercetin, Rutin | Antimicrobial Antidiarrhoeal |
| Tannins | Polymeric phenols (Mol. Wt. 500-3000) | Ellagitannin | Antimicrobial, Anthelmintic, Antidiarrhoeal |
| Saponins | Amphipathic glycosides | Vina-ginsenosides-R5 and -R6 | Antidiarrhoeal |
| Terpenoids and essential oils | Acetate units + fatty acids, extensive branching and cyclized | Capsaicin | Antimicrobial, Antidiarrhoeal |
| Alkaloids | Heterocyclic nitrogen compounds | Berberine, Piperine, Palmatine, Tetrahydropalmatine | Antimicrobial, Anthelmintic, Antidiarrhoeal |
| Lectins and Polypeptides | Proteins | Mannose-specific agglutinin, Fabatin | Antimicrobial |
| Glycosides | Sugar + non carbohydrate moiety | Amygdalin | Antidiarrhoeal |
| Coumarins | Phenols made of fused benzene and α-pyrone rings | Warfarin | Antimicrobial |

(Maniyar.Y, *et al.* 2010).

## 1.11.12 Methods of extraction

Variation in extraction methods usually depends upon:

- Length of the extraction period,
- Solvent used,
- pH of the solvent,
- Temperature
- Particle size of the plant tissues
- The solvent-to-sample ratio

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal. (Das K *et al.* 2010).

**Table 7: Mechanism of action of some phytochemicals**

| Phytochemicals | Activity | Mechanism of action |
|---|---|---|
| **Quinones** | Antimicrobial | Binds to adhesins, complex with cell wall, inactivates enzymes |
| **Flavonoids** | Antimicrobial<br>Antidiarrhoeal | Complex with cell wall, binds to adhesins<br>Inhibits release of autocoids and prostaglandins,<br>Inhibits contractions caused by spasmogens,<br>Stimulates normalization of the deranged water transport across the mucosal cells,<br>Inhibits GI release of acetylcholine |
| **Polyphenols and Tannins** | Antimicrobial<br>Antidiarrhoeal<br>Anthelmintic | Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation<br><br>Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water |

| | | |
|---|---|---|
| | | transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action<br><br>Increases supply of digestible proteins by animals by forming protein complexes in rumen, |
| **Coumarins** | Antiviral | Interaction with eucaryotic DNA |
| **Terpenoids and essential oils** | Antimicrobial<br>Antidiarrhoeal | Membrane disruption<br>Inhibits release of autocoids and prostaglandins |
| **Alkaloids** | Antimicrobial<br>Antidiarrhoeal<br>Anthelmintic | Intercalates into cell wall and DNA of parasites<br>Inhibits release of autocoids and prostaglandins<br>Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis |
| **Lectins and Polypeptides** | Antiviral | Blocks viral fusion or adsorption, forms disulfide bridges |
| **Glycosides** | Antidiarrhoeal | Inhibits release of autocoids and prostaglandins |
| **Saponins** | Antidiarrhoeal<br>Anticancer<br>Anthelmintic | Inhibits histamine release in vitro<br>Possesses membrane permeabilizing properties<br>Leads to vacuolization and disintegration of teguments |
| **Steroids** | Antidiarrhoeal | Enhance intestinal absorption of Na+ and water |

### 1.11.13 Medicinal use of *Thysanolaena maxima*

- Leaf past of *Thysanolaena maxima* with the leaf paste of *Litsea lancifolia* is given in case of Dysentery.
- Seeds of *Thysanolaena maxima* powdered and given to women before childbirth to facilitate delivery. The flour used as an abortifacient, contraceptive.
- Inflorescence paste mixed with a pinch of slaked lime is applied locally for treatment of boils or cancer.
- Young stem juice is applied on the eye when eyes become red and dirty. (Kharkongor P *et al.* 1981).

## 1.12 Antioxidants Activity Test

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the monophenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent.

Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.

Free radicals are highly charged and active particles which are made of unstable molecules or atoms due to their single and unbalanced electrons. The common free radicals are oxygenreactive species (ROS), namely, super oxide radical, hydroxyl radical, and peroxyl radical which can be internally produced by cellular metabolism, inflammation by immune cells and externally by radiation, pharmaceuticals, hydrogen peroxide, toxic chemicals, smoke, alcohol, oxidized polyunsaturated fats and cooked food. They are unstable and through chain reaction can attack vital biomolecules (DNA, lipids, proteins) in cells and body fluids. They also weaken the cells in our bodies leaving us vulnerable to disorders and diseases such as arteriosclerosis, coronary heart disease, stroke, hypertension, emphysema, diabetes, cataracts, rheumatoid arthritis, nephritis,

Alzheimer disease, cancer, AIDS, etc. Aging process is also a result of the oxidation by free radicals in the body. They are formed naturally, both internally by metabolism and externally by chemicals. These include alcohol consumption, drugs, toxic metals, emotional stress, smoking, pesticides, herbicides and air pollutants.

Fortunately, nature provides us with plenty of "protecting molecules" or the so called "antioxidants" which can trap or destroy free radicals and subsequently protect us from damage due to the oxidative stress. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (byproducts) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, macular degeneration, diabetes, cancer etc. are all contributed by oxidative damage.

## 1.12.1 Natural Antioxidants

There are two groups of natural antioxidants.

- The first group is our body enzymes such as superoxide dismutase (SOD), catalysts, glutathione peroxidase. Wheat and barley grain products are rich in SOD.
- The other group is nutrient antioxidants which are vitamin E, vitamin C and beta-carotene (the pre-form of vitamin A).

In addition, there are still numerous other antioxidants such as bioflavonoids, carotenoids (such as lutein and lycopene) and phenolic compounds. Selenium is also an important mineral antioxidant. Selenium is commonly found in onions, garlic, mushrooms, whole grain cereals, particularly in the wheat germ and rice bran.

## 1.12.2 The Antioxidant Process

Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. That is why there is a constant need to replenish our antioxidant resources. How they work can be classified in one of two ways:

**Chain-breaking**

When a free radical releases or steals an electron, a second radical is formed. This molecule then turns around and does the same thing to a third molecule, continuing to generate more unstable products. The process continues until termination occurs -- either the radical is stabilized by a chain-breaking antioxidant such as beta-carotene and vitamins C and E, or it simply decays into a harmless product.

**Preventive**

Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxides prevent oxidation by reducing the rate of chain initiation. That is, by scavenging initiating radicals, such antioxidants can thwart an oxidation chain from ever setting in motion. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron. The effectiveness of any given antioxidant in the body depends on which free radical is involved, how and where it is

generated, and where the target of damage is. Thus, while in one particular system an antioxidant may protect against free radicals, in other systems it could have no effect at all. Or, in certain circumstances, an antioxidant may even act as a "pro-oxidant" that generates toxic oxygen species.

## 1.13 Antimicrobial Screening

The main objective of performing the antibacterial screening is to determine the susceptibility of the pathogenic microorganisms to test compound which, in turn is used to selection of the compound as a therapeutic agent. In general, antimicrobial screening in-vitro is undertaken in following two steps:

**I) Primary assay**

It is essentially a qualitative or semi qualitative test that indicates the sensitivity or resistance of microorganisms to the compound. However this technique cannot be used to distinguish between bacteriostatic and bactericidal agents (Reiner, 1982). The primary assay can be performed in vitro by disk diffusion assay method, which includes

- Plate diffusion test
- Streak test

The plate diffusion test utilizes different concentrations of a test compound absorbed on sterile filter paper disks on the same plate whereas the streak test permits the determination of the antibacterial effect of a test compound on several microorganisms simultaneously and is suitable for the estimation of the spectrum of the activity. However, the plate diffusion test is commonly used.

**ii) Secondary assay**

It quantifies the relative potency such as minimum inhibitory concentration (MIC). The lowest concentration of an antimicrobial agent required to inhibit the growth of the microorganisms in vitro is referred to as minimum inhibitory concentration (MIC). It is done by serial dilution technique. (Reiner, 1982).

## 1.13.1 Antimicrobial drug

Antimicrobial drug/Antibiotics are the greatest contribution at the present century at therapeutic. Antibiotics are special kind of chemotherapeutic agent usually obtained from living organism. The term chemotherapeutic agent mean "All chemical substance that destroy all kind of cell wall such as bacterial cell wall, viral cell wall even human cell wall. "Antibiotics one kind of chemotherapeutic agent, but it does not destroy the humancell wall, it destroy the bacterial & viral cell wall. So all antibiotics are chemotherapeutic agent but all chemotherapeutic agents are not antibiotic. The word antibiotic come to refer to a metabolic of one microorganism that is very small amount is detrimental or inhibitory to their microorganism. The term antibiosis was first defined by vuillemin in 1889.The first systematic search for & study of antibiotics made by "Gratia & both about 1924. In 1929 Alexander Fleming discovers one kind of Antibiotics named by penicillin from the penicillium tree. Characteristics of Antibiotic: To be useful as chemotherapeutic agent antibiotics must have the following qualities:

- They should have the ability to destroy or inhibit many different species of pathogenic microorganism.
- They should prevent the ready development of resistant forms of the parasites.
- They should not be produced undesirable side effects in the host, such as sensibility or allergic reaction, never damage or irritation of the kidneys & gastrointestinal tract (G.I.T).
- They should not eliminate the normal microbial flora of the host. (Cui, Guo and Xiao, 2011)

### 1.13.1.1 Classification of Antibiotics

Antibiotic drug are classified in several way: For example, some are bactericidal & other are bacteriostatic. Here the term Bactericidal mean "Stop the Bacterial growth & it also kill the bacteria", and bacteriorstat mean "stop the bacterial growth but cannot kill the bacteria."

Antibiotic may be grouped on the basis of chemical structure.

- Sulfonamide & relative drugs
- Diaminopyrimidines.
- Quinolones
- ß- lactam antibiotics
- Nitromemzene derivatives
- Amino glycosides.
- Polypeptide Antibiotics.
- Nitrofuran derivatives etc.

### 1.13.1.2 Antibiotics & their mode of action

The major points of attack of antibiotics on microorganism include:

- Inhibition of cell wall synthesis Drug- penicillin, Bacitracin.
- Damage to the cytoplasmic membran. Drug- ploymxins, Hamycin.
- Inhibition of nucleic acid & protein synthesis, Drug-Tetracyclines, Clidamycin.
- Inhibition of specific enzyme systems. Drug-Pyridine, Pyrimidine.
- Interfere with DNA synthesis. Drug-Acyclovir.
- Interfere with intermediary metabolism. Drug-Sulfonamides, PAS (Para amino salicylic acid).

### 1.13.1.3 Uses of Antibiotics Drug that promote Resistance

- Inappropriate treatment of cold and other viral infection.
- Indiscriminate prophylaxis.
- Overuse of potent, broad-spectrums antibiotics.
- Administering doses that are too small or not continuing therapy long enough to eliminate the most resistant microbes.

Antibiotics resistance can be prevent by following:-

- Limiting the use of newer antibiotics so long as the currently used are effective.
- Avoidance of indiscriminate use of antibiotics.
- Using Antibiotics combinations in selected circumstances e.g- tuberculosis, leprosy

- Constant monitoring of resistance patterns in a hospital or community.

The possible causes of failure of antibiotics therapy are:

- The possible causes of failure of antibiotics therapy are:
- Antibiotics resistance to microorganism.
- Failure of selection of the best drugs, which in specific for specific microorganism.
- Sub optimal use of antibiotics.
    a) Inadequate dose
    b) Interval between doses too long.
    c) Duration of cause's too short
    d) Unsuitable route.
- Treatment begun too late to save patient
- Super infection by other pathogens.
- Undrained pus, retained infection foreign body, dead tissue.

### 1.13.1.4 Misuses of antibiotics

- Treatment of untreatable infection: The majority of the diseases causes by viruses will not respond to anti-infective agent. Thus antimicrobial therapy of measles, chickenpox, mumps & upper respiratory infection-90% is totally ineffective.
- Therapy of fever of undetermined origin.
- Improper doses.
- Reliance on chemotherapy with omission of surgical drainage.
- Lack of adequate bacteriological information.

Factors to be consider in selecting antibiotics drug:-

- Clinical symptoms & site of infection.
- Identity of the pathogen-samples for laboratory analysis must be collected before any chemotherapy begins.
- Drug toxicity.
- Cost
- Prior history drug allergy.

### 1.13.1.5 Rational use of antibiotics

- Use appropriate antibiotics with adequate bacteriological information.
- Antibiotics should be used in proper dose & for appropriate duration.
- Combination of antibiotic should be used where single drug is an ineffective or to overcome the chance of microbial resistance to antibiotics.
- A bactericidal antibiotic should not be used with a bacteriostatic antibiotic at the same time (antibiotic antagonism).
- Broad spectrum antibiotics should not be used indiscriminately.
- Consider the patient condition during selection of antibiotics (e.g-in renal failure Ciprofloxacin is contraindicated).
- Should not use the newer member of group of antibiotics so long as the currently used drug is effective.

# Chapter: 2

# LITERATURE REVIEW

## 2.1 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 IC50 of 250µg/ml. The overall result shows that, the plant have interesting antibacterial and antioxidant activity

## 2.2 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)

## 2.3 Chemical Composition and Nutritive Value of Kuchi (*Thysanolaena maxima*) Grass

In this study, investigated the chemical composition and Nutritive value of *Thysanolaena maxima* which is on DM basis was 15.31 CP, 3.82 EE, 23.58 CF, 47.69 NFE, 9.60 TA, 0.61 Ca and 0.21 P ,12.07 per cent DCP, 50.89 per cent TDN, 3.22 NR (Nutritive ratio), 35.96 per cent SE, 2239Kcal/kg DE and 1832 Kcal/kg ME. (Bhuyan, Das and Baruah, 1988)

## 2.4 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)

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

Two grass species, *T. maxima* and *V. zizanioides*, were used to conduct the experiments. Prior to testing, all grasses were acclimatized in the greenhouse for 2– 3 months (temperature, 27–29°C; approximately 70 % relative humidity; 17,568-lx light intensity; and 12/12-h photoperiod).

The 100 % survival rates of *T. maxima* and *V.zizanioides* grown on Pb mine tailings and the absence of symptom toxicity indicated both plants' ability to withstand a high contamination of Pb.It appears that *T. maxima*, reported to withstand Pb concentration up to 100,000 mg kg−1 (Rotkittikhunet al. 2006), is similarly suited to grow in a wide range of habitats, including degraded mining areas.

## 2.7 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 thesixth year. The observations were not extended beyond the fourth year due to disarrayed patterns in vigor loss.

## 2.8 Use of *Thysanolaena maxima* in treatment of Dysentery

Leaf past of *Thysanolaena maxima* with the leaf paste of *Litsea lancifolia* is given in case of Dysentery

## 2.9 Use of *Thysanolaena maxima* to facilitate Delivery

Seeds of *Thysanolaena maxima* powdered given to women before childbirth to facilitate delivery. The flour used as an abortifacient, contraceptive.

## 2.10 Use of *Thysanolaena maxima* to for treatment of boils or cancer.

Inflorescence paste mixed with a pinch of slaked lime is applied locally for treatment of boils or cancer.

## 2.11 Use of *Thysanolaena maxima* to treatment of eye

Young stem juice is applied on the eye when eyes become red and dirty.

## 2.12 Use of *Thysanolaena maxima* to treatment of flatulence and improves digestion.

Soft part of a young leaf and flower buds are eaten raw (No particular dosage) to cure flatulence and improves digestion.

## 2.13 Use of *Thysanolaena maxima* to treatment of tuberculosis

Pills prepared from the leaves are taken twice daily for the treatment of tuberculosis and also used in cuts, wounds.

## 2.14 *Thysanolaena latifolia*

- Use: Young leaves and stem tips are used to feed cattle and buffaloes. Its large inflorescences are used in making brooms. The grass is occasionally planted for ornamental purposes and as a hedge.
- Properties: N concentration 1.2%. The in vitro DM digestibility of leaves ranged from 40% to 60% (Falvey et al. 1981).

# Chapter: 3

# MATERIALS & METHODS

## 3.1 Preparation of Plant Extract for Experiments

### 3.1.1 Collection & Preparation of Plant Material

*Thysanolaena maxima* plant was collected in the month of June, 2014 from Chittagong Hill tracts during rainy season when weeds were in their maximum densities. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24hours at considerably low temperature for better grinding. The dried leaves was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

### 3.1.2 Washing and Drying of *Thysanolaena maxima* Plant

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

### 3.1.3 Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 158 gm which was measured using electronic balance and it was found to be 158 gm.

### 3.1.4 Extraction of the Dried Powdered Sample

The fine powder of *T.maxima* leaves was dissolved in 1000 ml methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

### 3.1.5 Filtration of the Extract

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

### 3.1.6 Solvent Evaporation

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

## 3.2 Principle of a Rotary Evaporator

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


**Figure 4: Drying of extract using rotary evaporator**

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

## 3.3 Antioxidant Assay of *Thysanolaena maxima*

### 3.3.1 Principle

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The presence of reluctant such as antioxidant substances in the samples causes the reduction of the

Fe3+/ferricyanide complex to the ferrous form. Therefore, Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986).

### 3.3.2 Materials required

**Table 8: Materials Required for Anti-oxidant Assay of *Thysanolaena maxima***

| **Reagents** | |
|---|---|
| **Name** | |
| **Potassium ferricyanide** | May and Backer, Dagenham, UK |
| **Trichloro Acetic acid** | Fine Chemicals, India |
| **Ascorbic acid** | Fine Chemicals, India |
| **Ferric Chloride (FeCl3)** | Standard SD Fine chem. Ltd., Biosar, India |
| **Glass Wares** | |
| **Name** | Quantity |
| **Test Tubes** | 10 |
| **Micropipette (100 – 1000 µL)** | 1 |
| Pipette with pumper (1 mL) | 1 |
| **Metal Wares** | |
| Name | Quantity |
| **Spatula** | 1 |
| **Non – fragile Materials** | |
| **Name** | Quantity |
| **Micropipette Tips** | 2 |
| **Machinery** | |
| **Name** | Quantity |
| **Double Beam UV – Vis Spectrophotometer** | 1 |

### 3.3.3 Procedure

- 2.0 ml of each fraction and standard (ascorbic acid) in different concentrationswere taken in test tubes.
- 2.5 ml of Potassium ferricyanide [$K_3Fe(CN)6$] 1% solution was added into thetest tubes.
- Then the test tubes were incubated for 10 minutes at $50^0$C to complete reaction.
- 2.5 ml of trichloro acetic acid (10%) was added into the test tubes.
- The total mixture was centrifuged at 3000 rpm for 10 minutes.
- 2.5 ml supernatant solution was withdrawn from the mixture and mixed with2.5 ml of distilled water.
- 0.5 ml of ferric chloride (0.1%) solution was added.
- Then the absorbance of the solution was measured at 700 nm using aspectrophotometer (Shimadzu UV PC-1600) against blank.
- A typical blank solution contained the same solution mixture without plantextract or standard and it was incubated under the same conditions as the rest ofthe sample solution.
- The absorbance of the blank solution was measured at 700 nm against thesolvent used in solution preparation was also taken.

## 3.4 Antimicrobial assay of *Thysanolaena maxima*

### 3.4.1 Principle

The disk diffusion susceptibility method is simple and well-standardized. Bacterial inoculums are applied to the surface of a large agar plate. Antibiotic discs and disc of test materials are placed on the inoculated agar surface. Plates are incubated for 16–24hr at 35°C prior to determination of results. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The zones of growth inhibition are measured to the nearest millimeter around each of the antibiotic disks. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium (Barry, 1976).

This ability may be estimated by any of the following three methods:

- Disc diffusion method
- Serial dilution method45
- Bio autographic method

## 3.4.2 Disc Diffusion Method

The antibacterial assay was performed by disc diffusion technique (Bauer AW et al, 1988). Disc diffusion technique is highly effective for rapidly growing microorganisms. 0.50g or 500 mg and 0.25g or 250mg of sample (methanol extracts of *Thysanolaena maxima*) was dissolved in 10ml of methanol solvents to prepare stock solutions respectively. The concentrations of sample solution are 50 mg/ml and 25mg/ml (i.e. each µl contain 50 µg and 25ug of sample)). 20 µl of such solution was applied on sterile disc (5mm diameter, filter paper) and allowed to dry off the solvent in an aseptic hood. Thus, such discs contain 250µg and 600µg of crude extracts. To compare the activity with standard antibiotics, Kanamicin (30 µg/disc) was used. The extract of *Thysanolaena maxima* was tested against three Gram-negative (*Salmonella typhi, Salmonella paratyphi, Escherichia coli*) bacteria and one fungi Candida albicans. Briefly, in this study the test discs and standard disc were placed in a Petri dish seeded with particular bacteria and then left in a suitable container for extraction. The Petri dishes were then incubated at 37°C for overnight to allow the bacterial growth. The antibacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm. (Bauer AW et al, 1988).

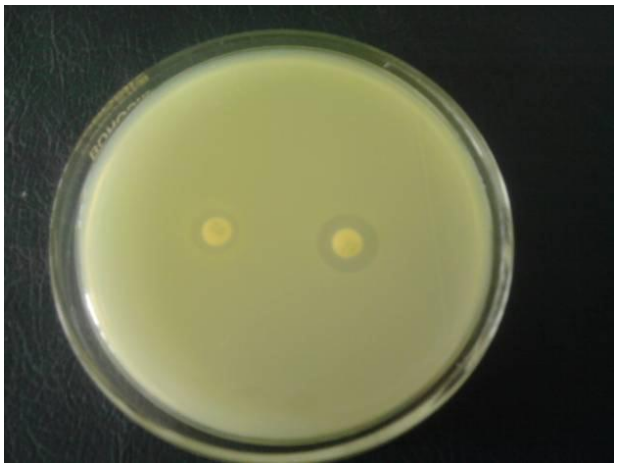

**Figure 5: Disc Diffusion Method**

### 3.4.3 Experimental Work

### 3.4.3.1 Materials Required

**Sample:** Concentrated Crude Methanolic Extract of Thysanolaena maxima Plant

**Bacterial Culture**: Bacterial strain

### Strains of Microorganism

- *Bacillus sereus*
- *Bacillus megaterium*
- *Bacillus subtilis*
- *Salmonella paratyphi*
- *Salmonella typhi*

### 3.4.3.2 Reagents

- Nutrient Agar (Micromaster, Germany)
- Methanol (Merck, Germany)
- Ethanol (Merck, Germany)
- Sodium chloride (Merck, Mumbai)

### 3.4.3.3 Apparatus

- Laminar Air Flow Cabinet
- Incubator
- Autoclave
- Hot Air Oven
- Electronic Balance

### 3.4.3.4 Equipment

- Micropipette
- Micropipette Tips

- Reagent Bottle
- Petri dishes
- Eppendorf tube
- Vial Pipette and Pipette pumper
- Inoculating Loop
- Sterile Forceps
- Spreader
- Filter paper
- Spatula
- Candle

### 3.4.3.5 Test Organisms

The microbial strains used for the experiment were collected as pure cultures from University of Dhaka. Both gram-positive and gram-negative bacteria were taken for the test, are listed in the following table.

**Table 9: List of the test pathogenic Bacteria**

| Serial No. | Name of the test organisms |
|---|---|
| **Gram Positive** | |
| **1.** | *Bacillus sereus* |
| **2.** | *Bacillus megaterium* |
| **3.** | *Bacillus subtilis* |
| **Gram negative** | |
| **1.** | *Salmonella paratyphi* |
| **2.** | *Salmonella typhi* |

### 3.4.3.6 Culture Medium and their composition

The Nutrient agar was used as a culture media for the in vitro antimicrobial activity testing.

**Table 10: Composition of nutrient agar medium**

| **Ingredients** | Amount |
|---|---|
| **Bacto peptone** | 0.5 gm |
| **Sodium chloride** | 0.5 gm |
| **Bacto yeast extract** | 1gm |
| **Bacto agar** | 2.0 gm |
| **Distilled water q.s.** | 100 ml |

### 3.4.3.7 Methods

### 3.4.3.7.1 Sterilization of Petri Dishes

Petri dishes having 130 mm diameter were used in this test. The Petri dishes were placed in the hot air oven at 150ºc temperature for 15 minutes for sterilization. After sterilization, the Petri dishes were transferred inside the laminar air flow cabinet to avoid contamination.

### 3.4.3.7.2 Media preparation and sterilization

The composition of the supplied agar medium was 28g per 1000 ml and thus the amount required for this test was calculated by unitary method. 5.6 gm nutrient agar was weighted and taken in the reagent bottle to prepare 200 ml of agar solution. Then distilled water was added up to 200 ml and the reagent bottle was put in autoclave machine at a temperature of 121ºc for 15 minutes at about 1.30 hours for sterilization.

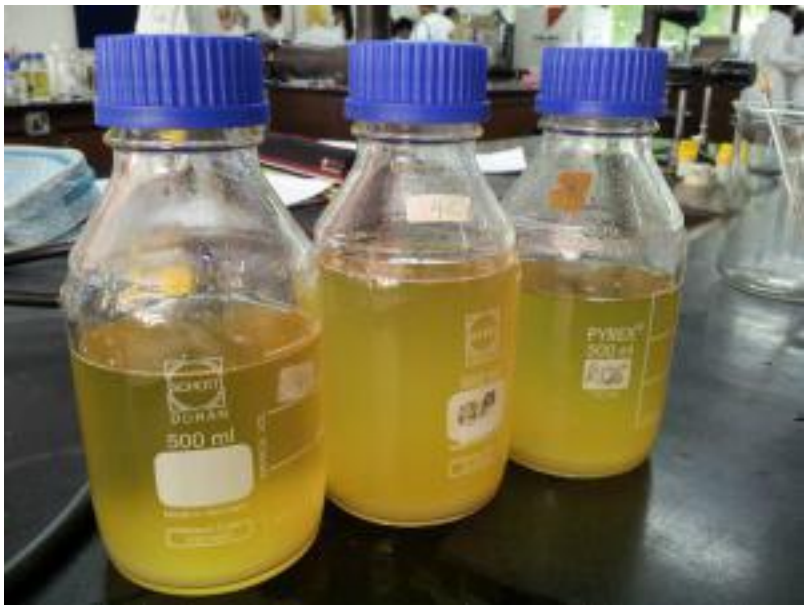


**Figure 6: Nutrient Agar in Culture Bottle**

### 3.4.3.7.3 Sterilization of Tips and Eppendorf tube

The micropipette tips and eppendorf tubes were placed in autoclave machine at a temperature of 121$^{o}$c for 15 minutes at about 1.30 hours, for sterilization.

### 3.4.3.7.4 Preparation of Subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 370 C for their optimum growth. These fresh cultures were used for the sensitivity test.

### 3.4.3.7.5 Preparation of Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized Petri dishes. The

Petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

### 3.4.3.7.6 Stock Solution Preparation

To prepare the stock solution of samples of 50 µg/disc concentration, 0.025 gm. sample was dissolved in 10 ml methanol in the test tube. Then the solution was shaken to dissolve the sample properly. Similarly, to prepare the stock solution of samples of 250 egg/disc and 600 µg/disc concentrations, 0.125 gm and 0.25 gm samples were dissolved in 10 ml methanol in two different test tubes respectively. Then the solutions in the test tubes were shaken to dissolve the sample properly.

### 3.4.3.7.7 Preparation of the Isotonic Solution

A 0.9% isotonic solution had to be prepared. This was prepared by weighing 0.9 g of Sodium chloride (Nacl) and by dissolving the measured Sodium chloride in 100 ml of distilled water. The isotonic solution was also autoclaved at 121°c for 15 minutes.

### 3.4.3.7.8 Dilution of the Test Micro-organisms

Previously cultured Petri dishes of the test microorganisms were assembled. At first, an inoculating loop was sterilized in a Bunsen burner. Then it was used to scrape a small colony of a specific species of microorganism from its culture. Now, the microorganism on the loop was transferred to a sterilized eppendorf tube, already containing 1 ml of isotonic solution. Then, the inoculating loop was resterilized and used to transfer another species of microorganism to a fresh eppendorf tube already filled with isotonic solution. In this way all the microorganisms were transferred to fresh eppendorf tubes and thus were made ready for the test. The eppendorf tubes were then applied on a vortex mixer for proper mixing of microorganism with the isotonic solution.

### 3.4.3.7.9 Preparation of Discs

### 3.4.3.7.9.1 Standard Discs

These were used as positive control to ensure the activity standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, gentamycin (30□g/disc) standard disc was used as the reference.

### 3.4.3.7.9.2 Preparation of Paper Discs

Filter paper disc (6 mm diameter) was prepared from filter paper by using punch machine. Then it was sterilized in autoclave machine. After sterilization, each disc was impregnated with 6 µl sample solution by using micropipette (20 µl) and residual solvents were completely evaporated in air.

**Figure 7: Filter paper discs**

### 3.4.3.7.10 Preparation of Agar Plate

According to the name of bacteria Petri dishes were marked. Agar medium was dispensed into each Petri dish to get 3-4 mm depth of agar media each. After pouring the agar medium, all Petri dishes were kept in room temperature so that the medium can properly solidify.

### 3.4.3.7.11 Inoculation of microorganisms

1ml diluted bacterial suspension in the eppendorf tube was transferred on agar plate by micropipette (100-1000 µl) after solidification of the agar medium. By using spreader the bacterial suspension was spread on agar medium. Paper discs containing samples of three different concentrations were placed on to nutrient agar medium. Standard disc (Gentamicin, 30µg) were used as positive and placed on to the agar medium.

### 3.4.3.7.12 Diffusion and Incubation

The sample discs, the standard antibiotic discs and were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in an incubator at 370 C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium.

### 3.4.3.7.13 Determination of Antimicrobial activity by measuring the Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

# Chapter: 4

# RESULTS & DISCUSSION

## 4.1 Result of Antimicrobial Screening

The extract of methanol was not so active against the entire test organism. The methanol extract (600 µg/disc) of *Thysanolaena maxima* showed antibacterial activity against Gram positive and Gram negative bacteria.

**Table 11: The Antibacterial activity (in vitro) of *Thysanolaena maxima* Methanol extract of and standard Kanamycin discs.**

| **Serial No.** | Name of the test organism | Diameter of the zone of inhibition(in mm) | |
|---|---|---|---|
| | | Methanol extract ( 600µg/disc) | Kanamycin (30µg/disc) |
| **Gram positive bacteria** | | | |
| **1.** | *Bacillus sereus* | 8 | 30 |
| **2.** | *Bacillus megaterium* | - | 28 |
| **3.** | *Bacillus subtilis* | - | 34 |
| **Gram negative bacteria** | | | |
| **4.** | *Salmonella paratyphi* | 8 | 17 |
| **5.** | *Salmonella typhi* | - | 18 |

## 4.2 Discussion

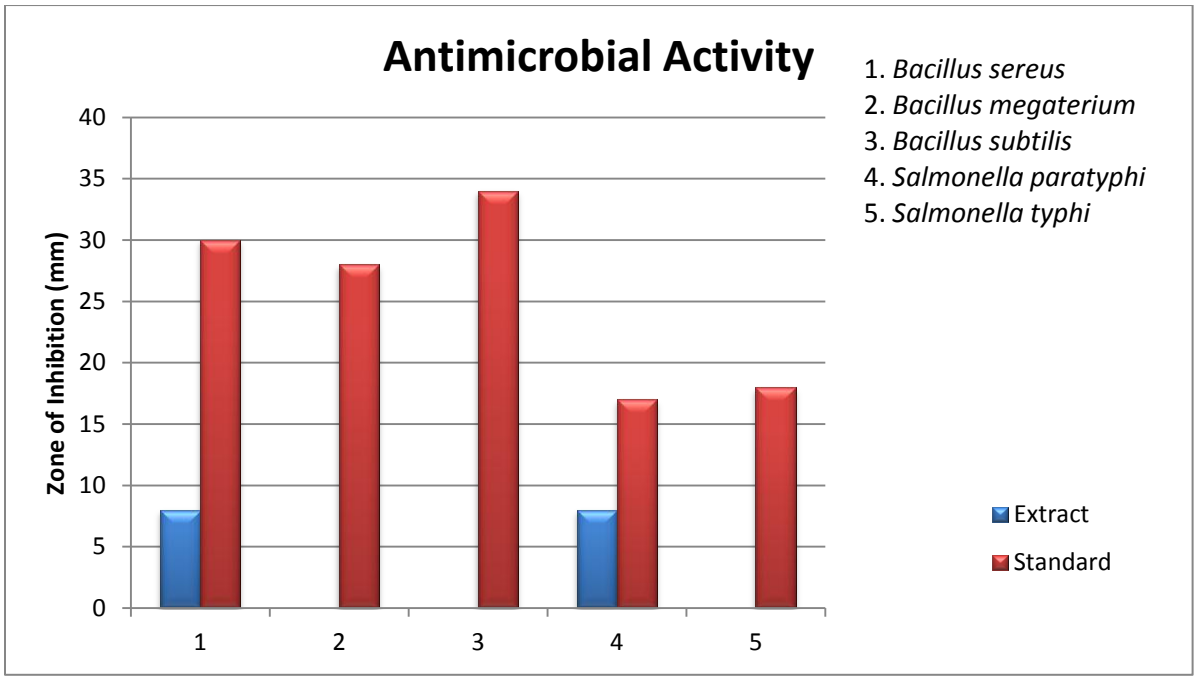


**Figure 8: Comparison of antimicrobial activity between standard and extract**

Methanolic extract of *Thysanolaena maxima* leaves extract showed very low antimicrobial activity when compared to reference standard drug Kanamycin. Since we have used Kanamycin as a reference standard in our experiment, it showed antimicrobial activity against tested bacteria, whereas, methanolic extract of *Thysanolaena maxima* showed very low activity against tested bacteria. None of the zone of inhibition of methanolic extract of *Thysanolaena maxima*is equal to Kanamycin against any bacteria as shown in the Figure: 8 even 3 of tested bacteria showed no antimicrobial activity.

## 4.3 Result of Antioxidant Activity

**Table 12: Concentration and Absorbance of *Thysanolaena maxima* and Ascorbic acid**

| Concentration (µg/ml) | Absorbance (at 700 nm) | |
|---|---|---|
| | ***Thysanolaena maxima*** | **Ascorbic acid** |
| 0 | **0** | **0** |
| 50 | **0.116** | **0.237** |
| 100 | **0.27** | **0.29** |
| 150 | **0.311** | **0.386** |
| 200 | **0.349** | **0.489** |

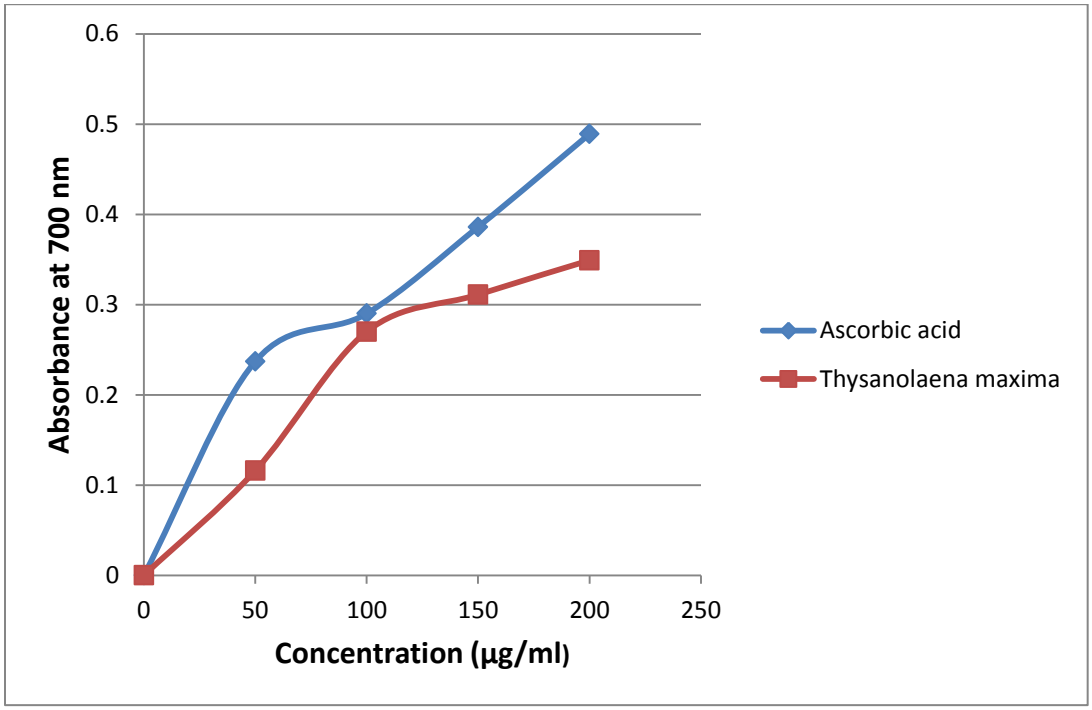


**Figure 9: Comparison between absorbance of extract (*Thysanolaena maxima*) and standard (Ascorbic acid) over different concentrations**

## 4.3 Discussion

Fig. 9 shows the reductive capabilities of the plant extract compared to Ascorbic acid determined using the potassium ferricyanide reduction method. The reducing power of extract of *Thysanolaena maxima* was very potent and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe3+ ions, which had a lesser reductive activity than the standard of Ascorbic acid.

# Chapter: 5

# CONCLUSION

## 5.1 Conclusion

As the literature review suggests, the presence of several phytochemical compounds in *Thysanolaena maxima* makes the plant pharmacologically active. The present study showed that it has very good antioxidant activity that could make it a potent drug against free radical mediated diseases.

The methanol extract showed that low antimicrobial activity when compared to reference standard drug Kanamycin.

So, further investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be tested. It will help in the development of new novel and safe drugs for the treatment of various diseases.

# Chapter: 6
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