"A Pharmacological Investigation on the Central Nervous System (CNS) Activity of Petroleum Ether Extract of *Syzygium samarangense* Leaves"

A thesis report submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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DECLARATION BY THE CANDIDATE

I, **Fabia Shazzad**, hereby declare that the dissertation entitled "A Pharmacological Investigation on the Central Nervous System (CNS) Activity Of Petroleum Ether Extract Of *Syzygium samarangense* Leaves", submitted by me to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Honors) is a confide record of original research work carried out by me under the supervision and guidance of **Marjana Khalil**, Lecturer, Department of Pharmacy, East West University.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled "A Pharmacological Investigation on the Central Nervous System (CNS) Activity Of Petroleum Ether Extract Of *Syzygium samarangense* Leaves", submitted to the Department of Pharmacy, East West University, Dhaka, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy, was carried out by Fabia Shazzad, **ID No.2014-1-70-076** under my supervision and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/Diploma.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation entitled “A Pharmacological Investigation on Tthe Central Nervous System (CNS) Activity of Petroleum Ether Extract of *Syzygium samarangense* Leaves” is a genuine research work carried out by **Fabia Shazzad**, under the supervision of **Marjana Khalil** (Lecturer, Department of Pharmacy, East West University, Dhaka). I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in this connection are duly acknowledged.

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Prof. Dr. Chowdhury Faiz Hossain

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This Research Paper is dedicated to

My Beloved Parents

ABSTRACT

Syzygium samarangense, plant of Myrtaceae family has been used as a traditional medicine for the cure of many diseases in different parts of the world such as stomatitis, diarrhea, diabetes etc. The objective of the current study was to evaluate the Central Nervous System (CNS) activity of Petroleum Ether extract of the leaves of *Syzygium samarangense plant* in *Swiss albino* mice models. The leaves were powdered and then soaked in Petroleum Ether to prepare the extract. At two doses, 100 mg/kg and 200 mg/kg body weight respectively, the activity was tested by using two experiments namely, Open field test and Hole board test. In both the experiments, significant ($p<0.001$) decrease in locomotion activity in the mice models was observed in a dose-dependent manner when compared with the standard drug, Diazepam. Therefore, it can be said that this plant can be a potential candidate for the development of drugs acting on the Central Nervous System (CNS) in near future.

Keywords: *Syzygium samarangense*, CNS activity, Open field, Hole board, petroleum ether, locomotion.

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Chapter One: Introduction

1.1 Overview

Medicinal plants, medicinal herbs or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals. World Health Organization (WHO) has provided a definition of medicinal plants, that is "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drug".

Almost every family in rural Amazonian villages has their own family medicinal plants garden. Some medicinal plants are wild crafted, meaning that they are harvested in the wild by people who are skilled at plant identification. Sometimes, plants cannot be cultivated, making wild crafting the only way to get them and some people believe that wild plants have more medicinal properties. Wild crafting can also be done to gather herbs for home use, with people seeking them out to use in their own medicinal preparations. Other plants may be cultivated. One of the advantages of cultivation is that it allows for greater control over growing conditions, which can result in a more predictable and consistent crop. Cultivation also allows for mass production, which makes plants more commercially viable, as they can be processed in large numbers and priced low enough that people will be able to afford them.

Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex. Medicinal plants are widely used to treat disease in non-industrialized societies, not least because they are far cheaper than modern medicines. Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. Roughly 50,000 species of higher plants have been used medicinally. This represents by far the biggest use of the natural world in terms of number of species. When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Nearly, twenty-five per cent of all of our prescription drugs are

derived from plants, many of them from tropical rainforests; and as many as 70% of our pharmaceuticals are modeled after constituents found in plants. Even today, 80% of the world's population relies on botanical medicines as their primary means of health care.

The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steroids, phenols glycosides and tannins.

The information obtained from extracts of medicinal plants makes pharmacological studies possible. If the active ingredients are characterized, then the mode of action of plants producing therapeutic effects can also be better investigated. Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens.

Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.

There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry.

1.2 History of the role of plants as sources of medicines

- **Prehistoric times**:

Plants, including many now used as culinary herbs and spices, have been used as medicines from prehistoric times.

- Spices have been used partly to counter food spoilage bacteria, especially in hot climates and especially in meat dishes which spoil more readily.
- Angiosperms (flowering plants) were the original source of most plant medicines.
- Human settlements are often surrounded by weeds useful as medicines, such as nettle, dandelion and chickweed.
- Humans were not alone in using herbs as medicines: some animals such as non-human primates, monarch butterflies and sheep ingest medicinal plants to treat illness.
- Plant samples from prehistoric burial sites are among the lines of evidence that Paleolithic peoples had knowledge of herbal medicine. For instance, a 60 000-year-old Neanderthal burial site, "Shanidar IV", in northern Iraq has yielded large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies. A mushroom was found in the personal effects of *Ötzi the Iceman*, whose body was frozen in the Ötztal Alps for more than 5,000 years. The mushroom was probably used to treat whipworm.

- **Ancient times**

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

- In ancient Sumeria, hundreds of medicinal plants including myrrh and opium are listed on clay tablets.

- The ancient Egyptian Ebers Papyrus lists over 800 plant medicines such as aloe, cannabis, castor bean, garlic, juniper and mandrake.
- From ancient times to the present, Ayurvedic medicine as documented in the Atharva Veda, the Rig Veda and the Sushruta Samhita has used hundreds of pharmacologically active herbs and spices such as turmeric, which contains curcumin.
- The Chinese pharmacopoeia, the *Shen nong Ben Cao Jing* records plant medicines such as chaulmoogra for leprosy, ephedra, and hemp. This was expanded in the Tang Dynasty *Yaoxing Lun*.
- In the fourth century BC, Aristotle's pupil Theophrastus wrote the first systematic botany text, *Historia plantarum*.
- In the first century AD, the Greek physician Pedanius Dioscorides documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*; it remained the authoritative reference on herbalism for over 1500 years, into the seventeenth century.

- **Middle Ages**

- ✓ In the Early Middle Ages, Benedictine monasteries preserved medical knowledge in Europe, translating and copying classical texts and maintaining herb gardens.
- ✓ Hildegard of Bingen wrote *CausaeetCurae* ("Causes and Cures") on medicine.
- ✓ In the Islamic Golden Age, scholars translated many classical Greek texts including Dioscorides into Arabic, adding their own commentaries.Herbalism flourished in the Islamic world, particularly in Baghdadand in Al-Andalus.
- ✓ Among many works on medicinal plants, Abulcasis (936–1013) of Cordoba wrote *The Book of Simples* and Ibn al-Baitar (1197–1248) recorded hundreds of medicinal herbs such as *Aconitum*, nux vomica, and tamarind in his *Corpus of Simples*.

- ✓ Avicenna included many plants in his 1025 *The Canon of Medicine*.
- ✓ Abu-Rayhan Biruni, Ibn Zuhr, Peter of Spain and John of St Amand wrote further pharmacopoeias.
- ✓ Following the Dark Ages there began a period of the encyclopedists and herbalists. The monasteries of Northern Europe produced large compendiums of information regarding plants, much of which was false. They stressed the medicinal value and folklore of plants. About the same time there appeared a "Doctrine of Signatures." This superstitious doctrine suggested that all plants possessed some sign, given by the Creator, which indicated the use for which they were intended. A plant with heart-shaped leaves was good for heart ailments; the liverleaf with its 3-lobed leaves was good for liver problems, etc. Many of the common names of plants owe their origin to this superstition. Names such as heartease, dogtooth violet, Solomon's seal and liverwort are examples.

- **Early Modern**

- ➢ The Early Modern period saw the flourishing of illustrated herbals across Europe, starting with the 1526 *Grete Herball*. John Gerardwrote his famous *The Herball or General History of Plants* in 1597, based on Rembert Dodoens and Nicholas Culpeper published his *The English Physician Enlarged*.
- ➢ Many new plant medicines arrived in Europe as products of Early Modern exploration and the resulting Columbian Exchange, in which livestock, crops and technologies were transferred between the Old World and the Americas in the 15th and 16th centuries.
- ➢ Medicinal herbs arriving in the Americas included garlic, ginger, and turmeric; coffee, tobacco and coca travelled in the other direction.
- ➢ In Mexico, the sixteenth century *Badianus Manuscript* described medicinal plants available in Central America.

- The Arabs introduced numerous new plants in pharmacotherapy, mostly from India, a country they used to have trade relations with, whereas the majority of the plants were with real medicinal value, and they have persisted in all pharmacopoeias in the world till today. The Arabs used aloe, deadly nightshade, henbane, coffee, ginger, strychnos, saffron, curcuma, pepper, cinnamon, rheum, senna, and so forth. Certain drugs with strong action were replaced by drugs with mild action, for instance, *Sennae folium* was used as a mild laxative, compared to the purgatives *Heleborusodorus* and *Euphorbium* used until then.
- Pharmacology and Pharmacognosy owe their beginnings to the earlier beliefs and knowledge about medicinal plants. The interest in medicinal plants was especially pronounced among the early botanists who were often physicians (Veeresham, 2012).

Traditional Medicines

According to WHO, Traditional Medicine is 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. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts and their various products as items of medicine. But the method of treatment and application of the medicament are greatly influenced by the religious beliefs of the different tribes and their concept of natural and supernatural causes of diseases.

Among the largest ethnic group, the ***Bangalees*** on the main land, there are two distinct forms of Traditional medicine practice:

- One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:
- ***Folk medicine,*** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting, bone-setting, hot and cold baths, therapeutic fasting and cauterisation.
- ***Religious medicine,*** which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods, etc.

✓ ***Spiritual medicine***, which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

The other is the improved and modified form based on the following two main traditional systems:

✓ The ***Unani-Tibb or Graeco-Arab system*** which has been developed by the Arab and Muslim scholars from the ancient Greek system, and

✓ The ***Ayurvedic system*** which is the old Indian system based on the *Vedas*, the oldest scriptures of the Hindu saints of the Aryan age (Liao and Wang, 2015).

Official Status of Tradition Medicinal in Bangladesh

Unani and Ayurvedic systems of medicine were officially recognised by the Government of Bangladesh immediately after independence and at the same time a Board of Unani and Ayurvedic systems of medicine was constituted. After the introduction of a National Drug Policy in 1982, Unani and Ayurvedic drugs have been brought under the control of the Drugs Administration Department of the Ministry of Health and Family Welfare by legislation to control and regulate the commercial manufacturing and marketing of quality

Unani and Ayurvedic drugs. The Board of Unani and Ayurvedic systems of medicine performs the following specific functions: registration of the traditional medicine practitioners, recognition of the relevant teaching institutions, holding of qualifying examinations, publication of text books, standardisation of Unani and Ayurvedic drugs, preparation and publication of Pharmacopoeias/Formularies and undertaking research and development programs. The Board has by this time published two National Formularies: - one for Unani and the other for Ayurvedic drugs, which have already been approved by the Government. They are now in use as official guides for the manufacture of all recognised Unani and Ayurvedic medicinal preparation (Khan and Rashid, 2006).

1.3 The importance of plants to humans

Plants have also been used in the production of stimulant beverages (e.g. tea, coffee, cocoa, and cola) and inebriants or intoxicants (e.g., wine, beer, kava) in many cultures since ancient times, and this trend continues till today. Tea (*Camellia sinensis* Kuntze) was first consumed in ancient China (the earliest reference is around CE 350), while coffee (*Coffea arabica* L.) was initially cultivated in Yemen for commercial purposes in the 9th century. The Aztec nobility used to consume bitter beverages containing raw cocoa beans (*Theobroma cacao* L.), red peppers, and various herbs. Nowadays, tea, coffee, and cocoa are important commodities and their consumption has spread worldwide. The active components of these stimulants are methylated xanthine derivatives, namely caffeine, theophylline, and theobromine, which are the main constituents of coffee, tea, and cocoa, respectively.

The most popular inebriants in society today are wine, beer, and liquor made from the fermentation of fruits and cereals. Wine was first fermented about 6000–8000 years ago in the Middle East, while the first beer was brewed around 5000–6000 BCE by the Babylonians. The intoxicating ingredient of these drinks is ethanol, a by-product of bacterial fermentation, rather than secondary plant metabolites. Recent studies have shown that a low to moderate consumption of red wine is associated with reduction of mortality due to cardiovascular disease and cancer.

Benefits of plants to humans are in following ways as-

- **Food**: Plants are the main source of food for humans. The fruits and other parts of plants are consumed by humans. They provide the complete food comprising of carbohydrates, fats, proteins, vitamins and minerals. This is the prime importance of plants to humans.

- **For Vitamin D synthesis**: Vitamin-D is a type of vitamin which does need to be supplied from external source. This is because it can be synthesized from ergosterol and cholesterol by irradiation with sunlight. When the light fall on the skin, the ergosterol below the skin converts to vitamin-D. So if someone stays away from sunlight for long, he tends to develop vitamin-D deficiency.

- **Maintains Body clock or Circadian Rhythm:** There is a clock inside both plants and animals which keep them active during day and relaxed at night. This clock is controlled by light. Plants broaden their leaves during day time starting from sunrise till sunset. Even we can notice leaves and few flowers like sunflower which bent towards light through the day. Also the plants grow in the direction of light as auxin a plant growth hormone is regulated by light.

- **Light and plant development:** Plants grow towards sky as they are guided by light. The plant growth hormone namely Auxin is responsible for it. The hormone is present the apical parts (tips) of growing regions. Based on the availability of light, it directs the growth. That is the plant grows towards light (Miller, 2014).

1.3.1 Drug discovery from plant sources

The branch of medical science dealing with the drug plants themselves is known as Pharmacognosy. It is concerned with the history, commerce, collection, selection, identification and preservation of crude drugs and raw materials. The action of drugs is

Pharmacology. Worldwide there are several thousand plants that have been and are still being used for medical purposes. Many of these are restricted in use by native people who have long resided in any given area.

The Pure Food and Drug Act of 1906 in the United States have standardized most of the truly valuable drug plants. Such drugs are referred to as "official." Details about these plants may be found at the United States Pharmacopoeia, the Homeopathic Pharmacopoeia and the National Formulary, and various other sources in the United States and Europe.

Some examples of drugs which are discovered from plants are given below:

Table 1.1: Example of some drugs discovered from plants

Plant Roots	Bark of plants, stems and woods	Plant leaves	Flowers	Fruits and seeds	Lower Plants	Others
Aconite Colchicum Gentian Goldenseal Ginseng Ipecac Jalap Podophyllum Rhubarb Valerian	Cascara Curare Quinine Ephedrine Guiacum Oleoresins	Aloe Belladonna Cocaine Buchu Digitalis Lobelia Stramonium Wormeood	Chamo mile Hops Santonin	Colocynth Croton Oil Nux vomica Opium Psyllium	Penicillin Streptom ycin Chlorom ycetin Aureomy cin Neomyci n Terramyc in	Agar Ergot Kelp Lycopodium Male furn

New drug discovery is facing serious challenges due to reduction in number of new drug approvals coupled with exorbitant rising cost. Advent of combinatorial chemistry provided new hope of higher success rates of new chemical entities (NCEs). However, even this scientific development has failed to improve the success rate in new drug discovery. This scenario has prompted us to come out with a novel approach of integrated drug discovery, where Ayurvedic wisdom can synergize with drug discovery from plant sources. Initial steps in new drug discovery involve identification of NCEs, which can be either sourced through chemical synthesis or can be isolated from natural products through biological activity guided fractionation. The sources of many of the new drugs and active ingredients of medicines are derived from natural products. The starting point for plant-based new drug discovery should be identification of the right candidate plants by applying Ayurvedic wisdom, traditional documented use, tribal non-documented use and exhaustive literature search. Frequency analysis of the ingredients of the ancient documented formulations and analysis of their Ayurvedic attributes may provide an in-depth idea of the predominance of particular Ayurvedic characteristics based on which appropriate candidate plants may be selected for bioactivity-based fractionation. The integration of Ayurvedic wisdom with drug discovery also brings the need for a paradigm shift in the extraction process from sequential to parallel extraction. Bioassay-guided fractionation of the identified plant may lead to standardized extract or isolated bioactive drug compound as the new drug. This integrated approach would lead to saving of cost and time, coupled with enhanced success rate in drug discovery (Katiyar et al., 2012).

1.3.2 Medicinal plant part utilization

For medicinal preparations, people mostly use above ground plant parts (76%), followed by belowground parts (17%) and whole plants (7%). Of the above ground parts, leaves are used most frequently (25%), followed by roots and fruits (20% each), bark (16%), whole plants (9%), flowers (4%), latex (4%) and seed (2%). The paste and juice made from leaves and barks are used in medicine, while fruits are eaten raw (Gurib-Fakim, 2014).

1.3.3 Plants used as sources of some significant drugs

With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. At one time, it was thought that ultimately all the plant drugs would be obtained from synthetic sources. However, in spite of phenomenal progress in the development of new drugs from synthetic sources and the appearance of antibiotics as major therapeutic agents, plants continue to provide basic raw materials for some of the most important drugs.

According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine.

- Mandrake was prescribed for pain relief
- Turmeric possesses blood clotting properties
- Roots of the endive plant were used for treatment of gall bladder disorders.
- Paclitaxel from *Taxus brevifolia* used for the treatment of lung, ovarian and breast cancer.
- The alkaloid, forskolin from *Coleus forskohlii* and phytochemicals from *Stephania glabra*, are now being rediscovered as adenylate cyclase and nitric oxide activators, which may help in preventing conditions including obesity and atherosclerosis.
- Apomorphine is a semi synthetic compound derived from morphine (*Papaver somniferum*) used in Parkinson's disease.
- Cannabidiol obtained from cannabis plant (*Cannabis sativa*) and Capsaicin active compound from *Capsicum annuum*are used as pain relievers.

  It has been found that in highly developed countries like the United States more than 100 chemical constituents of definite structure derived from 41 species of plants were used in modern medicine. It has also been estimated that in addition to these active constituents, more than 96 crude extracts were also used in the United States (Raffauf, 1960).

1.3.4 Some Modern Medicines from Plant sources

Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is currently available. It has been estimated that more than 400 traditional plants or plant derived products have been used for the management of type 2 diabetes across geographically. Galegine, a substance produced by the herb *Galega officinalis,* provides an excellent example of such a discovery. Experimental and clinical evaluations of galegine provided the pharmacological and chemical basis for the discovery of metformin which is the foundation therapy for type 2 diabetes. Plant derived agents are also being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world.

So, it is anticipated that plants can provide potential bioactive compounds for the development of new leads to combat various diseases. (Shah et al., 2014)

1.3.5 Drug Development from Plant Sources

Traditional medicinal preparations are generally supplied as crude extract of a medicinal plant. Plant extracts possess a number of chemical constituents, each of them may expert some effect on the living body. 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 (*Vincaa rosea*) which was formerly prescribed for treating diabetes.

The efficient hypotensive drug, Reserpine, was developed from *Rauwolfia serpentina* which was previously provided as an antidote to snake-bites and in the treatment of lunatic patient.

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 (Fokunang and Ngameni, 2011).

1.3.6 Methods of drug development

Drug development is an expensive practice. So, careful phytochemical analysis and pharmacological screening and if promising clinical tests are required.

Pharmacology is the study of the therapeutic value and/or potential toxicity of chemical agents on biological systems. It targets every aspect of the mechanisms for the chemical actions of both traditional and novel therapeutic agents. In its entirety, pharmacology embraces knowledge of the sources, chemical properties, biological effects and therapeutic uses of drugs.

Phytochemical analysis refers to the extraction, analysis and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

Pharmacological studies range from those that examine the effects of chemical agents on sub-cellular mechanisms, to those that deal with the potential hazards of pesticides and herbicides, to those that focus on the treatment and prevention of major diseases with drug

therapy. It is estimated that only 500 medicinal plant species had been recorded in Bangladesh out of approximately 1900 species regarded as having medicinal value.

The way of developing drugs from plants involves several stages, which include:

- Selection and correct identification of the proper medicinal plant.
- 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. (Pandey et al., 2010)

1.4 Importance of medicinal plants as Alternative Medicine

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical semi-synthesis. When a plant is designated as medicinal, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purposes. World Health Organization (WHO) has provided a definition of medicinal plants, which is “A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis”.

In the US, almost 1800 medicinal plant species are commercially available. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published and very likely a much larger number of the world's flowering plant species have been used medicinally. Sometimes the figure of 70,000 medicinal plant species is cited, but this includes many algae, fungi and micro-organisms that are not really plants as the word is understood by botanists. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines. A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredient.

This is similar to the one occurring in Bangladesh. Bangladesh is an Asian country where only 20 % of the people can be provided with modern healthcare services while the rest 80 % are dependent on traditional plant-based systems. In the Plant Kingdom, medicinal plants form the largest single grouping of plants. It is estimated that 30,000 species worldwide fall in this group, of which around 33% are trees.

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug administration or European Food Safety Authority to have medicinal effects. (Plants as a Source of Medicine, 2015)

1.4.1 Value of Medicinal Plants

Many of the plants could be used as stimulants, poisons, hallucinogens or as medicine because of the presence of unique or rich biological-active plant chemicals (i.e. Chemical compounds that have a biological effect on another organism). Chemicals that make a plant valuable as medicinal plant are:

- ✓ Alkaloids (compounds has addictive or pain killing or poisonous effect and sometime help in important cures).
- ✓ Glycosides (use as heart stimulant or drastic purgative or better sexual health).
- ✓ Tannins (used for gastrointestinal problems like diarrhea, dysentery, ulcer and for wounds and skin diseases).
- ✓ Volatile/essential oils (enhance appetite and facilitate digestion or use as antiseptic and insect repellent properties).
- ✓ Fixed oils (present in seeds and fruits could diminish acidity).
- ✓ Gum-resins and mucilage (possess analgesic property that suppress inflammation and protect affected tissues against further injury and cause mild purgative).
- ✓ Vitamins and minerals (Fruits and vegetables are the sources of vitamins and minerals and these are used popularly in herbals). (Badawi, 2012)

1.4.2 Importance of Medicinal Plant in Drug Discovery

Development of new drug is a complex, time-consuming and expensive process. The time taken from discovery of a new drug to its reaching the clinic is approximately 12 years, involving more than 1 billion US$ of investments in today's context. Essentially, the new drug discovery involves the identification of new chemical entities (NCEs), having the required characteristic of drug ability and medicinal chemistry. These NCEs can be isolated from natural products. More than 80% of drug substances were purely natural products or were inspired by the molecules derived from natural sources (including semi-synthetic analogs).

- ➢ Morphine was isolated from opium produced from cut seed pods of the poppy plant (*Papaver somniferum*) approximately 200 years ago.
- ➢ Few drugs developed from natural sources have undoubtedly revolutionized medicine, like:
- ➢ Antibiotics (e.g. penicillin, tetracycline, erythromycin), antiparasitics (e.g. avermectin).

- Antimalarials (e.g. quinine, artemisinin), lipid control agents (e.g. lovastatin).
- Immunosuppressant for organ transplants (e.g. cyclosporine, rapamycins).
- Anticancer drugs (e.g. paclitaxel, irinotecan).

Clinical trials are ongoing on more than 100 natural product derived drugs and at least 100 molecules/compounds are in preclinical development stage. Cancer and infections are the two predominant therapeutic areas for which the drug discovery program is based on natural products, but many other therapeutic areas also get covered, such as cardiovascular, gastrointestinal, inflammation etc.

The botanical sources are known to provide the following classes of NCEs for drug discovery processes:

- Bioactive compounds for direct use as drug, e.g. digoxin.
- Bioactive compounds with structures which themselves may act as lead compounds for more potent compounds, e.g. paclitaxel from *Taxus* species.
- The novel chemophore which may be converted into drug compounds with/without chemical analoging.
- Pure photochemical for use as marker compounds for standardization of crude plant material or extract.
- Pure photochemical which can be used as pharmacological tools.
- Herbal extracts as botanical drugs, e.g. green tea extract (Badawi, 2012).

1.5 Traditional Use of Medicinal Plants

Many clinical and animal studies document the efficacy of hawthorn as a cardio tonic. Cardio tonics help to improve blood supply to the heart, increase the tone of the heart muscle, stimulate cardiac output, dilate coronary arteries, stabilize blood pressure, prevent atherosclerosis (the accumulation of arterial plaque), and prevent or help improve congestive heart failure. Many herbs used for cardiovascular health, such as hawthorn and gingko, have antioxidant properties, which may help prevent hardening of the arteries or other circulatory insufficiencies. Some herbs used for cardiovascular health are commonly

taken to lower cholesterol. Garlic is one notable example, and a number of clinical studies have shown that garlic is effective in moderately reducing serum cholesterol. Clinical research indicates that ginger is a very effective herb for nausea, indigestion, and minor gastric upsets. Ginger is also effective for morning sickness in the early stages of pregnancy and for motion sickness. Peppermint oil has demonstrated clinical efficacy for irritable bowel syndrome. Many herbs are liver protective and restorative-they can help to protect a healthy liver and restore function to a liver that has suffered impaired functions due to disease or injury, such as cirrhosis, hepatitis, or exposure to hepatotoxic agents. Adaptogenic herbs, such as ginseng, owe much of their activity to stimulation of pituitary and adrenal activity. Many plants are diuretics. They can help eliminate disease-carrying microorganisms from the urinary tract, and they can help prevent kidney stone formation and bladder inflammation resulting from bladder irritation-whether or not it's due to microbial infection. Others are effective urinary tract disinfectants. One that has been studied clinically and found effective for both prevention and treatment of urinary tract infections is cranberry, which may be taken as cranberry juice or in the form of concentrated cranberry juice solids. Some nursing women use herbs to induce milk production during lactation, or conversely, to reduce milk production during weaning. For example, Fenugreek is an herb that has been successfully used to induce lactation. Black cohosh and red clover are effective for treating menopausal symptoms. Anti-inflammatory botanicals, of which there are many (examples include ginkgo, ginger, hawthorn, and St. John's wort) are useful in suppressing various immune functions involved in the inflammatory response. Extracts of immune-stimulating medicinal mushrooms, such as reishi or turkey tail can be used as adjunct therapies to help maintain immune functions during radiation and chemotherapy. Fruit and vegetables rich in antioxidants are best as antioxidant supplements. Green tea, in its natural form or as a concentrated supplement. Dark chocolate contains many of the same beneficial compounds, known as catechins. One application of botanical medicines in this area is to lower blood sugar in individuals who may be diabetic or pre-diabetic. Popular botanical medicines thought to have this effect include: Ginseng, Ayurvedic medicine, Green tea.

All cultures have a history of herbal medicine use, usually making use of the plants found closest to home. Even today in the times of advanced technology and medical science still

depend on plants for their healing. Western culture, however, is predominantly excited by the new and upcoming and the novel and perhaps most importantly the patentable. This means that the good, tried and tested tools of survival become relegated to historical anecdote.

But herbal medicines the original human health care products are still fully present and available to our lives if you look out for them.

Common herbs and spices – including ginger, turmeric and garlic, and cinnamon and rosemary as well as fenugreek seeds and leaves, artichoke leaf extract, yarrow, and holy basil all may help lower cholesterol. For lowering blood pressure, herbs and spices including cloves, ground Jamaican allspice, cinnamon, sage, marjoram, tarragon, and rosemary are beneficial. Thyme tincture can outperform conventional acne treatments.

Until the beginning of 1900s medicinal plants from all over the world were fully monographed in all pharmacopoeias as legitimate medicinal ingredients. They are now presented in relatively small numbers but that is slowly changing as we rediscover the true medicinal value of plants. European laws continue to restrict not only what can be sold, but what can be said about traditional herbal remedies insisting on the randomized trial being the only source of legitimate information.

It is good, then, to see some scientists acknowledging that ancient investigation is research and that traditional use, or herbs as often passed down orally as in written form – can also help us understand the uses and relevance of herbs in our lives. (Ross, 2005)

1.6 *Syzygium samarangense*

Table 1.2: Taxonomy of *Syzygium samarangense*

Kingdom:	Plantae
Class:	Magnoliopsida
Order:	Myrtales
Family:	Myrtaceae
Genus:	*Syzygium*
Species:	*Syzygium samarangense*

***Figure 1.1: Syzygium samarangense* plant (1)**

Member of the Myrtaceae is botanically identified as *Syzygium samarangense*. Merr. & Perry (syns. *S. javanicum* Miq.; *Eugenia javanica Lam*. in part; *E. alba* Roxb.).

Its various vernacular names are:

- samarang rose apple, *djamboesemarang* (Indonesia);
- *jambu* ayerrhio (Malaya);
- *pinijambu (Ceylon);*
- *jumrool*, *jamrul*, or *amrool*(India);
- *chompukao*, or *chompukio* (Thailand);
- *makopa* (Philippines);
- *cashu di Surinam*, or *Curacaoseappel* (Curacao);
- wax apple,
- wax jambu and
- water apple, generally.

***Figure 1.2: Syzygium samarangense* plant (2)**

The tree 16 to 50 ft (5-15 m) tall, has a short trunk 10 to 12 in (25-30 cm) thick and open, widespreading crown and pinkish-gray, flaking bark. The opposite leaves are nearly sessile, elliptic-oblong, rounded or slightly cordate at the base; yellowish to dark bluish-green; 4 to 10 in (10-25 cm) long and 2 to 4 3/4 in (5-12 cm) wide; very aromatic when crushed. Flowers, borne in drooping panicles of 3 to 30 at the branch tips or in smaller

clusters in the axils of fallen leaves, are fragrant, yellowish-white, 3/4 to 1 1/2 in (2-4 cm) broad, 4-petalled, with numerous stamens 3/5 to 1 in (1.5-2.5 cm) long. The waxy fruit, usually light-red, sometimes greenish-white or cream-colored, is pear-shaped, narrow at the base, very broad, flattened, indented and adorned with the 4 fleshy calyx lobes at the apex; 1 1/3 to 2 in (3.4-5 cm) long, 1 3/4 to 2 1/8 in (4.5-5.4 cm) wide. The skin is very thin, the flesh white, spongy, dry to juicy, subacid and very bland in flavor. There may be 1 or 2 somewhat rounded seeds 3/16 to 5/16 in (0.5-0.8 cm) wide, or none.

***Figure 1.3: Syzygium samarangense* flowers**

The tree is indigenous from Malaya to the Andaman and Nicobar Islands where there are wild trees in the coastal forests. It was introduced into the Philippines in prehistoric times and is widely grown throughout those islands. It is common in Thailand, Cambodia, Laos, Vietnam and Taiwan, frequently cultivated in India and in Zanzibar and Pemba, but primarily as an ornamental, seldom for its fruits which are little valued. It was introduced into Jamaica before 1903 and also into Surinam and the islands of Curacao, Aruba and Bonaire. A few trees have been grown in Israel but have borne sparsely.

***Figure 1.4: Syzygium samarangense* leaves**

The Java apple is extra-tropical, growing only at the lower altitudes–up to 4,000 ft (1,220m)–in India. It does best in parts of the Philippines that have a long dry season.

The soil must be fertile or the crops will be small and the fruit quality poor.

The trees grow spontaneously from seed. Preferred types are reproduced by layering, budding onto their own rootstocks, or onto seedlings of *S. densiflorum* A. DC., (the beautiful Wild Rose Apple of Malaya, which has edible flowers, undesirable fruits, but is not attacked by termites). Sometimes the Java apple is grafted onto the cultivated Rose Apple (q.v.).

If planted in orchards, the trees are spaced 26 to 32 ft (8-10 m) apart and are given a minimum of attention.

Figure 1.5: A section of *Syzygium samarangense* fruit

In Ceylon, the fruits are ripe from March to May; in India, the tree blooms in March and April and the fruit ripens in May and June; in Java, flowering occurs from April to June and fruiting from June to August. The Java apple is a heavy bearer on good soil. When 5 years old it may yield a crop of 700 fruits. In Malaya, the greenish fruits are eaten raw with salt or may be cooked as a sauce. They are also stewed with true apples. The pink fruits are juicier and more flavorful and suitable for eating out-of-hand or cooking without accompaniments except sugar.

Figure 1.6: Syzygium samarangense fruits

Other Uses-

Wood: The wood is red, coarse, hard; used for constructing huts in the Andaman and Nicobar Islands.

Medicinal Uses: The flowers are astringent and used in Taiwan to treat fever and halt diarrhea. Investigators have found their principal constituent to be tannin. They also contain desmethoxymatteucinol, 5-*O*-methyl-4'-desmethoxymatteucinol, oleanic acid and B-sitosterol. They show weak antibiotic action against *Staphylococcus aureus*, *Mycobacterium smegmatis*, and *Candida albicans.*

Other nutritional values: The nutrient composition of *S. samarangense* fruit per 100 g edible portion (Leung et al. 1972) was reported as: water 91.5 g, energy 30 kcal, protein 0.4 g, fat 0.1 g, carbohydrate 7.8 g, fibre 0.8 g, ash 0.2 g, Ca 17 mg, P 9 mg, Fe 0.3 mg, Na 2 mg, K 105 mg, b-carotene 0 mg, thiamin 0.03 mg, riboflavin 0.01 mg, niacin 0.3 mg, ascorbic acid 13 mg. Another analysis conducted in Australia reported that wax jambu had the following food value per 100 g edible portion (Wills 198): water 90.3%, protein 0.7 g, fat 0.2 g, glucose 2.1 g, fructose 2.4 g, dietary fibre 1.9 g, malic acid 0.10 g, citric acid 0.12 g, oxalic acid 0.02 g, energy 94 kJ, vitamin C 8 mg, thiamin 0.02 mg, riboflavin 0.04 mg, niacin 0.5 mg, K 38 mg, Na 1 mg, Ca 13 mg, Mg 5 mg, Fe 0.8 mg and Zn 0.1 mg. A total of 39 volatile constituents were identified in wax jambu*(S. samarangense*) (Wong and Lai 1996). The volatiles of wax jambu were characterized by the presence of a large number of C9 aldehydes and alcohols. A triterpene, methyl 3-epi-betulinate in its native form and 4,6-dihydroxy-2-methoxy- 3, 5 -dimethyl chalcone along with ursolic acid, jacoumaric acid and arjunolic acid were isolated from the aerial parts of *Syzygium samarangense* (Srivastava et al. 1995). Various parts of the plant have been reported to have bioactive compounds and to exhibit antioxidant, anticancer, antiviral, antimicrobial, spasmolytic, antihyperglycaemic, protease inhibition, anti-amnesiac and immunomodulatory activities (Lim, 2012).

1.7 Aim and Objective of the study

The purpose of this study was to evaluate pharmacological effect of Petroleum Ether extract of leaves of *Syzygium samarangense* plant on the Central Nervous System (CNS).

The aim was -

- Assessment of general locomotor levels and anxiety in Swiss albino mice model by Open Field Test.
- Analysis of neophilia, anxiety and stress responses in Swiss albino mice model by Hole Board Experiment.

The principle objective of this study was to explore the possibilities of deriving medicinal agents from *Syzygium samarangense* plant leaves for the treatment of various diseases.

Chapter Two: Literature Review

2.1 Cytotoxic chalcones and antioxidants from the fruits of a *Syzygium samarangense*

Bioassay-guided fractionation of the methanolic extracts of the pulp and seeds of the fruits of *Syzygium samarangense* led to the identification of four cytotoxic compounds and eight antioxidants on the basis of HPLC-PDA analysis, MS and various NMR spectroscopic techniques. Three *C*-methylated chalcones, 2′,4′-dihydroxy-3′,5′-dimethyl-6′-methoxychalcone, 2′,4′-dihydroxy-3′-methyl-6′-methoxychalcone and 2′,4′-dihydroxy-6′-methoxychalcone were isolated and displayed cytotoxic activity against the SW-480 human colon cancer cell line. Also a number of known antioxidants were obtained including six quercetin glycosides: reynoutrin, hyperin, myricitrin, quercitrin, quercetin and guaijaverin,one flavanone: (*S*)-pinocembrin and two phenolic acids: gallic acid and ellagic acid.

The edible fruits of *Syzygium samarangense* represent potential benefits for human health because they are a rich dietary source of polyphenolic antioxidants. In addition, the seeds are a rich source of the cytotoxic chalcones **1-3**, especially true for compound **1**, since it is present in high concentration (35.0 mg per kg fresh weight). (Simirgiotis et al., 2008)

2.2 An Extract from *Syzygium samarangense* affects Glycogenesis and Glycolysis Pathways in Tumor Necrosis Factor-α-Treated FL83B Mouse Hepatocytes

FL83B mouse hepatocytes were treated with tumor necrosis factor-α (TNF-α) to induce insulin resistance to investigate the effect of a wax apple aqueous extract (WAE) in insulin-resistant mouse hepatocytes. The uptake of 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2 NBDG), a fluorescent D-glucose derivative, was performed and the metabolism of carbohydrates was evaluated by examining the expression of glycogenesis or glycolysis-related proteins in insulin-resistant hepatocytes. The results show that WAE significantly improves the uptake of glucose and enhances glycogen content in insulin-resistant FL83B mouse hepatocytes. The results from Western blot

analysis also reveal that WAE increases the expression of glycogen synthase (GS), hexokinase (HXK), glucose-6-phosphate dehydrogenase (G6PD), phosphofructokinase (PFK) and aldolase in TNF-α treated cells, indicating that WAE may ameliorate glucose metabolism by promoting glycogen synthesis and the glycolysis pathways in insulin-resistant FL83B mouse hepatocytes.

The study investigated the effects of WAE on the metabolism of carbohydrates in TNF-α-induced insulin-resistant FL83B mouse hepatocytes. The results show that WAE improves glucose uptake in TNF-α-treated FL83B cells. Furthermore, WAE increases expression of GS, HXK, G6PD, PFK and aldolase, suggesting increased glycolysis and gluconeogenesis and WAE increases glycogen storage. These findings suggest that wax apple fruit may mitigate the hyperglycemia in Type 2 DM patients; therefore, it has the potential to be developed into a functional food or dietary supplement that prevents and/or alleviates DM. (Shen, Chang and Chang, 2013)

2.3 Physiochemical and Phytochemical Properties of *Syzygium samarangense* as affected by Growth Regulator Application

This study represents the effects of growth regulators on the physiochemical and phytochemical properties of the wax apple fruit. Net photosynthesis, sucrose phosphate synthase (SPS) activity, peel color, fruit firmness, juice content, ph value, total soluble solids (tsss) and the sugar acid ratio were all significantly increased in growth regulators (pgrs) treated fruits. The application of gibberellin (GA_3), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D) significantly reduced titratable acidity and increased total sugar and carbohydrate content compared to the control. The 50 mg/L GA_3, 10 mg/L NAA, and 5 mg/L 2,4-D treatments produced the greatest increases in phenol and flavonoid content; vitamin C content was also higher for these treatments. PGR treatment significantly affected chlorophyll, anthocyanin and carotene content and produced higher phenylalanine ammonia lyase (PAL) and antioxidant activity levels. There was a positive correlation between peel color and TSS and antioxidant activity and both phenol and flavonoid content and PAL activity and anthocyanin formation. A taste panel assessment

was also performed and the highest scores were given to fruits that had been treated with GA_3 or auxin. The study showed that application of 50 mg/L GA_3, 10 mg/L NAA and 5 mg/L 2, 4-D once a week from bud development to fruit maturation increased the physiochemical and phytochemical properties of wax apple fruits.

From the study, it is concluded that the pgrs (GA_3, NAA, and 2,4-D) can improve the physiochemical and phytochemical status of wax apple fruits. Nevertheless, it must be emphasized that the positive effects of pgrs on the quality of wax apple are dependent on types, dose, and environmental conditions. 50 mg/L GA_3 treatment produced greater increases in physiochemical and phytochemical nutrition than the 100 and 20 mg/L GA_3 treatments. For NAA, the 10 mg/L NAA treatment had the greatest effect on physiochemical and phytochemical nutrition. For 2,4-D, another synthetic auxin, the most promising results were obtained with a low concentration of 5 mg/L and treatments in excess of 10 mg/L actually produced adverse effects on physiochemical and phytochemical properties. From experiments performed under field conditions, it was also concluded that the 50 mg/L GA_3, 10 mg/L NAA and 5 mg/L 2,4-D treatments show particular promise for enhancing the physiochemical and phytochemical quality of wax apple fruits (Khandaker et al., 2012).

2.4 Fraction from *Syzygium samarangense* Fruit Extract Ameliorates Insulin Resistance via Modulating Insulin Signaling and Inflammation Pathway in Tumor Necrosis Factor α-Treated FL83B Mouse Hepatocytes

In this study, mouse FL83B cells were treated with tumor necrosis factor-alpha (TNF-α) to induce insulin resistance and then co-incubated with a fraction from wax apple fruit extract (FWFE). This fraction significantly increased the uptake of the nonradioactive fluorescent indicator 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG) in insulin resistant cells. Western blot analysis revealed that, compared with the TNF-α-treated control group, FWFE increased the expression of the insulin receptor (IR), insulin receptor substrate-1 (IRS-1), protein kinase B (Akt/PKB), phosphatidylinositol-3 kinase (PI3K) and glucose transporter 2 (GLUT-2) and increased IR tyrosylphosporylation, in

insulin resistant FL83B cells. However, FWFE decreased phosphorylation of c-Jun *N*-terminal kinases (JNK), but not the expression of the intercellular signal-regulated kinases (ERK) in the same cells. These results suggest that FWFE might alleviate insulin resistance in TNF-α-treated FL83B cells by activating PI3K-Akt/PKB signaling and inhibiting inflammatory response via suppression of JNK, rather than ERK activation.

The study investigated the effects of FWFE on glucose uptake and the expression of insulin and inflammatory signal transduction-related proteins in TNF-α-induced insulin resistant FL83B mouse hepatocytes. This postulated that it can alleviate insulin resistance via inhibiting the intracellular JNK inflammatory signaling cascades, restoring the PI3K-Akt/PKB insulin signaling pathway and then enhancing glucose uptake in TNF-α-treated FL83B mouse hepatocytes. Therefore, FWFE might have potential use in the development of anti-diabetic drugs, health foods and dietary supplements (Shen, Chang and Chang, 2012).

2.5 The Effects of *Syzygium samarangense* on Alcohol-Induced Liver Injury

The work aimed at studying the effects of *Syzygium samarangense* on alcohol-induced liver injury in mice. The animals were treated daily with alcohol and fruit juice for fifteen days. Chronic treatment with alcohol increased the levels of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TBIL), triglyceride (TG), malondialdehyde (MDA) and decreased total protein (TP). Histopathological evaluation also showed that ethanol induced extensive fat droplets in hepatocyte cytoplasm. *Syzygium samarangense* normalized various biochemical parameters. These results strongly suggest that treatment with *Syzygium samarangense* could protect liver from the injury of alcohol.

Treatment with *Syzygium samarangense* can protect the liver from damages of alcohol and the mechanism of the protective effect might be related to the enhancement of the antioxidant system. Intake of *Syzygium samarangense* with alcohol consumption can be recommended. In addition, *Syzygium samarangense* can also be further developed as a

functional food or drug for the prevention and treatment of alcoholic liver disease (Zhang et al., 2016).

2.6 Vescalagin from Pink *Syzygium samarangense* alleviates Hepatic Insulin Resistance and Ameliorates Glycemic Metabolism Abnormality in Rats Fed a High-Fructose Diet

This study investigates the ameliorative effect of vescalagin (VES) isolated from Pink wax apple fruit on hepatic insulin resistance and abnormal carbohydrate metabolism in high-fructose diet (HFD)-induced hyperglycemic rats. The results show that in HFD rats, VES significantly reduced the values of the area under the curve for glucose in an oral glucose tolerance test and the homeostasis model assessment of insulin resistance index. VES significantly enhanced the activity of hepatic antioxidant enzymes while reducing thiobarbituric acid-reactive substances in HFD rats. Western blot assay revealed that VES reduced hepatic protein expression involved in inflammation pathways while up-regulating expression of hepatic insulin signaling-related proteins. Moreover, VES up-regulated the expression of hepatic glycogen synthase and hepatic glycolysis-related proteins while down-regulating hepatic gluconeogenesis-related proteins in HFD rats. This study suggests some therapeutic potential of VES in preventing the progression of diabetes mellitus. (Huang et al., 2016)

2.7 Prolyl Endopeptidase Inhibitors from *Syzygium samarangense*

Compounds isolated from the hexane extract of the leaves of Syzygium samarangense (Blume) Merr. & L. M. Perry were tested for inhibitory activity against the serine proteases: trypsin, thrombin and prolyl endopeptidase. The compounds were identified as an intractable mixture of α-carotene and β-carotene (1), lupeol (2), betulin (3), epi-betulinic acid (4), 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (5), 2′-hydroxy-4′,6′-dimethoxy-3′- methylchalcone (6), 2′,4′-dihydroxy-6′-methoxy-3′,5v-dimethylchalcone (7), 2′,4′-

dihydroxy- 6′-methoxy-3′-methyldihydrochalcone (8) and 7-hydroxy-5-methoxy-6,8-dimethylflavanone (9). Hydrogenation of compounds 5, 6 and 7 yielded compound 8, 2′-hydroxy-4′,6′-dimethoxy- 3′-methyldihydrochalcone (10) and 2′,4′-dihydroxy-6′-methoxy-3′,5-dimethyldihydrochalcone (11), respectively. The hydrogenated products of compounds 6 and 7 were also tested for enzyme inhibitory activity. In addition, β-sitosterol (12) and β-D-sitosterylglucoside (13) were also isolated. Compounds 3-8 and 10 exhibited significant and selective inhibition against prolyl endopeptidase among three serine proteases. (Amor et al., 2004)

2.8 Spasmolytic Flavonoids from *Syzygium samarangense*

The hexane extract of Syzygium samarangense (Ss.Hex) dose-dependently (10D1000 μg/ml) relaxed the spontaneously contracting isolated rabbit jejunum. Four rare C-methylated flavonoids with a chalcone and a flavanone skeleton were isolated from Ss.Hex and were subsequently tested for spasmolytic activity. All flavonoids, identified as 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (1), 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (2), 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (3) and 7-hydroxy-5-methoxy-6,8-dimethylflavanone (4), showed dose-dependent spasmolytic activity in the rabbit jejunum with IC_{50} values of 148.3 ± 69.4, 77.2 ± 43.5, 142.4 ± 58.6 and 178.5 ± 37.5 μg/ml (mean ± SEM), respectively. The dihydrochalcone derivative of compound 1, 2′-hydroxy-4′,6′-dimethoxy-3′- methyldihydrochalcone (5), when tested for spasmolytic activity, did not significantly relax the smooth muscle relative to the other compounds. Verapamil, a standard spasmolytic, has an IC_{50} value of 0.16 ± 0.04 μg/ml. This is the first report of the relaxant activity of chalcones, specifically of compounds 1-3. (Amor et al., 2005)

2.9 Chemical composition and antioxidant activity of essential oil from *Syzygium samarangense* flower-bud

This study aimed to investigate the chemical composition of essential oil from Syzygium samarangense (BL.) Merr.et Perry flower-bud and its antioxidant activity for the first time. The chemical composition of essential oil from Syzygium samarangense (BL.) Merr.et Perry flower-bud was analyzed using GC-MS method. Its antioxidant activities were measured by DPPH•, ABTS+• radical-scavenging and Cu2+ reducing power assays. RESULTS: GC-MS analysis revealed that there were 40 chemical components in the essential oil. Its IC50 values were 2740.93±122.77, 523.62±78.9 and 138.59±2.64 μg/mL, respectively for scavenging DPPH•, ABTS+• and Cu2+ reducing power. Five compounds are considered as the main components in the essential oil, including caryophyllene (7.950%), decahydro- 4'-methyl-1-methylene-7-(1-methylethenyl)-naphthalene (7.346%), 1,2,3,4,4',5,6,8'-Octahydro-4',8-dimethyl-2-(1-methylethenyl)-naphthalene (7.292%), 1,2,4,5-tetramethyl benzene (7.187%), and caryophyllene oxide (6.319%). The comparison on IC50 values between the essential oil and positive controls demonstrated that the essential oil exhibits moderate antioxidant ability. (Gao, Hu and Li, 2012)

2.10 Bioactivities of Triterpenes and a Sterol from *Syzygium samarangense*

Cycloartenyl stearate, lupenyl stearate, sitosteryl stearate and 24-methylenecycloartanyl stearate from the air-dried leaves of *Syzygium samarangense* exhibited potent analgesic and anti-inflammatory activities at effective doses of 6.25 mg/kg body weight and 12.5 mg/kg body weight, respectively. The sample also exhibited negligible toxicity on zebrafish embryonic tissues. There were incidences of mortality upon direct exposure of sample to dechlorionated embryos, but higher mortality and aberration were observed during intact chorion treatment. (Raga et al., 2011)

2.11 Protective Effects of Vescalagin from Pink *Syzygium samarangense* Fruit against Methylglyoxal-Induced Inflammation and Carbohydrate Metabolic Disorder in Rats

The unbalance of glucose metabolism in humans may cause the excessive formation of methylglyoxal (MG), which can react with various biomolecules to form the precursor of advanced glycation end products (ages). Vescalagin (VES) is an ellagitannin that alleviates insulin resistance in cell study. Results showed that VES reduced the value of oral glucose tolerance test, cardiovascular risk index, ages and tumor necrosis factor-α contents while increasing C-peptide and D-lactate contents significantly in rats orally administered MG and VES together. The preventive effect of VES on MG-induced inflammation and carbohydrate metabolic disorder in rats was thus proved. On the basis of the experiment data, a mechanism, which involves the increase in D-lactate to retard AGE formation and the decrease in cytokine release to prevent β-cell damage, is proposed to explain the bioactivities of VES in antiglycation and in the alleviation of MG-induced carbohydrate metabolic disorder in rats. (Chang, Shen and Wu, 2013)

2.12 Anti-proliferative and apoptotic activities of *Syzygium samarangense* fruits extract against human a549 lung cancer cell lines

The intention of this study was to check the anti-proliferative and apoptotic activity of *Syzygium samarangense* fruits methanolic extract against of a549 cell lines. The methanol extract at different concentrations were tested against a549 human lung cancer cell lines for cell viability or cytotoxicity by assay and the hallmark of the apoptosis was analyzed by DNA fragmentation method. The morphological changes resulted due to apoptosis were investigated by Propidium Iodide (PI) staining technique. The results showed that the tested extracts showed strong and decreased cell viability in a concentration-dependent manner. Values represented that the anti-proliferative activity was found with a minimum concentration of 21.86µg/ml. The presence of ladders of DNA fragments in the DNA

fragmentation assay indicates a biochemical hallmark of intrinsic apoptotic cell death. Altered cell morphology after treatment with the extract demonstrated that cells experienced apoptosis. (Mollika, 2013)

Chapter Three: Materials and Methods

3.1 Collection and Preparation of *Syzygium samarangense* extract

3.1.1 Collection of the *Syzygium samarangense* leaves

The leaves of *Syzygium samarangense* were collected from the Botanical Garden located at Mirpur - 1, Dhaka, Bangladesh.

3.1.2 Preparation of *Syzygium samarangense* extract

3.1.2.1 Drying of the collected leaves of *Syzygium samarangense*

The plant materials were washed with water properly to remove the adhering dirt. All unwanted plant parts were discarded. The leaves were then spread on large polythene bags and placed for shadow drying for about 1 week. The leaves were turned upside down after every 1 day for proper drying of both sides of the leaves.

3.1.2.2 Grinding and Storage of the Dried Sample

The dried leaves were ground to a coarse powder with the help of a high capacity mechanical grinder (Grinding Mill). This causes breakdown of plant parts into smaller pieces, thus exposing the internal cellular structure of the plant parts. This facilitates the penetration of solvents into the cells of the plant parts to extract the chemical constituents. Before grinding of the plant sample, the grinder was thoroughly cleaned to make sure that no contamination occurred by the remnant of the previously triturated materials.

After grinding, the powdered sample was kept in clean closed glass containers till extraction. The net weight of dry powder was 900gm.

3.1.2.3 Maceration of the Dried Powdered Sample

From the total amount of powder, gm powder was soaked in Petroleum Ether for the further processes of extraction.

The powder was soaked in 1 Litre of Petroleum Ether for 7 days. The preparation was kept in an amber colored bottle. The bottle was regularly shaken to facilitate the complete exhaustion of the chemical constituents into the solvent.

3.1.2.4 Filtration and Retrieval of the extract

After the completion of maceration process, the solution was filtered in three consecutive steps. At first, the filtration was done by using sterile cotton cloth, then by sterile cotton filter and lastly by No. 1 Whatman filter papers. Later on, the solvent was evaporated completely by Heidolph Rotary Evaporator. The yield was collected in a beaker and preserved in the refrigerator with the mouth sealed with plastic.

3.2 Standard Drug

Diazepam was used for this study purpose which was supplied from Square Pharmaceuticals Ltd.

3.3 Research Animal

For the research purpose, 30 *Swiss albino* mice were collected from ICDDRB. The average weight of the mice were 20-25 gm. Optimum environmental conditions were maintained to rear the mice. The conditions were 12-hours light/dark cycle, 55-65% relative humidity, and 24.0 ± 2.0°C temperature. Also, the mice were supplied with ample food-pellets supplied by Animal Research Facility, ICDDRB and filtered water.

3.4 Ethical Approval

Institutional Animal Ethical Committee approved the guidelines which were followed forcarrying out the study.

3.5 Pharmacological Study of Plant Extract

CNS Depressant activity was studied in mice model to determine the medicinal activity of *S. samarangense* leaf extract.

The CNS action of *Syzygium samarangense* leaves extract was observed by comparing with the standard Diazepam in the experimental rodents. CNS depressant activity was assessed by using two techniques. They were:

1. Open Field Test and
2. Hole Board Experiment

3.5.1 Method design of CNS Experiments

For both the experiments, 24 mice were selected randomly and then divided into 4 groups. Each group consisted of 6 mice and they were termed Group 1 to Group 4.

Group 1 – Control (Distilled Water)

Group 2 – Standard (Diazepam)

Group 3 – Petroleum Ether 100 mg/kg

Group 4 – Petroleum Ether 200 mg/kg

Before the experiment, the mice were weighed and marked accordingly. The dose of the sample and the standard drug were administered per body weight. A specific treatment was set for each group.

3.5.2 Preparation of standard and sample solution

For the preparation of Petroleum Ether extract solution at doses 100 mg/kg and 200 mg/kg per body weight of mice, the extract was weighed based on the weight of the experimented mice and sonicated in a unidirectional way by the addition of 3 ml of distilled water. A small amount of CMC was slowly added as a suspending agent for proper mixing. To stabilize the suspension, it was stirred adequately.

For the preparation of positive control group, Diazepam (1mg/kg) was taken and a 3 ml suspension was prepared.

Table 3.1: Test samples used in the estimation of CNS activity of *Syzygium samarangense* plant

Group	Treatment	Dose	Route of Administration
Group 1 (Control)	Distilled Water	10 ml/kg	Orally
Group 2 (Standard)	Diazepam	1 mg/kg	Orally
Group 3 (Extract)	SSPE	100 mg/kg	Orally
Group 4 (Extract)	SSPE	200 mg/kg	Orally

3.5.3 Open Field Test

Figure 3.1: Open Field Instrument

The Open Field test was performed according to Gupta using a cubic box measuring 1m x 1m x 1m. (Gupta et al, 1971)

The top of the cube was uncovered. The rodent was placed in the middle of the bottom surface of the box and its movements were recorded over the course of minutes to hours as it moved around and explored the arena.

The procedure for assessment of CNS depressant effect of the Pet Ether extract of leaves of *Syzygium samarangense* by Open Field test is described below:

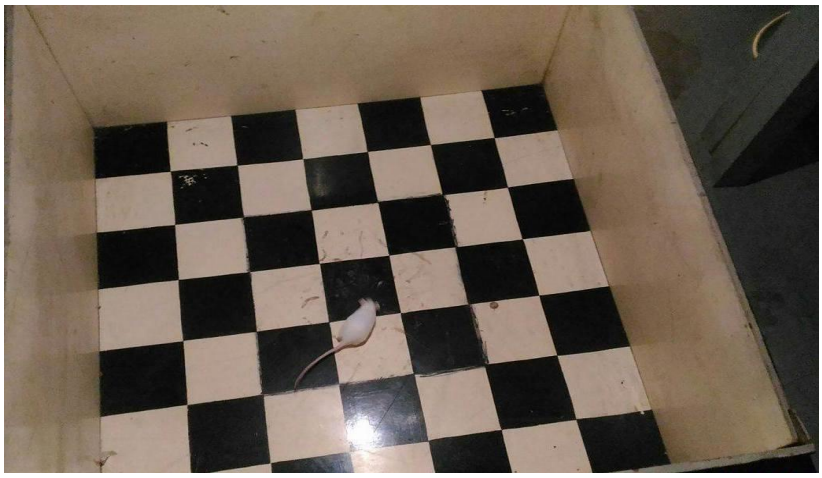

Figure 3.2: Open Field Experiment

- Mice were weighed and categorized into 4 groups of each consisting of 6 mice
- By a rodent-feeding needle, sample and standard solutions were administered per oral. This was done at 0 minute
- The number of squares visited by the mice at 0 minute was counted for 3 minutes
- The number of squares visited by the mice after 30 and 60 minutes were also counted for a period of 3 minutes each
- The data was recorded for calculation.

3.5.4 Hole Board Experiment

Figure 3.3: Hole Board Instrument

The Hole Board experiment was done following Takagi's method on a quadripedal holed board with an enclosure of 40 cm x 40 cm x 35 cm and the test chamber holes having the diameter of 3 cm each. (Takagi et al., 1971)

The rodent was placed in the center of the board and its movements were recorded over the course of minutes to hours as it moved around and explored the arena.

The procedure for assessment of CNS depressant effect of the Pet Ether extract of leaves of *Syzygium samarangense* by Hole Board experiment is described below:

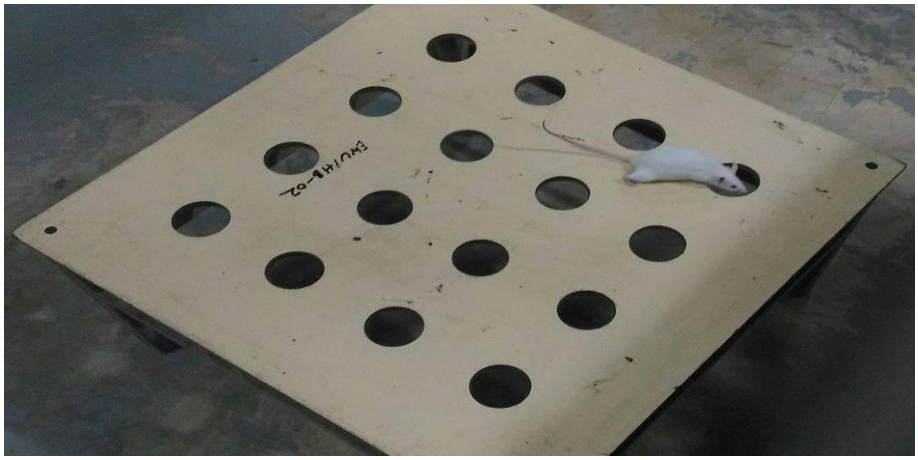

Figure 3.4: Hole Board Experiment

- Mice were weighed and categorized into 4 groups of each consisting of 6 mice.
- By a rodent-feeding needle, sample and standard solutions were administered per oral. This was done at 0 minute.
- The number of holes visited by the mice at 0 minute was counted for 3 minutes.
- The number of holes visited by the mice after 30 and 60 minutes were also counted for a period of 3 minutes each.
- The data was recorded for calculation.

Chapter Four: Results and Discussion

4.1 Results

4.1.1 Open Field Test

At doses 100 mg/kg and 200 mg/kg, experimental leaf extracts were administered to mice. As a result, the movements of the mice got reduced significantly ($p < 0.05/ 0.01/ 0.001$) in a dose-dependent manner. From Table 4.1.1, significant levels of decrease in movement of mice after 30 and 60 minutes of extract solution administration can be observed. The standard drug, Diazepam, also exhibited significant decrease in locomotion in the mice model after 30 and 60 minutes of administration respectively.

Table 4.1 Data of CNS Activity test of *Syzygium samarangense* extract by Open Field Test

Group	Treatment	Dose, Route	Number of Movements		
			0 min	30 min	60 min
Group – 1 (Control)	Distilled Water	10 ml/kg, p.o	137.5 ± 44.64	126 ± 32.78	159.67 ± 15.47
Group –2 (Standard)	Diazepam	1 mg/kg, p.o	137.5 ± 44.64	36.17 ± 26.09***	68.83 ± 39.40***
Group – 3 (Extract)	SSPE	100 mg/kg	68 ± 33.02*	51.33 ± 28.27***	10 ± 3.58***
Group – 4 (Extract)	SSPE	200 mg/kg	54.67 ± 20.11**	62 ± 31.00**	54.5 ± 25.00***

SSPE refers to *Syzygium samarangense* in Petroleum Ether extract. Values were expressed as Mean ± SD (n=6); One-Way Analysis of Variance (ANOVA) trailed by Dunnett's Test. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ were considered significant.

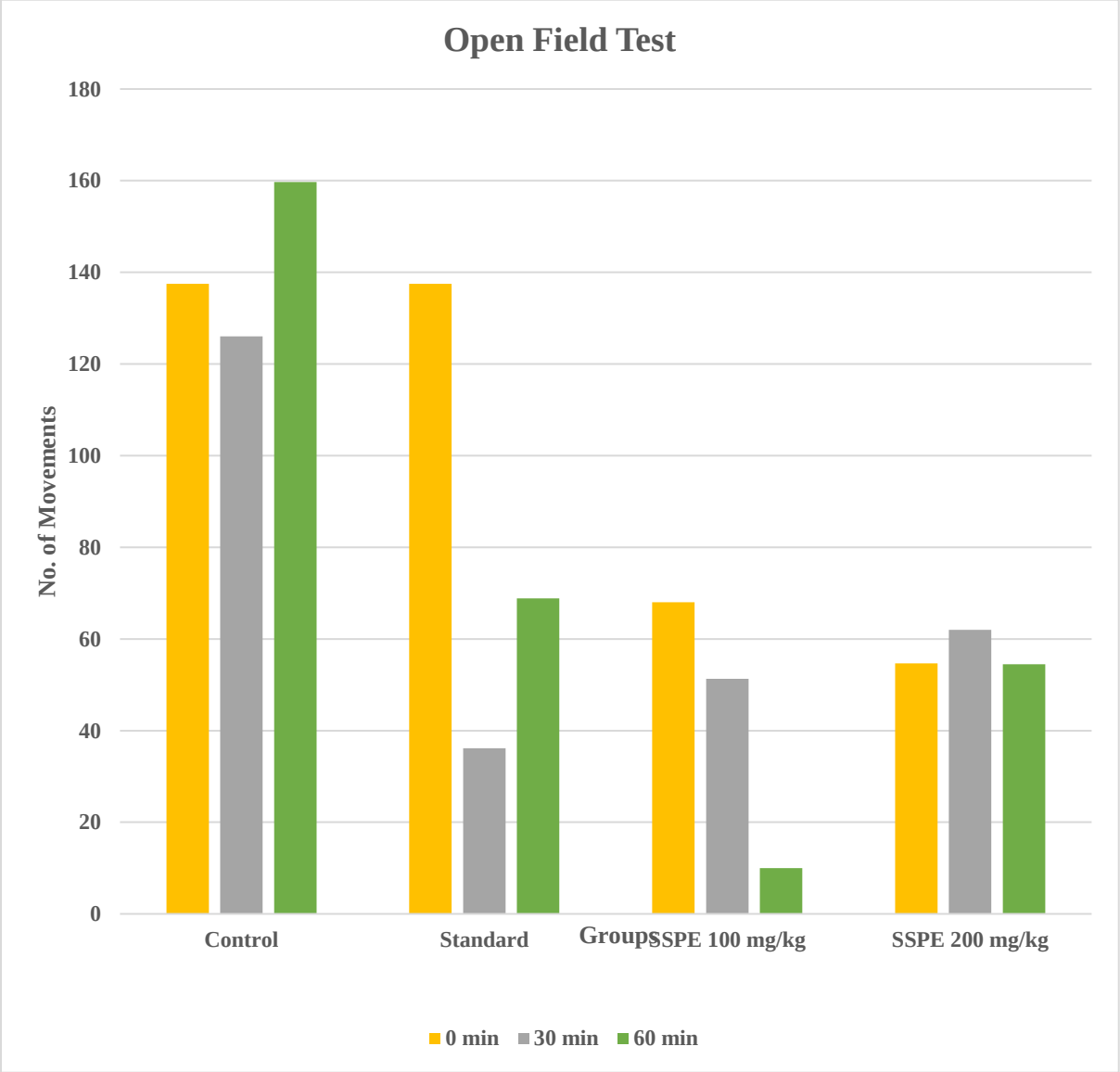

Figure 4.1: Graph of Open Field Test with Petroleum Ether extract of *Syzygium samarangense* leaves

4.1.2 Hole Board Experiment

At doses 100 mg/kg and 200 mg/kg, experimental leaf extracts were administered to mice. As a result, the movements of the mice got reduced significantly ($p < 0.05/ 0.01/ 0.001$) in a dose-dependent manner. From Table 4.1.2, significant levels of decrease in locomotion of mice after 30 and 60 minutes of extract solution administration can be observed. The standard drug, Diazepam, also exhibited significant decrease in locomotion in the mice model from after 30 and 60 minutes of administration respectively.

Table 4.2: Result of CNS Activity of *Syzygium samarangense* extracts by Hole Board Experiment

Group	Treatment	Dose	Number of Movements			
			-30 min	0 min	30 min	60 min
Group – 1 (Control)	Distilled Water	10 ml/kg	20.83 ± 7.03	23.67 ± 5.16	18.33 ± 2.80	19.17 ± 2.93
Group – 2 (Standard)	Diazepam	1 mg/kg	10.67 ± 3.56	8.00 ± 5.54***	7.50 ± 1.87***	4.00 ± 1.90***
Group – 3 (Extract)	SSPE	100 mg/kg	22.00 ± 4.90	13.00 ± 4.52*	9.83 ± 2.40**	5.83 ± 1.60***
Group – 4 (Extract)	SSPE	200 mg/kg	48.33 ± 8.69***	13.17 ± 3.19*	10.50 ± 5.90*	5.83 ± 1.60***

SSPE refers to Syzygium samarangense in Petroleum Ether. Values were expressed as Mean ± SD (n=6); One-Way Analysis of Variance (ANOVA) trailed by Dunnett's Test. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ were considered significant.

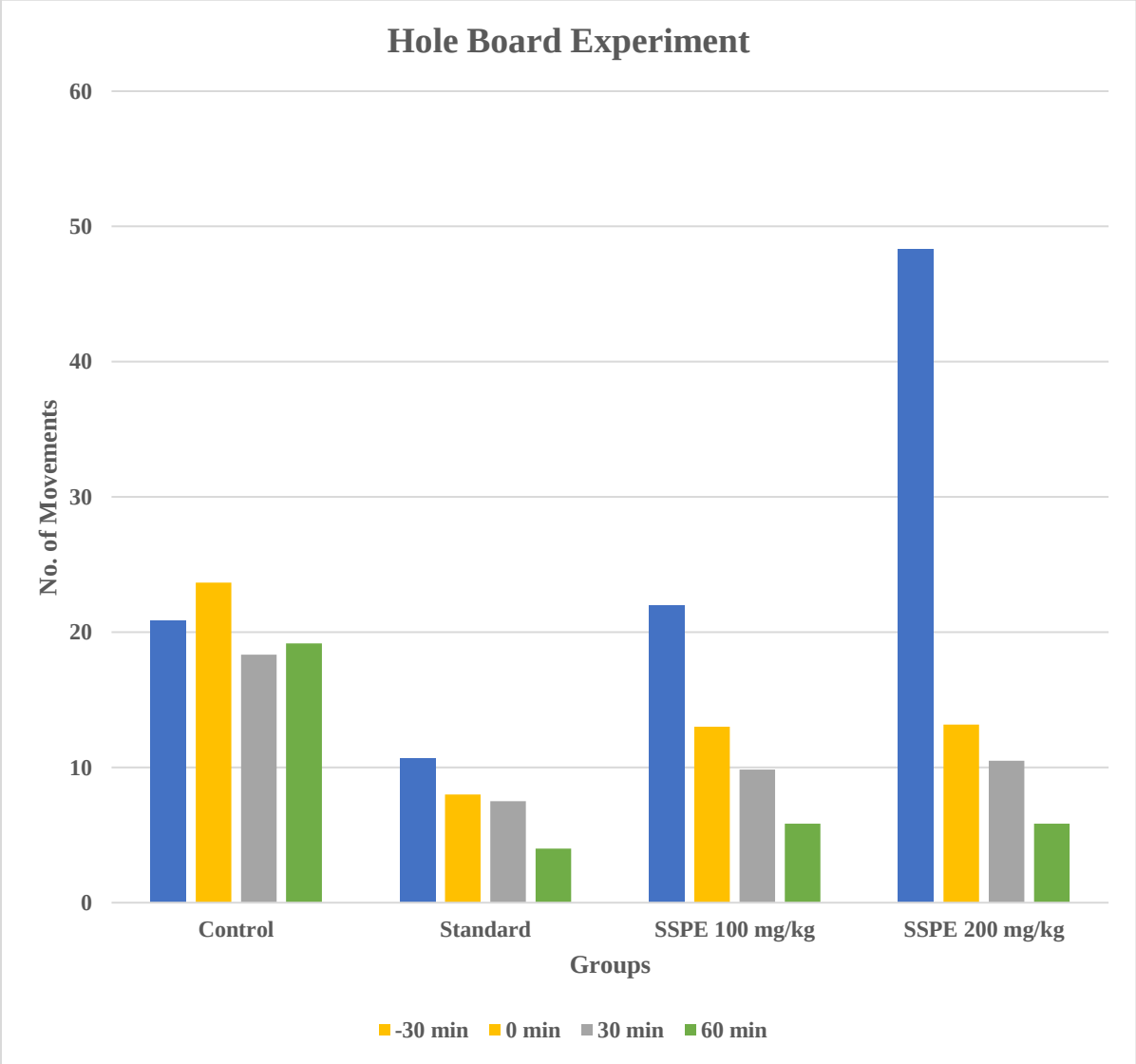

Figure 4.2: Graph of Hole Board Experiment with Petroleum Ether extract of *Syzygium samarangense* leaves

4.2 Discussion

These two experiments namely, Open field test and Hole board test were performed to evaluate the Central Nervous System (CNS) activity of Petroleum Ether extract of *Syzygium samarangense* plant leaves.

From the above tables, Table 4.1 and 4.2, it can be observed that the extract significantly decreased the locomotion in mice model after 30 and 60 minutes of observation respectively. Locomotion is a potential parameter for the assessment of both the CNS stimulatory or depressant effect of a plant extract. Increase in this activity indicates the CNS stimulatory effect and decrease in the same is indicative of CNS depressant activity. So, it can be said that the Petroleum Ether leaf extracts of *Syzygium samarangense* plant exhibits CNS depressant activity. We know that, the Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Various anxiolytic, muscle relaxant, sedative-hypnotic drugs elucidate their action through $GABA_A$, thus it is possible that extracts of *Syzygium samarangense* may act by potentiating GABA-ergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extract (Kolawole et al., 2007).

Some studies suggest that compounds like flavonoids, phenolic acids, cytotoxic chalcones, saponins and tannins are useful in many CNS disorders. Earlier investigation on phytoconstituents suggests that many flavonoids were found to be ligands for the $GABA_A$ receptors in the central nervous system; which led to the assumption that they can act as benzodiazepine-like molecules (Verma et al., 2010).

Phytochemical investigations of *Syzygium samarangense* show that the leaves are rich in tannins and flavonoids mostly (Peter et al., 2011) which may be responsible for its CNS depressant activity. Also, a study was conducted stating that the methanolic extract of the bark of *Syzygium samarangense* also possess a very potent CNS depressant action. (Mollika, 2013) So, perhaps, the common constituents between the two plant parts might be exerting the same CNS depression activity.

Chapter Five: Conclusion

Products derived from natural resources as plant extracts have always been used as traditional medicines around different parts of the world. Various pharmacological studies always reveal the potential medicinal properties of plants of our surroundings. Different data on the traditional uses of plants encourage the isolation of secondary metabolites that leads to new lead compounds. With the increasing demands of inventing new drugs, the pharmacological assay of natural plant resources play an important role in traditional drug discovery. The study of traditional medicinal plants is increasing in a significant rate with the view to invent and establish newer therapy lines. Petroleum Ether extract of *Syzygium samarangense* plant leaves was assessed for CNS activity. Upon observation of the results, it can be said that the extract produced significant ($p<0.001$) Central Nervous System (CNS) depressant effect at doses 100 mg/kg and 200 mg/kg compared to the control group. Thus, it can be concluded that the study served its purpose and further investigations should be carried out to isolate and identify more active compounds present in the plant that are responsible for pharmacological activity in the development of novel and safe drugs.

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