A Pharmacological Investigation on CNS Activity of Methanolic Extract of *Syzygium samarangense* Leaves

"This dissertation is submitted for the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy"

Submitted by

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DECLARATION BY THE CANDIDATE

I, Md. Mohibul Alam, hereby declare that this dissertation, entitled **"A Pharmacological Investigation on CNS Activity of Methanolic Extract of *Syzygium samarangense* Leaves"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy, is a genuine & authentic research work carried out by me. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled **"A Pharmacological Investigation on CNS Activity of Methanolic Extract of *Syzygium samarangense* Leaves"** is a research work done under my guidance and supervision by Md. Mohibul Alam (ID: 2014-1-70-006), in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled **"A Pharmacological Investigation on CNS Activity of Methanolic Extract of *Syzygium samarangense* Leaves"** is a bona fide research work done by Md. Mohibul Alam (ID: 2014-1-70-006), in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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DEDICATION

~ This research paper is dedicated to my beloved Parents

and to those who were selflessly kind to me ~

Abstract

Syzygium samarangense (Family: Myrtaceae) has been widely used as a traditional medicine for the cure of diarrhea, amenorrhea, diabetes, stomatitis, and many other ailments in various countries. Multiple researches have been conducted to identify its various chemical constituents and also several pharmacological activities of the components. The current study was performed to evaluate the CNS effects of Methanolic extracts of the leaves of *Syzygium samarangense* in *Swiss albino* mice model. The leaves were powdered and then soaked in Methanol to prepare the extract. Two doses: 100 mg/kg and 200 mg/kg body weight were selected to test the activity by using the Open Field Test and Hole Board Experiment. In case of both the experiments, significant ($p<0.001$) decrease in locomotor activity in the mice model was observed in a dose-dependent manner when compared with the standard drug, Diazepam. Thus, it can be said that this plant can be a potential candidate for the development of Depressant drugs acting on the Central Nervous System (CNS) in near future.

Keywords: *Syzygium samarangense*, Myrtaceae, CNS activity, Open field, Hole board

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List of Abbreviations

CMC	Carboxy Methyl Cellulose
CNS	Central Nervous System
GABA	Gamma Aminobutyric Acid
$GABA_A$	Gamma Aminobutyric Acid Type A
gm	Gram(s)
SSM	Methanolic Extract of *Syzygium samarangense*
mg	Milligram(s)
µg	Microgram(s)
min	Minute(s)
ml	Millilitre(s)
p.o.	Per Oral
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Medicinal Plants – An Inception

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. 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.

The earliest historical records of herbs are found from the Sumerian civilisation, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt describes over 850 plant medicines, while Dioscorides documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*, forming the basis of pharmacopoeias for some 1500 years.

Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes, the alkaloids, glycosides, polyphenols, and terpenes.

Medicinal plants are widely used to treat disease in non-industrialized societies, not least because they are far cheaper than modern medicines. And innumerable drugs manufactured these days have been originated from various medicinal plants.

Global sales from exported drugs and medicines by country in 2016 totaled the US $318.6 billion. Overall, the value of drugs and medicine exports were up by an average 0.4% for all exporting countries since 2012 when drugs and medicines shipments were valued at $317.3 billion. Year over year, there was a -1.5% decline from 2015 to 2016. Among continents, European countries accounted for the highest dollar value worth of drugs and medicine exports during 2016 with shipments amounting to $251.9 billion or 79% of the global total. In second place were North American exporters at 9.8% while 9.4% of worldwide drugs and medicine shipments originated from Asia. (Workman, 2017)

1.1.1 The Value of Medicinal Plants in Our Lives

Plants are fundamental to life. For millennia the plants, animals, rocks, and trees were the only pharmaceutical giants we had. Like all living things on Earth, every one of us is still a shareholder in Nature – the greatest pharmacy on Earth.

Plants are the most formidable chemists. They are constantly producing an arsenal of chemical compounds, in order to respond to different challenges and threats in their environment. They materialise chemical compounds that make them impervious to particular climatic conditions, certain microorganisms, bacteria, viruses, insects, numerous animals, including us.

We humans are still learning and re-learning how to harness the self-healing ability of plants, in order to enhance or rebalance the health of our own body, mind and spirit.

It is this ever expanding and evolving field of knowledge that inspires research and re-education. Throughout human history we have learned a lot from plants and we have continually endeavored to pass this knowledge on to the next generation. (Vilinac, 2017)

1.1.2 Long-established Uses of Medicinal Plants

- 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. (Jepson and Craig, 2007)
- 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. (Chantre and Lairon, 2002)
- Numerous clinical and animal studies document the efficacy of hawthorn as a cardiotonic. Cardiotonics 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. (Frishman et al., 2005)
- 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. (Langmead and Rampton, 2001)
- Adaptogenic herbs, such as ginseng, owe much of their activity to stimulation of pituitary and adrenal activity. (Gaffney et al., 2001)
- 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. (Gabay, 2002)
- 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. (Geller and Studee, 2005)
- Dark chocolate contains many of the same beneficial compounds, known as Catechins. (Engler and Chen, 2004)
- Fruit and vegetables rich in antioxidants are best as antioxidant supplements.
- Green tea, in its natural form or as a concentrated supplement.
- 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. (Taking Charge of Your Health & Wellbeing, 2017)

1.1.3 Historical Pieces of Evidence of Uses of Medicinal Plants

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

- **In 2500 BC**

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

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

- **In 1550 BC**

The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc. According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense.

- **In 800 BC**

In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to. Some of them were given the names after mythological characters from these epics; for instance, Elecampane (*Inula helenium* L. Asteraceae) was named in honor of Elena,

who was the centre of the Trojan War. As regards the plants from the genus *Artemisia*, which were believed to restore strength and protect health, their name was derived from the Greek word *artemis*, meaning "healthy."

- **In 500 BC**

Herodotus referred to castor oil plant, Orpheus to the fragrant hellebore and garlic, and Pythagoras to the sea onion (*Scilla maritima*), mustard, and cabbage.

- **In 459-370 BC**

The works of Hippocrates contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (*Centaurium umbellatum Gilib*) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents.

- **In 371-287 BC**

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

In 25 BC-50 AD

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

In 77 AD

In ancient history, the most prominent writer on plant drugs was Dioscorides, "the father of Pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army. Circa 77 AD he wrote the work "De Materia Medica." This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic *materia medica* until the late Middle Ages and the Renaissance. Of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic effect. In addition to the plant description, the names in other languages coupled with the localities where they occur or are grown are provided. The plants having a mild effect are dominant, but there are also references to those containing alkaloid or other matter with strong effect (fragrant hellebore, false hellebore, poppy, buttercup, jimson weed, henbane, deadly nightshade). Dioscorides' most appreciated domestic plants are as follows: willow, chamomile, garlic, onion, marshmallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore). Chamomile (*Matricaria recucita* L.), known under the name Chamaeleon, is used as an antiphlogistic to cure wounds, stings, burns, and ulcers, then for cleansing and rinsing the eyes, ears, nose, and mouth. Owing to its mild carminative action, it is particularly appropriate for usage with children. Dioscorides deemed that it had abortive action, on which he wrote, "The flower, root, and the entire plant accelerate menstruation, the release of the embryo, and the discharge of urine and stone, provided that they are used in the form of an infusion and baths." This untrue belief was later embraced by both the Romans and the Arabs; hence the Latin name *Matricaria*, derived from two words: *mater* denoting "mother," i.e. matrix, denoting 'uterus'. Dioscorides differentiated between a number of species from the genus *Mentha*, which were grown and used to relieve headache and stomach ache. The bulbs of sea onion and parsley were utilized as diuretics, oak bark was used for gynaecological purposes, while white willow was used as an antipyretic. As maintained by Dioscorides, *Scillae bulbus* was also applied as an expectorant, cardiac

stimulant, and antihydrotic. It is worth underscoring that Dioscorides pointed to the possibility of forgery of drugs, both the domestic ones such as opium forged by a yellow poppy (*Glaucium flavum*) milk sap and poppy, and the more expensive oriental drugs, transported by the Arab merchants from the Far East, such as iris, calamus, caradmomum, incense, etc.

- **In 23 AD-79**

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

- **In 131 AD-200**

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

- **In the seventh century AD**

The Slavic people used *Rosmarinus officinalis, Ocimum basilicum, Iris germanica*, and *Mentha viridis* in cosmetics, *Alium sativum* as a remedy and *Veratrum album, Cucumis sativus, Urtica dioica, Achilea millefolium, Artemisia maritime* L., *Lavandula officinalis, Sambuci flos* against several injuries insects, i.e. lice, fleas, moths, mosquitos, and spiders and *Aconitum napellus* as a poison in hunting.

In the Middle Ages

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

In 742 – 814 AD

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

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

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

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

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

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

While the old peoples used medicinal plants primarily as simple pharmaceutical forms—infusions, decoctions and macerations—in the Middle Ages, and in particular between 16th and 18th centuries, the demand for compound drugs was increasing. The compound drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug the theriac was produced from a number of medicinal plants, rare animals, and minerals, it was highly valued and sold expensively.

In 18th century

In his work *Species Plantarium* (1753), Linnaeus (1707-1788) provided a brief description and classification of the species described until then. The species were described and named without taking into consideration whether some of them had previously been described somewhere. For the naming, a polynomial system was employed where the first word denoted the genus while the remaining polynomial phrase explained other features of the plant (e.g. the willow Clusius was named *Salix pumila angustifolia antera*). Linnaeus altered the naming system into a binominal one. The name of each species consisted of the genus name, with an initial capital letter, and the species name, with an initial small letter.

Early 19th century

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

In late 19th and early 20th centuries

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

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

In present days

Almost all pharmacopoeias in the world—Ph Eur 6, USP XXXI, BP 2007-proscribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine). For the sake of adequate and successfully applied therapy, knowledge of the precise diagnosis of the illness as well as of medicinal plants, i.e. the pharmacological effect of their components is essential. Plant drugs and phyto-preparations, most commonly with defined active components, verified action and, sometimes, therapeutic efficiency, are applied as therapeutic means. In the major European producer and consumer of herbal preparations—Germany, rational phytotherapy is employed, based on applications of preparations whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs.

With the new Law on Drugs and Medical Devices dated September 2007 and enacted in the Republic of Macedonia, dry or sometimes fresh parts of medicinal plants (herbal substances) may be used for preparation of herbal drugs, herbal processed products, and traditional herbal drugs. Herbal substances may also be utilized for manufacture of

homeopathic drugs, which are stipulated in the current law, too. In the Republic of Macedonia herbal preparations are dispensed without a medical prescription, as "over the counter" (OTC) preparations. (Petrovska, 2012)

1.1.4 Opportunities of Drug Development from Natural Products

Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Nature has been a source of therapeutic agents and a significant number of modern drugs have been developed from natural sources, many based on their use in traditional medicine. Over the last century, a remarkable number of top selling drugs have been derived from natural products (Vincristine from *Catharanthus roseus*, morphine from *Papaver somniferum*, quinine and quinidine from *Cinchona spp)*. Nowadays, approximately 40% of the modern drugs have been developed from natural source. More precisely, 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60-80% of antibacterial and anti-cancer drugs were from natural origin. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancer had natural origin. In 2001, eight (simvastatin, pravastatin, amoxicillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporine and paclitaxel) Of the 30 top - selling medicines were natural products or their derivatives. (Cragg and Newman, 2013)

In light of all these facts, natural product drug discovery process failed to generate little respect. As drug discovery has emerged into a highly competitive era in which the quality of chemical collections and the time taken from assay to drug development are crucial factors in the success of a company, combinatorial chemistry has become the darling of the pharmaceutical industry, bringing with it the promise of new level of chemical diversity. But this adoption of new strategy by the pharmaceutical companies gained little momentum.

Biotechnology companies working in the fields of combinatorial biosynthesis, genetic engineering and met genomic approaches to identify novel natural product lead molecules have met with limited success. These disappointments have led the pharmaceutical industry to consider whether natural product chemical diversity can or

will continue to generate valuable templates for drug development. (Katiyar *et al.,* 2012)

Natural products offer a potentially infinite source of chemical diversity unparalleled to any synthetic chemical collection or combinatorial chemistry approach. In addition to that, these potent natural product compounds can have astounding chemical structures that can lead to unexpected, alternative medicinal chemistry programs based on important biological targets.

In the past few years, new natural products with a wide variety of chemical classes have been reported in the scientific literatures. Moreover, a total of 19 natural product based drugs were approved for marketing worldwide in between the year 2005 to April 2010, among which 7 being classified as natural products, 10 semi-synthetic natural products and 2 natural product derived drugs. (Decorte, 2016)

1.1.5 Traditional Uses of Medicinal Plants in Bangladesh

The rural population of Bangladesh has traditionally depended on folk medicinal healers for treatment of their ailments. These healers use medicinal plants as their primary source of medicinal formulations. Rural patients are more dependent on traditional or folk medicinal healers for treatment of urinary tract infections (UTIs) and sexually transmitted diseases (STDs) for a number of reasons including lack of access to modern medical facilities, clinging to traditional approaches, and finally hesitancy to relate this form of illnesses in front of unknown doctors. Since the traditional healer usually resides in the same village or in an adjoining area, the patient is more comfortable in seeking them for treatment. An ethnomedicinal survey was conducted among the traditional healers of various ethnic groups and in several regions of the country to obtain information on medicinal plants used to treat UTIs and STDs. Interviews were conducted in the local dialect or language about plant parts used, ailments treated, formulations, and dosages.

- Thirty-one species were reported by traditional healers as being used for UTIs, including leucorrhea, frequent or infrequent urination, cloudy urination and burning sensations during urination.
- Ten species were reported to be used against STDs like syphilis and gonorrhea. (Hossain et al., 2010)

Folk medicinal practitioners (Kavirajes) of Bangladesh are consulted for treatment of various ailments by a substantial segment of the rural and urban population of the country. The major element that distinguishes the folk medicinal practitioners from other forms of medical practices is their use of simple formulations of medicinal plants for treatment. The plant(s) used by the Kavirajes for treatment of any specific ailment vary considerably in the various parts of the country, and such differences exist even among Kavirajes of adjoining villages.

An ethnomedicinal survey was conducted among the Kavirajes of two villages, namely Babla and Terbaria, which lies in Tangail district in the central portion of the country. Each village had one practicing Kaviraj. Leaves constituted the major plant part used, being used 48.7% of the time. From the number of plants used, it appeared that gastrointestinal tract disorders formed the major complaint of the patients with 5 plants used for treatment of various complaints like constipation, diarrhea, indigestion, and loss of appetite. Four plants each were used for treatment of pain, and skin disorders (scabies, eczema), and as blood purifier. Four plants were used for treatment of diseases in cattle.

Among other ailments treated by the Kavirajes were tuberculosis, sexual disorders, urinary problems, infections, fever, hepatic disorders, kidney problems, pneumonia, stomach stones, diabetes, swellings, debility, helminthiasis, hypertension, vitamin C deficiency, tumor, and poisoning. One plant was used to maintain the body in good health and so served as a preventive measure instead of a curative effect. Since a number of allopathic medicines have been derived from medicinal plants, the plants reported in the survey can, following scientific inquiry, form novel sources of newer drugs. (Mollik et al., 2009)

1.1.6 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. (Ghani, 1998)

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 (*Vinca rosea*) 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 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).

1.1.7 Storage of Herbal Medicine

The herbs available in most stores come in several different forms: teas, syrups, oils, liquid extracts, tinctures, and dry extracts (pills or capsules). Teas can made from dried herbs left to soak for a few minutes in hot water, or by boiling herbs in water and then straining the liquid. Syrups, made from concentrated extracts and added to sweet-tasting preparations, are often used for sore throats and coughs. Oils are extracted from plants and often used as rubs for massage, either by themselves or as part of an ointment or cream. Tinctures and liquid extracts are made of active herbal ingredients dissolved in a liquid (usually water, alcohol, or glycerol). Tinctures are typically a 1:5 or 1:10 concentration, meaning that one part of the herb is prepared with 5 to 10 parts (by weight) of the liquid. Liquid extracts are more concentrated than tinctures and are typically a 1:1 concentration. A dry extract form is the most concentrated form of an herbal product (typically 2:1 to 8:1) and is sold as a tablet, capsule, or lozenge.

No organization or agency regulates the manufacture or certifies the labeling of herbal preparations. This means you cannot be sure that the amount of the herb contained in the bottle, or even from dose to dose, is the same as what is stated on the label. Some herbal preparations are standardized, meaning that the preparation is guaranteed to contain a specific amount of the active ingredients of the herb. However, it is still important to ask companies making standardized herbal products about their product's guarantee. It is important to talk to your doctor or an expert in herbal medicine about the recommended doses of any herbal products. (Ernst, 2011)

1.1.8 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 keep pleasant taste, color and odor.
- To maintain stability.

(Ghani, 1998)

1.1.9 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.

(Ghani, 1998)

1.1.10 Necessity of Herbal Drug Research in 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% people suffering from 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)

1.2 Plant Profile

1.2.1 Botanical Name

Syzygium samarangense (Blume) Merr. & L. M. Perry

1.2.2 Synonyms

- *Eugenia javanica*
- *Eugenia samarangensis*
- *Jambosa javanica*
- *Jambosa samarangensis*
- *Myrtus javanica*
- *Myrtus samrangensis*

(Theplantlist.org, 2017)

1.2.3 Taxonomic Hierarchy of *Syzygium samarangense*

Domain: Eukaryota

Kingdom: Planate

Division: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Species: *Syzygium samarangense*

(Itis.gov, 2017)

1.2.4. Vernacular Names

Table 1.1: List of Vernacular Names of *Syzygium samarangense*

Bangla	Jamrul
English	Wax apple, Love apple, Java apple, Mountain apple, Cloud apple, Water apple
Indonesian	Jambu air
Jamaican	Jamaican apple, Otaheti apple
Malay	Water guava
Malayalam	Chambekka
Philippines	Makopa
Sri Lankan	Jumbu
Taiwan	Belfruit
Thai	Chomphu
Vietnamese	Man

(Peter et al., 2011)

1.2.5. Plant Morphology

1.2.5.1 Stem

The tree, 16 to 50 ft. (5-15 m) tall, has a short trunk 10 to 12 inches (25-30 cm) thick, and open, wide spreading crown, and pinkish-gray, flaking bark.

Figure 1.1: Stem of *Syzygium samarangense*

1.2.5.2 Leaves

The opposite leaves are nearly sessile, elliptic-oblong, rounded or slightly cordate at the base; yellowish to dark bluish-green; 4 to 10 inches (10-25 cm) long and 2 to 4 3/4 in (5-12 cm) wide; very aromatic when crushed.

Figure 1.2: Leaves of *Syzygium samarangense*

1.2.5.3 Flowers

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.

Figure 1.3: Flowers of *Syzygium samarangense*

1.2.5.4 Fruits

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, sub-acid and very bland in flavor.

Figure 1.4: Fruits of *Syzygium samarangense*

1.2.5.5 Seeds

There may be 1 or 2 somewhat rounded seeds 3/16 to 5/16 inches (0.5-0.8 cm) wide, or none.


Figure 1.5: Seeds of *Syzygium samarangense*

1.2.6 Biology

Shoot growth proceeds in flushes which are more or less synchronous, depending on the climate. The juvenile period lasts for 3-7 years. Bearing of clonal trees starts after 3-5 years.

There are definite flowering seasons, often two, sometimes three in a year, but the timing varies from year to year. *Syzygium samarangense* commonly flowers early or late in the dry season; the flowers appear to be self-compatible and the fruit ripens 30-40 days after anthesis. (Orwa et al., 2009)

1.2.7 Ecology

The trees are at home in fairly moist tropical lowlands up to 1200 m elevation. *Syzygium samarangense* grows best in areas with a fairly long dry season. This does not mean that this species is drought resistant. The species require a sufficient water supply and are often planted along streams or ponds. (Orwa et al., 2009)

1.2.8 Traditional Claims of *Syzygium samarangense* Plant Parts

- **Leaves:** It is used as astringent, to treat fever and halt diarrhea. Powdered leaves are used for cracked tongues. Juice of leaves is used in baths and lotion. It is also used in diabetes, cough and headaches.
- **Fruits:** It is used in diabetes, stomatitis aphthosa, diuretic, emmenagogue, abortifacient and febrifuge. Decoction of fruits is used in fever.
- **Root-bark:** The root bark decoction is used in dysentery and amenorrhea and also used as abortifacient.
- **Root:** It is used as diuretic and is given to alleviate edema. Malayans use powdered dried root preparations for itching.
- **Bark:** Juice of bark is used to treat wounds and the bark is used as astringent in mouthwash preparations for the treatment of thrush.
- **Stem:** Decoction of stem is used to treat diarrhea

(Peter et al., 2011)

1.3 Aim and Objective of the study

The purpose of this study was to evaluate pharmacological effect of Methanolic extract of leaves of *Syzygium samarangense* plant on the Central Nervous System. The aim was to-

- Assess general locomotor levels and anxiety in *Swiss albino* mice model by Open Field Test
- Analyze neophilia, anxiety, and stress responses in *Swiss albino* mice model by Hole Board Experiment

Traditionally, this plant has been used to treat a variety of diseases leading to the fact that it must have some potential medicinal properties. Thus, the principle objective of this study was to explore the possibilities of deriving medicinal agents from this plant for the treatment of various diseases.

CHAPTER 2

LITERATURE REVIEW

2.1 Pharmacological Studies

2.1.1 Immunomodulatory activity

A pharmacological investigation was performed with the sixteen flavonoids isolated from acetone extract leaves of *Syzygium samarangense* for immunomodulatory effects. Human Peripheral Blood Mononuclear Cells (PBMC) were used as test models and cell proliferation was assessed by ^{3}H-thymidine uptake. Four of the flavonoids exhibited suppression of PBMC cells through cytotoxic effect in a dose-dependent manner. The inhibitory mechanisms might have involved the impairment of IL-2 and IFN-γ production. (Kuo, Yang and Lin, 2004)

2.1.2 Anticholinesterase activity

In a study, it was found that a Dihydrochalcone flavonoid, 2', 4'-dihydroxy-6'-methoxy-3', 5'-dimethyl-dihydrochalcone, isolated from *Syzygium samarangense* has been potent in demonstrating anti-cholinesterase activity. The chemical showed 98.5% inhibitory activity against acetylcholinesterase and 68.0% inhibitory activity against butyrylcholinesterase at concentrations 0.25mM and 0.20mM respectively. (Amor, Villaseñor and Nawaz, 2005)

2.1.3 Antioxidant activity

A study investigated antioxidant activity of seven plants from Myrtaceae family which also included *S. samarangense*. The fruits underwent various chemical assay and in the DPPH assay, *Syzygium samarangense* fruit showed a moderate antioxidant effect in IC_{50} value being 76.8 µg/mL. (Reynertson, Basile and Kennelly, 2005)

2.1.4 Anti-diarrheal activity

A study was performed to justify the use of *Syzygium samarangense* in hypermotility condition of the gut. Dose-dependent studies were performed using the hexane extract of the plant. It was found that the different doses exhibit relaxation of frequent

contractions in rabbit jejunum. Several flavonoids were isolated among which 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone proved to be the most potent in exerting spasmolytic effect. The study indicated that the presence of compounds with spasmolytic and calcium antagonist activity might be responsible for the use of this plant in diarrhea. (Ghayur et al, 2006)

2.1.5 Anti-microbial activity

Research was carried out to evaluate the antimicrobial properties of *Syzygium samarangense* fruit against certain bacterial and fungal strains using disc diffusion method. The petroleum ether and Methanolic extracts of S. samarangense fruit exhibited significant inhibition. They were also effective against both Gram Positive and Gram Negative bacteria. (Ratnam and Raju, 2008)

2.1.6 Antihyperglycemic activity

Antihyperglycemic activity was investigated in *Syzygium samarangense* and two other native plants of Bangladesh. Methanolic extracts of the leaves of the plants were used for the study. The extracts were administered in mice at different doses one-hour prior to glucose administration and blood glucose levels of the experimental mice were measured 2 hours later using the glucose oxidase method. The Methanolic leaf extract of *S. samarangense* showed to be the most potent among the three, being able to reduce the serum glucose level by 59.3% compared to the standard, Glibenclamide that reduced only 57.3% with respect to their controls. Thus, Methanolic extract of leaves of *S. samarangense* exhibited potent Antihyperglycemic activity. (Shahreen et al, 2012)

2.1.7 Cytokine Inhibitory activity

In order to observe pharmacological activity of Aurentiacin chalcone isolated from *Syzygium samarangense*, a pharmacological study was performed in an inflammatory animal model. Intraperitoneal injection of Aurentiacin suppressed the release of pro-

inflammatory cytokines. The result suggested that Aurentiacin showed anti-inflammatory activity related to the inhibition of NF-κB activation. (Kim et al., 2012)

2.1.8 Analgesic activity

A study was done to assess the analgesic effect of Methanolic extract of *Syzygium samarangense* leaves. The evaluation was done by using acetic acid induced writhing and formalin tests. The extract significantly ($p<0.05$) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose-dependent manner. (Mollika et al, 2014)

2.1.9 Anti-inflammatory activity

Pharmacological study was performed to evaluate the anti-inflammatory effect of Methanolic extract of *Syzygium samarangense* leaves. The study was done using carrageenan induced hind paw edema in mice model. The extract displayed a significant ($p<0.05$) inhibition of carrageenan induced paw edema after 4 hrs in a dose dependent manner. (Mollika et al, 2014)

2.1.10 Hepatoprotective activity

In an investigation, fruits of *Syzygium samarangense* and two other plants were used on alcohol-induced liver injury in mice. Chronic treatment with alcohol showed elevation of various parameters in the mice that led to damage in the hepatocytes. *Syzygium samarangense* fruit normalized various biochemical parameters. This indicated that *S. samarangense* might possess hepatoprotective effect that could cure liver injuries due to alcohol. (Zhang et al., 2016)

2.2 Chemical Constituents

Investigators have found several chemical constituents upon performing various assays of different *Syzygium samarangense* plant parts.

- **Leaves** contain
  - Lupeol (triterpenoid);
  - Betulin (triterpenoid);
  - Epi-betulinicacid (triterpenoid);
  - 2, 4-dihydroxy-6-methoxy-3-methylchalcone;
  - 2-hydroxy-4, 6-dimethoxy-3-methylchalcone;
  - 2, 4-dihydroxy-6-methoxy-3, 5-dimethylchalcone;
  - 2, 4-dihydroxy-6-methoxy-3-methyldihydrochalcone;
  - 7-hydroxy-5-methoxy-6, 8-dimethylflavanone;
  - 2-hydroxy-4, 6-dimethoxy-3-methyldihydrochalcone;
  - 2, 4-dihydroxy-6-methoxy-3, 5-dimethyldihydrochalcone;
  - Sitosterol;
  - Alpha-carotene and
  - Beta-carotene.

- **Leaf oil** is largely composed of
  - Monoterpenes (30% Sesquiterpenes, 9% Caryophyllene).

- **Aerial parts** contain
  - Ursolic acid,
  - Jacoumaric acid
  - Arjunolic acid,
  - Mearnsitrin,
  - 2-C-Methyl-5-*O*-Galloylmyricetin-3-*O*-α-l-Rhamnopyranoside,
  - Desmethoxymatteucinol,
  - 4, 6-Dihydroxy-2-Methoxy-3, 5-Dimethylchalcone,
  - Methyl 3-*epi*-betulinate,
  - Oleanolic acid,
  - Desmethoxymatteucinol,

- 5-*O*-Methyl-4-desmethoxymatteucinol,
- Oleanic acid.

✓ Quercetin glycosides are also present in this plant which include

- Reynoutrin,
- Hyperin,
- Myricitrin,
- Quercitrin,
- Quercetin and
- Guaijaverin.

✓ It also contains Flavanone - (*S*)-pinocembrin, and Phenolic acids- Gallic acid and Ellagic acid.

(Peter et al., 2011)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Collection and Preparation of the Plant 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 720gm (approximately).

3.1.2.3 Maceration of the Dried Powdered Sample

From the total amount of powder, 240gm powder was soaked in Methanol for the further processes of extraction.

The powder was soaked in 1 Litre of Methanol 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.

Figure 3.1: Heidolph Rotary Evaporator

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 ICDDR, B. 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, ICDDR, B and filtered water.

Figure 3.2: *Swiss albino* mice

3.4 Ethical Approval

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

3.5 Pharmacological Study of Plant Extract

CNS 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 activity was assessed by using two techniques. They were:

- Open Field Test and
- Hole Board Experiment

3.5.1 Method Design of CNS Depressant 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 – Methanol 100 mg/kg

Group 4 – Methanol 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 Methanolic 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 (1 mg/kg) was taken and a 3 ml suspension was prepared.

Table 3.1: Test samples used in the estimation of CNS Depressant 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)	SSM	100 mg/kg	Orally
Group 4 (Extract)	SSM	200 mg/kg	Orally

3.5.3 Open Field Test

Gupta's open field method (Gupta et al., 1971) was followed to carry out Open field test. The box was half square meter as well as divided into squares each. On the other hand the box was black and white colour like a chess board. The apparatus had a wall which was 40cm in height. For 3 minutes, each square was counted which was visited by mice. Also, during the study period, several results were taken on 0, 30, and 60 minutes.

Figure 3.3: Open Field Test Apparatus

The flow chart of the procedure for assessment of CNS effect of the Methanolic extract of leaves of *Syzygium samarangense* by Open Field test is shown below:

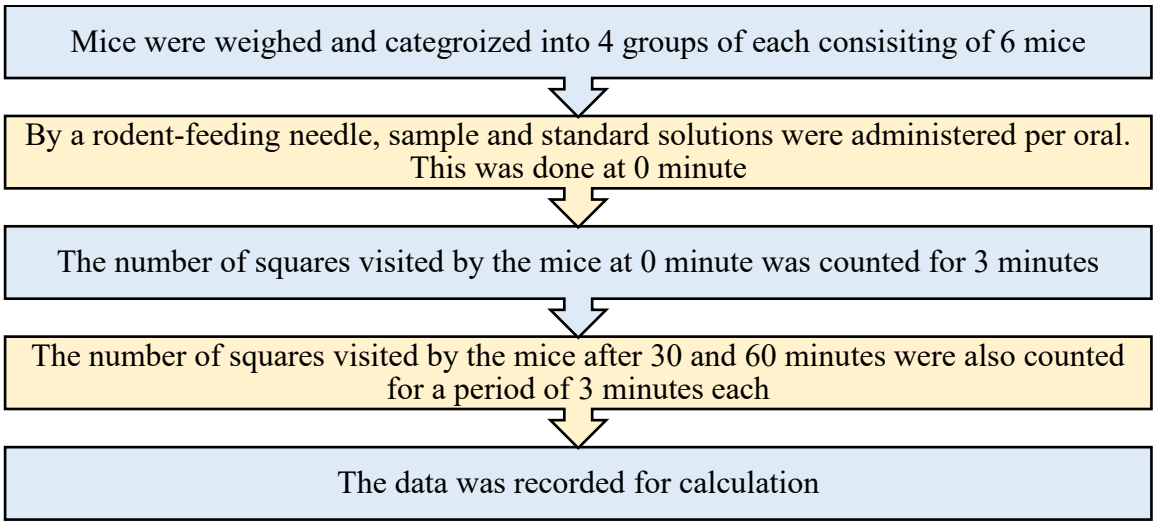

Figure 3.4: Flow chart showing the process of Open Field test for the determination of CNS activity of Methanolic extract of *Syzygium samarangense* leaves in *Swiss albino* mice model (Gupta et al., 1971)

3.5.4 Hole Board Experiment

The main purpose of Hole Board Experiment is to analyze the locomotor and exploratory effects of the extract by using the Hole board on mice. Takagi's method (Takagi et al., 1971) was followed to examine the test. The box where the hole-board test was tested, a size of 30 x 20 x 14 cm was measured. In the middle of the box there were 16 holes cut in the wooden base, each having a diameter of 3cm.

Figure 3.5: Hole Board Experiment Apparatus

The flow chart of the procedure for assessment of CNS effect of the Methanolic extract of leaves of *Syzygium samarangense* by Hole Board experiment is shown below:

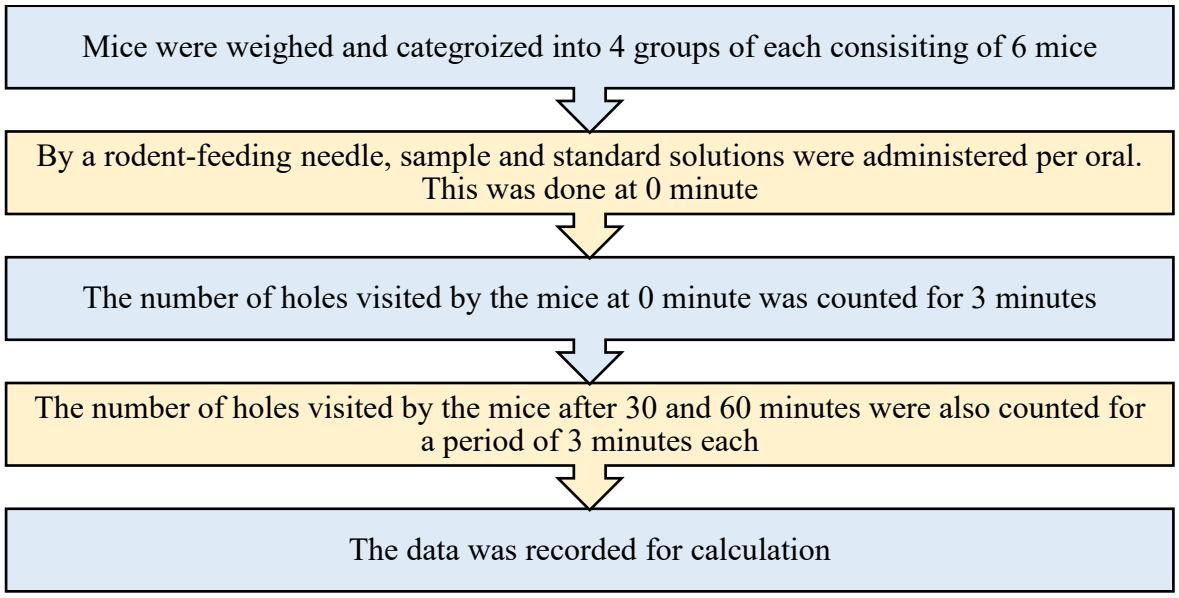

Figure 3.6: Flow chart showing the process of Hole Board Experiment for the determination of CNS activity of Methanolic extract of *Syzygium samarangense* leaves in *Swiss albino* mice model (Takagi et al., 1971)

CHAPTER 4

RESULTS

&

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, 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.

Table 4.1: Data of Open Field Test to determine CNS Activity of Methanolic extract of *Syzygium samarangense* leaves in *Swiss albino* mice model

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)	SSM	100 mg/kg, p.o	48.83 ± 43.57**	40.33 ± 27.91***	36.83 ± 24.62***
Group- 4 (Extract)	SSM	200 mg/kg, p.o	71 ± 66.66*	33.5 ± 19.01***	13.83 ± 6.85***

SSM refers to *Syzygium samarangense* in Methanol. 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.

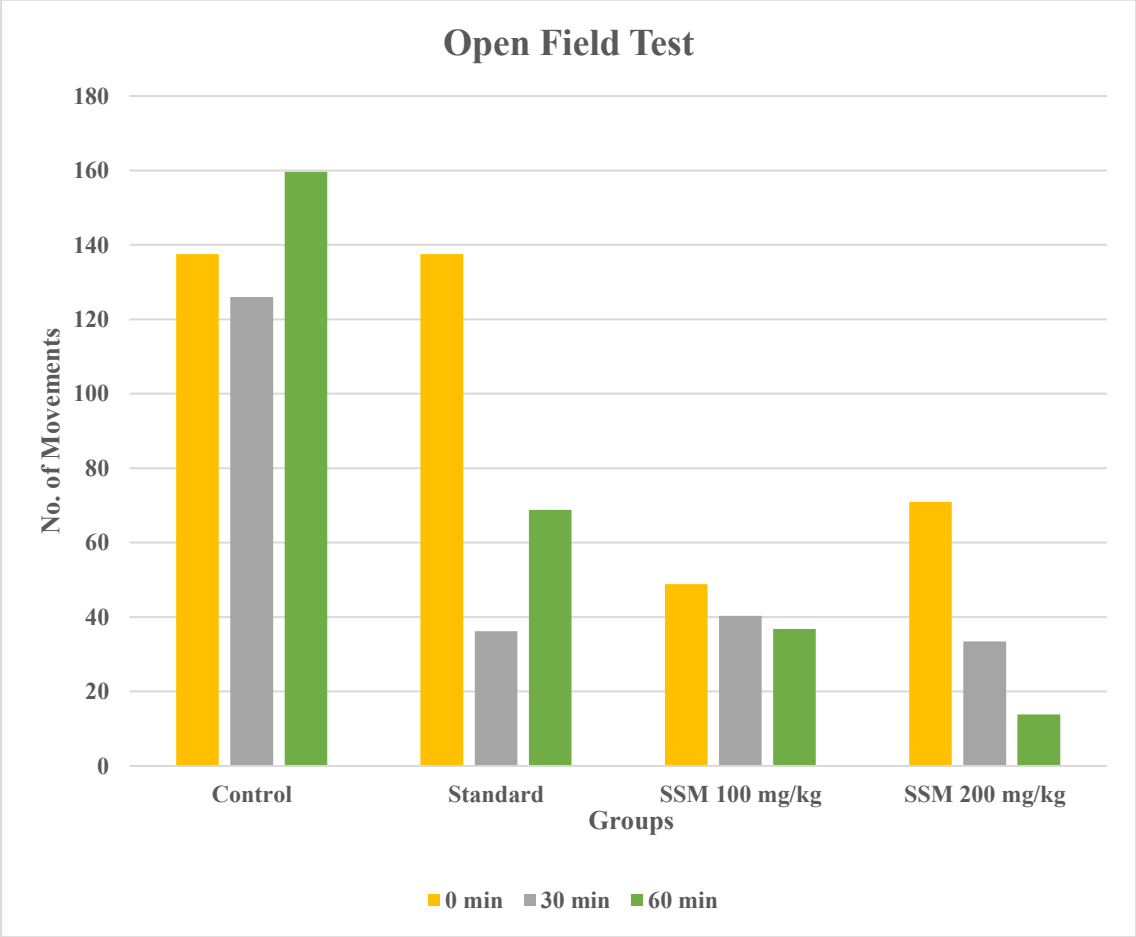

Figure 4.1: Graph for Open-Field Test for the determination of CNS activity of Methanolic extract of *Syzygium samarangense* leaves in *Swiss albino* mice model

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.

Table 4.2: Data of Hole Board Experiment to determine CNS Activity of Methanolic extract of *Syzygium samarangense* leaves in *Swiss albino* mice model

Group	Treatment	Dose, Route	Number of Movements			
			-30 min	0 min	30 min	60 min
Group – 1 (Control)	Distilled Water	10 ml/kg, p.o	20.83 ± 7.03	23.67 ± 5.16	18.33 ± 2.80	19.17 ± 2.93
Group – 2 (Standard)	Diazepam	1 mg/kg, p.o	10.67 ± 3.56	8.00 ± 5.54***	7.50 ± 1.87***	4.00 ± 1.90***
Group – 3 (Extract)	SSM	100 mg/kg, p.o	16.67 ± 3.83	10.67 ± 4.23**	7.83 ± 4.07***	6.17 ± 2.86***
Group- 4 (Extract)	SSM	200 mg/kg, p.o	36.83 ± 12.35*	16.33 ± 6.02	6.17 ± 2.32***	2.83 ± 1.94***

SSM refers to *Syzygium samarangense* in Methanol. 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.

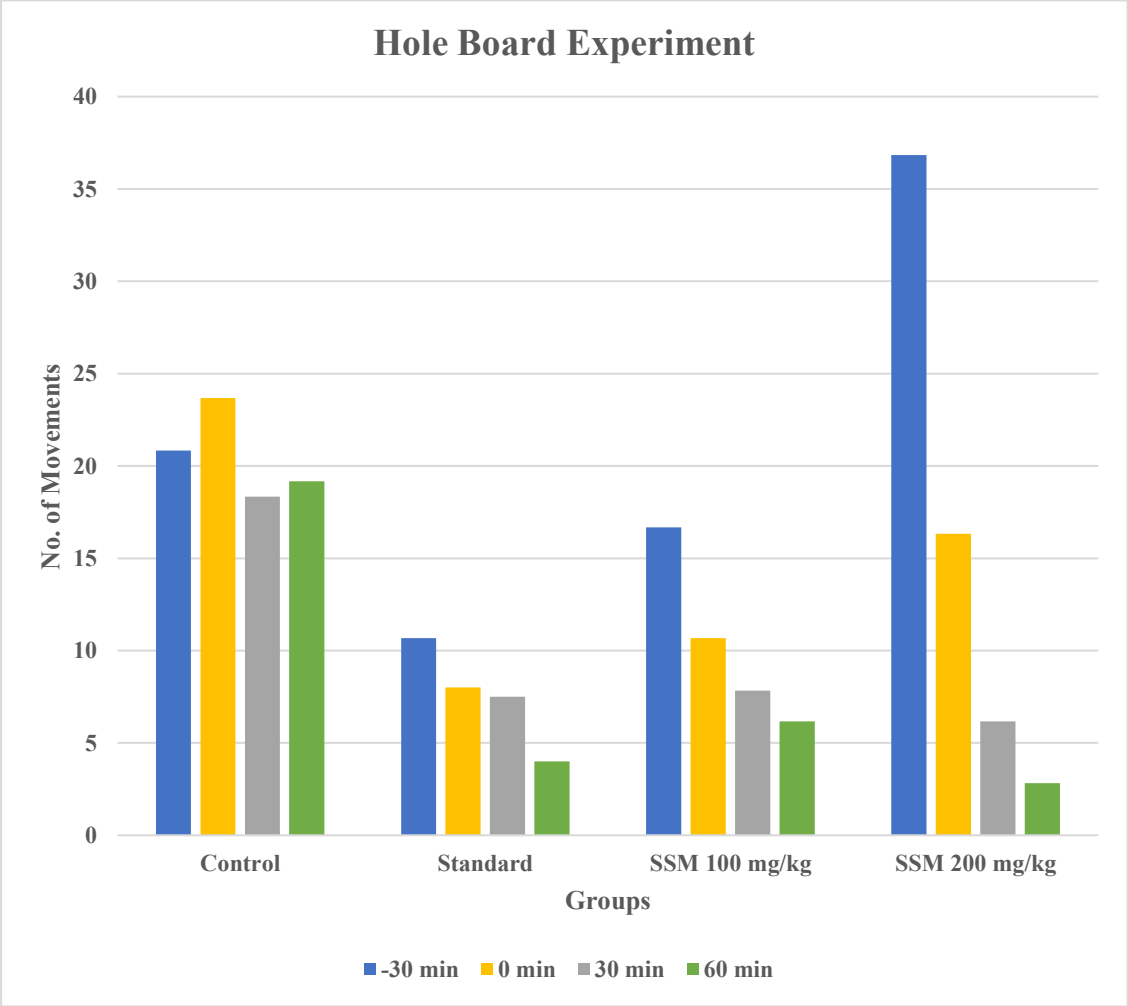

Figure 4.2: Graph for Hole Board Experiment for the determination of CNS activity of Methanolic extract of *Syzygium samarangense* leaves in *Swiss albino* mice model

4.2 Discussion

The studies were performed to evaluate the CNS depressant activity of Methanolic extract of *Syzygium samarangense* leaves. From Table 4.1 and 4.2, we can observe that the extract showed a significant decrease in locomotion in mice model after 30 and 60 minutes of observation.

Locomotor activity is considered as a potential parameter for assessing CNS stimulatory or depressant effect of a plant extract. Increase in locomotor activity indicates CNS stimulatory effect and decrease in the same is indicative of CNS depressant activity. Thus, it can be said that Methanolic leaf extracts of *Syzygium samarangense* exhibit CNS depressant activity.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs exert their action through $GABA_A$, therefore 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, saponins and tannins are useful in many CNS disorders. Earlier investigation on phytoconstituents suggests that many flavonoids and neuroactive steroids 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 possesses 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 5

CONCLUSION

Traditional medicines are mostly utilized by means of the natural products isolated from natural resources such as plant extracts. Pharmacological studies always reveal the potential medicinal properties of plants of our surroundings. Ethnobotanical data on the traditional uses of plants encourage the isolation of secondary metabolites leading to new lead compounds. With the increasing demands of inventing new drugs, the pharmacological assay of natural plant resources plays a non-parallel role in traditional drug discovery. Day by day the study of traditional medicinal plants is increasing in significant rate with the view to invention and establishment of new therapy line. With that in mind, the Methanolic extract of *Syzygium samarangense* leaves was assessed for CNS activity. Upon observation of the results, it can be said that the extract produced significant ($p<0.001$) CNS depressant effect at doses 100 mg/kg and 200 mg/kg compared to the control group. Thus, it can be concluded by stating 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.

CHAPTER 6

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