Neuropharmacological Investigation of Fruits of *Aphanamixis polystachya* (Meliaceae) on Mice Model

This thesis paper is submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Certificate

This is to certify that "Neuropharmacological Investigation of Fruits of *Aphanamixis polystachya* (Meliaceae)" on mice model. This research work done by Sakib Uddin Ahmed (ID# 2008-1-70-011), under the guidance and supervision of Farjana Khatun, Lecturer, Department of Pharmacy, East West university, Dhaka.

Sufia Islam 17.07.2012

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This is to certify that "Neuropharmacological Investigation of Fruits of *Aphanamixis polystachya* (Meliaceae)" on mice model and submitted to the department of pharmacy, East West University, Dhaka, in partial fulfillment of the requirements for the degree of bachelor of pharmacy (B.Phrm) was carried out by Sakib Uddin Ahmed (ID#2008-1-70-011) under guidance of supervision Farjana Khatun and that no part of this study has been submitted for any other degree.

Farjana Khatun

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ABSTRACT

Plants and other natural substances have been used as the rich source of medicine. The list of drugs obtained from plant source is fairly extensive. *Aphanamixis polystachya* plant is reported in ethnopharmacological as medicinal plant.

The neuropharmacological activity was evaluated by Hole cross, Hole board and Elevated plus-maze test and data were analyzed by using SPSS analytical method.

For hole cross experiment *n*-hexane and methanol soluble fraction at dose of 200 and 400 mg/kg body weight, the decreased the locomotor activity of the experimented animals. Among these sample, *n*-hexane fraction of *A. polystachya* fruits at dose of 200 mg/kg body weight mostly decrease the frequency of moment of the mice through the hole. It was also observed that methanol fraction at dose of 400 mg/kg body weight highly decreased the locomotor activity of the test animals. In the hole board experiment ethyl acetate at dose of 200 and methanol at dose of 400 mg/kg body weight showed to some extent anxiolytic activity with compare to positive control grope. On the other hand *n*-hexane fractions and methanol at dose of 200 mg/kg body weight did not showed any anxiolytic activity. On the other hand for elevated plus maze test, methanol and ethyl acetate soluble fraction may have anxiolytic activity. Whereas, *n*-hexane at dose of 400 mg/kg body weight did not have any anxiolytic activity.

Keywords: *Aphanamixis polystachya*, CNS depressant, Anxiolytic activity, Soxhlet, Hole Cross, Hole Board, Elevated Plus Maze.

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Chapter-1
Introduction

1.1 Medication system in primeval age

Nobody knows exactly where medicinal plants were used for the first time. Surely the search of some remedy was something that occurred simultaneously in all the cultures, fruit of the desire of the man to heal, for magical-religion reasons or because of some preparation that provide a great temporary happiness. Most times the discoveries were simply results of the search of new types of foods. But the ancestors had to verify the new species were foods or poison by test. Knowledge on the medicinal plants, before the birth of writing was transmitted orally. It is known the first written text on the use of medicinal plants is about 4000 years old appears in a Sumerian clay tablet that recorded plant remedies for various illnesses in 2200 B.C. The Egyptians were the first formally recognized civilizations to practice medicine in a systematic and well-documented manner. Herbal remedies and surgical treatments were widely used. They used antiseptic to aid which was the willow leaves and bark. They used spice powders and soaked ribbons in mummification. Imhotep was practicing these around 2725 B.C. As evident from the Papyrus Ebers (Written about 1500 BC), they possessed a good knowledge of the medicinal properties of hundred of plants. Many of the present day important plant drugs like henbane (Hyoscyamus spp.), mandrake (Mandragora officinarum), opium (latex of the mature but unripe fruits of Papaver somniferum), pomegranate (fruits of Punica granatum), castor oil (oil of Ricinus communis seed), aloe (juice of leaves of Aloe spp.), onion (Allium cepa), hemlock (Conium spp.), cannabis (Cannabis sativa), senna (Cassia senna), and many other were in common use in Egypt about 4500 years ago. As far as records go, it appears that Babylonians (about 3000 BC) were aware of a large number of medicinal plants and their properties. The Chinese and Indian cultures also had extensive lists of plants useful for healing. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the *Rig Veda* (4500-1600 BC). It supplies various information of the medicinal use of plants in the Indian subcontinent (Hill, AF., 1972).

The *Susruta Samhita*, probably written before 1000 BC, deals with details of surgery and therapeutics, while the comprehensive Indian Herbal, *Charaka Samhita*, written about the same period, deals mainly with medicinal agents and cites more than 500 medicinal plants with their medicinal properties and uses.(Hill, AF., 1972). *Charaka Samhita is the first book of Ayurveda*

that has description of plant medicines used in the *Ayurvedic* system and were described about 1200 BC with a list of 127 plants (Obianwu, 1984). The Pun-tsao contains thousands of herbal cures attributed to Shen-nung, (2000B.C) China's legendary that contains medicinal effects of 365 plants. The earliest Chinese Pharmacopoeia, the Pen Tsao, appeared around 1122 BC. This authoritative work describes the use of Chaulmoogra oil to treat leprosy. It also recorded the medicinal uses of *Ephedra* species for the first time. Jivaka was the most celebrated doctor in India during the Buddha's time (563-483 BC). He removed all animal drugs from Ayurveda and introduced Mogha system of medicine (Ghani, A., 2003).

The beginning of the Renaissance saw a revival of herbalism, the identification of medicinally useful plants. This coupled with the invention of the printing press in 1450 ushered in the Age of Herbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses of plants, but also included much misinformation and superstition. The Doctrine of Signatures, for example, held that the medicinal use of plants could be ascertained by recognizing features of the plant that corresponded to human anatomy. The practice of medicine using medicinal plants flourished most during the Greek civilization when historical personalities like Hippocrates (born in 460 BC) and Theophrastus (born in 370 BC) practiced herbal medicine. The material medica of the great Greek physician Hippocrates (460-370 BC) consists of some 300-400 medicinal plants which included opium, mint, rosemary, sage and verbena. The far ranging scientific work of Aristotle (384-322 BC), a Greek philosopher, included an effort to c The encyclopedic work of Dioscorides (1st Century AD), De Materia Medica (Published in 78 AD), was the forerunner of all modern pharmacopoeias and an authoritative text on botanical medicine. This work featured about 600 medicinal plants. Two of the 37 volumes of books written by Pliny De Elder (23-70 AD) were devoted to medical botany and these included a large number of medicinal plants. Galen (131-200 AD), wrote about 500 volumes of books describing hundreds of recipes and formulations containing a large number of medicinal plants and animal products. (Claus, E.P *et al*., 1965). This doctrine, as expatiate by Galen, formed the basis of both allopathic and homeopathic systems of medicines practiced today and found the properties of the various medicinal herbs known at that time (Sofowora, A., 1982).

1.2 Medicinal plants

Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals or can produce therapeutic action called medicinal plant. It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites, like alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, resins, lactones, quinones, volatile oils, etc and contain minerals and vitamins, possess medicinal properties (Sofowora, A., 1982).

Accordingly, World Health Organization (WHO) has formulated a definition of medicinal plants in the following way: "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs"(Sofowora, A., 1982).

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal plants" (Ghani, A., 2003).
The history of use of medicinal plants by human beings to treat diverse ailments goes back to thousands of years ago. Today Medicinal plants constitute an important natural wealth of a country**.** They also play a significant role in providing primary health care services to the rural people (Sofowara, A., 1982; Hill, A.F., 1989).

Now the advent of modern or allopathic medicine has somehow diminished the role of medicinal plants in favor of synthetic drugs, even a number of modern drug discoveries have been based on medicinal plants used by indigenous people (Balick, J.M. and P.A. Cox, 1996). In recent years, because of the costs as well as serious side-effects of a number of modern drugs, attention has turned back to medicinal plants as a source for discovery of newer drugs with less cost and side effects. It has been reported that about 64% of the total world population is using traditional medicine to satisfy their health-care needs (Cotton, C.M., 1996).

1.3 Medicinal plants used in traditional medicine

The active principles in medicinal plants are chemical compounds known as secondary plant. Products which are found from plants, plant parts and plant products of all descriptions. Medicinal properties are invariably used as principal components or ingredients of various traditional medicines. The number of with medicinal properties included in the *Materia Medica* of traditional medicine in this subcontinent at present stands at about 2000 (Chopra *et al*., 1958). More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. This number of the indigenous medicinal plants is in the increase with the discovery and introduction of newer plants every day. In the traditional systems at the present time almost every plant and herb growing in the country has ascribed to it some medicinal virtues and is used either as principal therapeutic agent or as necessary associate (excipient) in medicinal preparations to increase the potency of the principal ingredients and probably stability of the preparations (Ghani, A., 2003).

More than 250 of such medicinal plants are now in common use in the preparation of traditional medicine in Bangladesh, which include plants like *Rauvolfia serpentine (*for treating high blood pressure, instantly, insomnia),*Terminalia arjuna (*for treating heart diseases*), Andrographis paniculata (*for the cure of fever and liver diseases*), Allium sativum (*for reducing blood cholesterol*), Coccinea indica (*for management of diabetes*), Abroma augusta (*for curingurogenital and female diseases*), Catharanthus roseus (*for the treatment diabetes*), Centella asiatica* (for treating diarrhea and dysentry*), Bacopa monniera (*for increasing longevity of life and as tonic of brain*)* and many others. It is estimated that more than one thousand metric tons of medical plants are annually required by the industries involved in the manufacture of traditional medicine in Bangladesh (Mian & Ghani., 1990).

1.4 Contributions of medicinal plants to modern medicine

Medicine is the art and science of the diagnosis and treatment of disease or any drug or remedy. The World Health Organization defines drugs as “Any substance used in a pharmaceutical

product that is intended to modify or explore physiological system or pathological states for the benefit of the recipient" (Korolkovas, A., 1980).

In other, medicine refers to a preparation or compound containing one or more drugs or therapeutic agents, which is used in the treatment, cure and mitigation of various diseases and external injuries of man and other animals. The preparation may also contain substances other than the drugs. The drugs within the preparation are the active therapeutic agents that cure the disease or heal the wound or injury. Plants have been providing the human being with the basic necessities of life, that is, food, fuel and shelter, from the very beginning of their existence, and for their continued living and sustenance on this earth. In addition, the medicinal plants, the ones that possessed some various medicinal properties, have been serving them as the major sources of therapeutic agents for maintenance of their health. These medicinal plants were used by the early human beings, as are done now, in variety of forms, such as in the entire form, and as powders, pastes, juices, infusions and decoctions for the treatment of their diseases and ailments. These various converted forms of the medicinal plants may thus be very conveniently and genuinely called medicinal preparations or medicaments. This way, the medicinal plants formed an integral part of the health management practices and constituted imported items of medicines used in the treatment of diseases from the very early days human civilizations. And in the course of their uses as medicinal agents, the medicinal plants have contributed substantially to the gradual development of medicines to their present state (Korolkovas, A., 1980).

Medicines that are used today are not definitely the same ones as those that were used in the ancient times or even in the recent past. Modification, improvement, sophistication and newer discoveries are continuously changing the type, quality, presentation and concept of medicinal preparations. As therapeutic uses of plants continued with the progress of civilization and development of human knowledge, scientists endeavored to isolate different chemical constituents from plants, put them in biological and pharmacological tests and thus have been able to identify and isolate therapeutically active compounds, which have been used to prepare modern medicines. In course of time, their synthetic analogues have also been prepared. In this way, ancient uses of *Datura* plants have led to the isolation of hyoscine, hyoscyamine, atropine and tigloidine, *Cinchona* bark to quinine and quinidine, *Rauvolfia serpentina* to reserpine and

rescinnamine, *Digitalis purpurea* to digitoxin and digoxin, Opium to morphine and codeine, *Ergot* to ergotamine and ergometrine, *Senna* to sennosides, *Catharanthus roseus to vinblastine and vincristine*- mention a few (Korolkovas, A., 1980).

Billions of dollars are spent for developing a new drug every year, but little is spent to know their exact pattern of use, and how much devastation it is causing at the user level. Isolation of the natural analgesic drug morphine from Opium, the latex of *Papaver somniferum* capsules, in 1804 is probably the first most important example of natural drugs which plants have directly contributed to modern medicine. Isolation of other important plant derived drugs of modern medicine rapidly followed and many useful drugs have since been discovered and introduced into modern medicine. Drugs like strychnine from *Strychnos nuxvomica (*1817*),* emetine from *Cephaelis ipecacuanha* (1817), caffeine from *Camellia sinensis* (1819), quinie from *Cinchona spp.* (1820) and colchinine from *Colchicum autumnale* (1820) constitute some example of such early drugs (Ghani, A., 2003). The list of the plant derived medicinal substances occurring in modern medicine is very long now. About 100 such drugs of defined structures are in common use today throughout the world and about half of them are accepted as useful drugs in the industrialized counties. It is estimated that more than 25% (currently the figure is claimed to be 36%) of all prescription drugs used in the industrialized countries contain active principles that are still extracted from plants (Farnsworth &Morris., 1976).

These include drugs like atropine, colchicines, deserpidine, digoxin, L-dopa, emetine, ephedrine, ergometrine, ergotamine, hyoscine, papaverinr, hyoscyamine, lanatosides, lobeline, morphine, narcotine, ouabain, papain, physostigmine, picrotoxin, pilocarpine, pseudo-ephedrine,quinidine, quinine, rescinnamine, reserpine, sennosides, sparteine, strophanthin, strychnine, theophylline, theobromine, vinblastine, vincristine, etc. Other plant derived drugs which are used widely but not generally in the western medicine include anabasine, andrographolide, arecoline, berberine, brucine, cannabinol, caphaeline, cocaine, curcumin, glycyrrhizin, hesperidine, hydrastine, nicotine, palmitine, quercetin, rutin, santonin, vincamine, yohimbine, etc. In addition to those, there are other plant derived chemical substances of known structures that are used as drugs or necessary components of many modern medicinal preparations. These include camphor, capaicin, cucalyptol, menthol, minor cardiac glycosides, various volatile oils, etc. These are only

a few examples of a vast number of drugs that are derived from plants (Farnsworth &Morris., 1976).

The importance of medicinal plants becomes more patent at the present time in developing countries. It is estimated that 40% people are depended on plants in China 70% & 80% people in Pakistan. In technology advanced as the United States it is estimated that 60% of population use medicinal plants habitually to fight certain ailments. In Japan there are official medicines (Farnsworth &Morris., 1976).

1.5 The medicinal plants contribution in the new world

Just before of modern medicine: At the early of modern medicine the Muslim physicians were done a great job. The Arabian Muslim physicians, like Al-Razi and Ibn Sina (9^{th} to 12^{th} century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use. Al Razi's important books are: Qitab-al-Mansuri, Al-Hawai, Qitab-al-Muluki, Qitab-al-Judari-wal-Hasabah, Maan La Yahoduruho Tibb etc. The famous medical book, Al-Kanun, of Ibn Sina was the prescribed book of medicine in the schools of western medicine for several centuries (Mian & Ghani., 1990).

The use of medicinal plants in Europe in the 13^{th} and 14^{th} centuries was based on the Doctrine of Signatures or Similar developed by Paracelsus (1490-1541 AD), a swiss alchemist and physician. (Murray, 1995**).** The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca (*Erythroxylum* species) and tobacco (*Nicotiana tabacum*) was common in these countries in the 14^{th} and 15^{th} centuries. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the *Rig Veda* (4500-1600 BC). It supplies various information of the medicinal use of plants in the Indian subcontinent (Hill, 1972). Medicinal plants used by the Australian aborigines many centuries ago tremendously enriched the stock of medicinal plants of the world. The current list of the medicinal plants growing around the world includes more than a thousand items (Sofowora, A., 1982).

Modern prescription drugs: To make prescriptions easily understandable by the patients, Paracelsus (1493 AD) started to use German instead of traditional Latin language used in medicine. His book On Diseases of Miners was very important at that time. Nuremburg Pharmacopoeia was published in 1546. First London Pharmacopoeia published in 1618. Later on its name became the British Pharmacopoeia. Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally isolated from plants (Hill, A., 1972).

1.6 Family profile

The plants belong to the family Meliaceae, closely related to the mahogany family is a flowering plant family of mostly trees and shrubs (and a few herbaceous plants, mangroves) in the order Sapindales. The family includes about 50 genera and 550 species, with a pantropical distribution. The tree belongs to Meliaceae family is erect with slender or spreading branches. It has irregular or systematical crowns and can reach 30-90ft in height. They are characterised by alternate, usually pinnate leaves with 5 to 7 leaflets and without stipules and by syncarp, apparently bisexual (but actually mostly cryptically unisexual) flowers borne in panicles, cymes, spikes, or clusters. The flowers are small, white or pale-yellow. Most species are evergreen, but some are deciduous, either in the dry season or in winter. The fruits are oval, oval-oblong or nearly round and borne in cluster of 2 to 30. The fruit skin is grayish-yellow to pale brownish or pink, leathery, thin or thick and may contain milky latex. The fruits contain 5 or more segment of aromatic, translucent, juicy flesh with sweet to acid taste. Lansium is commonly propagated from

seeds. The seedlings are generally fairly uniform. They can also be vegetatively propagated by grafting (Pennington *et al*., 1975).

1.6.1 Species belong to this family

Table 1.1: Tree belongs to the family Meliaceae.

Plant name	Scientific name	Source
Neem tree	*Azadirachta indica*	India
Crabwood Tree	*Carapa procera*	South America and Africa
Cedrela	*Cedrela odorata*	Central and South America
Bead Tree	*Melia azedarach*	North America, Queensland, India and southern China
Senegal Mahogany	*Khaya senegalensis*	Tropical Africa
Mahogany	*Swietenia* species	Tropical Americas

Trees of the genus *Aphanamixis, Swietenia* and *Entandophragma,* commonly called mahogany, and of the genus *Cedrela* are economically important timber trees. The pithraj (genus *Aphanamixis*) and the neem tree (genus *Azadirachta*), grown throughout the Old World tropics, notably in India and Southeast Asia, is a source of timber and medicinal constitutes. (Pennington *et al*., 1975)

1.6.2 Selected species of *Aphanamixispolystachya*

Table1.2: Species of *Aphanamixis polystachya.*

A. polystachya	*A. pedicellata*	*A.perrottetiana*
A. amboensis	*A. pinatubensis*	*A.apoensis*
A. borneensis	*A. polillensis*	*A. polystachya*
A. pulgarensis	*A. rohituka*	*A. coriacea*
A. cucullata	*A. rubiginosa*	*A. schlechteri*

1.7 Morphology of *Aphanamixis polystachya*

Large canopy tree up to 25 m high, rarely to 35 m. Bole cylindrical or markedly fluted up to 100 cm diagram; often crooked or straight (bole up to c. 15 m long); buttresses present (buttresses 1-4 m high); spines absent; aerial roots aerial roots absent; stilt roots stilt roots absent; Bark brownish red or pale brown, rough, scaly or flaky; Subrhytidome (under-bark) red (bright red); less than 25 mm thick (5-6 mm thick); bark blaze consisting of one layer; strongly aromatic; pleasant; outer blaze red, markings absent, fibrous; inner blaze red, markings absent, fibrous; bark exudates (sap) present, white or milky, not readily flowing (spotty), color not changing on exposure to air, sticky; terminal buds not enclosed by leaves(Chatterjee *et al*., 1970).

1.8 Botanical feature of *Aphanamixis polystachya*

Leaves: Leaves spaced along branches, spiral leaves occurring singly at a node and arranged spirally up the branch let, a leaf made up from two or more leaflets; petiole present, not winged, attached to base of leaf blade, swollen (at base and inhabited by ants); leaves pinnate (un-branched with more than three leaflets); leaves with a terminal leaflet (the number of leaflets odd - imparipinnate), broadest below middle, 9.5-25.0 cm, 5.5-8.0 cm, leaflets opposite, asymmetric, terminal developing leaflet buds straight; venation pinnate, secondary veins open, prominent, intramarginal veins absent; leaves lower surface green, upper surface green, indumentum (hairs) absent; absent; domatia absent; stipules absent (Chandrasekharan *et al*., 1970).

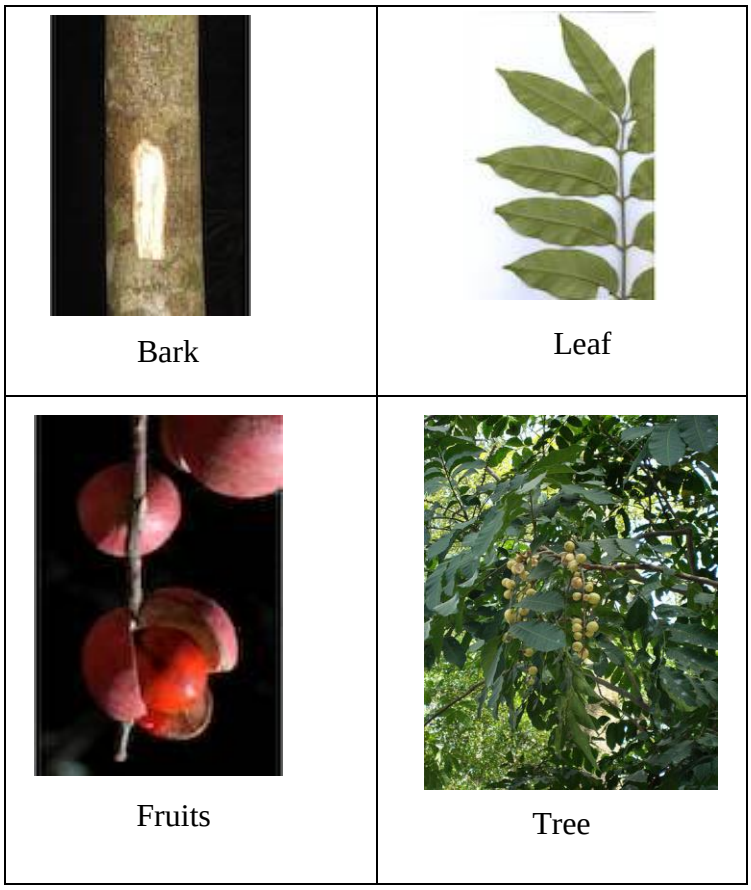

Figure 1.1: Different parts of Aphanamixis *polystachya*

Flowers: Inflorescence axillaries (sweetly aromatic), flowers on an unbranched axis, cones absent; flowers unisexual or bisexual, unisexual with male and female flowers on different plants, stalked (shortly), flowers with many planes of symmetry, 3.0-5.0 (-7.0) mm long, diameter small (up to10 mm diam.) (4-9 mm diam.); perianth present, with distinct sepals and petals whorls, inner perianth pale yellow or cream-coloured (sometimes tinged with red); 3, some or partly joined; stamens 3-8, present, joined (to form a staminal tube), at base joined to the perianth; ovary superior, carpals joined (Chandrasekharan *et al*., 1970).

Fruits: In frutescence arranged on un branched axis, fruit 20.0-40.0 mm long, yellow when young or pale red, not spiny, slightly fleshy, simple, dehiscent, capsule; seeds 1-3, much more than 10 mm long (17-20 mm long), not winged, narrow (longer than wide), seed 1-10 mm diam. (c. 6 mm diam.) (Chandrasekharan *et al*., 1970).

1.9 Botanical classification of *Aphanamixis polystachya*

Table 1.3: Botanical classification of *Aphanamixis polystachya*

Kingdom	Plantae
Phylum	Angiosperm
Class	Eudicots
Sub Class	Rosidae
Order	Sapindales
Family	Meliaceae
Genus	*Aphanamixis*
Species	*Aphanamixis polystachya*

1.10 Synonyms

Aglaia aphanamixis, Aglaia aphanamixis, Aglaia beddomei, Aglaia cochinchinensis, Aglaia janowskyi Aglaia polystachya, Alliaria cuneata, Amoora amboinensis, Amoora aphanamixis, Amoora aphanamixis, Amoora beddomei, Amoora cumingiana, Amoora elmeri, Amoora grandifolia.

1.11 Local names

Table 1.4: Local names

Bengali : Pithraj	**Hindi : Harin-hara**
English : Rohitaka tree	**Sanskrit: Janavallabha**

1.12 Botanical Distribution

The tree is a native of most of the hotter parts of India, Malay and Ceylon. It is distributed through the Sub-Himalayan tract from Gonda (Uttar Pradesh) eastwards to Bengal and Assam, Sikkim up to 1800 m; Chota Nagpur, Konkhan, Western Ghats an adjoining hills ranges, from Poona district southwards to Tinnevelly up to 1050 m and southwards up to Burma and the Andaman, Cambodia, Indochina, China (Agnihotri *et al*., 1987).

1.13 Useable parts

The bark is used as a strong astringent and the powdered bark is said to be a useful remedy for enlarged spleen, also useful in liver diseases, tumors, abdominal complaints. It was, of course, later found unsatisfactory in enlarged spleen and enlargement of liver of infants. Seeds are acrid with a sharp taste; refrigerant, laxative, anthelmintic; to cure ulcers, diseases of the eye and of the ear; lessen muscular pain. In Bengal, oil is expressed from the seeds which were used for various economic purposes. The seed oil is used as a stimulating liniment in rheumatism; the oil is also used as a cure of blood diseases and as a dressing for sores (Agnihotri *et al*., 1987).

1.14 Local use of *Aphanamixis polystachya*

Bark is used in spleen, liver diseases, tumour and abdominal complaints. Seed-oil is used in rheumatism. Ayurveda recommends the decoction of root bark in enlargement of glands, liver and spleen disorders and corpulence. In Ayurveda, Rohitaka infusion mixed with honey is given internally to tackle skin diseases of a fast spreading nature. The bark is used for a remedy for rheumatism, colds and chest pains. Leave and seeds: Extracts of leaves and seeds have insecticidal properties (Agnihotri *et al*., 1987).

1.15 Literature review of *Aphanamixis polystachya*

Phytochemical investigation

From the seeds and bark a number of glycosides have been reported. The seed oil was found to be comprised of the fatty acids: stearic, palmitic, oleic, a-linoleic, isomeric linoleic and a -linolenic acids. From the ethyl acetate extract of the seeds, three new glycosides, 3', 4', 5'-trihydroxyfla vonone-7- *O*- ß -D- xylopyranosyl-ß-D- arabinopyranoside, dihydrorobinetin-7-*O*-ß-D-glucopyranosyl-*O*-a-L-rhamnopyranoside; stigmasta-5, were isolated (Bhatta, SK., 1980).

The petroleum ether extract of the leaves yielded a neutral compound designated as aphanamixol which was characterized as eperu-13-en-8-, 15-diol (Bhatta, SK., 1980). The different extract of the bark yielded a number of constituents. These were identified as aphanamixinin. From the ethanolic extract of powdered stem bark a new saponin, poriferasterol-3-rhamnoside has been isolated and characterized Isolation and characterization of ethanolic extract from roots of *Aphanamixis polystachya* yields three new compounds limonoid and flavonoids along with aphanamixinin. Saponins, terpenoids and carbohydrates were found to be present in *Aphanamixis polystachya* (Shinkar AS., 2007). There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals (Xu, R *et al*., 1996). There is evidence of the presence of saponins in traditional medicine preparations, where oral administrations might be expected to lead to hydrolysis of glycoside from terpenoid (and obviation of any toxicity associated with the intact molecule (Asl, M. N., 2008). But as is often the case with wide-ranging commercial therapeutic claims for natural products such as the claims for organismal human benefit are often based on very preliminary biochemical or cell biological studies (Achnine, L., 2005).

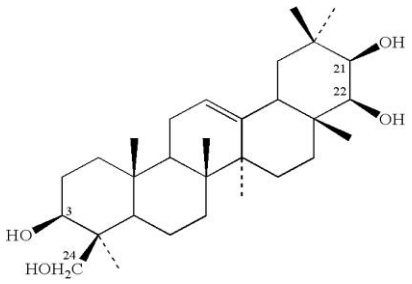

Figure 1.2: Saponins

Terpenoidsare usually the main constituents of essential oils of most plants offering a wide variety of pleasant scents from flowery to fruity, to woody or balsamic notes. For this reason terpenoids constitute a very important class of compounds for flavour and fragrance industries, in fact, in the US alone, the demand is forecast to grow 3.7 percent per year to $5.3 billion in 2012. The recent patents on production and extraction of terpenoids commonly used as natural flavoring compounds in food industries are reviewed in the present manuscript (Balandrin, M.F., 1985).

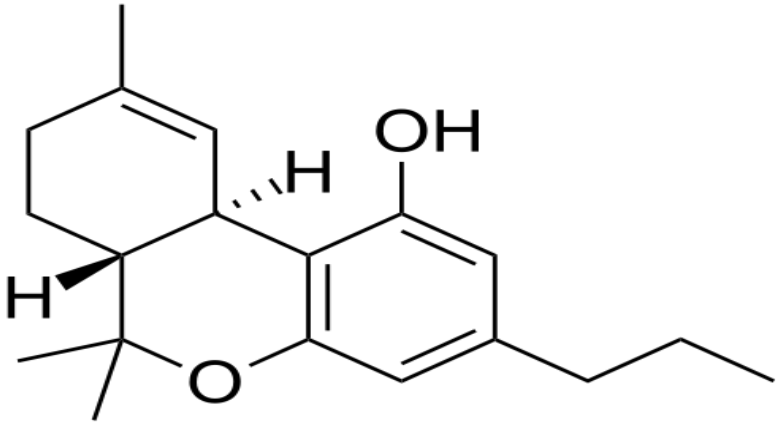

Figure 1.3: Terpenoids

Carbohydrates generally source of energy and it also play role in the beak down of fatty acids and preventing ketosis (Matthews, C.E. *et al*., 1999).

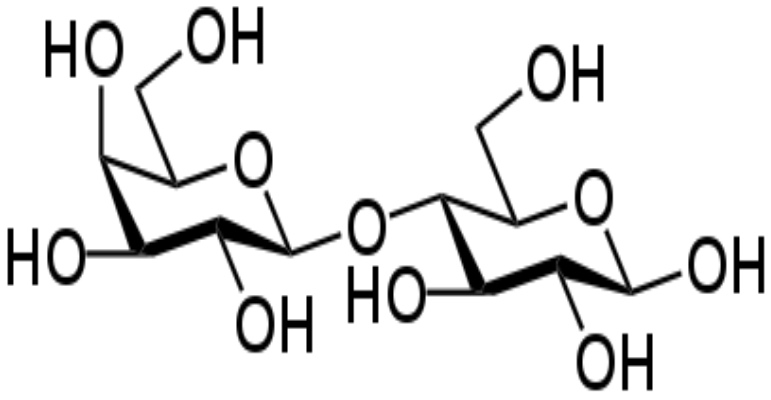

Figure 1.4: Carbohydrates

Pharmacological investigation

Phmacognostic study of stem bark of *Aphanamixis polystachya* (Wall.) Parke was carried out. Bark was found to be strong astringent. The bark was characterized by usually clean edges of cracks and fissures, short type of fracture, solitary or aggregated sclereids restricted to cortex (not in secondary phloem), right square and rectangular prisms, double pyramids or short prisms with pyramids on each end; slight characteristic smell; astringent taste; and by the absences of corky warts, lenticels, 2- seriate rays and rosette crystals (Sasnyal, M *et al*.,1981).

The ethanol extract of air-dried, powdered stem bark of *A.rohituka* yielded a new saponin. It was identified as poriferasterol-3-rhamnoside (Agnihotri, VK, 1987).

Oral administration of ethyl acetate soluble fraction of the alcoholic extract, induced a marked reversal of liver injury to the normal state in experimental rats (Rao, KN *et al*., 1993).

An ethyl acetate extract derived from the stem bark of *A. rohituka* exhibited antitumor activity on mice inocula ted with Dalton's lymphoma ascites cells (DLA) (Rabi, T *et al*., 1995).

Antihepatotoxic property of a resuspended residue of the alcoholic extract of *Amoora rohituka* was studied in rats with hepatic injur y induced by carbon tetrachloride. The results indicate that *A.rohituka* suspension possesses hepatoprotective activity (Gole, MK *et al*., 1997).

The extracts of two Bangladeshi medicinal plants *Toona ciliate* (stem bark) and *Amoora rohituka* (stem bark), along with siderin, a major coumarin from *T.ciliata* exhibited significant in vitro antibacterial activity. The extracts also demonstrated mild antifungal effect (Prasad, GC., 1999).

The ethanolic extract of *Aphanamixis polystachya* was tested in Swiss albino mice transplanted with ehrlich ascites carcinoma (EAC) and exposed to various doses of gamma-radiation (Jagetia, GC *et al*., 2005).

1.16 Rational and objective of the study

Rational of the study:

Plants and other natural substances have been used as the rich source of medicine. The list of drugs obtained from plant source is fairly extensive. Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medical facilities.

There are many significant research work done on bark, leave and root of *A.polystachya* plants and they have very good pharmacological activities. However, the literature review revealed that limited numbers of research work has been carried out on fruit extract. That's why the study was conduct to investigate the CNS depressant activity of different solvent soluble fraction (*n*-hexane solvent soluble, ethyl acetate solvent soluble and methanol solvent soluble) of *Aphanamixis polystachya* fruits using mice model.

Objective of the study:

To evaluate the CNS depressant activity of fruits of *Aphanamixis polystachya* by

- Investigate CNS activities by Hole cross method

- Study anxiety-related behavior by Elevated Plus maze test

- Observe sedative activities by Hole board method

Chapter-2
Materials and Method

2.1 Selection of plant part

The fresh fruits of the plant *Aphanamixis polystachya* were selected for chemical investigation.

2.2 Collection and identification of plant

The fresh fruit *Aphanamixis polystachya* was collected in the month of February, 2011 from Mymensing, Bangladesh. Collected fruit was identified by Dr. Bushra Khan, Chief scientist and taxonomist of Bangladesh National Herbarium. A duplicate specimen (DACB-35449) has been deposited in the Bangladesh National Herbarium.

2.3 Drying and Pulverization

The fresh fruit part of the plant *Aphanamixis polystachya* was washed with water to remove adhering dirt and then cut into small pieces and finally sun dried for 7 days. After complete drying, the entire portions were pulverized into a coarse powder with help of a grinding machine and were stored in an airtight container for further use. Before drying collected amount of fruit part was 400 gm and after drying remaining amount was 144.1 gm.

2.4 Hot Continuous Extraction (Soxhlet)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in extraction chamber of the Soxhlet apparatus. The extracting solvent in boiling flask is heated, and its vapors condense in condenser. The condensed extracting drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in extraction chamber rises to the top of siphon arm, the liquid contents of extraction chamber siphon into boiling flask and we collect our product. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale (Handa, S.S *et al*., 2008).

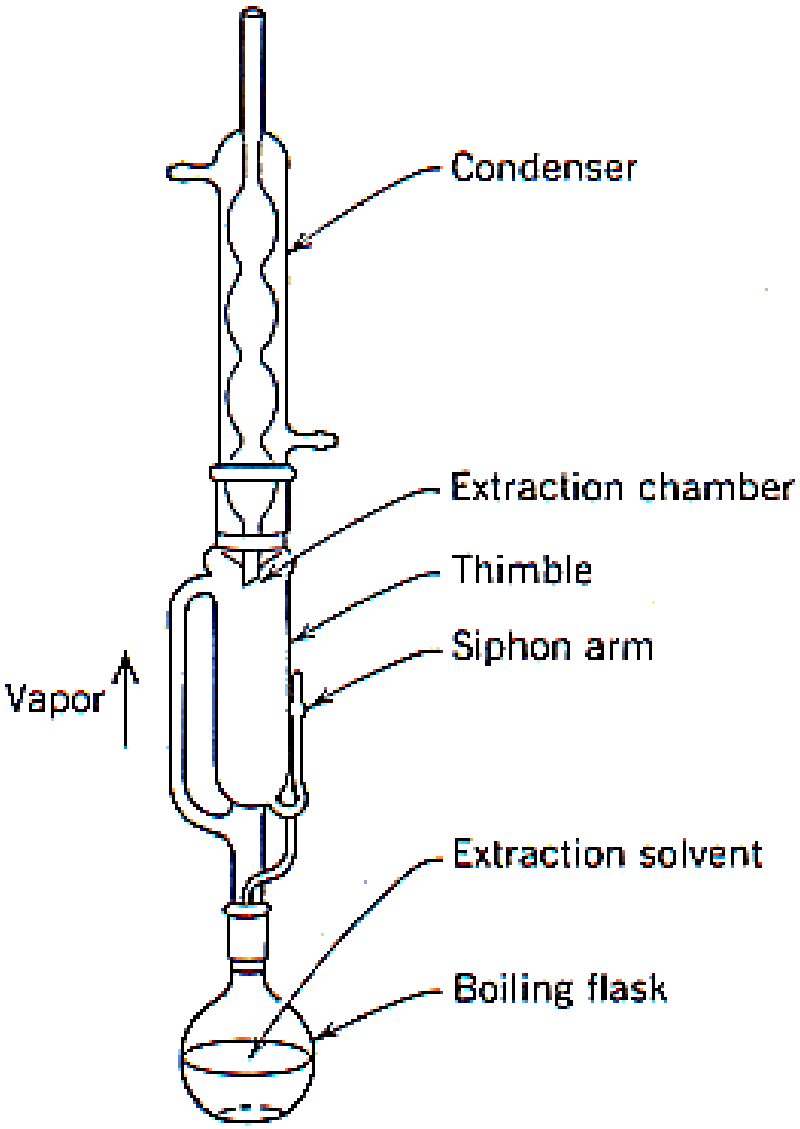

Figure2.1: Soxhlet apparatus.

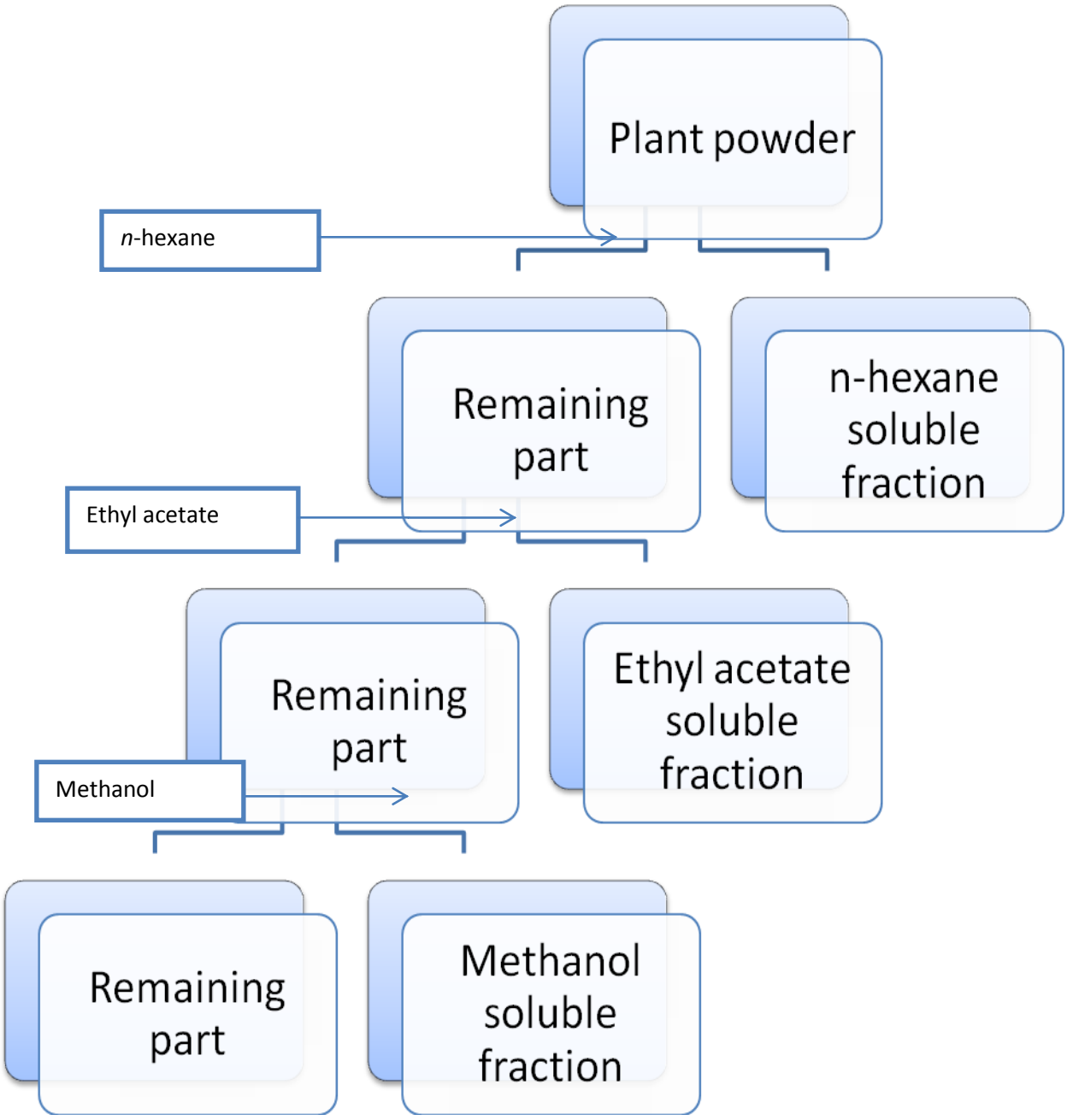

Figure 2.2: Flow chart of soxhlet extraction

2.5 Animal

For the experiment male Swiss albino mice of 3 - 4 weeks of age, weighing between 20 - 25 gm, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: (23.0 ± 2.0º), relative humidity: 55 - 65% and 12 h light/12 h dark cycle). The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethics committee, East West University.

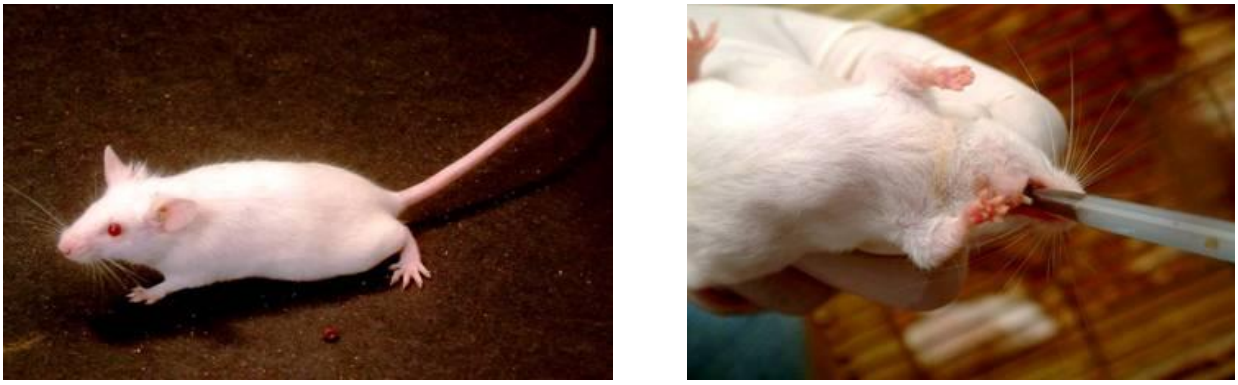

Figure 2.3: Swice albino mice.

2.6 Materials used in CNS depressant activity for Hole cross method

Chemicals	Instrument	Sources
- Diazepam - Normal saline -Tween 80	-Case (30 × 20 × 14)cm, 3 cm diameter, height 7.5 cm - Injection (100) unit - Needle - Syringe - Weight balance - Pipette - Vial (5ml)	- East West University lab (chemical and instrument)

2.7 Method of Hole cross test

The method was adopted as described by Takagi *et al*. (1971). A wooden partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the extract (Takagi *et al*., 1971).

2.8 Preparation of the test materials and standard

Aphanamixis polystachya 200mg/kg and 400mg/kg body weight were dissolved by additional of small amount of suspending agent Tween 80. Normal saline was slowly added to make up the volume up to 3 ml. To prepare the standard, diazepam 1mg/kg was dissolved in normal saline and made up the volume up to 3 ml. Normal saline (0.9%) was given to negative group.

2.9 Designing of the experiment

Forty mice were divided into eight groups and each group contains five mice. Group one received control 1% tween 80 in normal saline, Group two received standard 1 mg/kg body weight Diazepam, Group three and four received *n*-hexane extract of *Aphanamixis polystachya* at dose of 200 and 400 mg/kg body weight, Group five and six received Ethyl acetate extract of *Aphanamixis polystachya* at dose of 200 and 400 mg/kg body weight, Group seven and eight received Methanol extract of *A. polystachya* at dose of 200 and 400 mg/kg body weight.

2.10 Experimental procedure

At the beginning of the experiment, extracts of *Aphanamixis polystachya,* Diazepam and normal saline were administrated orally to all of the mice respectly. Mice were placed in a steel partition, fixed in the middle of the case. The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 3 minute at 0, 30, 60, 90 minute after oral administration of the drugs. (Hossain, M.M *et al*., 2009)

2.11 Materials used in anxiolytic activity for Elevated Plus Maze test

Chemicals	Instrument	Sources
Diazepam Normal saline Tween 80	Two open arms, 50 × 10 cm, and two closed arms, 50 × 10 × 40 cm Injection (100) unit Needle Syringe Weight balance Pipette Vial (5ml)	East West University lab (chemical and instrument)

2.12 Method of Elevated Plus Maze test

Anxiety-related behavior was measured by the elevated plus-maze test. The elevated plus-maze Consist of two open arms, 50 × 10 cm, and two closed arms, 50 × 10 × 40 cm. The maze was elevated to a height of 15 cm above the floor. Each mice was placed on the central platform facing a closed arm. During 5 min test period, the following measures were taken by an observer, number of open arms entries, percentage of time spent in open arms, number of closed arm entries, time spent in closed arms. Entering into an arm was noted only when all paws had crossed out central area. Also frequency of rearing and grooming time are measured (Kurt, M., 2004).

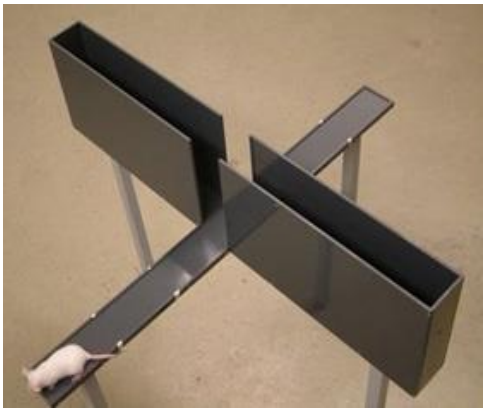

Figure 2.4: Elevated Plus Maze test apparatus

2.13 Preparation of the test materials and standard

Aphanamixis polystachya 200mg/kg and 400mg/kg body weight were dissolved by additional of small amount of suspending agent Tween 80. Normal saline was slowly added to make up the volume up to 3 ml. To prepare the standard, diazepam 1mg/kg was dissolved in normal saline and made up the volume up to 3 ml. Normal saline (0.9%) was given to negative group.

2.14 Designing of the experiment

Forty mice were divided into eight groups and each group contains five mice. Group one received control 1% tween 80 in normal saline, Group two received standard 1 mg/kg body weight Diazepam, Group three and four received *n*-hexane extract of *Aphanamixis polystachya* at dose of 200 and 400 mg/kg body weight, Group five and six received Ethyl acetate extract of *Aphanamixis polystachya* at dose of 200 and 400 mg/kg body weight, Group seven and eight received Methanol extract of *A. polystachya* at dose of 200 and 400 mg/kg body weight.

2.15 Experimental procedure

At the beginning of the experiment, *Aphanamixis polystachya*, diazepam and normal saline were administrated orally to all of the mice. . Each mice was placed on the central platform facing a closed arm. During 5 min test period, the following measures, number of open arms entries, percentage of time spent in open arms, number of closed arm entries, time spent in closed arms. Entering into an arm was noted only when all paws had crossed out central area. Also frequency of rearing and grooming time are measured.

2.16 Materials required for Hole board test

Drugs	Instrument	Sources
Diazepam Normal saline Tween 80	Case (30 × 20 × 14)cm, 3 cm diameter, height 7.5 cm Injection (100) unit Needle Syringe Weight balance Pipette Vial (5ml)	East West University lab (chemical and instrument)

2.17 Method of Hole board test

The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes (Perez *et al*., 1998). Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter. The mice were placed and released singly in the centre of the board, facing away from the observer. The number of holes explored and the duration of each of the explorations was shown in real time to the observer and at the end of the experiment (usually 5 min) all the information was stored in a file for post experiment study. After each trial the apparatus was wiped clean to remove traces of the previous assay. A decrease in the number of head-dips, the time spent during head-dipping reveals the sedative behavior of experimental mice (Perez *et al*., 1998).

2.18 Preparation of the test materials and standard

Aphanamixis polystachya 200mg/kg and 400mg/kg body weight were dissolved by additional of small amount of suspending agent Tween 80. Normal saline was slowly added to make up the

volume up to 3 ml. To prepare the standard, diazepam 1mg/kg was dissolved in normal saline and made up the volume up to 3 ml. Normal saline (0.9%) was given to negative group.

2.19 Designing of the experiment

Forty mice were divided into eight groups and each group contains five mice. Group one received control 1% tween 80 in normal saline, Group two received standard 1 mg/kg body weight Diazepam, Group three and four received *n*-hexane extract of *Aphanamixis polystachya* at dose of 200 and 400 mg/kg body weight, Group five and six received Ethyl acetate extract of *Aphanamixis polystachya* at dose of 200 and 400 mg/kg body weight, Group seven and eight received Methanol extract of *A. polystachya* at dose of 200 and 400 mg/kg body weight.

2.20 Experimental procedure

After administering plant extract each mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter. The mice were placed and released singly in the centre of the board, facing away from the observer. The number of holes explored and the duration of each of the explorations was shown in real time to the observer and at the end of the experiment all the information was stored in a file for post experiment study. After each trial the apparatus was wiped clean to remove traces of the previous assay.

2.21 Statistical Data

SPSS: Window 15, one way ANOVA, using dunnett-*t*. Where all the samples are compare to negative.

Chapter-3
Results and Discussion

3.1 Result of CNS depressant activity of *A. polystachya* by Hole Cross method

To Investigate the CNS depressant activity of *n*-hexane, methanol, ethyl acetate solvent soluble fraction of the fruit of *A. polystachya* studied in different doses of 200 and 400 mg/Kg body weight, using hole cross method. The average and standard error mean of crossing the hole by the dose of 200 mg/kg and 400 mg/kg respectively have been showed in Table 3.1. The result was found to statistically highly significant.

Table 3.1: Data obtained from Hole cross experiment

Group	**Average ± SEM**				
	0 min	**30 min**	**60 min**	**90 min**	**120 min**
Negative control 1%tween in saline	5±.40497	5.8±.40497	6.2±.40497	6.2±.40497	6±.40497
Positive control diazepam (1mg/kg)	7*±.40497	7±.40497	7.2*±.40497	7.8*±.40497	7*±.40497
***n*-hexane (200 mg/kg)**	3.8***± .40497	4***±.40497	4.2***±.40497	3***±.40497	2.8***±.40497
***n*-hexane(400 mg/kg)**	5*±.40497	4.8*±.40497	4.6*±.40497	4.8*±.40497	4.6*±.40497
EA(200 mg/kg)`	5.6±.40497	8±.40497	4.8±.40497	6.4±.40497	5.4±.40497
EA (400 mg/kg)	6.6±.40497	7±.40497	5±.40497	6.6±.40497	6.4±.40497
Methanol (200 mg/kg)	3.8***±.40497	4.6***±.40497	4.8***±.40497	4***±.40497	4.8***±.40497
Methanol (400 mg/kg)	2.8***±.40497	2.4***±.40497	1.6***±.40497	3***±.40497	1.8***±.40497

Values are expressed as Mean±SEM (n=5); * *p*<0.05, **: *p*<0.01, *: *p*<0.001, dunnett *t*-test as compared to negative control.**

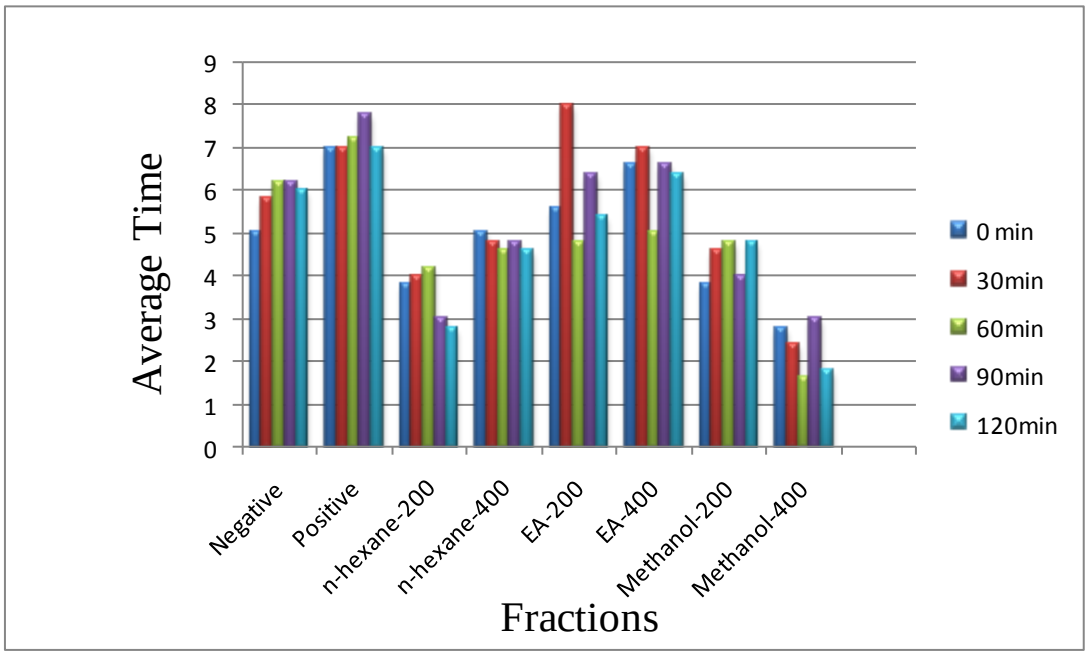

Figure 3.1: Average value of different solvent soluble fractions in Hole Cross experiment.

From the observation data it has been observed that in *n*-hexane and methanol soluble fraction at dose of 200 and 400 mg/kg body weight, the decreased the locomotor activity of the experimented animals. Among these sample, *n*-hexane fraction of *A. polystachya* fruits at dose of 200 mg/kg body weight mostly decrease the frequency of moment of the mice through the hole. It was also observed that methanol fraction at dose of 400 mg/kg body weight highly decreased the locomotor activity of the test animals.

The mean values of both fractions (n-hexane 200 and methanol 400 mg/kg) were compared to the value of negative control group and in the both case, the result was statistically highly significant ($p<0.001$).

Form this experiment it could be concluded that n-hexane and methanol fraction have the capability to depress the CNS and which may be due to the present of bioactive compounds to these fractions.

3.2 Result of sedative activity of *Aphanamixis polystachya* by Hole Board method

Anxiolytic property of *n*-hexane, methanol, and ethyl acetate solvent soluble fraction of the fruit of *A. polystachya* studied in different doses of 200 and 400 mg/Kg body weight, using hole board method. The fractions produced % inhibition of head dipping at doses of 200 and 400 mg/kg body weight respectively (Table 3.2 and Fig. 3.2). The result was found to statistically highly significant.

Table 3.2: Data obtained from Hole Board experiment.

Animal Group	**Frequency of Dipping**					**Mean±SEM**
	M1	**M2**	**M3**	**M4**	**M5**	
Negative Control 1% tween 80 in saline water	30	67	64	80	82	64.6±6.48267
Standard (Diazepam)	27	29	45	34	42	35.4***±6.48267
Methanol fraction-200mg	8	14	14	13	16	13***±6.48267
Methanol fraction-400	22	31	23	30	35	28.2***±6.48267
n-hexane-200mg	11	20	9	31	4	15***±6.48267
n-hexane-400mg	12	15	22	3	22	14.8***±6.48267
Ethyl acetate-200mg	15	30	21	27	16	21.8***±6.48267
Ethyl acetate-400mg	24	18	6	24	6	15.6***±6.48267

Values are expressed as Mean±SEM (n=5); * $p<0.05$, **: $p<0.01$, ***: $p<0.001$, dunnett *t*-test as compared to negative control.

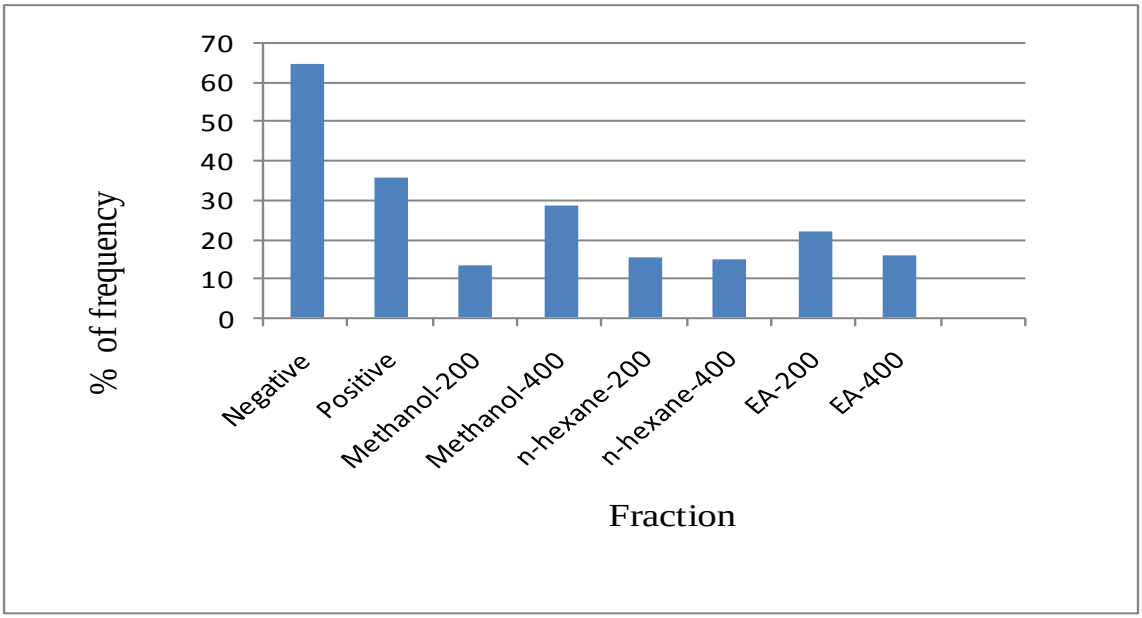

Figure 3.2: Average head dipping of mice for different solvent soluble fractions in Hole Board experiment.

From the obtained data it has been observed ethyl acetate 200 and methanol 400 mg/kg body weight showed to some extent anxiolytic activity with compare to positive control grope. On the other hand n-hexane fractions and methanol 200 mg/kg body weight did not showed any anxiolytic activity.

3.3 Result of anxiety activity of *Aphanamixis polystachya* by Elevated Plus Maze test

Anxiolytic property of *n*-hexane, methanol, and ethyl acetate solvent soluble fraction of the fruit of *A. polystachya* studied in different doses of 200 and 400 mg/Kg body weight, using Elevated plus maze experiment.

Table 3.3: Data obtained from Elevated Plus Maze experiment

Animal Group	**Mean ± SD (counts/5minutes)**			
	Open arm Duration	**Frequency of open arm**	**Closed arm Duration**	**Frequency of Closed arm**
Negative Control 1% tween 80 in saline water	2.6	0.8	257.8±11.39398	8.4±1.719
Positive control diazepam (1mg/kg)	2.4	0.2	261.8±11.39398	7.6±1.719
Ethyl acetate-400	14	2.6	217.4±11.39398	12.6±1.719
Ethyl acetate-200	6.4	1.8	240.4±11.39398	11.3±1.719
Methanol-400	8.2	2.6	203.2±11.39398	14.4±1.719
Methanol-200	16	3.2	207.6±11.39398	13.2±1.719
n-hexane-400	0	0	253.6±11.39398	8.2±1.719
n-hexane-200	2.6	1	249	9.4

Open arm duration, df=39, and F= 3.224, $p<0.01$, Open arm frequency, df=39, F=3.309, $p<0.001$, Closed arm duration, df=39, F=8.554, significant value=0.000, Closed arm frequency, F=4.5050, $p<0.001$.

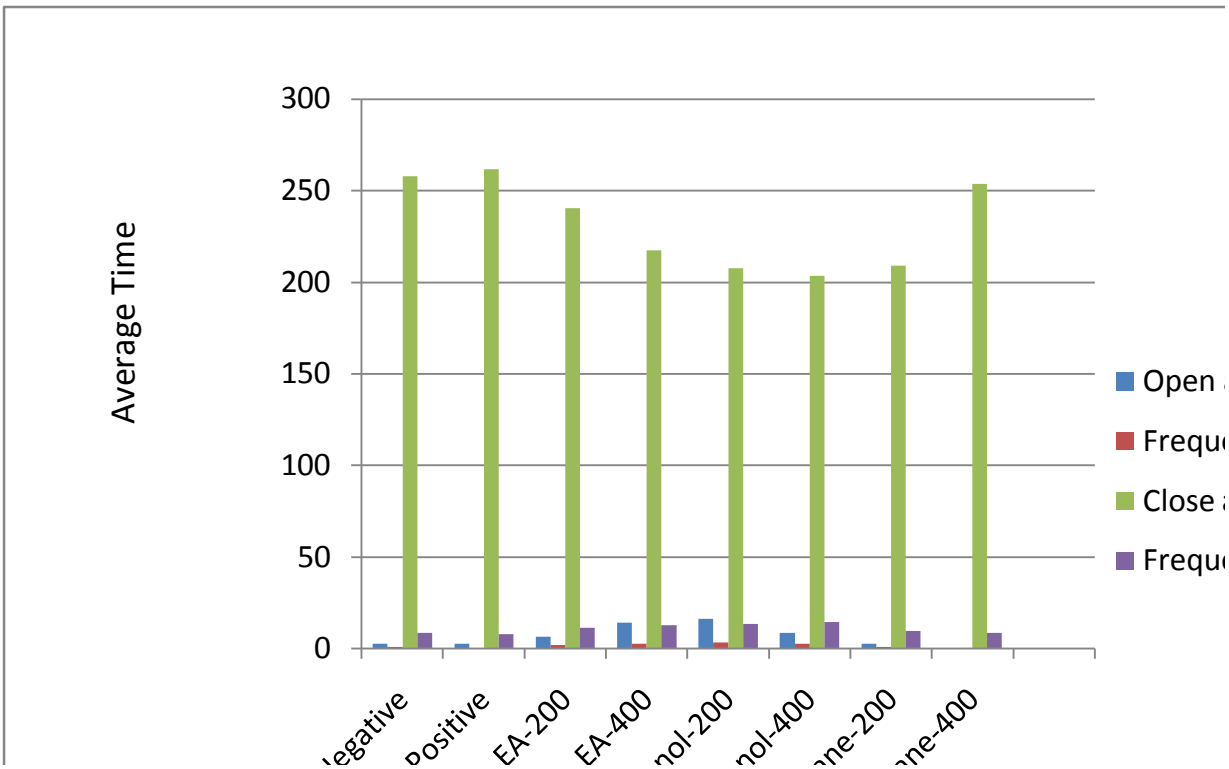

Figure 3.3: Average time duration in different arms and their frequency in Elevated Plus Maze experiment.

From the experiment it was observed that mice taken methanol and ethyl acetate soluble fraction at dose of 200 and 400 mg/kg body weight, stayed more time in open arm of Elevated Plus Maze apparatus in comparison to standard and negative control group. Moreover they were also stayed less time in closed arm of Elevated Plus Maze apparatus in comparison to standard and negative control group. The value obtained from these fraction were statistically significant ($p<0.05$).

On the other hand, n-hexane at dose of 400 mg/kg body weight showed higher staying value in open arm and did not stay in open arm of Elevated Plus Maze apparatus. The obtained value was compared to the valued of negative control group and found it was statistically highly significant ($p<0.01$).

From the observed result, it could be concluded that methanol and ethyl acetate soluble fraction may have anxiolytic activity. Where as n-hexane at dose of 400 mg/kg body weight did not have any anxiolytic activity.

Chapter-4
Conclusion

CONCLUSION

The results obtained in this study indicate that the *n*-hexane, ethyl acetate and methanol fractions of the fruit of *Aphanamixis polystachya* have significant CNS Depressant and Anxiolytic activities different *in vivo* animal model systems. The medicinal values of the plant fruit may be related to their constituent phytochemicals. So, further detailed investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be done. It will help in the development of novel and safe drugs for the treatment of different type of CNS disorders.

Chapter-5
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