In vivo Investigation of CNS Depressant and Anxiolytic Activity of Fruits of *Aphanamixis polystachya* (Meliaceae)

This thesis paper is submitted to the Department of Pharmacy, East West University in conformity with the Requirements for the degree of Bachelor of Pharmacy

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Certificate

This is to certify that "*In-vivo* Investigation of CNS Depressant and Anxiolytic Activity of Fruits of *Aphanamixis polystachya* (Meliaceae)" is a research work done by Mithun Mallik (ID: 2007-1-70-065) under the guidance and supervision of Farjana Khatun, Lecturer, Department of Pharmacy, East West university, Dhaka.

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Dedicated To My Loving Parents

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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 apparatus, Hole Cross, Hole Board, Elevated Plus Maze.

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Introduction

1.1 Introduction

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority to have medicinal effects. World Health Organization (WHO) has provided a definition of medicinal plants, that is "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs."(Sofowora,1982).

World Health Organization (WHO) reported that 80% of the world's population depend on medicinal plants for their primary health care. In the Plant Kingdom, Medicinal plants form the largest single grouping of plants. It is estimated that 30,000 species worldwide fall in this group, of which around 33% are trees (Abayomi, 1993).

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs (Lown, 1993).In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods (Herborn, 1998). The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins (Abayomi, 1993).

The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized (Abayomi, 1993).

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow *et al.*, 2003). Bacterial pathogens have evolved numerous defense

mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Scazzocchio *et al*., 2001).

There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El-seedi *et al*, 2002; Rojas *et al*., 2003; Duraipandiyan *et al*, 2006; Parekh and Chanda, 2007).

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines (Stuffness *et al*., 1982). Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry (Baker *et al*, 1995).

1.1.1 History of plants in medicine (Levetin *et al*., 2003)

The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies. The Pun-tsao contains thousands of herbal cures attributed to Shennung, China's legendary emperor who lived 4500 years ago. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. The Badianus Manuscript is an illustrated document that reports the traditional medical knowledge of the Aztecs.

Western medicine can be traced back to the Greek physician Hippocrates, who believed that disease had natural causes and used various herbal remedies in his treatments. Early Roman writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in *De Materia Medica.* Many of the herbal remedies used by the Greeks

and Romans were effective treatments that have become incorporated into modern medicine (e.g., willow bark tea, the precursor to aspirin). Dioscorides' work remained the standard medical reference in most of Europe for the next 1500 years.

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. For example, the red juice of bloodwort suggests that it should be used for blood disorders; the lobed appearance of liverworts suggests that it should be used to treat liver complaints; the "humanoid" form of mandrake root suggests that is should be used to promote male virility and ensure conception.

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 19^{th} 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 semisynthetic or wholly synthetic ingredients originally isolated from plants.

While Western medicine strayed away from herbalism, 75% to 90% of the rural population of the rest world still relies on herbal medicine as their only health care. In

many village marketplaces, medicinal herbs are sold alongside vegetables and other Wares. The People's Republic of China is the leading country for incorporating traditional herbal medicine into a modern health care system; the result is a blend of herbal medicine, acupuncture, and Western medicine. Plantations exist in China for the cultivation of medicinal plants, and thousands of species are thus available for the Chinese herbalist; prescriptions are filled with measured amounts of specific herbs rather than with pills or ointments. In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine, with its Muslim and Greek roots, is another widely practiced herbal tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices.

1.1.2 Traditional medicine

Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Traditional preparation comprises medicinal plants, minerals and organic matters etc. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record is evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years. About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra *et al*., 1956), which also forms a rich source of knowledge (Nadkarni, 1982; Jone, 1984). The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe *et al*., 1997). In India around 20,000 medicinal plant species have been recorded recently (Dev, 1997), but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000). Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care for

human alleviation because it has no side effects (Veale *et al*., 1992). Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources (Anesini *et al*, 1993).

The use of traditional medicine has increased in developed countries also, mainly due to the failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites. The adverse effects of chemical drugs, questioning of the approaches and assumptions of allopathic medicine, their increasing costs and greater public access to information on traditional medicine has also led to an increase in interest in alternative treatments (WHO 2002). Plant extracts have become a source of hope as a wide group of medicinal plant preparations are available that have been used over the centuries almost exclusively on the basis of empirical evidence. Hence, it has become necessary to revisit the importance of these herbal medicines (Murray, 1995).

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed alternative or complementary medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (Mian *et al*., 1990).

1.1.3 Trends of using traditional medicine

In some Asian and African countries, 80% of the population depend on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture). Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. Annual revenues in Western Europe

reached US$ 5 billion in 2003-2004. In China sales of products totaled US$ 14 billion in 2005. Herbal medicine revenue in Brazil was US$ 160 million in 2007 *(*Ghani, 2003).

1.1.4 Modern medicine from medicinal plants

Medicinal plants play a vital role for the development of new drugs. During 1950-1970 approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. From 1971 to 1990 new drugs such as ectoposide, E-guggulsterone, teniposide, nabilone, plaunotol, Z-guggulsterone, lectinan, artemisinin and ginkgolides appeared all over the world. 2% of drugs were introduced from 1991 to 1995 including paciltaxel, toptecan, gomishin, irinotecan etc. Plant based drugs provide outstanding contribution to modern therapeutics; for example: serpentine isolated from the root of Indian plant *Rauwolfia serpentina* in 1953, was a revolutionary event in the treatment of hypertension and lowering of blood pressure. Vinblastine isolated from the *Catharanthus rosesus* is used for the treatment of Hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymophosarcoma, small cell lung, cervical and breast cancer. (Farnsworth *et al*.,1977). Phophyllotoxin is a constituent of *Phodophyllum emodi* currently used against testicular, small cell lung cancer and lymphomas. Indian indigenous tree of *Nothapodytes nimmoniana* (*Mappia foetida)* are mostly used in Japan for the treatment of cervical cancer. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. Medicinal plants play an important role in the development of potent therapeutic agents. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. More than 64 plants have been found to possess significant antibacterial properties; and more than 24 plants have been found to possess antidiabetic properties (Arcamone *et al*., 1980), antimicrobial studies of plants (Perumal, 1998; Perumal Samy, 2006), plant for antiodotes activity - *Daboia russellii* and *Naja kaouthia* venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R.Br (Chatterjee *et al*., 2006). Which effectively

neutralized *Daboia russellii* venom induced pathophysiological changes (Alam, 1994). The present investigation explores the isolation and purification of another active compound from the methanolic root extract of *Hemidesmus indicus*, which was responsible for snake venom neutralization. Antagonism of both viper and cobra venom and antiserum action potentiation, antioxidant property of the active compound was studied in experimental animals. Recently, (Chatterjee *et al*., 2004) from this laboratory reported that an active compound from the *Strychnus nux vomica* seed extract, inhibited viper venom induced lipid peroxidation in experimental animals. The mechanism of action of the plant derived micromolecules induced venom neutralization need further attention, for the development of plant-derived therapeutic antagonist against snakebite for the community in need. However, the toxicity of plants has known for a long period of time, and the history of these toxic plants side by side with medicinal ones are very old and popular worldwide, they considered the major natural source of folk medication and toxication even after arising of recent chemical synthesis of the active constituents contained by these plants (Adailkan *et al*., 2001; Heinrich, 2000; Pfister, 2002). 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. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas (WHO 2002). Increasing interest by multinational pharmaceutical companies and domestic manufacturers of herbal-based medicines is contributing to a significant economic growth of the global medicinal plants sector. However, a large proportion of medicinal plant research is focused on nutraceuticals, chronic and metabolic disorders (diabetes, cardiovascular, etc.) and other diseases like HIV/AIDS, malaria, etc. Whereas, the common diseases of resource poor communities such as diarrhoeal diseases and acute respiratory tract infections (ARI) are often not addressed. Moreover, unlike the rural communities who use fresh/dried plant material or their crude extracts, the industry lays importance on isolation of active principles or standardized fractions since crude extracts are not patentable. However, it is often seen

that a crude extract is more active compared to the isolated active fractions. Among the main armaments in defense of the medicinal plants we have to mention the followings-

A future medicine bank to disease: There are approximately half million plants with flowers, most of which have not been investigated & which principles could be decisive in the treatment of present or future. Novel structures of biologically active compounds, isolated from plant sources, often prompt the chemists to synthesize similar or biologically more potent semi-synthetic compounds (Ghani, 2003).

Synergic medicine: Many application of isolated component has not had the wished effect due to turn out to be toxic. Thus the uses complement or damage or negative effects. Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant derived compounds with known biological activity (Ghani, 2003).

Support of official medicine: The treatment of vary complex disease can require in some cases the support of the medicinal properties of the plants or the derivatives that they provide. Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by the chemists for use as potent drugs (Ghani, 2003).

Preventive medicine: Finally we do not have to forget the preventive character that the plants have regarding the appearance of disease. In this sense the plants are better than the chemical remedies that are applied essentially when the disease has already appeared.

Homatropine (a synthetic tropane alkaloid similar to atropine), syrosingopine (a synthetic derivative of reserpinre), chloroquine (a synthetic derivative of quinine), dihydrmorphinone, methyl dihydromorphinone, oxy morphine, ethyl morphine, and n-allyl-normorphine (synthetic derivative of morphine) are some of the examples of such synthetic drugs which plants have contributed indirectly. Even in this age of synthetic drugs, there are some naturally occurring drugs, such as Digitalis glycosides used in cardiac complications and the Catharanthus alkaloids used in cancers, which have no

synthetic alternatives. In such cases, plants continue to remain as their principal and only sources *(*Ghani, 2003).

Artuso (1997) has outlined the entire process which includes formulating an appropriate strategy and he estimates that the entire process would take more than 10–20 years. This approach is very demanding since there is an estimated 250,000 species of higher plants present on this earth (Ayensu *et al*, 1978). However, this scenario would change with use of the high throughput advanced screening methods that are available today. Another approach than can prove to be a highly productive and cost effective in development of safe, effective and acceptable therapeutic agents is reverse pharmacology which is based on the documented therapeutic effects of plants in ancient texts. It focuses on aspects of the medicinal plant research: from collection of plant material, to efficacy and safety evaluation through preclinical studies and phytochemical standardization (Vaidya, 2006).

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,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 *et al*., 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 *et al*., 1976; Murray, 1995; Hill, 1989).

1.2 Family profile

The plants belong to the family Meliaceae, closely related to the mahogany family which 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 greyish-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)

Species belong to family Meliaceae

Table 1.1**:** Plants of 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)

Important species of *Aphanamixis* genera

A. polystachya
A. amboensis
A. apoensis
A. borneensis
A. coriacea
A. cucullata
A. cumingiana
A. davaoensis
A. decandra.
A. elmeri
A. grandifolia
A. humilis
A. lauterbachii
A. macrocalyx
A. myrmecophila
A. obliquifolia
A. pedicellata
A. perrottetiana
A. pinatubensis
A. polillensis
A. polystachya
A. pulgarensis
A. reticulosa.
A. rohituka
A. rubiginosa
A. schlechteri
A. sinensis
A. sumatrana
A. timorensis
A. trichanthera
A. tripetala
A. velutina

1.3 Plant Profile

1.3.1 Morphology

Aphanamixis polystachya tree is a deciduous tree native to India, growing to 20-30 m tall. Leaves are odd- or even- pinnate, 30-60 cm long, with 9-21 leaflets. Leaflets are oblong-elliptic, elliptic, or ovate, 17-26 × 4-10 cm with basal pair smallest, leathery when mature, with visible transparent tiny spots under sunlight. Base of the leaflets is oblique, margin entire. Flower clusters occur in leaf axils, less than a foot long. Flowers are 6-7 mm in diameter, with 3 bracteoles. Flowers have 5 nearly curcular sepals, 1-1.5 mm across. Petals are 3-7 mm in diameter, concave. Male flowers numerous in axillary panicles, female or bi sexual flowers larger than male in axillary or supra axillary solitary spikes. Staminal tube is spherical, smooth. Capsule is sort of ovoid, 2-2.5 × 2.5-3 cm, orangish when mature. Seeds are greyish brown. Flowering: May-September. Fruits are 25 mm diameter, pink-red-purplish, capsules and yellow when ripe, opening by 3 valves. Seeds are with scarlet aril. Seeds with orange-red aril. (Chatterjee *et al*., 1970).

1.3.2 Botanical classification

Table 1.2 **:** Broad Classification of *Aphanamixis polystachya*

Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Sapindales
Family	Meliaceae
Genus	Aphanamixis
Species	*A. polystachya*

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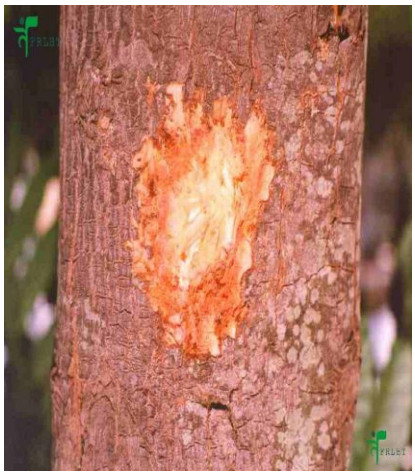
1.3.3 Ecology

Usually found on hillsides and ridges with sandy to clay soils. Also on limestone. In secondary forests usually present as a pre-disturbance remnant. In Viet Nam, this tree occurs in evergreen tropical rainforest. India, almost throughout. Pakistan, Nepal, Bhutan, Bangladesh, Sri Lanka and Myanmar. (Chandrasekharan *et al*., 1970).

1.3.4 Various parts of *Aphanamixis polystachya*



(1)

(2)

(3)

(4)

Figure 1.1**:** Bark (1), fruit (green,2), fruit (ripe,3), leaves(4)

1.3.5 Botanical descriptions

Table 1.3**:** Botanical descriptions of *Aphanamixis polystachya*.

Habit	Trees up to 20 m tall.
Trunk & Bark	Bark grey, fissured; blaze cream.
Branches and Branchlets	Branchlets terete, glabrous.
Leaves	Leaves compound, imparipinnate, alternate, spiral, clustered at twig ends, 50 (-90) cm long; rachis pulvinate, 30 cm or more long, often lepidote_scaly; leaflets opposite to subopposite, 4-8 pairs with terminal one, petiolule 0.4 to 1 cm long; lamina 7-18 (-22) x 3-6.5 (-10) cm, oblong-lanceolate, apex acuminate, base asymmetric, margin entire, coriaceous, glabrous; midrib slightly raised above, stout beneath; secondary_nerves slender; tertiary_nerves broadly reticulate.
Flower	Inflorescence panicles; flowers polygamous.
Fruit and Seed	Capsule, subglobose, to 3 cm across, coriaceous, pale reddish, 3-celled; seed 1, orange red.

1.3.6 Synonyms

Aglaia aphanamixis., Aglaia aphanamixis., Aglaia beddomei (Kosterm.), Aglaia cochinchinensis, Aglaia janowskyi, Aglaia polystachya., Alliaria cuneata, Amoora amboinensis, Amoora aphanamixis, Amoora aphanamixis, Amoora beddomei, Amoora cumingiana, Amoora elmeri.

1.3.7 Common name

Table 1.4**:** Common names of *Aphanamixis polystachya*

Bengali	Pitaraj
Hindi	Harinkhana
Manipuri	Heirangkhoi
Marathi	Raktharohida
Tamil	Malampuluvan, sem, semmaram
Telugu	Chevamanu, Rohitaka
Kuki	Sahala
Assamese	Hakhori bakhori
Sanskrit	Anavallabha, ksharayogya, lakshmi, lakshmivana, lohita

1.3.8 Useful part

Bark, seeds, fruits, root

1.3.9 Uses

The wood is used for construction purposes. Mashed leaves in water solution are effective antifeedants, able to protect crops against insect herbivory. Oil for making soap is extracted from the seeds. The recent studies on alternative medicine suggested that Pitaraj possesses considerable antitumor and antibacterial properties. Bark is a strong astringent, antimicrobial, used for the treatment of liver and spleen diseases, rheumatism and tumors. Methanol extract of the dried bark was checked for growth inhibitory activity against HeLa cells. The bark also possesses mild relaxant, cardiotonic, hepatoprotective and cholerectic activities and exhibits analgesic, immunosuppressive and antidiabetic properties. The roots are reputed in the indigenous system of medicine as a cure for diarrhea and dysentery. The stem extract shows hepatoprotective activities. The ground

leaves, bark and seeds in a 2.5% mixture provided some protection for wheat flour by reducing F1 progeny. (Agnihotri *et al*., 1987).

1.3.10 Chemical constituents

Chemical constituents isolated from extract of *Aphanamixis polystachya*

Table 1.5**:** Chemical constituents of *Aphanamixis polystachya*

1. Lyoniside	7. Thiopental sodium
2. Nudiposide	8. Two lignan glycosides
3. Sterol	9. Aphanamixinin
4. Ergosta-4,6,8,22-tetraen-3-one	10. Flavanone
5. Stigmasterol	11. Anthraquinone glycosides
6. Oleic	12. Linoleic acids

Fruit shell contains triterpene and eight new ring A-*seco* limonoids, aphanalides. Two new highly oxidized A,B-*seco* limonoids, aphapolynins A and B, were also isolated from the fruits of *Aphanamixis polystachya*. Leaves contain diterpene alcohol and beta-sitosterol. Seeds yield polystachin, an alkaloid, a glycoside and a saponin A chromone and 3 flavonoid glycosides have been reported from roots (Agnihotri *et al*., 1987).

1.4 Phytochemicals and pharmacological studies on *A. polystachya*

1.4.1 Chemical investigation

Aphanamixis polystachya or Amoora rohituka (family: Meliaceae; local names: Roina, Pitraj etc) is a medicinal plant of Bangladesh and various parts of *A. polystachya* give therapeutic activity. Different types of compound , for example limonoids, terpenoids, glycosides, alkaloidand a saponin have previously been isolated from this plant. Methanol extract of the dried bark contain stigmasterol, oleic, linoleic acid was isolated from n-hexane extract. A new ligan glycerol, 2-methoxy-2-hydroxy propanoic acid, 3-methyl-2-hydroxy pentanoic acid and 2,3,4-trihydroxy butanal. 2-methoxy-2-hydroxy propanoic acid were isolated from *Aphanamixis polystachya* sub-fractions of an acetone extract of seeds .This fraction was isolated and analysed by gas chromatography-mass spectrometry. (Samir Kumar Sadhu *et al*.,2006).

Aphanamixoid A (1), a limonoid with a new carbon skeleton, along with its biogenetically related limonoid aphanamixoid B (2), was isolated from the leaves and twigs of *Aphanamixis polystachya*. Compounds 1 and 2 showed cytotoxic activity against two tumor cell lines. (T. Rabi *et al*., 1995).

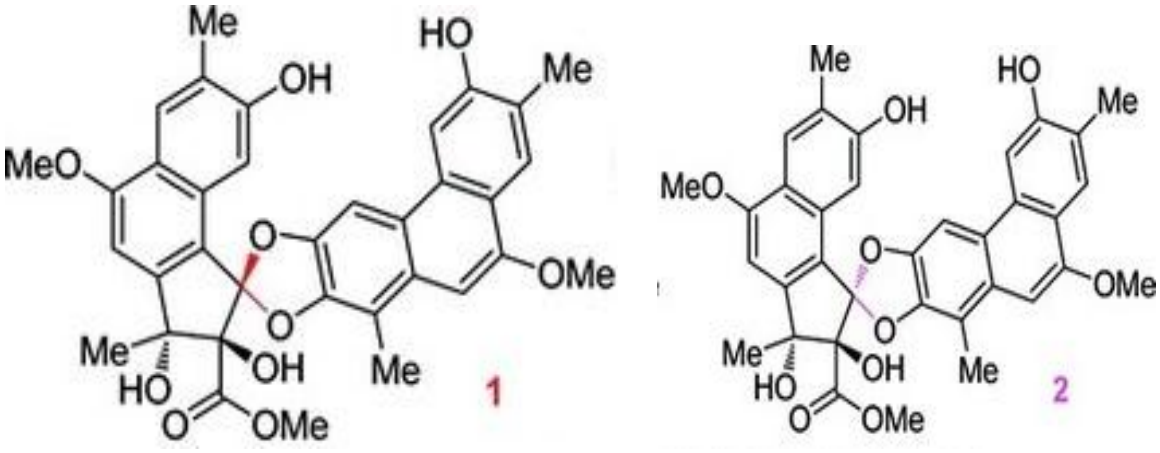
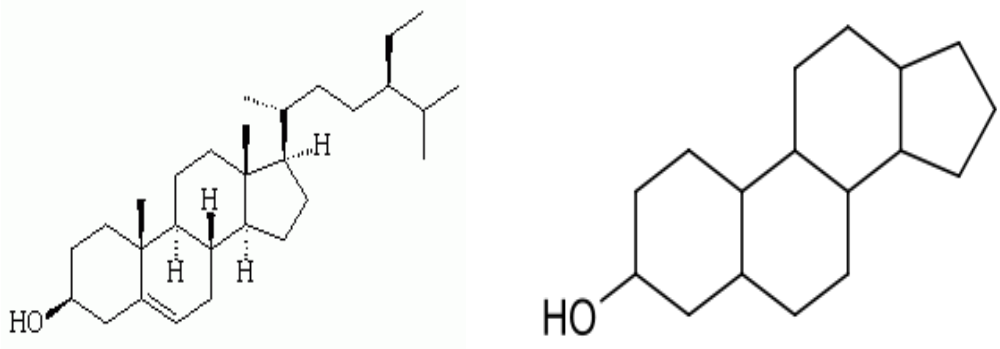


Figure 1.2**:** Structure of Aphanamixoid A (1) and Aphanamixoid B (2)

Phytosterols, more commonly known as plant sterols, isolated from *A. polystachya* and have been shown in clinical trials to block cholesterol absorption sites in the human intestine, thus helping to reduce cholesterol in humans. They are currently approved by the U.S. Food and Drug Administration for use as a food additive; however, there is some concern that they may block absorption, not only of cholesterol, but of other important nutrients as well. (Ostlund RE *et al*., 2003).



(1) (2)

Figure 1.3**:** Structure of Phytosterols (1) and Sterol (2)

Aphanamixis polystachya has been used in Asia as traditional medicines for a long time. Ergosta-4, 6, 8(14), 22-tetraen-3-one (ergone) is one of the chemical constitute isolated from *Aphanamixis polystachya* which possess cytotoxic activity. (Md. Mokarram Hossain *et al*., 2009)

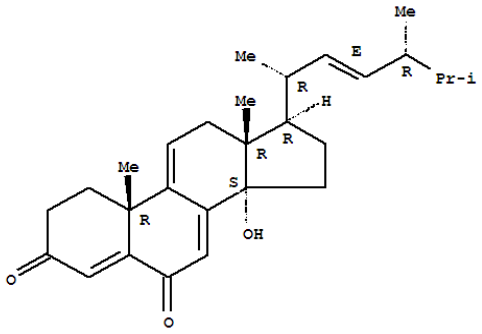


Figure 1.4**:** Structure of Ergosta-4,6,8(14),22-tetraen-3-one

The petroleum ether extract of the stem bark of *Aphanamixis polystachya* afforded two novel guaiane-derived sesquiterpenoids, 6β,7β-epoxyguai-4-en-3-one (1) and 6β,7β-epoxy-4β,5-dihydroxyguaiane (2) which possess promising antibacterial activity. (R. Chowdhury *et al*., 2010)

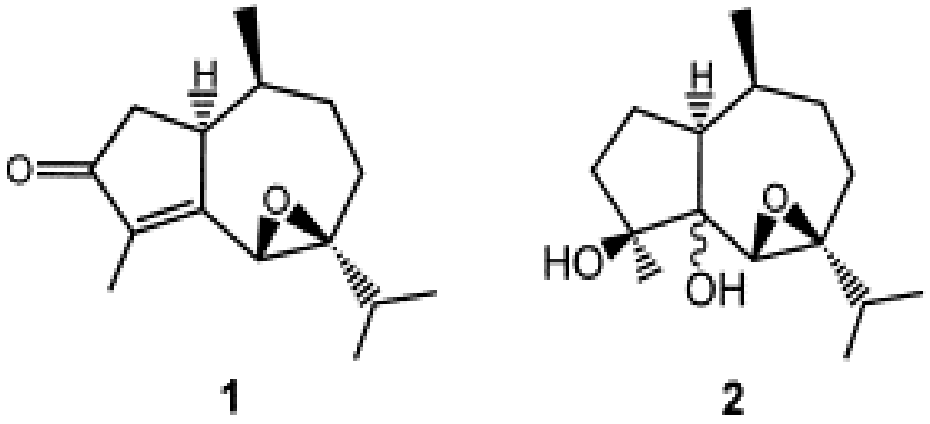


Figure 1.5**:** Structure of 6β,7β-epoxyguai-4-en-3-one (**1)** and 6β,7β-epoxy-4β,5-dihydroxyguaiane (**2**)

1.4.2 Pharmacological activity

The Superoxide radical scavenging activity of *A. polystachya* bark extracts was studied in compared with vitamin C. The methanol, aqueous and aqueous methanol extracts showed potent superoxide radical scavenging activity, as indicated by their IC_{50} values and 8.3 mg respectively compared to Vitamin C which showed IC_{50}, 125 µg. From a comparison of IC_{50} values *A. polystachya* extracts were found to be about 16 times more potent than that of vitamin C. (Kiselova *et al*., 2006)

A. polystachya bark extracts exhibited potent ABTS free radical scavenging activity compared to that vitamin C. The IC_{50} value indicated that the ABTS free radical scavenging activity of *A. polystachya* extracs was nearly 2 fold higher than vitamin C. (Agnihotri *et al*., 1987).

A. polystachya bark extracts exhibited superior ferric reducing antioxidant power as depicted compared to that of vitamin C. The EC_1 values indicated that the ferric reducing antioxidant potential of methanol extract was about 2 fold higher compared to vitamin C. (Awika *et al*., 2003).

Various experiment indicated that the extract of fruits of *A. polystachya* significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity is a measure of the level of excitability of the CNS (Mansur, 1980) and any decrease of this activity may be closely related to sedation resulting from depression of the central nervous system (Ozturk, 1996).

Administration of the crude extract of fruits of *A. polystachya* was produced a significant increase in the hypnotic effect induced by the thiopental sodium, in a dose dependent manner, thus suggesting a profound sedative activity (Kumar, 2008). (Chopra *et al*., 1956).

The methanol extract was also evaluated in the tail immersion, hot plate and acetic acid-induced writhing test for its analgesic activity. The hotplate method and tail immersion test are considered to be selective to examine compounds acting through opioid receptor, the *A. polystachya* extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. (Jain *et al*., 1985).

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

The effect of ethyl acetate fraction of *Aphanamixis polystachya* (EAP) was investigated on the radiation-induced chromosome damage in the bone marrow cells of Swiss albino mice exposed to various doses of gamma-radiation and EAP reduces radiation-induced chromosome damage. (Jagetia GC, Venkatesha VA., 2006)

1.5 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 and anxiolytic activity of fruits of *A. 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 Materials for extraction

Test samples

Test materials	
1.	*n*-hexane soluble solvent and methanol soluble solvent of
2.	Ethyl acetate soluble solvent
3.	Methanol soluble solvent

Glass wares

Materials		*Source*
1.	Quick fit flasks	East west University lab
2.	Conical flasks, Beakers, Test tubes, Funnels, Measuring cylinders, Pipettes etc.	East west University lab
3.	Pastur pipettes	East west University lab
4.	Automatic pipette puller	East west University lab

Solvents

Solvents		**Source**
1.	Methanol	Merck, Germany
2.	*n*- hexane	Merck, Germany
3.	Ethyl acetate	Merck, Germany

Filter aids

1.	Filter paper (what man no.1)
2.	Cotton (Martin cloth)
3.	Cotton (normal cloth)

Equipments

Equipments		*Source*
1.	Rotary vacuum evaporator	Rotary evaporator-HB4 basic IKA
2.	Electronic balance	Denver Instruments M-220
3.	Table-top UV detector (254 &366nm)	CAMAG
5.	Solvent distillation plant	University Instruments Lab
6.	Distilled water plant	University Instruments Lab

2.2 Materials for *in vivo* experimental test

2.2.1 Reagents, chemicals and equipments used for CNS depressants activity

Reagents and chemicals		**Source**
1.	Diazepam	Square Pharmaceutical Ltd, Bangladesh
2.	Tween-80 (as suspending agent)	
3.	Sterile normal saline solution (0.9% NaCl)	Beximco Infusion Ltd.

Equipments		**Source**
1.	Hole board & Hole cross box	Lab-5, East West university
2.	Sterile disposable syringe(1ml,100 division)	CHPL, India
3.	Tuberculin syringe with ball shaped end	Merck, Germany
4.	Electronic and digital balance	Denver Instruments M-220

2.2.2 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.

Materials used for animal houseing

Materials	
1.	Polyvinyl cages
2.	Soft wood shavings for bedding

Figure 2.1 : Swiss albino mice

2.3 Method of extraction

2.3.1 Selection of plants

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

2.3.2 Collection and identification of plants

The fresh fruit parts of the plant *Aphanamixis polystachya* were collected from the area of Mymensing district during the month of February, 2011. The *A. polystachya* was taxonomically identified by Dr. Bushra Khan, Chief Scientist and Taxonomist of Bangladesh. A duplicated specimen (DACB-35449) has been deposited in the Bangladesh National Herbarium.

2.3.3 Drying and pulverization

The fruit parts of the plant *Aphanamixis polystachya* were washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days and finally dried at 45ºC for 36 hours in an electric oven. 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.

2.3.4 Extraction of plant material

About 400 gm of fruit parts were washed, air dried and ground into course powder. It was extracted in soxhlet apparatus with alcoholic (95.0%), aqueous methanol (methanol: water, 50:50) and petroleum ether solvent for 36 hours. The solvents were then removed under pressure to yield brownish residue (yields sequentially: 12.51%, 05.82% and 02.41% w/w with respect to dried fruit part). The dried fruits were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 60°C.

The solvent was completely removed and obtained dried crude extract which was used for investigation.

2.3.5 Soxhlet apparatus

Soxhlet apparatus is consists of an extractor, a distillation still, a tubular condenser for the distillation still, a tubular condenser for the recovery of solvent from the marc, a receiver for collecting the condensate from the condenser, and a solvent storage tank. (Sherma, J., 2000).

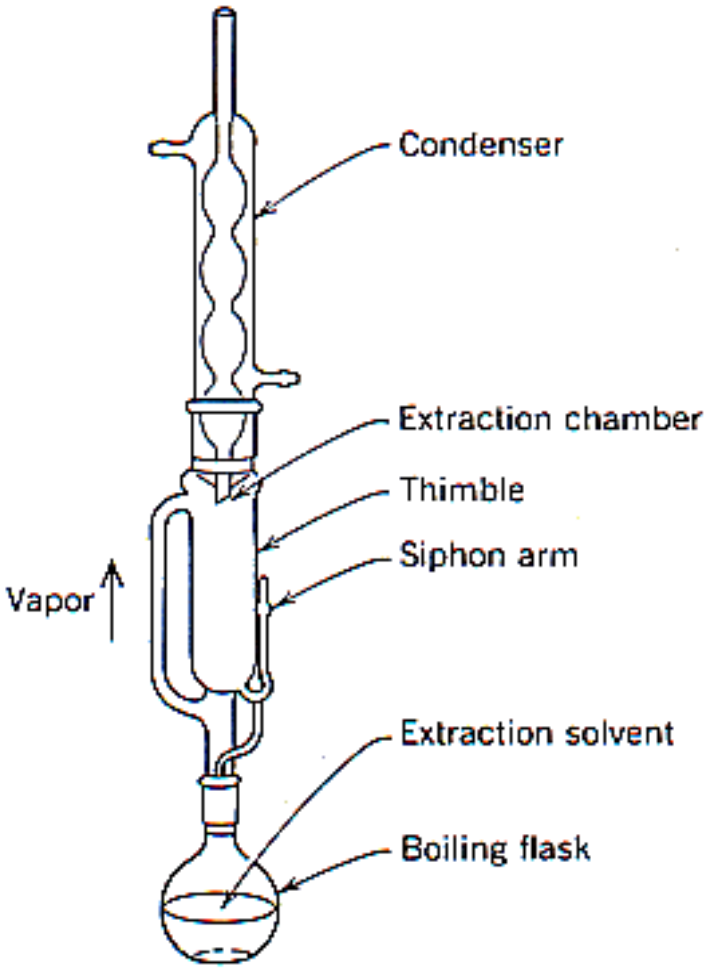


Figure 2.2**:** Soxhlet Apparatus.

2.3.6 Extraction through Soxhlet apparatus

This method involves bringing a material to be extracted (usually in solid form) into contact with the extraction solvent for a period of time, followed by separation of the solution from the solid debris. The plant material is fed into the extractor, and solvent is added until it reaches the siphon point of the extractor. Then, the extract is siphoned out into the distillation still, which is heated with steam. The solvent vapors go to the distillation condenser, get condensed and return to the extractor. The level of the solvent in the extractor again rises to the siphon point and the extract is siphoned out into the distillation still. In this way, fresh solvent comes in contact with the plant material a number of times, until the plant material is completely extracted. The final extract in the distillation still, which is rich in active principle, is concentrated and the solvent is recovered. (Sherma, J., 2000).

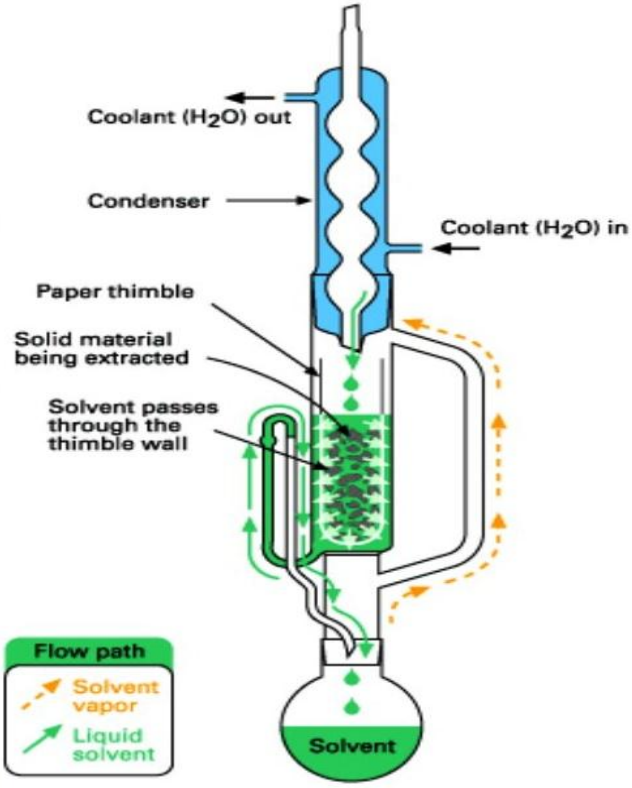


Figure 2.3**:** Extraction through Soxhlet apparatus.

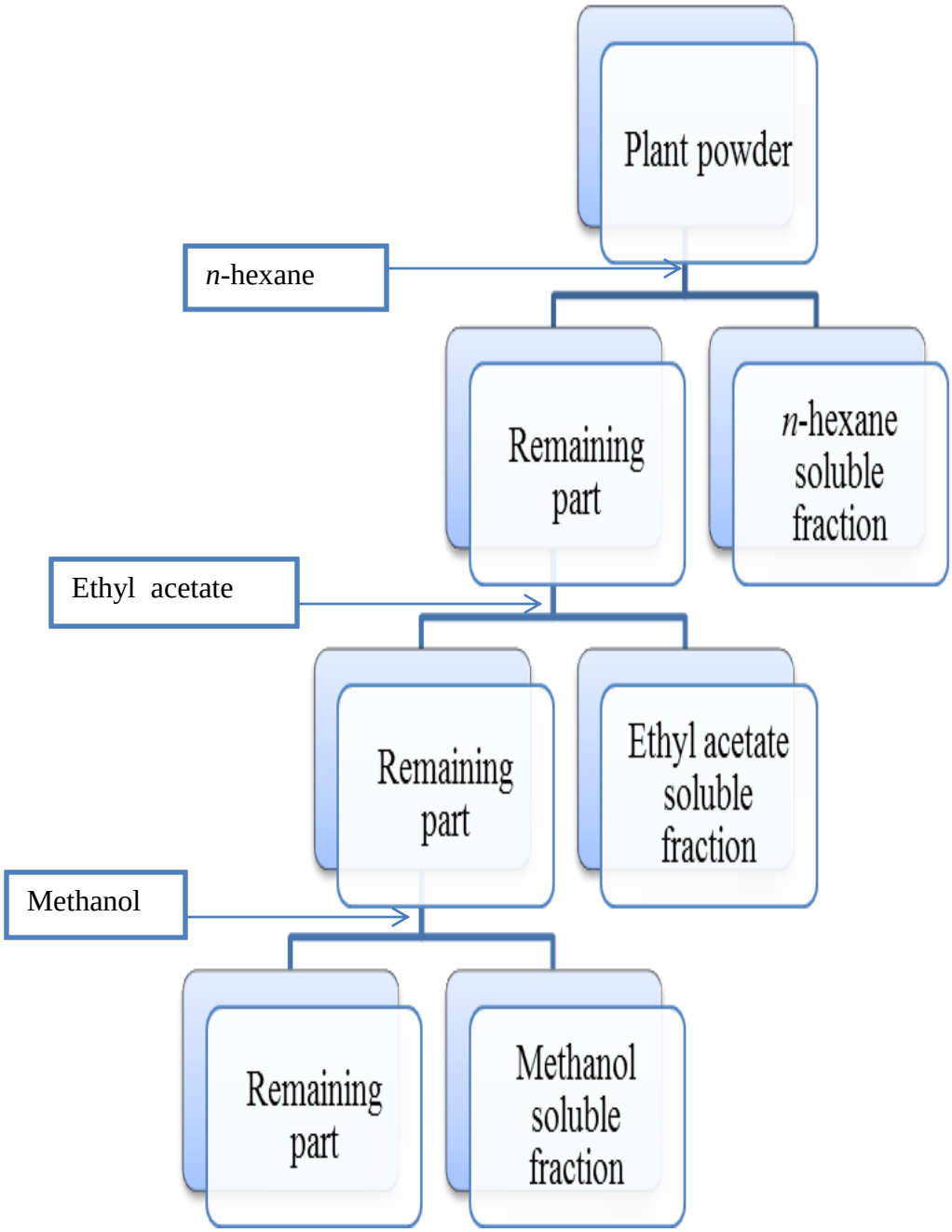


Figure 2.4**:** Schematic presentation of the extraction from fruits.

2.4 Method for *in vivo* hole cross , hole board and elevated plus maze test

2.4.1 Experimental design

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

2.4.2 Preparation of test materials

In order to administer the crude extract at dose of 400 mg/kg body weight of mice, 200 & 400 mg/kg of the extract was measured and was triturated unidirectional way by the addition of small amount of suspending agents Tween-80. After proper mixing of extract with normal saline and then slowly added normal saline 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 in negative group.

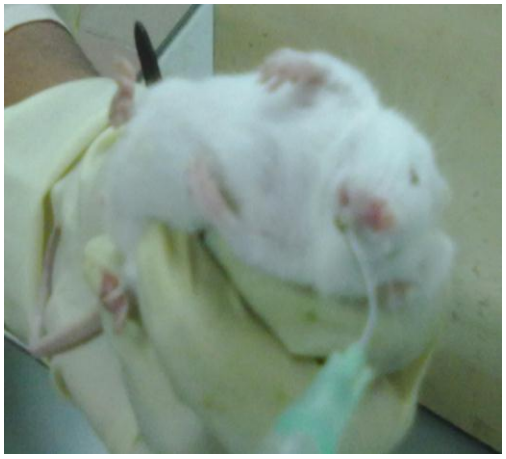


Figure 2.5 **:** Oral administration of test sample

2.5 Procedure of CNS depressant effect of the plant *A. polystachya*

2.5.1 The Hole Cross method

The hole cross method, as described by Takagi (Takagi *et al.*, 3) was adopted for screening CNS depressant activity in mice. A wood 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 center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of *Aphanamixis polystachya* extract at the doses of 200 & 400 mg/kg.

Figure 2.6**:** Crossing the hole of hole of cross apparatus.

2.5.2 Elevated Plus Maze test

Animals were randomly allocated to four experimental groups (n= 5 each). Group 1 and 2 were named as negative and positive control and 2 other groups were termed as treated group. The test groups received ethanolic extract of fruit of *Aphanamixis polystachya* at the dose of 200 and 400mg/kg body weight respectively. Group 1 received 1% Tween 80 solution. Group 2 got administration of Diazepam (as a standard drug) at 1mg/kg body weight. Drug or vehicle was injected intraperitoneally in a volume of 0.5ml/kg. Tests were performed 30 min after injections. (Navarro JF *et al*., 2006).

2.5.2.1 Apparatus and behavioral test

Plus-maze test consisted of two open arms (30 × 5 cm, surrounded by a 0.25-cm-high border) and two closed arms (30 × 5 cm, surrounded by 25-cm-high walls) with the two pairs of identical platform, which emerged from a central platform (5 × 5 cm), positioned opposite each other. The apparatus was elevated 40 cm above the floor. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Each session lasted 5 min, being recorded by a video camera. After each test, the maze was thoroughly cleaned. Behavioral analysis was performed by watching the recorded videos later. A number of classical parameters were collected during the session: (a) Open arm duration: the total amount of time the mouse spent in the open arms; (b) Closed arm duration: the total amount of time the mouse spent in the closed arms; (c) Central platform duration: the total amount of time the mouse spent in the central platform; (d) Open arm frequency: the frequency of mouse entry with all four paws into the open, unprotected arms; (e) Closed arm frequency: the frequency of mouse entry with all four paws into the closed, protected arms, and (f) Total number of entries in the arms.Likewise, different ethological measures were also quantified: (a) Rearings: a body stance in which the animal sets his forepaws onto the wall of a closed arm while keeping his rear legs on the floor; (b) Stretched attend posture (SAP): a body posture in which the mouse stretches forward and then retracts to its original position without moving the feet, and (c) Grooming: Itching of the face by the front legs. (Navarro JF *et al*., 2006).

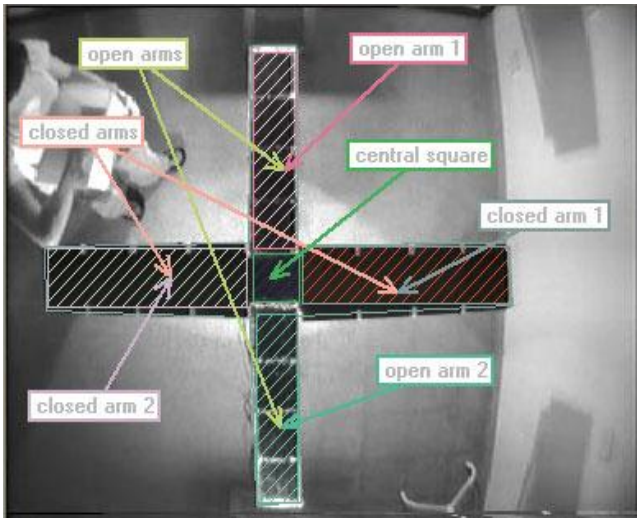


Figure 2.7**:** Elevated Plus Maze test apparatus.

2.5.3 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). The animals were randomly grouped into four groups each containing five mice. Group one served as the control group and was treated with 1% Tween 80 solution *i.p.* Group two got administration of Diazepam (as a standard drug) at 1mg/kg body weight. Drug or vehicle was injected intraperitoneally in a volume of 0.5ml/kg. Groups three and four were treated with the extract of fruit of *Aphanamixis polystachya* at the dose of 200 and 400mg/kg body weight respectively. 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. (Aiyelero OM *et al*., 2012; Perez GRM *et al*., 1998)

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.**

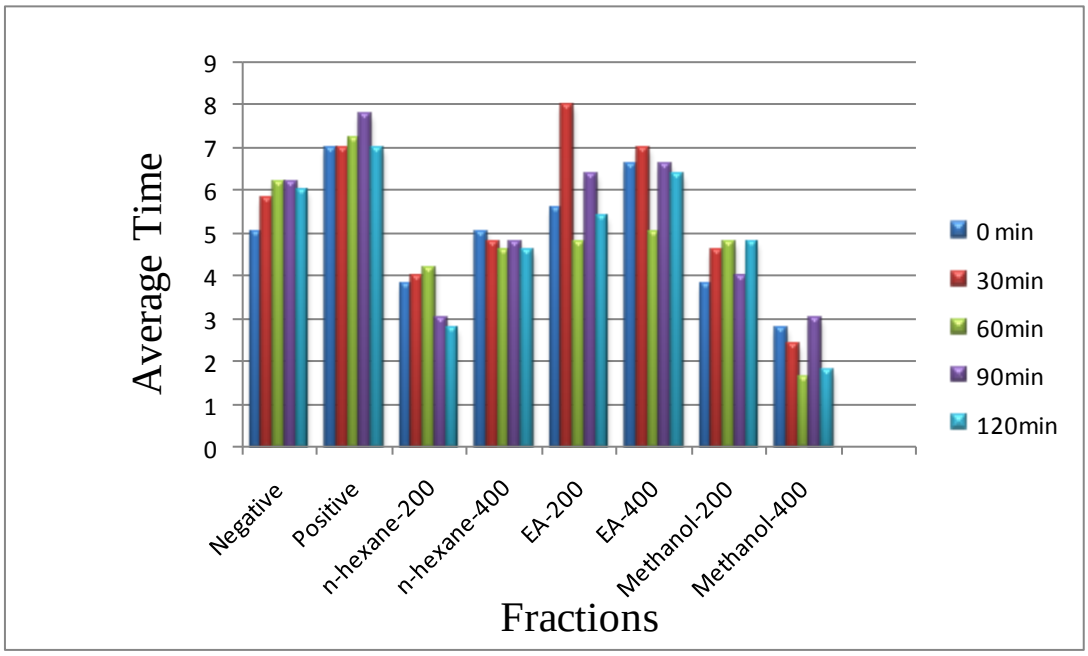


Figure 3.1: Average value of different solvent soluble fractions in Hole Cross experiment.

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.**

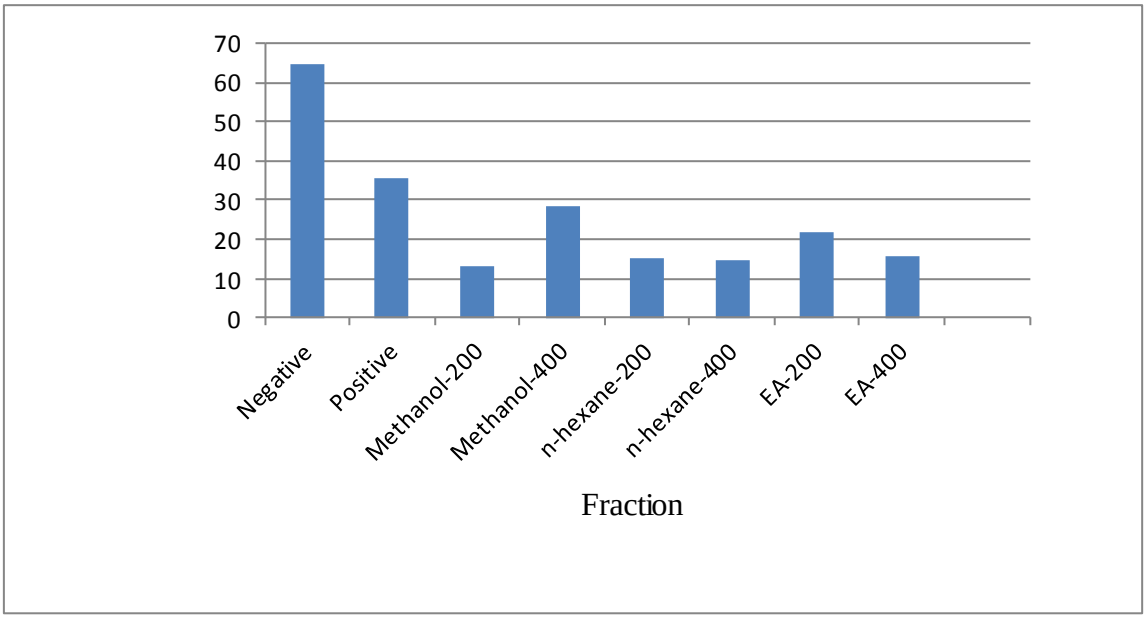


Figure 3.2: Average head dipping of mice for different solvent soluble fractions in Hole Board experiment.

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, $p<0.001$.

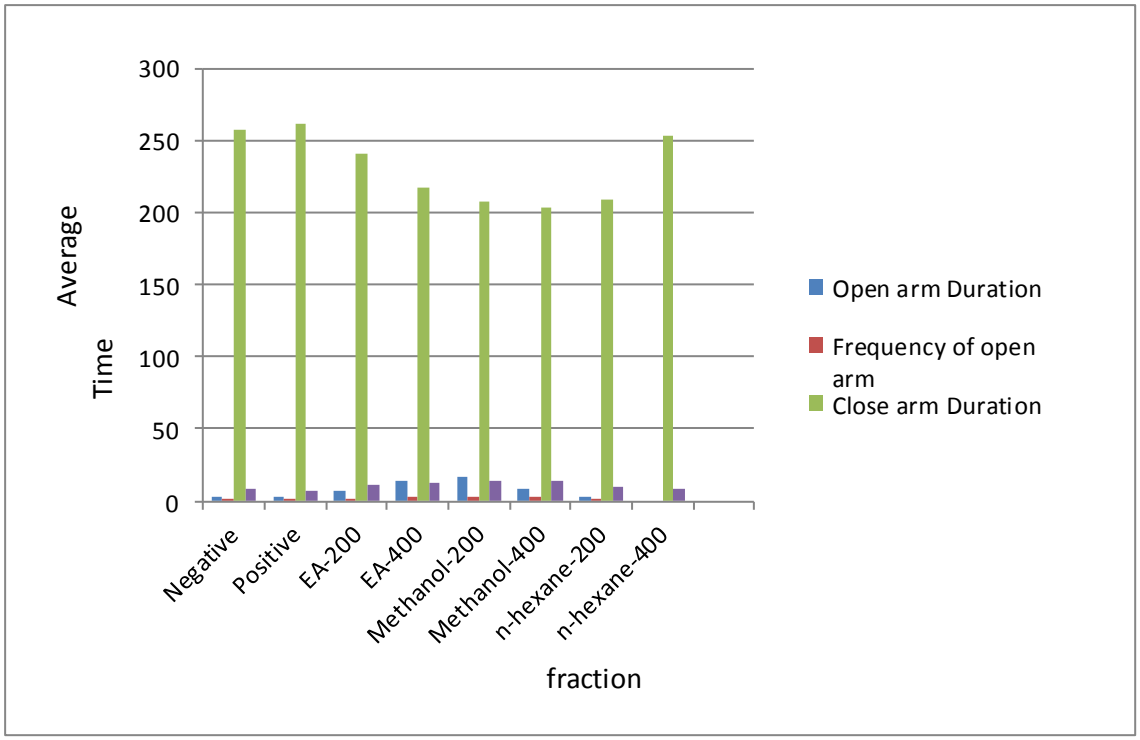


Figure 3.3: Average time duration in different arms and their frequency in Elevated Plus Maze experiment.

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 types of CNS disorders.

Chapter-5
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