Determination of CNS Depressant and Analgesic Activity of Bark of *Artocarpus chama*

A Dissertation Submitted to the Department of Pharmacy, East West University, in the Partial Fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

Submitted by

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Declaration by the Research Candidate

I, **Md. Rezaul Karim Rabby,** hereby declare that the dissertation entitled **"Determination of CNS depressant and Analgesic activity of Bark of *Artocarpus chama*"** submitted by myself to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2017, under the supervision and guidance of **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University and the dissertation has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

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This is to certify that the thesis **"Determination of CNS depressant and Analgesic activity of Bark of *Artocarpus chama*"** submitted to the Department of Pharmacy, East West University, Dhaka-1212, in partial fulfilment of the requirement for the Degree of Bachelor in Pharmacy, was carried out by **Md. Rezaul Karim Rabby**, ID: 2014-1- 70-020.

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Abstract

Phytochemical screening of *Artocarpus chama* revealed that the plant contains flavonoid, alkaloid. Flavonoid has anti-inflammatory and CNS depressant activity. The study was conducted to evaluate CNS depressant activity by open field and hole board test and analgesic activity by acetic acid induced writhing test and formalin induced pain method of bark of *Artocarpus chama.* In the study, it was found that the open field test of bark extract of methanol and pet ether has shown significant ($p<0.001$) decrease in locomotor activity in mice model compared to standard drug, diazepam. In case of Acetic acid induced writhing test, methanol and pet ether extracts at a dose of 400 mg/kg showed significant ($p<0.001$) decrease in writhing compared to standard drug, indomethacin. ACBM at a dose of 200 mg/kg and 400 mg/kg showed inhibition about 41.14% and 75.92% respectively. ACBP at a dose of 200 mg/kg and 400 mg/kg showed inhibition about 56.52% and 61.54% respectively. Formalin induced pain test, the bark extracts at a dose of 200 mg/kg and 400 mg/kg prevent the licking and biting activity of mice in a dose depending manner in the late phase compared to standard drug, ibuprofen. ACBM showed good inhibition about 42.32% in late phase at a dose of 200 mg/kg, ACBM at a dose of 400 mg/kg showed good inhibition about 58.36% in late phase. ACBP at a dose of 200 mg/kg and 400 mg/kg showed good inhibition in late phase about 66.04% and 51.19% respectively. It can be concluded that the plant might have CNS depressant activity and also analgesic activity.

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List of Abbreviations

ACBM	*Artocarpus chama* Bark in Methanol
ACBP	*Artocarpus chama* Bark in Pet ether
ICDDR, B	International Centre for Diarrhoeal Disease Research, Bangladesh
µg	Micrograms
ml	Milliliters
cm	Centimeters
mm	Millimeters
kg	Kilograms
g	Grams
min	Minute
WHO	World Health Organization

Dedication

DEDICATED TO MY PARENTS

Chapter 1

Introduction

1.1 Overview

Herbal medicines are those which are prepared directly from plants. It may be plants leaf, root, bark, etc. Herbal medicine is also known as traditional medicine and it is still widely practiced. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on scientific method. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs (Ahmed *et al*., 2001).

A natural product is a chemical compound or substance produced by a living organism—that is, found in nature. Natural products can also be prepared by chemical synthesis (both semi synthesis and total synthesis) (Ahmed *et al*., 2001).

Natural products sometimes have therapeutic benefit as traditional medicines for treating diseases, yielding knowledge to derive active components as lead compounds for drug discovery. Although natural products have inspired numerous U.S. Food and Drug Administration-approved drugs, drug development from natural sources has received declining attention by pharmaceutical companies, partly due to unreliable access and supply, intellectual property concerns, seasonal or environmental variability of composition, and loss of sources due to rising extinction rates. Synthetic and semi synthetic drug is also detrimental to health, because these drugs can be carcinogenic as well as costly (Ahmed *et al*., 2001).

1.2 Herbal medicine

Usually ‘herb’ means plant or plant parts used for treatment of different kinds of diseases in human or animal. It can be any part of a plant like seeds, berries, bark, root, leaf, fruits and even flowers. Herbal medicine involves the use of herbs for the betterment of our health and also to treat different kind of diseases.

Traditionally herbal medicine has been used to treat diseases, with a written history of 5000 years old. Even now 75% of the world population still rely on herbal medicine for cure, mitigation and prevention (AHG n.d.). Herbal medicine is used for a variety of reasons such as for the treatment of allergies, asthma, eczema, premenstrual syndrome, rheumatoid arthritis, fibromyalgia, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome and cancer. There are different physical forms of an herbal medicine that are sold out in stores, such as teas, syrups, oils, liquid

extracts, tinctures and dry extracts. Teas are prepared by soaking the dry herb in a hot water, syrups are prepared from concentrated extracts and added to sweet testing preparations, oils are directly extracted from plants, tinctures are prepared by dissolving the active ingredient directly in a liquid such as water, alcohol or glycerol (AHG n.d.). Herbal medicine is not scientifically proven but people use it by traditional belief. There is no specific ingredient which is giving the beneficial effect, it may be combination of many other ingredient. Environment has great impact on the activity of the plant such as temperature, humidity, dew point, nature of the soil, amount of raining in a country all of these has a great influence on the plant. (AHG n.d.). People of America use herbal medicine as capsule, supplementary, extracts, tinctures and also as teas. Some of known herbal medicine which are used for-

- Aloe is used for sunburns, minor burn, skin irritation and inflammation.
- Arnica is used for bruises, sprains, sore muscles and joints
- Chamomile tea is ingested for upset stomach, heartburn, indigestion and colic
- Echinacea is ingested for colds, flu, sore throat
- Garlic is ingested to possibly reduce cholesterol and blood pressure, treat fungal infections and colds
- Ginger is ingested for nausea and motion sickness and as an anti-inflammatory
- Mullein is ingested for chest congestion and dry, bronchial coughs
- Passionflower is ingested for non-sedating relaxation
- Peppermint tea is ingested for indigestion, nausea and other digestive problems
- Peppermint oil (in enteric-coated capsules) is ingested for irritable bowel syndrome and other chronic intestinal ailments
- Tea tree oil is applied topically for fungal infections such as athlete's foot and fungal infections of the toenails and fingernails
- Turmeric is ingested to combat inflammation and protect against cancer and Alzheimer's disease

- Valerian is ingested for sleeping problems (Weil n.d.).

Despite of the beneficial effect, herbal medicine might have side effects too. Side effects become more common if there is a mixture of herb and drugs. For example, if we mix anticoagulant drug with ginkgo which is natural blood thinner. So, this kind of drugs should be avoided before surgery otherwise it could affect our blood pressure and heart rate. On the other hand, herbs which causes sedation should be avoided at least 14-15 days before surgery. Special caution should be taken in case of pregnancy, it is better to avoid any herb in pregnancy especially in the first trimester. In some consideration like 1000 mg of ginger in capsule or candied forms is beneficial for morning sickness. But without the advice of a profession practitioner one should not take any kind of herbs since it may harm the fetus. Women who are breastfeeding should avoid herbs for the first four to six months of baby's life (Weil n.d.). Although the herbal medicines are not scientifically proven, the use of herbal medicine is still taught in medical and pharmacy schools. Practitioner are also very much interested in knowing the beneficial and adverse effect of herbal medicines.

1.3 Therapeutically active ingredient isolated from nature

Herbal medicines or drugs are derived from plants, it can be plants different parts like root, barks, leaf, etc. The active constituent of this plants is used to develop a drug such as anti-inflammatory, analgesic, CNS depressant, CNS stimulant, cardiovascular drugs, anti-diabetic, anti-obesity, anti-malarial, anti-viral, and anti-neoplastic agents. But all the time it is not necessary that the active constituent will be beneficial to us, it might be harmful too. In that case, those active constituents are neglected. This may be due to the complex nature of plants as they contain a number of phytoconstituents. There are a limited number of pharmaceutical company who manufacture drugs this may be due to the high cost involved in isolation and identification of pure compounds, difficulty in collection, the complex nature of plants. In the conventional drug discovery process, a single pure active constituent is isolated, purified, and standardized. If the herbal contains multiple active constituent then it can be separated by DNA fingerprinting, high-pressure thin layer chromatography (HPTLC), and liquid chromatography–mass spectroscopy (LCMS). (Sardana, 2012)

Table 1.1: Some of the drugs that are derived from the natural sources

Drugs	Action	Plant source
Acetyldigoxin	Cardiotonic	*Digitalis lanata* (Grecian foxglove, woolly foxglove)
Allantoin	Vulnerary	Several plants
Atropine	Anticholinergic	*Atropa belladonna* (deadly nightshade)
Benzyl benzoate	Scabicide	Several plants
Caffeine	CNS stimulant	*Camellia sinensis* (tea, also coffee, cocoa and other plants)
Camphor	Local anaesthetic	*Erythroxylum coca* (coca plant)
Codeine	Analgesic, antitussive	*Papaver somniferum* (poppy)
L-Dopa	Anti-parkinsonism	Mucuna species (nescafe, cowage, velvetbean)
Digitoxin	Cardiotonic	*Digitalis purpurea* (purple foxglove)
Digoxin	Cardiotonic	*Digitalis purpurea* (purple or common foxglove)
Ephedrine	Sympathomimetic, antihistamine	*Ephedra sinica* (ephedra, ma huang)
Hyoscyamine	Anticholinergic	*Hyoscyamus niger* (black henbane, stinking nightshade, henpin)
Menthol	Rubefacient	Mentha species (mint)

Morphine	Analgesic	*Papaver somniferum* (poppy)
Nicotine	Insecticide	*Nicotiana tabacum* (tobacco)
Papavarine	Smooth muscle relaxant	*Papaver somniferum* (opium poppy, common poppy)
Physostigmine	Cholinesterase inhibitor	*Physostigma venenosum* (Calabar bean)
Pilocarpine	Parasympathomimetic	*Pilocarpus jaborandi* (jaborandi, Indian hemp)
Quinidine	Antiarrhythmic	*Cinchona ledgeriana* (quinine tree)
Quinine	Antimalarial	*Cinchona ledgeriana* (quinine tree)
Reserpine	Antihypertensive, tranquilizer	*Rauvolfia serpentina*
Strychnine	CNS stimulant	*Strychnos nux-vomica* (poison nut tree)
Theophylline	Diuretic, bronchodilator	*Theobroma cacao* and others (cocoa, tea)
Vinblastine	Antitumor, Antileukemic agent	*Catharanthus roseus* (Madagascar periwinkle)
Vincristine	Antitumor, Antileukemic agent	*Catharanthus roseus* (Madagascar periwinkle)

(Helmenstine, 2017)

1.4 History of mankind using medicinal plants

1.4.1 Pre-history

Archaeological evidence indicates that humans were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Plant samples that were collected from prehistoric burial sites claim that Paleolithic peoples had knowledge of herbal medicine, for instance, a 60,000-year-old Neanderthal burial site, “Shanidar IV”, in northern Iraq has yielded large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies. Medicinal herbs were found in the personal effects of Ötzi the Iceman, whose body was frozen in the Ötztal Alps for more than 5,000 years. These herbs appear to have been used to treat the parasites found in his intestines. In Mesopotamia, the written study of herbs dates back over 5,000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants (such as myrrh and opium) (The Master Herbalist, 2015).

1.4.2 Ancient history

In primordial Egypt, the primordial Egyptians wrote the Ebers Papyrus around 1500 BC which contains information about 850 medicinal plant including garlic, juniper, cannabis, castor bean, aloe, and mandrake. Herbs used by Egyptian healers were mostly indigenous in origin, although some were imported from other regions like Lebanon. Other than papyri, evidence of herbal medicine has also been found in tomb illustrations or jars containing traces of herbs. In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 4,000 BC. Earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charakaand Sushruta during the 1st millennium BC. The *Sushruta Samhita* attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources. By 2,000 BC, Chinese started prescribing to herbal medicines. In China, seeds likely used for herbalism have also been found in the archaeological sites of Bronze Age China dating from the Shang Dynasty. The first Chinese pharmacopoeia was first written by emperor Shennong. The “Shennong Ben Cao Jing”. The “Shennong Ben Cao Jing” lists 365 medicinal plants and their uses – including Ephedra (the shrub that introduced the

drug ephedrine to modern medicine), hemp, and chaulmoogra (one of the first effective treatments for leprosy). Succeeding generations augmented on the *Shennong Bencao Jing*, as in the *Yaoxing Lun(Treatise on the Nature of Medicinal Herbs)*, a 7th-century Tang Dynasty treatise on herbal medicine. The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century BC, and one by Krateuas from the 1st century BC. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates 460BC (e.g. *De herbis et curis*) and – especially – Galen (e.g. *Therapeutics*), provided the pattern for later western medicine. *De Materia Medica* remained the authoritative reference of herbalism into the 17th century. Similarly, important for herbalists and botanists of later centuries was Theophrastus' *Historia Plantarum*, written in the 4th century BC, which was the first systematization of the botanical world (The Master Herbalist 2015).

1.4.3 Middle ages

Benedictine monasteries were the primary source of medical knowledge in Europe and England during the Early Middle Ages. However, most of these monastic scholars' efforts were focused on translating and copying ancient Greco-Roman and Arabic works rather than developing new ideas and information's. Many information on medicine by Greek and Roman were preserved by hand copying of manuscripts in monasteries. he monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. At the same time, folk medicine in the home and village continued uninterrupted, supporting numerous wandering and settled herbalists. Among these were the "wise-women" and "wise men", who prescribed herbal remedies often along with spells, enchantments, divination and advice. It was not until the late Middle Ages that women and men who were knowledgeable in herb lore became the targets of the witch hysteria. One of the most famous women in the herbal tradition was Hildegard von Bingen. A 12th-century Benedictine nun, she wrote a medical text called *Causae et Curae* (The Master Herbalist 2015).

1.4.4 Early modern era

The15th, 16th, and 17th century was the golden period of herbal medicine because many of the books were re-written in English which helps many people to understand. The first herbal to be published in English was the anonymous *Grete Herball* of 1526. The two best-known herbals in English were *The Herball or General History of Plants* (1597) by John Gerard and *The English Physician Enlarged* (1653) by Nicholas Culpeper. Gerard's text was basically a pirated translation of a book by the Belgian herbalist Dodoens and his illustrations came from a German botanical work (The Master Herbalist 2015).

1.5 Usage of medicinal plants in India

About 80% of the world's population relies solely or largely on traditional remedies for their healthcare needs. Today, about 70,000 to 80,000 plant species are used for medicinal or aromatic purposes globally. India with its ecological, geographical and climatic diversities is perhaps the richest nation with a vast herbal medicinal wealth (About 15000-20000 plants have good medicinal value). In India the therapeutic use of herbs dates back to the Vedic period. The Rigveda has documented about 67 medicinal plants, Yajurveda 81 species and Atharvaveda 290 species. With the increasing esteem of herbal medicine and Ayurveda, use of medicinal plants is expected to rise globally. The popularity of herbs has increased because of side effects of synthetic drugs, development of resistance to many drugs like antibiotics, public awareness, population explosion, insufficient supply of drugs, and high cost of synthetic drugs.

Medicinal plants used in India such as- Amla, Ashok, Aswagandha, Brahmi, Chirata, Kalmegh, Sandal wood, Sarpa gandha, Tulsi, henna, Ghritkumari, Nim, Vasa, Nageswar, Kantakari. (Liveayurved n.d.)

1.6 Usage of medicinal plants in Bangladesh

Traditional medicines are those medicine which are being used unchanged over the past year, that's why they are called traditional medicine. It has been used for the treatment of physical and psychological diseases. Most of the times, the type, preparation, and uses of traditional medicines are largely influenced by folklore customs and the cultural habits, social practices, religious beliefs and, in many cases, superstitions of the people

who prescribe or use them. First use of traditional medicine was found in Rigveda, the oldest repository of knowledge in this subcontinent, Later Ayurveda, developed from the Vedic concept of life, becomes the important source of all medicines. Traditional medicine includes not only medicinal substances of natural origin but also items like magic, charms, incantations, religious verses, spiritual methods, amulets, sacrifices, folklore customs, and even physical and mental tortures. For these reasons, the forms of traditional medicine practiced today vary from highly organized and long-established Chinese, Ayurvedic and Unani systems to various Folk medical practices, such as herbalism, spiritualism, and religious medical practices. But most of the medicines are still practiced in the same way as were practiced in the past, they are collectively called traditional medicine. Traditional medicine composed of material and non-material component. The material component composed of parts or organs of plants and their products. They also consist of animal organs, minerals and other natural substances. The non-material components, which constitute important items of religious and spiritual medicines, include torture, charms, magic, incantations, religious verses, amulets and rituals like sacrifices, appeasement of evil spirits, etc. Diagnosis of disease were done by the symptoms of patient mainly from physical and psychological symptoms. The symptoms are determined by directly asking the patient. Traditional medicines are directly applied on the patient externally or internally, Treatments in traditional medicine are carried out by internal and external application of medicaments, physical manipulation of various parts of the body, performing rituals, psychological treatment, and also by minor surgery. Some of the older forms of traditional medicines, particularly the religious, spiritual and folkloric ones, are still used in Bangladesh. However, Ayurvedic and Unani medicines are widely used in Bangladesh as they have gone through extensive change through modernization. They are now practiced along with allopathic medicines as an alternative and supplementary system of medicine in Bangladesh. Medicinal products of Unani and Ayurvedic are prepared by modern pharmaceutical technology. These medicines are dispensed as broken pieces or coarse, as fine powders, as different sizes of pellets, as compressed tablets, as liquid preparations, as semi solids and in the form of creams and ointments neatly packed in appropriate sachets, packets, aluminum foils, plastic or metallic containers and glass bottles. The containers and bottles are appropriately labelled with dosing, usage, indications, contraindications, etc.

There are about two dozen registered herbal pharmaceuticals in Bangladesh. Of which about four big pharmaceuticals (Sadhana, Sakhti, Kundeswari and Hamdard) are now producing more than 80 percent of the traditional remedies. There is at least one traditional medicine shop in every market. These are controlled by Bangladesh Unani and Ayurvedic Board. Both the Ayurvedic and Unani systems of traditional health care have firm roots in Bangladesh and are widely practiced all over the country. Traditional medicine plays a very important role in Bangladesh, particularly at the primary health care level, as an estimated 70 to 75% people of the country still use traditional medicine for management of their health problems.Both Ayurvedic and Unani medicines have strong roots in Bangladesh, and it is widely practiced in Bangladesh. There are about 6,000 registered and 10,000 unregistered practitioners who practiced these systems in Bangladesh. There are about 15 government recognized and funded educational institutions which teaches about these systems. Among these systems, there are 10 institutions which teaches about Unani systems and 5 institutions which teaches about Ayurvedic systems. These institutions offer a four-year diploma course and six-month internship training. There are about 400 students in the institutions. Since the 1989-90 academic session a Government Unani and Ayurvedic Degree College, affiliated to the university of Dhaka, has been established in Dhaka. This college offers a five-year degree course and one-year internship training in an attached 100-bed Traditional Medical Hospital (LIVEAYURVED n.d.).

1.7 Traditional usage of *Artocarpus chama*

- The juice of stem bark (5-10 mL, 3-4 times daily) was given orally in the treat-

  ment of Diarrhea
- *Artocarpus* species (Moraceae) provide a variety of prenylated flavonoids and a Limited number of stilbenoids with interesting biological activities, such as cytotoxicity, antibacterial effects.
- Roots were used for treating dysentery (Ahmed *et al.*, 2012).

1.8 Family of *Artocarpus chama*

Artocarpus chama lies under the Moraceae family. The Moraceae are often called the mulberry family or fig family. They are a family of flowering plants comprising about 38 genera and over 1180 species. Most are widespread in tropical and subtropical regions, less so in temperate climates. The only synapomorphy within Moraceae is presence of laticifers and milky sap in all parenchymatous tissues, but generally useful field characters include two carpels sometimes with one reduced, compound inconspicuous flowers, and compound fruits. The family includes well-known plants such as the fig, banyan, breadfruit, mulberry, and Osage-orange. The 'flowers' of Moraceae are often pseudanthia (reduced inflorescences) (Wikipedia, 2017).

1.9 Genus of *Artocarpus chama*

Artocarpus is a genus of approximately 60 trees and shrubs of Southeast Asian and Pacific origin, belonging to the mulberry family, Moraceae. Most species of Artocarpus are restricted to Southeast Asia; a few cultivated species are more widely distributed, especially *A. altilis* (breadfruit) and *A. heterophyllus* (jackfruit), which are cultivated throughout the tropics. The extracts and metabolites of Artocarpus particularly those from leaves, bark, stem and fruit possess several useful bioactive compounds and recently additional data are available on exploitation of these compounds in the various biological activities including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, tyrosinase inhibitory and cytotoxicity. All Artocarpus species are laticiferous trees or shrubs that are composed of leaves, twigs and stems capable of producing a milky sap. The fauna type is monoecious and produces unisexual flowers; furthermore, both sexes are present within the same plant. The plants produce small, greenish, female flowers that grow on short, fleshy spikes. Following pollination, the flowers grow into a syncarpous fruit, and these are capable of growing into very large sizes. The stipulated leaves vary from small and entire (*Artocarpus integer*) to large and lobed (*Artocarpus altilis*), with the cordate leaves of the species *A. altilis* ending in long, sharp tips (Wikipedia, 2017).

1.10 Description of the plant

1.10.1 Plant Name

Artocarpus chama

1.10.2 Common Names

Sam (Asam), Chapalish (English), Chapalash (Hindi). (COL, 2017)

1.10.3 Synonyms of this plant

Artocarpus chama Buch. -Ham, *Artocarpus chama* Buch. -Ham. ex Wall, *Artocarpus melinoxylus* Gagnep, *Ficus chrysophthalma* (Miq.) Miq, *Saccus chaplasha* (Roxb.) Kuntze, *Urostigma chrysopthalmum* Miq. (COL, 2017)

1.10.4 Vernacular name

Chaplash

1.10.5 Classification

Kingdom- Plantae

Phylum- Tracheophyta

Class - Magnoliopsida

Order - Rosales

Family – Moraceae

Genus- *Artocarpus*

Species –*Artocarpus chama.* (GBIF n.d.)

1.11 Morphology of the plant

Trees to 40 m tall, deciduous. Bark black, gray, or brown, coarse. Branchlets furrowed when dry, 4-8 mm thick, pubescence rust-colored to reddish yellow, hairs long and spreading to bend. Stipules amplexicaul. Leaves spirally arranged; petiole brown, 1.5-4.5 cm, densely pubescent; leaf blade elliptic, oblong, or ovate, 13-37 × 6- 21 cm, abaxially densely rust-colored to grayish white pubescent but more densely so along veins, adaxially glabrous or with sparse bent hairs, base broadly cuneate to rounded, margin entire or ± crenate, apex acute to shortly acuminate; secondary veins 9-18 on each side of midvein, apically curved, and joined together near margin, tertiary veins reticulate and with dark brown glandular points. Inflorescences axillary, solitary. Male inflorescences ellipsoid, ovoid, or clavate, 1.2-2.3 × 1-1.8 cm; bracts shield-shaped; pedicel ca. 2 mm, shortly pubescent. Female inflorescences globose to ellipsoid; bracts peltate. Style exerted. Male flowers: calyx lobes 2 or 3, ca. 5 mm, margin ciliate; filaments short; anthers ellipsoid. Fruiting syncarp yellow when young then rust-colored brown, ± globose, 5-6 cm in diam.; peduncle 1.5-4.5 cm, with short brown hairs; persistent calyx separating near top, with several persistent bracts. Drupes ellipsoid, ca. 10 × 6 mm (EOL 2013).

1.11.1 Habit

Tree

1.11.2 Habitat

Evergreen forest in the humid tropical zone or in areas with a relatively mild monsoon climate.

1.11.3 Distribution

Bangladesh, Bhutan, India, Laos, Malaysia, Myanmar, Sikkim, Thailand (EOL 2013).

1.11.4 Cultivation Details

A plant of the moist to wet tropics where there is a distinct dry season, it is found at elevations up to 1,650 meters. It grows best in areas where annual daytime temperatures are within the range 22 - 32°c, but can tolerate 10 - 40°c. It prefers a mean annual rainfall in the range 3,000 - 4,000mm, but tolerates 2,000 - 5,500mm. Mature trees grow best in full sun or light shade, though younger trees require some shade Grows best in a fertile, medium to heavy, well-drained soil. Prefers a pH in the range 5 - 6.5, tolerating 4 - 7.5. Wood production may be 15 - 40 cubic meters per hectare per year. (Fern 2014).

1.11.5 Edible Uses

The subglobose fruit can be 6 - 10cm in diameter. (Fern 2014)

1.12 Physical anatomy of the plants

Figure 1.1: Leaf of *Artocarpus chama*

Figure 1.2: Bark of *Artocarpus chama*

Figure 1.3: Fruit of *Artocarpus chama*

1.13 Some medicinally important plants under Moraceae family

1.13.1 Artocarpus Heterophyllus Lamk

Taxonomic description: A medium-sized to large evergreen tree, with rather short trunk and large, dense, rounded crown. Leaves 10-20 cm long, elliptic, entire, thickly coriaceous. Flower heads embraced by spathaceous, deciduous, stipular sheaths; male cylindric, 5-15 cm long. Fruits large, 30-75 cm long, round to oblong, tubercles (Rahman and Khanom, 2013).

Local name: Khanthal.

Habit: Tree

Flowering season: February-July.

Fruits: Well developed young fruits are cooked as vegetables. Pulp of ripe fruits is eaten fresh, sometimes made into various delicacies. The rind of the fruit is also a good cattle feed. The unripe fruits are astringent, carminative and tonic; the ripe fruit is laxative, oleaginous, tonic, fattening and aphrodisiac (Rahman and Khanom, 2013).

Leaf: Leaf ash is useful in healing ulcer. Young leaves are used to treat skin diseases, asthma and diarrhea (Rahman and Khanom, 2013).

Roots: Roots are used internally in diarrhea (Rahman and Khanom, 2013).

Seeds: The seeds are cooked as vegetables, eaten after boiling or roasting. The seeds are diuretic, aphrodisiac and constipating. Roots are used in diarrhea.

Latex: The latex of the plant is applied externally to glandular swelling and abscesses to promote suppuration (Rahman and Khanom, 2013).

1.13.2 *Artocarpus lacucha* Buch-Ham

Taxonomic description: A medium-sized, deciduous tree with large dense spreading crown. Leaves 10-30 cm long, coriaceous, oblong, elliptic or subovate, entire. Flowers in axillary globose, shortly pedunculate heads. Fruit 5-7.5 cm across, lobulated, yellow when ripe (Rahman and Khanom, 2013).
Local name: Dewa, Bonkanthal.

Habit: Tree

Flowering season: April-June.

Fruits: Fruits are edible.

Leaf: Alcoholic extract of the leaves possesses good antibacterial properties.

Seeds: Seeds are popular as a purge. In case of breast-feeding babies, 3-4 seeds are made into paste and mixed with mother's milk, and administered to cure constipation.

Roots: A yellow dye is obtained from root.
Bark: An infusion of the bark is applied for small pimples and cracked skin. The bark finely powdered is applied to sores to draw out the purulent matter

1.13.3 Ficus Benghalensis *L.*

Taxonomic description: A large spreading, evergreen or semi-deciduous tree of low stature (trunk), sending down many aerial roots from the branches. All parts contain white latex. Leaves coriaceous, 10-20 cm long, ovate or elliptic, entire. Receptacles about 2 cm diam., sessile, in pairs, axillary, globose, puberulous, red when ripe.

Local Name: Bot, Botgach.

Habit: Tree

Flowering season: January to December.

Ethno-botanical uses:

Whole plant: The tree is worshipped by the Hindus in various parts of the Indian sub-continent. The young maids worship the tree for getting good husband. The numerous hanging roots are considered symbolic other matted hair of long departed Guru. The

ethnic people of Bangladesh also consider the tree sacred and as an abode of their gods.

Latex: Latex of the plant is aphrodisiac, tonic, vulnerary and maturing; used in toothache, dysentery, diarrhea, piles and diabetes; applied externally to cracked or inflamed soles, to alleviate rheumatic pains and lumbago. It lessens inflammations.

Roots: The aerial root is styptic and aphrodisiac. Tips of the hanging roots are given for obstinate vomiting (Rahman and Khanom, 2013).

Bark: An infusion of the bark is tonic; used for the treatment of diabetes. Bark extract is anti-diabetic in rabbit; decoction showed strong anti-fertility activity in man and women (Rahman and Khanom, 2013).

Leaf: The leaves are vulnerary; useful in biliousness; warm leaves are applied to abscesses. An infusion of the young buds is useful in diarrhea and dysentery.
Seeds: Seeds are cooling and tonic.

1.13.4 *Ficus Elastica* Roxb ex Hornem

Taxonomic description: A medium to large evergreen tree with milky latex. Trunk and young shoots glabrous, crown very dense, branches spreading, with or without narrow aerial hanging roots. Leaves simple, alternate, petiolate, petioles 3-6 cm long, lamina oblong 22-25 by 14-16 cm, thick glossy above, base cuneate to obtuse, margin entire, apex shortly acuminate, lateral nerves almost parallel, stipules large, rosy or pinkish brown when young. Male flowers small pedicellate, sepal 3-4 ovate, stamen 1, anthers 2. Female flowers sessile, sepals 4. Free ovary with subterminal long styles entire, apex shortly acuminate, lateral nerves almost parallel, stipules large, rosy or pinkish brown when young. Male flowers small pedicellate, sepal 3-4 ovate, stamen 1, anthers 2. Female flowers sessile, sepals 4. Free ovary with subterminal long styles.

Local name: Rubber Gachh, Attah Bar.

Habit: Tree

Flowering season: March-April.

Ethno-botanical uses:

Latex: The latex contains rubber, which can be used for tiers, foot wears toys, gloves.

This latex is also an irritant to the eyes and skin and can be fatal if taken internally. Latex used for parasitic worms.

Leaf: The very young leaf tips have been eaten as vegetables.

Root: Decoction of aerial rootlets used for wounds, cuts and sores.

Bark: Bark is astringent and used as styptics for wounds.

1.13.5 Ficus Hispida *L.*

Taxonomic description: A very hispid small deciduous tree. Leaves 10-30 cm long, membranous, ovate, oblong or sub obovate, apiculate or shortly and abruptly acuminate, the lower surface hispid-pubescent, the upper hispid-scabrid. Receptacles 1.3-2.5 cm across, turbinate, obovoid or sub pyriform, hispid, yellowish when ripe.

Local name: Dumur, Kakdumur.

Habit: Small tree/ large shrub

Flowering season: April-September

Ethno-botanical uses:

Whole plant: All parts of the plant are cooling, astringent to the bowels and anti-dysentery; useful in ulcers, biliousness, psoriasis, anemia, piles, jaundice, hemorrhage of the nose and mouth.

Fruits: Fruits are astringent, tonic, emetic and lactagogue. Fruits are prescribed for diabetic patients. Fruits are also cooling and astringent. To stop menstrual hemorrhage, root juice along with rice-soaked water.

Bark: Barks are purgative and emetic, lactagogue and tonic.

Seeds: Seeds are purgative and emetic, lactagogue and tonic.

1.14 Study Objective

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

- ✓ To determine the CNS depressant activity by open field and hole-board test.
- ✓ To determine analgesic activity by writhing and formalin test.

Chapter 2

Literature Review

2.1 Chemical constituents isolated from *Artocarpus chama*

The preliminary phytochemical screening revealed that the methanol and pet ether fraction of fruits possess the presence of various bioactive components like flavonoids, alkaloids and carbohydrates (Ahmed *et al*., 2012).

2.2 Quantitative analysis of the phytochemical constituent of fruits of *Artocarpus chama*

2.2.1 Determination of total phenol content of fruits of *Artocarpus chama*

The pet ether extract of the fruits of *A. chama* Buch. was found to contain large amount of phenolics, 178.08 ± 2.05 mg/g Gallic acid equivalent(GAE) while methanolic extract contain moderate amount, 41.12 ± 1.83 mg/g GAE using Folin- Ciocalteau method (Ahmed *et al.*, 2012).

2.2.2 Determination of total flavonoid content of fruits of *Artocarpus chama*

The total flavonoid content of pet ether and methanol extracts were found to be 24.95 ± 0.36 and 25.71 ± 0.59 mg/g quercetin equivalent, respectively. These results suggested that the antioxidant activities of *A. chama* might be due to its flavonoid content since *A. chama* roots contains variety of prenylated flavonoids e.g. isoprenylated flavones, flavones. (Yong-Hong *et al.*, 2004)

2.3 Antioxidant property of the fruits of *Artocarpus chama*

2.3.1 $DPPH^{-}$ radical scavenging activity

In DPPH radical scavenging assay both pet ether and methanol extract exhibited a concentration-dependent antiradical activity by inhibiting DPPH radical. Ascorbic acid, which is a well-known antioxidant, showed higher degree of free radical-scavenging activity than that of the plant extract at each concentration points. The IC_{50} value of the crude pet ether and methanol extract were 27.64 µg/ml and 39.08 µg/ml,

respectively, while the IC_{50} value for the reference ascorbic acid was 12.70 µg/ml (Ahmed *et al.*, 2012).

2.3.2 Cupric reducing antioxidant capacity (CUPRAC)

It was observed that at concentration level of 200 µg/ml, the reducing capacity of pet ether, methanol extract and ascorbic acid was 0.3115, 0.3545 and 0.744 µg/ml, respectively (Ahmed *et al.*, 2012).

2.3.3 Reducing power antioxidant capacity

The reducing power capabilities of the plant extracts compared to ascorbic acid. Both extracts displayed good reducing power which was found to rise with increasing concentrations of the extracts. At 200 µg/ml concentration level, the absorbance of standard ascorbic acid, pet ether extract and methanol extract were 1.01, 0.52 and 0.60, respectively. Both the plant extracts showed almost similar reducing power capacity (Ahmed *et al.*, 2012).

2.4 Quantitative analysis of the phytochemical constituent of seeds of *Artocarpus chama*

2.4.1 Determination of total phenol content of seeds of *Artocarpus chama*

The methanolic extract of seeds of *Artocarpus chama* was found to contain high amount of phenolics, 61.04 mg/g gallic acid equivalent using Follin-ciocalteau method (Ahmed *et al*., 2013).

2.4.2 Determination of total flavonoid content of seeds of *Artocarpus chama*

The total flavonoid content of methanolic extract was found to be 33.71 mg/g quercetin equivalent. Results told that the antioxidant activities of *A. chama* probably for its flavonoid content (Ahmed *et al*., 2013).

2.5 Antioxidant property of the seeds of *Artocarpus chama*

2.5.1 $DPPH^{-}$ radical scavenging activity

The IC_{50} value of the crude methanol extract was 54.29 ± 1.98 µg/ml, while the IC_{50} value for the reference ascorbic acid was 14.56. at concentration level of 200 µ g/ml, the reducing capacity of methanol extract and ascorbic acid is 0.324 and 0.744, respectively (Ahmed *et al*., 2013).

2.5.2 Cupric reducing antioxidant capacity (CUPRAC)

At concentration level 200 µg/ml, the reducing capacity of methanol extract and ascorbic acid was 0.324 and 0.744 respectively (Ahmed *et al*., 2013).

2.5.3 Reducing power antioxidant capacity

At concentration level 200 µg/ml, the absorbance of standard ascorbic acid and methanol extract was 1.01 and 0.56 respectively (Ahmed *et al*., 2013).

Chapter 3

Methodology

3.1 Preparation of the Plant Sample

3.1.1 Collection and Proper Identification of the Plant Sample

At first with the help of a comprehensive literature review *Artocarpus chama* selected for this investigation. The barks were collected from Botanical garden, Dhaka, Bangladesh during the month of December.

3.1.2 Preparation of plant sample

The experimented plant barks were separated from undesirable plant parts and dried in the sun shed for 5 days. After completion of drying, dried leaves were pulverized into coarse powder by suitable grinding machine. Powders were kept in clean airtight glass containers for further use. Here the grinder was properly cleaned so that contamination with previous ground material on other foreign matter can be avoided. The weight of leaf powder was 256 gm. Finally, it was placed in dry and cool area until experiment begins.

3.1.3 Extraction of powdered sample

Two glass jars were washed properly and then rinsed with methanol and pet ether as well as dried. After that the dried barks powder were put into the jar and methanol and pet ether were poured into it up to 1-inch height above the sample surface. The container was sealed with its content and kept for 4 days with occasional shaking and stirring. This shaking was done to get better extraction.

3.1.4 Procedure of Evaporation in Rotary Evaporator

- ✓ After the filtration process two parts were obtained namely ‘residual part’ and ‘filtered part or filtrate’.
- ✓ The filtered part, which contains the substance soluble in methanol was putted into a 1000ml round bottom flask and then the flask was place in a rotary

evaporator.

- ✓ The evaporation was done at 50-degree Celsius for methanol and pet ether.
- ✓ The number of rotation per minute was selected as 60 rpm. The pressure of the vacuum pumper machine was 6bar.
- ✓ The water flow through the distillation chamber was also provided in a satisfactory flow rate.
- ✓ When the evaporation seemed to be satisfactory, then the methanol and pet ether extract were collected in a 50mL beaker.
- ✓ The extractions were collected from the evaporating flask and the solvents were collected from the receiving flask.
- ✓ The evaporator flask was rinsed by methanol and pet ether in case of the extract of methanol and pet ether extract.
- ✓ Then the beaker was covered with aluminium foil paper and kept for 60 minutes.
- ✓ Finally, the concentrated methanol and pet ether plant extract were found and stored in the laboratory refrigerator from which the extracts were used for many chemical investigations.

The extracts of methanol and pet ether were chosen for investigation and was labelled as-ACBM and ACBP (Methanolic and Pet ether extract of *Artocarpus chama* respectively)

3.2 Drugs

Diazepam, Ibuprofen and Indomethacin were used for current study

3.3 Experimental animal

For the pharmacological investigation 32 Swiss albino mice were collected from ICDDRB, Dhaka, Bangladesh. The average weights of the mice were 16 to 18 gm. Standard environmental situation was maintained to keep the mice. The condition was

55-65% relative humidity, 12 hours light/dark cycle and 24.0 ± 0°C temperature. Also, sufficient amount of food and water was supplied all the time.

Figure 3.1: *Swiss albino* mice

3.4 Identification of animals during experiment

Each group consists of six mice and hence it is difficult to identify and observe at time six mice receiving same treatment. Thus, it was important to identify individual animal of a group during the treatment. The animals were individualized by marking: marked as M_1=mice 1, M_2=mice 2, M_3=mice 3 and so on with different colors.

3.5 Pharmacological investigation of plant extracts

The following pharmacological investigations were done to determine the medicinal effect of the experimented extracts:

- ✓ CNS depressant activity and
- ✓ Analgesic activity

3.5.1 Study of CNS Depressant effect of ACBM and ACBP extracts

CNS Depressant drugs are the agents which slow down the activity of brain. These types of drugs are prescribed by doctor for the treatment of panic attack, anxiety, insomnia etc. Mostly CNS Depressants activate GABA neurotransmitter. This helps

in decreasing brain activity. The CNS depressant action of *Artocarpus chama* plant extracts were observed by comparing with the standard diazepam in the experimented rodents. CNS depressant activity was determined by using two techniques.

They are:

- ✓ Open field technique
- ✓ Hole board technique

3.5.1.1 The Design of the CNS depressant Experiments

In both methods 36 mice were chosen randomly and then divided into 6 groups. They were group G_1 to G_6 where 6 mice were in each group. A particular treatment was given to each group. Before this specific treatment, weight of every mouse was measured accurately as well as marked. Also, the dosage of the sample and standard were also settled according to body weight.

Group-G_1- ACBM 200 mg/kg

Group-G_2- ACBM 400 mg/kg

Group-G_3- ACBP 200 mg/kg

Group-G_4- ACBP 400 mg/kg

Group-G_5 Standard (Diazepam)

Group-G_6- Control (5 % CMC in distilled water)

3.5.1.2 Reagents, Chemicals and Equipment

Table 3.1: Reagents, Chemicals and Equipment used for CNS depressant test

Reagents Chemicals and Equipment	Source
Diazepam	Square Pharmaceuticals Ltd.
5 % CMC-low viscous (as suspending agent)	BDH Chemicals Ltd
Distilled water	Arrowhead
Sterile disposable syringe (1ml, 100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and Digital balance	Denver Instruments M-220/USA

3.5.1.3 Preparation of drug and chemical solution

At first standard which is considered to be diazepam at 1 mg/kg dose was prepared. Then required amount of diazepam was dissolved in water and according to body weight was given to each mouse orally.

Crude extract was also prepared at a dose of 200mg/kg and 400mg/kg by body weight of mice. Then doses were measured as well as 5% CMC (low viscous) was added to every preparation. Then dose was administered according to their body weight.

Table 3.2: Test samples used in the estimation of CNS Depressant activity of A. *chama* plant

Group	Treatment	Dose	Route of administration
Group 1 (Extract)	ACBM	200 mg/kg	Orally
Group 2 (Extract)	ACBM	400 mg/kg	Orally
Group 3 (Extract)	ACBP	200 mg/kg	Orally
Group 4 (Extract)	ACBP	400 mg/kg	Orally
Group 5 (Standard)	Diazepam	1 mg/kg	Orally
Group 6 (Control)	5 % CMC in Distilled Water	10 ml/kg	Orally

3.5.1.4 Open field test

Gupta's open field method (Gupta *et al*., 1971) was followed to carry out Open field test. The box was half square meter as well as divided into squares each. On the other hand, the box was black and white color like a chess board. The apparatus had a wall which was 40cm in height. For 3 minutes, each square was counted which was visited by mice. Also, during the study period, several results were taken on 0, 30, 60, 90 and 120 minutes.

The procedure for evaluation of CNS depressant effect of *Artocarpus chama* plant by open field test is given below:

i. At first mice were weighed and after that categorized into 6 groups where 6 mice were in each group
ii. Then by a long needle which was attached with ball shaped end, sample and standards were administered orally. This was done at 0 minutes.
iii. Number of squares which was visited by mice at 0 Minute was counted for 3 minutes
iv. Eventually after 30, 60, 90 and 120 minutes the number of times all the

mice traveled from one compartment to another was counted for a duration of 3 minutes and afterwards the data was recorded for the two extracts of the plant.

3.5.1.5 Hole Board Test

The method described by (Takagi *et al.*, 1971) was implemented for this study. Again, 36 mice were equally divided into 6 groups. The control group received 5% CMC in distilled water (10 ml/kg body weight), the standard group received Diazepam (1 mg/kg body weight) and the experimental groups received crude extract at 200 mg/kg and 400 mg/kg body weight. 16 holes, each of 4 cm in diameter, were made at a plane plate of a woody table at a height of 1 foot from the ground. The number of poking and Deeping of mice through the hole was counted for a period of 3 minute after 0, 30, 60, 90, and 120 min of oral administration of the extract.

The procedure for evaluation of CNS depressant effect of *Artocarpus chama* plant by Hole board test is given below:

i. At first mice were weighed and after that categorized into 6 groups where 6 mice were in each group
ii. Then by a long needle which was attached with ball shaped end, sample and standards were administered orally. This was done at 0 minutes.
iii. Number of holes which was visited by mice at 0 Minute was counted for 3 minutes
iv. Eventually after 30, 60, 90 and 120 minutes the number of times all the mice gave pocking and dipping was counted for a duration of 3 minutes and afterwards the data was recorded for the two extracts of the plant.

3.5.2 Analgesic activity of *Artocarpus chama* plant extracts

Drug which is used to relieve pain is called analgesic drug. These drugs are also known as painkiller. The analgesic test was done by two methods. These two methods are: -

- Acetic acid induced writhing technique
- Formalin induced pain technique.

3.5.2.1 Design of the analgesic experiment

36 mice were chosen anyway and divided into 6 groups where the groups were from G_1 to G_6 as well as 6 mice were in each group. Each group got a specific treatment. Before the treatment, each mouse was weighed properly as well as marked. Then the dosage of the test sample and control materials was also settled according to body weight.

Group-G_1- ACBM 200 mg/kg

Group-G_2- ACBM 400 mg/kg

Group-G_3- ACBP 200 mg/kg

Group-G_4- ACBP 400 mg/kg

Group-G_5 Standard (Indomethacin/ Ibuprofen)

Group-G_6- Control (5 % CMC in distilled water)

3.5.2.2 Acetic acid-induced writhing technique

Acetic acid induced writhing test is a technique where analgesic behavior is observed. In this method (Ahmed *et al.*, 2001) intra-peritoneally acetic acid was administered to the mice so that pain sensation generates. Here, indomethacin was considered as standard. At first the normal saline, extracts at a dose of 200 mg/kg and 400 mg/kg as well as standard drug were administered orally. After 30 minutes, the solution of 0.7% v/v acetic acid was administered intraperitoneally. After administration of solution of acetic acid, no writhing was counted for 5 minutes. After 5 minutes, writhing was counted for 15 minutes. For that each mouse was placed on observation table and noticed the number of writhing of mice. The mice did not give full writhing all the time. They gave half writhing also. So, two incomplete writhing were counted as one complete writhing.

3.5.2.2.1 Reagents, Chemicals and Equipment

Table 3.3: Reagents, Chemicals and equipment used for acetic acid induced analgesic test

Reagents Chemicals and Equipment's	Source
Acetic acid	Eurosoft fabric softener
Indomethacin	Square Pharmaceuticals Ltd.
5 % CMC- low viscous (as suspending agent)	BDH Chemicals Ltd
Distilled water	Arrowhead
Sterile disposable syringe (1ml, 100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and digital balance	Denver Instruments M-220/USA

3.5.2.2.2 Preparation of drug and chemical solution

For the preparation of standard which is indomethacin at a dose of 10 mg/kg, specific quantity of this drug was suited. Then required amount of indomethacin was dissolved in water and then according to body weight was given to each mouse orally.

Crude extract was also prepared at a dose of 200mg/kg and 400mg/kg by body weight of mice. Then doses were measured as well as 5% CMC (low viscous) was added to every preparation. Then dose was administered according to their body weight.

Table 3.4: Test samples used in the estimation of Analgesic activity of *A. chama* plant

Group	Treatment	Dose	Route of administration
Group 1 (Extract)	ACBM	200 mg/kg	Orally
Group 2 (Extract)	ACBM	400 mg/kg	Orally
Group 3 (Extract)	ACBP	200 mg/kg	Orally
Group 4 (Extract)	ACBP	400 mg/kg	Orally
Group 5 (Standard)	Indomethacin	10 mg/kg	Orally
Group 6 (Control)	5 % CMC in Distilled Water	10 ml/kg	Orally

3.5.2.2.3 Procedure of analgesic activity of *Artocarpus chama* extract by acetic acid induced writhing technique

i. At first weight of the mice were measured as well as split into 6 groups where 6 mice were in each group

ii. Later at 0 hour orally standard and samples were administered with the help of ball shaped ended needle.

iii. Then acetic acid was induced to each mouse of group (G_1 to G_6) intraperitoneally after 30 minutes

iv. To confirm the acceptable absorption of administered specimen, 30 minutes interval was given

v. Afterwards 5 minutes of induced acetic acid, the writhing for each mouse were counted for 15 minutes

vi. Finally, the documented numbers of writhes were compared with standard group mice (Ahmed *et al.*, 2001).

3.5.2.3 Formalin induced pain method

Formalin test is another method (Sharma *et al.*, 2010) by which analgesic activity is also observed. In this case, formalin injection is induced to mice's right hind paw. As a result, biphasic pain is produced. For standard, we choose ibuprofen which has pain sensation inhibition control. After that the standard was compared with test samples and control.

3.5.2.3.1 Reagents, Chemicals and Equipment

Table 3.5: Reagents, Chemicals and equipment's used for formalin induced analgesic test

Reagents Chemicals and Equipment's	Source
Formalin	Nazifa chemical corporation
Ibuprofen	Square Pharmaceuticals Ltd.
5 % CMC- low viscous (as suspending agent)	BDH Chemicals Ltd
Distilled water	Arrowhead
Sterile disposable syringe (1ml, 100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and digital balance	Denver Instruments M-220/USA

3.5.2.3.2 Preparation of drug and chemical solution

For the preparation of standard which is ibuprofen at a dose of 100 mg/kg, specific quantity of this drug was suited. Then required amount of ibuprofen was dissolved in water and then according to body weight was given to each mouse orally.

Crude extract was also prepared at a dose of 200mg/kg and 400mg/kg by body weight of mice. Then doses were measured as well as 5 % CMC (low viscous) was added to

every preparation. Then dose was administered according to their body weight.

Table 3.6: Test samples used in the estimation of Analgesic activity of *A. chama* plant

Group	Treatment	Dose	Route of administration
Group 1 (Extract)	ACBM	200 mg/kg	Orally
Group 2 (Extract)	ACBM	400 mg/kg	Orally
Group 3 (Extract)	ACBP	200 mg/kg	Orally
Group 4 (Extract)	ACBP	400 mg/kg	Orally
Group 5 (Standard)	Ibuprofen	100 mg/kg	Orally
Group 6 (Control)	5 % CMC in Distilled Water	10 ml/kg	Orally

3.5.2.3.3 Procedure of analgesic activity of *Artocarpus chama* extract by formalin

i. The mice were weighed and then split into 6 groups where 6 mice were in each group

ii. Later at 0-hour standard and samples were administered to mice of group (G_1 to G_6) orally by a needle which contained a ball shaped end

iii. 20 µl of 1% formalin was induced to each mouse in the right-hand paw after 30 minutes

iv. In first phase which is after the administration of formalin, numbers of licking and biting by mice are counted. The duration of first phase is 0 to 300 seconds.

v. In second phase, numbers of licking and biting by mice are also counted and the time was 15-30 minutes.

3.5.2.3.4 Counting of licking and biting of paws

Because of induced formalin, mice bite or lick the wounded paw and this was determined by help of stopwatch

3.6 Statistical Analysis

Total values which were obtained from the experiments are represented as mean ± standard error of the mean (SEM). Statistically obtained data was estimated by using ANOVA (Analysis of variance) and post-hoc Dunnett's test which was associated with SPSS program (SPSS 17.0, USA). Results were calculated by using Microsoft Excel 2016. Dissimilarity between the means of the various groups were measured significantly at $p < 0.05$, $p < 0.01$ and $p<0.001$.

Chapter 4

Results and Discussion

4.1 Results of CNS Depressant activity of plant extracts on mice

For the analysis of CNS depressant activity, the open field method and hole cross method were followed according to Gupta's method (Gupta *et al*., 1971) and Takagi's method (Takagi *et al*., 1971) respectively. Both tests were done on mice to find out CNS depressant activity. The results of these two methods are shown below:

4.1.1 Results of Open Field Test

At 200 mg/kg and 400 mg/kg dose, experimental barks extracts were administered to mice. As a result, the movements of mice were reduced in a dose depending manner. Also, it was comparable with diazepam (standard). This movement lowering effect of extract on mice was observed at 30 min interval from 0 minute up to 120 minutes. The extracts caused reduction in movement and this may be connected to CNS depression, as reduction or depression of movement is common to most antipsychotics.

Table 4.1: Data of CNS activity test of *A. chama* plant extracts by Open field method

Group	Treatment	Dose	Number of movement					
			-30 min	0 min	30 min	60 min	90 min	120 min
Group 1 (Extract)	ACBM	200 mg/kg	106.83 ± 6.28	61.50 ± 11.79**	55.83 ± 11.94**	39.17 ± 9.77***	18.17 ± 8.79***	14.33 ± 5.95***
Group 2 (Extract)	ACBM	400 mg/kg	94.33 ± 19.42	31.33 ± 4.86***	30.33 ± 4.13***	31.33 ± 5.74***	16.83 ± 3.97***	13.83 ± 2.23***
Group 3 (Extract)	ACBP	200 mg/kg	83.00 ± 12.92	36.67 ± 14.21***	35.83 ± 6.03***	34.17 ± 7.03***	29.50 ± 4.97***	23.33 ± 5.87***
Group 4 (Extract)	ACBP	400 mg/kg	96.00 ± 25.53	32.83 ± 18.64***	32.67 ± 8.64***	31.33 ± 5.74***	23.33 ± 10.16***	26.67 ± 6.42***
Group 5 (Standard)	Diazepam	1 mg/kg	137.50 ±	137.50 ± 18.23	36.17 ± 10.65***	36.00 ± 8.11***	35.67 ± 10.87***	30.33± 15.95***
Group 6 (Control)	5% CMC in distilled water	10 ml/kg	130.83 ± 24.10	137.50 ± 18.23	126.00 ± 13.38	159.67 ± 6.32	162.50 ± 10.34	153.00 ± 9.41

ACBM and ACBP refer to *A. chama* Bark in Methanol and Pet Ether respectively. Every value is conveyed as mean ± SEM (n=6); One-Way Analysis of Variance (ANOVA) trailed by Dunnet's test. Results were calculated by using Microsoft Excel 2016. ***p<0.001; **p<0.01; *p<0.05 are considered to be significant.

4.1.2 Result of Hole Board Test

In this technique, the barks extract at 200 mg/kg and 400 mg/kg dose showed continuous decrease of movement in the experimental animals from 0 minute to 120 minutes.

Table 4.2: Data of CNS Depressant activity test of *A. chama* plant extracts by Hole- board method

Group	Treatment	Dose	Number of movement					
			-30 min	0 min	30 min	60 min	90 min	120 min
Group 1 (Extract)	ACBM	200 mg/kg	14.33 ± 1.20	8.17 ± 0.95	7.00 ± 1.46	6.17 ± 1.11	5.50 ± 0.67	2.33 ± 1.05
Group 2 (Extract)	ACBM	400 mg/kg	25.50 ± 6.33	11.17 ± 1.66	7.83 ± 1.80	7.33 ± 0.42	5.33 ± 1.26	5.33 ± 0.56
Group 3 (Extract)	ACBP	200 mg/kg	18.00 ± 1.83	8.67 ± 2.60	7.33 ± 1.31	6.50 ± 1.48	6.33 ± 0.76	6.33 ± 2.23
Group 4 (Extract)	ACBP	400 mg/kg	24.67 ± 5.45	14.50 ± 4.86	5.50 ± 1.31	3.33 ± 0.33**	3.17 ± 0.79*	4.00 ± 0.97
Group 5 (Standard)	Diazepam	1 mg/kg	17.50 ± 3.70	5.50 ± 1.84*	5.17 ± 0.87	4.00 ± 0.77*	3.67 ± 0.61*	3.33 ± 0.33
Group 6 (Control)	5 % CMC in Distilled Water	10 ml/kg	20.83 ± 2.87	15.50 ± 1.12	11.50 ± 1.54	9.33 ± 1.33	9.50 ± 1.84	7.33 ± 1.48

ACBM and ACBP refer to *A. chama* Bark in Methanol and Pet Ether respectively. Every value is conveyed as mean ± SEM (n=6); One-Way Analysis of Variance (ANOVA) trailed by Dunnet's test. Results were calculated by using Microsoft Excel 2016. ***p<0.001; **p<0.01; *p<0.05 are considered to be significant.

4.2 Result of analgesic activity of plant extracts on mice

For the experiment of analgesic activity, two methods were followed. They were acetic acid induced writhing method and formalin induced pain method.

4.2.1 Formalin-induced pain method (biphasic pain)

The analgesic activity by formalin induced pain method was determined by counting paw licking and biting events. The bark extract of the experimental plant at a dose of 200mg/kg and 400 mg/kg prevent the licking and biting activity of mice in a dose depending manner in the late phase compared to standard which was Ibuprofen. ACBM showed good inhibition about 42.32% in late phase at a dose of 200 mg/kg, ACBM at a dose of 400 mg/kg showed good inhibition about 58.36% in late phase. ACBP at a dose of 200 mg/kg and 400 mg/kg showed good inhibition in late phase about 66.04% and 51.19% respectively. These events are given in the following table:

Table 4.3: Data of Formalin induced analgesic activity test of *A. chama* plant extracts

Group	Treatment	Dose (mg/kg)	Early phase	Late phase	Inhibition %(Early phase)	Inhibition %(Delayed phase)
Group1 (Extract)	ACBM	200 mg/kg	53.00 ± 8.87	56.33 ± 4.84**	8.28%	42.32%
Group2 (Extract)	ACBM	400 mg/kg	74.00 ± 6.07	40.67 ± 6.47***	10.30%	58.36%
Group 3 (Extract)	ACBP	200 mg/kg	54.33 ± 3.31*	33.17 ± 4.25***	34.14%	66.04%
Group 4 (Extract)	ACBP	400 mg/kg	82.50 ± 3.62	47.67 ± 3.31***	0.00%	51.19%
Group 5 (Standard)	Ibuprofen	100 mg/kg	28.33 ± 4.22***	40.83 ± 7.43***	65.66%	58.19%
Group 6 (Control)	5 % CMC in Distilled Water	10 ml/kg	82.50 ± 10.97	97.67 12.41	-	-

ACBM and ACBP refer to *A. chama* Bark in Methanol and Pet Ether respectively. Every value is conveyed as mean ± SEM (n=6); One-Way Analysis of Variance (ANOVA) trailed by Dunnet's test. Results were calculated by using Microsoft Excel 2016. ***p<0.001; **p<0.01; *p<0.05 are considered to be significant.

4.2.2 Acetic acid induced writhing test (peripheral pain)

In this test, the antinociceptive effects of plant *Artocarpus chama* were investigated by administering 200 mg/kg and 400 mg/kg dose to rodents. By applying this test, it was seen that there was significant effect of plant extract compare to standard drug (Indomethacin). Among the sample of crude extracts Group 2 and Group 8 showed better results compared to standard which was Indomethacin. ACBM at a dose of 200 mg/kg and 400 mg/kg showed inhibition about 41.14% and 75.92% respectively. ACBP at a dose of 200 mg/kg and 400 mg/kg showed inhibition about 56.52% and 61.54% respectively.

Table 4.4: Data of Analgesic (Writhing) activity test of *A. chama* plant extracts

Group	Treatment	Dose	Number of writhing	Percentage of inhibition
Group 1 (Extract)	ACBM	200 mg/kg	29.33 ± 3.55**	41.14%
Group 2 (Extract)	ACBM	400 mg/kg	12.00 ± 0.22***	75.92%
Group 3 (Extract)	ACBP	200 mg/kg	39.33 ± 2.92	56.52%
Group 4 (Extract)	ACBP	400 mg/kg	12.83 ± 2.07***	61.54%
Group 5 (Standard)	Indomethacin	10 mg/kg	6.5 ± 1.88***	86.96%
Group 6 (Control)	5 % CMC in Distilled Water	10 ml/kg	49.83 ± 5.1	-

ACBM and ACBP refer to *A. chama* Bark in Methanol and Pet Ether respectively. Every value is conveyed as mean ± SEM (n=6); One-Way Analysis of Variance (ANOVA) trailed by Dunnet's test. Results were calculated by using Microsoft Excel 2016. ***p<0.001; **p<0.01; *p<0.05 are considered to be significant.

4.3 Discussion

Diazepam being a benzodiazepine class of drugs, it has sedative action. It increases neuronal membrane permeability to chloride ions by binding to stereospecific benzodiazepine receptors on the postsynaptic GABA neuron and enhancing the GABA inhibitory effects resulting in hyperpolarization and stabilization (Drugbank, 2017). The methanol and pet ether extract of plants gave action compared to diazepam, the movement of mice decreased in a dose dependent manner in open field. So, it indicates that the plant may have depressant action. As phytochemical screening revealed that the plant contains flavonoid and flavonoid has CNS depressant activity so it might be flavonoid that is giving the depressant activity. Before oral absorption flavone undergoes deglycosylation either by lacto phloridzin hydrolase or cytostatic beta glucosidase. The absorbed aglycone is then conjugated by methylation, sulphatation or glucuronidation. Both the aglycones and the conjugates can pass the blood- brain barrier. In the CNS several flavones bind to the benzodiazepine site on the $GABA_A$-receptor (Jager and Sabby, 2011). In hole board test, it is found that the movements of the mice are decreasing, So, it also indicates that the plant has depressant activity. The results are also similar to diazepam. It can be concluded that the plant has depressant activity. From the analgesic activity results, formalin-induced test is the most commonly used test to assess peripherally acting analgesics. Licking and biting generated by parenteral administration of formalin in mice, are due to profound pain of endogenous nature which recur for a prolonged period of time. Biting and licking is an explicit response to the intense pain induced by irritant principles via nociceptors characterized by episodes of biting and licking of mice's paw. The signals transmitted to central nervous system in response to pain due to irritation, because release of mediators such as prostaglandins which contributes to the increased sensitivity to nociceptors (Shivaji, 2012). The increase in prostaglandin production further enhances the vascular permeability. The decrease in the number of biting and licking assumes decrease of prostaglandins synthesis which results in significant analgesic activity, in formalin test ibuprofen was considered as standard, it inhibits synthesis of prostaglandin in body tissues by inhibiting cox-1 and cox-2 (JBC, 2015), the plant extracts showed decreasing leaking and biting compared to standard, it may be due to the presence of phytochemical constituents which is reducing the number of licking and biting, so the methanol and pet ether extracts of barks showed analgesic activity. In case

of writhing test, writhing is an overt response to the intense pain induced by irritant principles via nociceptors characterized by episodes of retraction of abdomen and stretching of hind limbs. The signals transmitted to central nervous system in response to pain due to irritation, cause release of mediators such as prostaglandins which contributes to the increased sensitivity to nociceptors (Gawade, 2012). Indomethacin was considered as standard drug, it reversibly inhibits the cox-1 and cox-2 enzymes (Drugbank, 2017), thus resulting in reduced synthesis of prostaglandin precursors, the methanol fraction of the barks showed good writhing compared to standard, it might be flavonoid which is giving analgesic activity as the plant contain these constituents. Some flavonoids are reported to possess significant analgesic activity. Some flavonoids can significantly interfere with inflammatory mediators (Ullah *et al*., 2014). Certain members of flavonoids significantly affect the function of the immune system and inflammatory cells. A number of flavonoids such as hesperidin, apigenin, luteolin, and quercetin are reported to possess analgesic effects. Flavonoids may affect specifically the function of enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases. The inhibition of kinases is due to the competitive binding of flavonoids with ATP at catalytic sites on the enzymes. These enzymes are involved in signal transduction and cell activation processes involving cells of the immune system. It has been reported that flavonoids are able to inhibit expression of isoforms of inducible nitric oxide synthase, cyclooxygenase, and lipooxygenase, which are responsible for the production of a great amount of nitric oxide, prostanoids, leukotrienes, and other mediators of the inflammatory process such as cytokines, chemokines, or adhesion molecules Flavonoids also inhibit phosphodiesterases involved in cell activation.

From our best knowledge, this is the first report of CNS depressant and analgesic activity of *Artocarpus chama bark*. Now it can be concluded on the basis of results obtained from investigation that the plant may be useful as CNS depressant and analgesic agent. But our work was only preliminary effort. It will require additional detailed advanced investigation.

Chapter 5
Conclusion

We can find thousands of plants which are medicinally useful, *Artocarpus chama* is one of them. The plant showed analgesic and CNS activity. For determining the effects CNS (Open field and Hole board) And Analgesic (Formalin induced pain and Writhing test) test were done. The results of ACBM 200mg/kg, ACBM 400 mg/kg and ACBP 200 mg/kg, ACBP 400 mg/kg of CNS and Analgesic test are statistically significant. But it was only preliminary testing, we did not do phytochemical testing it is required further comprehensive exploration as well as depiction of active compounds and necessitates preformulation studies for expansion of a potential dosage form.

Chapter 6

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