CERTIFICATE

I do hereby declare that the Project Report entitled " PHARMACOLOGICAL INVESTIGATIONS OF *Melocanna baccifera (Roxb).*" presented to the Department of Pharmacy, East west University Bangladesh, is the outcome of the investigations performed by me under the supervision of Amran hawlader, Department of Pharmacy, East West University. I also declare that no part of this Project Report has been or is being submitted else where for the award of any Degree or Diploma.

Md. Amran Howlader
Senior Lecturer, Department of Pharmacy
East West University Bangladesh
(Instructor)

APPROVAL

The Project Report entitled " PHARMACOLOGICAL INVESTIGATIONS ON *Melocanna baccifera (roxb)"* submitted by Meharun Nessa, ID: 2006-3-70-003 to the Department of Pharmacy, East West University, has been accepted as satisfactory for the partial fulfillment of the requirement of the degree of Bachelor of Pharmacy (Hons.) and approved as to its style and contents.

Sufia Islam 27.06.2011

Sufia Islam Ph. D
Chairperson & Associate Professor
Department of Pharmacy
East West University

ACKNOWLEDGEMENT

I would like to convey my gratitude to Almighty Allah then to my honorable Supervisor, Md. Amran howlader, Senior Lecturer Department of Pharmacy, East West University,for his adroit supervision, benevolent guidance, positive counseling and constant encouragement. I am really indebted to her for providing invaluable collection of relevant research articles which helped me a lot in writing literature review part; for her valuable input to make the discussion sound significantly and finally, for her sincere and expert proof checking of the whole draft.

It is my pleasure to express my deep gratitude and warm regards to the honorable Chairperson & Associate Professor Sufia Islam Ph. D , Department of Pharmacy, for her heartiest co-operation and good wishes.

I am also grateful and to my family, specially to my parents and all of my respectable teachers of Pharmacy department for there support and inspirations.

Special thanks, to my all of project partners and all of my friends for their great support during my project period. I want to give thanks to the authority of the East West University Bangladesh for providing huge facilities in the laboratory in the due time.

Finally, I am profoundly indebted to the Almighty for providing me the strength and perseverance to carry out the whole work in time. Lastly I wish the cumulative progress of East West University in the forth-coming future.

ABSTRACT

The aim of present study was to examine cytotoxic, neuropharmacological and analgesic and anti-inflammatory activity of the ethanolic leaves extract of *Melocanna baccifera (roxb)*. In Brine shrimp lethality bioassay the extract showed strong cytotoxicity where the LC_{50} value of the leave extract was 177.86µg/ml. The neuropharmacological activity was evaluated by using hole cross and open field tests where not significant activity and no exploratory behavior was observed in the leavs extract treated mice as was in mice which were administered reference sedative drug diazepam. The analgesic activity was evaluated using acetic acid induced writhing test at the doses of 50mg/kg, 100mg/kg and 200mg/kg body weight. The anti-inflammatory activity was measured by using Carrageenan- induced paw oedema.

Key Words: Cytotoxic, Neuropharmacological, Brine shrimp, Analgesic, Diazepam, Anti - inflammatory

DEDICATED

TO MY PARENTS

LIST OF CONTENTS

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

Contents **Page no.**

Chapter 1: Introduction 1-10

1.1Medicinal Plant 2

1.1.1 Reason of choose medicinal plants as therapeutic purpose 3

1.1.2 Medicinal Plants: Indirect contribution to modern synthetic drugs 3

1.2 Contribution of Plant as Medicine 4

1.3 Crude drug 5

1.4 Current Status of Medicinal Plant 7

1.4.1 National Status 7

1.4.2 International Status 7

1.5. Rationale of the Work 8-9

1.6 Plant information 10

1.7 Study protocol 10

Chapter 2: Plant Review 11-23

2.1 Common name 12

2.2 Botanical classification 12

2.3 Description 14

2.4. Flowering and Fruiting 15

2.5 Geographic distribution 16

2.6 Anatomy and Fibre Characteristics 18

2.7Chemistry 18

2.8 Physical and Mechanical properties 19

2.9 Uses 19

2.10 Leptocanna chinensis 19

2.11 Taxonomy 20

2.12 Uses 20

2.13 Schizostachyum chinense 21

Chapter 3: Methods and Materials 24-39

3.1 Plant materials 25

3.1.1 Identification of the plant 25

3.2 Collection, drying and pulverization of plants parts 27

3.2.1 Plant collection 27

3.2.2 Plant drying 27

3.2.3 Grinding 27

3.3 Extraction of leaves 27

3.3.1 Over all extraction process 31

3.4 Materials used in the study 32

3.4.1 List of apparatus used 32

3.4.2 List of glass ware used 34

3.4.3 List of reagent used 35

3.4.4 Animal used 35

3.4.5 Animal feed used 36

3.4.6 Material used for animal house 38

Chapter 4: Methods 40-70

Cytotoxicity test

4.1. Brine Shrimp Lethality Bioassay 42

4.1.1 Principle 43

4.1.2 Materials 43

4.1.3 Preparation of Seawater 44

4.1.4 Hatching of brine shrimps 44

4.1.5 Preparation of test solutions 46

4.1.6 Counting of Nauplii and Analysis of Data 46

Anti-inflammatory

5.1 Introduction 48-50

5.2 Carrageenan-induced paw oedema 50

Neuropharmacological Screening

6.1 Neuropharmacological Screening of *Melocanna bacccifera R.* 52

6.1.1 Objectives 52

6.1.2 Study Design 53

6.2 Hole Cross Test 53

6.3 Open Field Test 55

Analgesic Activity Test

7.1 Analgesic Activity Test of Leaf Extract of *Melocanna baccifera R.* 58

7.2 Analgesic Activity Test by Acetic Acid Induced Writhing Test 59

7.2.1 Objectives 59

7.2.2 Principle 59

7.2.3 Experimental Animal 61

7.2.4 Experimental Design 61

7.2.5 Preparation of the Test Materials 61

7.2.6 Mechanism of Writhing Test 63

7.2.7 Study Design 63

7.2.8 Counting of Writhing 65

Antioxidant activity

8.1 Antioxidant activity : DPPH assay 69

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

8.1.1 **Introduction** **69**

8.1.2 **Principle** **69**

8.1.3 **Materials & Method** **69**

8.1.4 **Materials** **70**

8.1.5 **Method** **70**

Chapter 5: Results **71-94**

9.1 **Results for brine shrimp** **72**

9.2 **Discussion** **74**

10.1 **Results of CNS depressant for hole cross** **75**

10.1.1. **Data of Hole Cross Test** **75**

10.1.2 **Stastical Analysis** **78**

10.3 **Discussion** **80**

11.1 **Data of open field test** **81**

11.1.1 **Stastical analysis for open field** **84**

11.2 **Discussion** **85**

11.3 **Results of Analgesic Activity** **86**

11.3.1. **Data of Analgesic Activity** **86**

11.3.2 **Stastical analysis** **89**

11.4 **Discussion** **90**

12.1 **Result of Anti Inflammatory Test** **91**

12.2 **Stastical analysis** **93**

12.3 Discussion 94

Chapter 6: Conclusion 95-96

Chapter 7: References 97-100

Chapter 8: Appendix 101-102

LIST OF TABLES

Tables Page no.

Tables	Page no.
Table 1.1: Examples of Crude Drugs and Their Therapeutic Uses	6
Table 1.1 List of Apparatus Used in the Experiment	33
Table 1.2 List of Glassware Used	34
Table 1.3 List of Reagents Used for the Experiment	35
Table 1.4 Detail information of the mice used for the Experiment	36
Table 1.5 Type of Food Used for the Mice	36
Table1.6 Materials Used for Animal House	38
Table 1.7 Material for Brine Shrimp Lethality Bioassy	38
Table 1.8 Material for Antioxidant Activity test	39
Table-7.1: Experiment profile to assess the effect of leaves extract of *Melocanna baccifera (Roxb)* on acetic acid induced writhing of mice	65
Table 9.1 Cytotoxic potential of ethanolic leaves extract	72
Table 10.1: Primary data table for hole cross test of leaf extract of *Melocanna baccifera (*Roxb)	76
Table 10.1.2: Effect of leaves extract of *Melocanna baccifera* on hole cross test	77
Table 11.1: Primary data table for open filed test of of *M. baccifera*	81
Table 11.2: Effect of leaves extract of *Melocanna baccifera R.* on open field test	82
Table 11.3: Data table of Acetic acid induced writhing test of leaves extract of *M.baccifera*	86
Table 11.4: Results of Acetic acid induced writhing test	87
Table 12.1 Data of anti inflammatory test	91

LIST OF FIGURES

Figures	Page no.
Figure 2.1. Steam of *Melocanna baccifera (Roxb*	13
Figure2.2: Tree of *Melocanna baccifera (Roxb)*	17
Figure2.3: Leaves of Schizostachyum	22
Figure 3.1: Accession code of *Melocanna baccifera* on herbarium sheet	26
Figure 3.2: Grinding by using blender	28
Figure 3.3: Hot extraction of leaves of *Melocanna baccifera* by using soxhlet extractor	29
Figure 3.4: Leaves extract of *Melocanna baccifera (Roxb) Kurz.*	30
Flow Chart 1.1 The Overall Extraction Process of *Melocanna baccifera.*	31
Figure 3.5 *Swiss albino* mice	37
Figure 4.1: Hatching of brine shrimps in laboratory	45
Figure 6.1: Hole cross test for Neuropharmacological activity on mice	54
Figure 6.2: Open field test for Neuropharmacological activity on mice	56
Figure 7.1: Synthesis of prostaglandins and leukotrienes	60
Figure 7.2: Injecting mice intraperitoneally with acetic acid	62
Figure 7.3: Schematic representation of acetic acid induced writhing of mice for investigation of analgesic activity	64
Figure 7.4: Full writhing given by mice	66
Figure 7.5: Half writhing given by mice	67
Figure 9.1: Graphical presentationof *Melocanna baccifera* toward Brine Shrimp nauplii	73
Figure 10.1: Bar diagram presentation of the results of Hole Cross Test of leaves extract of *Melocanna baccifera*	78
Figure 11.1: Bar diagram presentation of the results of Open Field Test of leaves extract of *Melocanna baccifera*	83
Figure 11.2: Bar diagram showing the result of writhing test by leaves extract of *M. baccifera.*	88

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

Chapter 1

Introduction

INTRODUCTION

1.1 Medicinal Plants

A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. This definition of medicinal plant has been formulated by WHO (World Health Organization). (Sofowora, 1982).

Medicinal plants can be defined as the plants whose roots, leaves, seeds, bark, or other constituent possess therapeutic, tonic, purgative, or other pharmacologic activity when administered to higher animals. Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has been now established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties (Ghnai, 2005). In our opinion, we should continue recognizing all those plants as medicinal which have been traditionally used for over the years and are still being used for therapeutic purposes, some with spectacular reputation, until their efficacy is proved otherwise scientific analysis and clinical evaluation.

A number of important modern drugs and natural products and most of the traditional medicines are derived from medicinal plants. The treatment of disease by the use of plants was the beginning of pharmacotherapy or treatment of diseases by means of drugs (Ghani, 1998). Drug, substance that affects the function of living cells, used in medicine to diagnose, cure, prevent the occurrence of disease and disorders, and prolong the life of patients with incurable conditions. The list of medicinal plants growing around the world includes more than a thousand items (Ghani, 1998).

1.1.1. Reasons to Choose Medicinal Plants for Therapeutic Purpose

- Many people believe that plants are less toxic and safer than manufactured drugs,
- many people believe that plants are more natural than manufactured drugs,
- medicinal plants can be made at home and are less expensive than manufactured drugs and
- in developing countries, medicinal plants often are more accessible than manufactured drugs.

1.1.2 Medicinal Plants: Indirect Contribution to modern synthetic drugs

Plants have contributed and are still contributing to the development of modern synthetic drugs and medicine in a number of ways as stated below:

- Novel structures of biologically active chemical compounds, isolated from plant sources, often prompt the chemist to synthesis similar or better semi-synthetic compounds.
- Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant-derived compounds with known biological activity.
- Various analogues and derivatives of plants constituents with similar or better pharmacological actions and therapeutic properties are often prepared by chemists for use as potent drugs.

1.2 Contribution of Plant as Medicine

Therapeutic uses of plants continued with the progress of civilization and development of human knowledge, scientists endeavored to isolate different chemical constituents from plants, put them to biological and pharmacological tests and thus have been able to identify and isolate therapeutically active compounds. The 19th century saw the scientific revolution in medicine. The first isolation and crystallization of an active drug from a natural source was the accomplishment of a pharmacist's assistant Sertuner (1783-1841), who obtained pure morphine from natural opium in 1803. Pure quinine was isolated from cinchona bark in 1820. 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 nux-vomeca* (1817), emetine from *Cephaelis ipecacuanha* (1817), caffeine from *Thea sinensis* (1819), quinine from *Chcinchona spp.* (1820) and colchicines from *Colchicum autumnale* (1820) constitutes some example of such early drugs.

Facilitated by the rapid development of technology of isolation and characterization process that is chromatographic and spectroscopic methods, a large number of therapeutically active plant constituents have been isolated during last two decades (Ghani, 1998).

1.3 Crude Drug

The substance of natural origin both from plant and animal source possesses therapeutic properties and pharmacological actions. These substances in the natural state comprise whole plants, their morphological or anatomical parts, saps, secretions etc. whole animals, their anatomical parts, glands or other organs, extracts, secretions of their organs. These drugs are used as therapeutic agents in many traditional medicinal preparations in everywhere (Ghani, 1998).

A crude drug is a natural drug of plant, animal or mineral origin which has undergone no treatment other than collection and drying, that is the quality or appearance of the drug has not been advanced or improved by any physical or chemical treatment (Ghani, 1998).

In Bangladesh numerous crude drugs are prepared from local plant and animal sources while many more are imported from foreign countries for use in the preparation of Unani, Ayurvedic and Homeopathic medicines. Many of them are also used in Hekimi, Kaviraji and Folk medicine practices in the country. Some of the official crude drugs available in Bangladesh are Abroma bark, Acacia, Aloes, Amlaki, Arjuna, Asoka bark, Asparagas, Babchi, Black Cumin, Calotropis, Capsicum, Cassia fruit, Castor, Catharanthus, Chaulmoogra, Cinnamon, Colocynth, Colophony, Coriander, Eucalyptus, Fenugreek, Garlic allium, Ginger, Henna, Herpestis, Hydrocotyle, Indian Ipecac, Indian Sarsaparilla, Indian Senega, Indian Squill, Kalamegh, Kurchi bark, Lemongrass, Linseed, Myrobalan, Neem, Nux-vomica, Papaya, Peppermint, Rauwolfia, Sesame, Stramonium, Turmeric, Vasaka, and Withania (Ghani, 1998).

Table 1.1: Examples of Crude Drugs and Their Therapeutic Uses (Ghani, 1998)

Drugs	Plant Source	Therapeutic Use
Digitoxin, digoxin	*Digitalis purpura, Digitalis lanata*	Cardiotonic
Morphine	*Papaver somniferum*	Sedative, narcotic, analgesic
Quinine, quinidine	*Cinchona sp.*	Antipyretic, antimalarial
Vinblastin, vincristine	*Catharanthus roseus*	Anticancer
Paclitaxel	*Texus brevifolia*	Anticancer
Theophylline	*Camellia sinensis*	Smooth muscle relaxant
Reserpine, Rescinnamine	*Rauwolfia sp.*	Hypotensive, vasodilator
Menthol	*Mentha piperita*	Anti-pruritic, antiseptic
Colchicine	*Colchicum autumnale*	Anti-gout, anti-arthritic
Hyoscine,Hyoscyamine	*Datura, Hyoscyamus, Scopolia,Duboisiaspp.*	Parasympatholytic, Mydriatic
Papaverine	*Papaver somniferum*	Smooth muscle relaxant
Pilocarpine	*Pilocarpus jaborandi*	Parasymparhomimetic, cholinergic
Theobromine	*Theobroma cacao*	Smooth muscle relaxant,

1.4 Current Status of Medicinal Plant

1.4.1 National Status

- Status of medicinal plants in Bangladesh-
- About 500 medicinal plants have been reported to occur in Bangladesh
- Almost 80% of rural populations are dependent on medicinal plants for their primary health care
- The local people conserve traditional knowledge through their experience and practices, which is handed down orally without any documentation
- The over exploitation of wild medicinal plants has become a threat to its extinction
- In Bangladesh there are no systematic cultivation processes of conservation strategies about medicinal plants
- There is no government policy or rules and regulations about the medicinal plants cultivation, conservation, and marketing and
- There are almost 422 herbal medicinal companies using medicinal plants as raw materials mostly by importing from abroad (www.mapbd.com).

1.4.2 International Status

[illegible] enumeration of the WHO from the late 1970 listed 21,000 medicinal species. However, in China alone 4,941 of 26,092 native species are used as drugs in Chinese traditional medicine (Duke, 1985), an astonishing 18.9 percent. If this proportion is calculated for other well-known medicinal floras and then applied to the global total of [illegible] flowering plants species, it can be estimated that the number of plant species used for medicinal purpose is more than 50,000 (www.mapbd.com).

1.5 Rationale of the Work

The use of medicinal herb in the treatment and prevention of disease is attracting attention by scientists' worldwide (Sofowora, 1982). This is corroborated by World Health Organization in its quest to bring primary health care to the people. The plant kingdom has long serve as a prolific source of useful drugs, foods, additives, flavoring agents, colorants, binders, and lubricants. As a matter of fact, it has been estimated that about 25% of all prescribed medicine today are substances derived from plants. Chemicals that make a plant valuable as medicinal plant are:

1. Alkaloids (Compound has addictive or pain killing or poisonous effect and sometimes help in important cures),
2. Glycosides (Use as heart stimulant or drastic purgative or better sexual health),
3. Tannins (Used for gastrointestinal problems like diarrhea, dysentery, ulcer, wounds, and for skin diseases),
4. Volatile/ Essential oils (Enhance appetite and facilitate digestion or use as antiseptic/ insecticide and insect repellant properties),
5. Fixed oils (present in seeds and foods can diminish gastric or acidity),
6. Gum-resins and mucilage (possess analgesic property that suppress inflammation and protect affected tissues against further injury and cause mild purgative, and
7. Vitamins and minerals (Fruits and vegetable are the sources of vitamins and minerals, and these are used popularly in herbals (www.*Life.umd.edu*).

According to WHO, medical plants are accessible, affordable and culturally appropriate source of primary health care for more than 80% of Asia's population (Hossain M.Z.). Bangladesh is an Asian country where only 20% of the people can be provided with modern healthcare services while the rest 80% are dependent on traditional plant-based systems. The use of traditional medicine is increasing in developing countries. This is probably due to the escalating in population, to the government supports to the forms of indigenous medicine, and finally, to the patriotic desire to revive and maintain the traditional culture. There are several studies on the botanical aspects of the plants of Bangladesh.

No accurate result has been published regarding the number if medicinal plants in Bangladesh. Due to favorable climate, abundant rainfall and fertile soil, plants are sufficient in our country. Almost 5000 plants spp found in Bangladesh, about 1000 spp are said to have medicinal qualities. Recent study has identified about 550 medicinal plants in Bangladesh (Yusuf, 1994).The chemical ingredients and uses of 449 medicinal plants have been enlisted (Ghani, 1998). According to concerned authorities, much more medicinal plant species are still waiting to be enlisted as the important medicinal plants of Bangladesh. Day by day phytochemical studies of medicinal plants have got a rapid place with technological advancement. Many chemical compounds of diversified nature from plants often played an important role to give a new direction for laboratory synthesis of many new classes of drug molecules.

1.6 Plant information

Melocanna is a genus of tropical clumping bamboo (tribe Bambuseae of the family Poaceae). It comprises 3 species, found in East Asia. The genus is similar to Bambusa. The 48-year cycle of M. baccifera in northeastern India is responsible for the mautam phenomenon of bamboo flowering, followed by a plague of rats and famine.

1.7. Study Protocol

Due to the availability of the genus *Melocanna Baccifera(Roxb)* in Bangladesh and depending on the tenability of laboratory facilities, the present project work has been designed to evaluate the following activities of *Melocanna* Linn. –

- Phytochemical screening of the crude ethanolic extract of bark of *Melocanna Baccifera(Roxb)* L.
- Evaluation of possible cytotoxic property of the crude bark extract of *Melocanna Baccifera(Roxb)* L. using Brine shrimp lethality bioassay.
- Observation of neuropharmacological activity of the bark extract of *Melocanna Baccifera(Roxb)* L. using rodent behavioral models such as hole cross and open field test.
- Evaluation of analgesic activity of bark extract of *Melocanna Baccifera(Roxb)* L. using acetic acid induced writhing test and tail immersion method and

Chapter 2
Plant Review

2.1. COMMON NAMES

Melocanna

Melocanna is a genus of tropical clumping bamboo (tribe Bambuseae of the family Poaceae). It comprises 3 species, found in East Asia. The genus is similar to Bambusa. The 48-year cycle of M. baccifera in northeastern India is responsible for the mautam phenomenon of bamboo flowering, followed by a plague of rats and famine.

2.2. BOTANICAL CLASSIFICATION OF *Melocanna baccifera*

Scientific classification

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Monocots
(unranked):	Commelinids
Order:	Poales
Family:	Poaceae
Subfamily:	Bambusoideae
Supertribe:	Bambusodae
Tribe:	Bambuseae
Subtribe:	Melocanninae
Genus:	Melocanna Trin.
Species:	*Melocanna baccifera*

Figure 2.1: Steam of *Melocanna baccifera (Roxb).*

2.3. Description

Evergreen bamboo, clump diffuse. Culms 10-20 m high, 3-7 cm diameter, green when young, straw coloured when old; longest internodes 20-25 cm long. Culm-sheaths 10-15 cm long, yellowish green when young and yellowish brown on maturity, brittle, striate, truncate or concave at the tip, glabrous or sparsely with whitish appressed hairs on the back. Ligule very short with undulated or toothed margin, auricles small, sub-equal, membranous, fringed with silvery bristles; blade deciduous, usually 15-30 cm long, 2-3 cm broad, subulate. Young shoots smooth, light purple or purplish green; ligule with long hairs, soon caducous, blades linear, green. Leaves 15-30 cm long, 2.5-5 cm broad, oblong lanceolate, apex acuminate, leaf sheath thick, ligulate; auricles very small with silvery bristles. Inflorescence a large compound panicle of one-sided drooping, spicate branches, bearing clusters of 3 to 4 spikelets in the axils of short, blunt, glabrous bracts, empty glumes 2-4. Palea glabrous, convolute, mucronate, acuminate not keeled. Lodicules 2, narrow, linear-oblong, obtuse and erose-fimbriate at the tip. Stamens free at base or irregularly joined, filaments flat; anthers yellow,notched at the apex; ovary ovoid; style elongate, divided in to 2-4 hairy recurved stigmas. Caryopsis very large, fleshy, pear-shaped, the stalk is inserted at the thick end and the apex terminates in a curved beak.

The species can be recognised easily by diffused clump habit, having culm-sheath straight for about two-third of the way up, then once or twice transversely waved with acuminate flagelliform blade (Alam, 1982).

Chromosome number 2n = 72.

2.4 FLOWERING AND FRUITING

Flowering has been reported during 1863, 1866, 1892, 1893, 1900-1902, 1910-1912, 1933 and 1960 (Chatterjee, 1960; Vaid, 1972). Sporadic flowering was reported in Cachar and Manipur in 1967 (Nath, 1968). Sharma (1992) reported flowering at FRI, Dehra Dun. Flowering and fruiting was observed at Pune during 1993. Length of flowering period according to Gamble (1896) is 30 years, according to Kurz (1876) is 30-35 years, according to Troup (1921) is about 45 years. Culms and rhizomes die after flowering. *Profuse natural regeneration has been observed.*

Seed is green, smooth, sessile, very large having a mid length and diameter of 6.9- 7.2 cm and 4.1-4.3 cm, respectively; obliquely ovoid, thick fleshy, onion shaped and the apex *terminating in a curved beak. There is no endosperm in the ripe seed, but it has a* 7-13 mm thick white to creamy coloured fleshy pericarp filled with starch just below the green surface of the seed. A more or less round shaped white-coloured embryo with a broad fleshy cotyledonary body is present inside the seed cavity. The fruit is not a true caryopsis, it can be termed as a bacciform caryopsis. Vivipary is observed.

2.5. Geographic Distribution

The species is distributed in India, Bangladesh and Myanmar, cultivated in many Asian countries. In India, it is mainly seen in Assam, Manipur, Meghalaya, Mizoram, Tripura, West Bengal and other parts of Eastern India in the plains and low hills (Biswas et al., 1991). Also found in Singtam, East Sikkim. Seen in cultivation in Maharashtra and parts of Karnataka. It grows almost equally on the well-watered sandy clay loam, alluvial soil and on the well drained residual soils consisting of almost pure sand even at the summits of the low sand stone hills. It springs up in practically pure patches where natural forests have been cleared for agricultural purposes (McClure 1966).

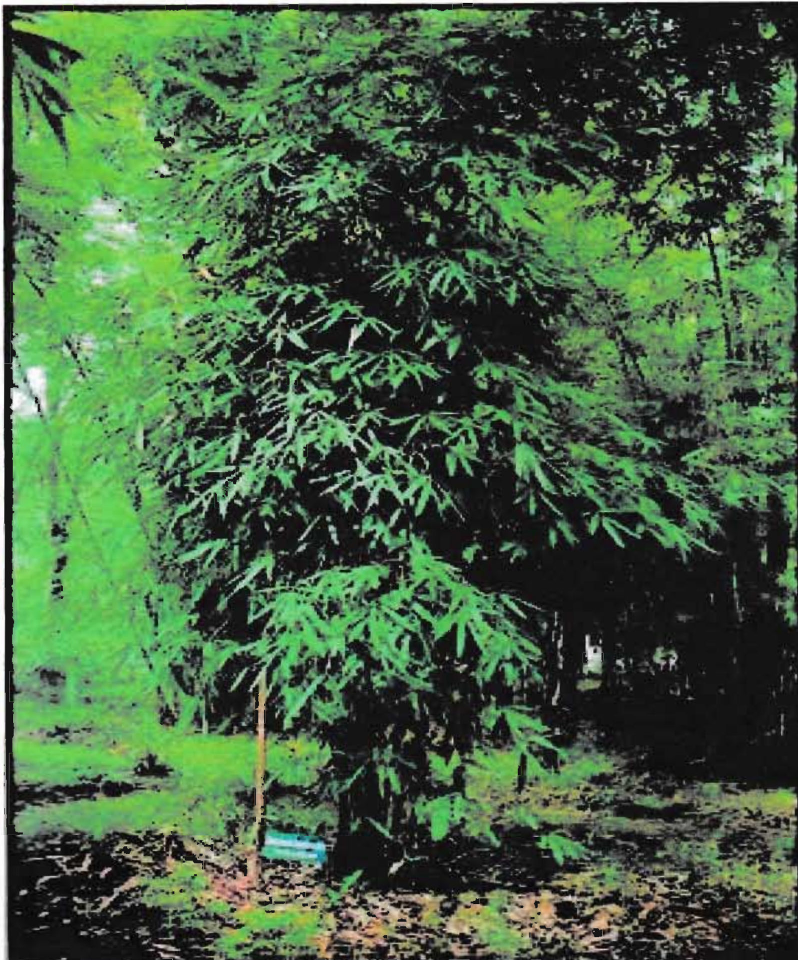


Figure 2.2: Tree of *Melocanna baccifera (Roxb)*

[illegible] ANATOMY AND FIBRE CHARACTERISTICS

The epidermis is made up of long cells alternating with short cells longitudinally. The long epidermal cells uniform in width (about 7.4 µm) with undulating walls, vary in length from 16.5 µm-122 µm. The cell wall thick and septa-like partitions absent. One pair of short cells alternate with an epidermal cell, two pairs of short cells present occasionally. The cork cells small and rectangular or reniform. Silica cells very small, angular or rectangular. The average number of short cell couples is 1894 per mm2. Bicellular and [illegible]-like hairs common often occurring in place of short cells. Spines present, few, mostly solitary, as many as 22 per microscopic field (0.17 mm2). The average number of stomata 10 per field (Ghosh and Negi, 1960). In culm macerates three fibre types are [illegible] very thick, thick, and thin walled. Septate fibres absent, fibre tips pointed, blunt or [illegible] and wall lamellation 4-7-layered. Slenderness ratio 142.2, flexibility ratio 75.6, Runkel ratio 0.8, fibre length 2.68 mm; fibre diameter 14.37 µm, lumen diameter 4.08 µm, wall thickness 5.15 µm, parenchyma 20 per cent (Singh et al., 1976).

[illegible] CHEMISTRY

Proximate chemical analysis showed ash 1.9 per cent, cold water solubles 3.25 per cent, hot water solubles 6.4 per cent, alcohol benzene solubles 1.43 per cent, ether solubles [illegible] per cent, caustic soda solubles 18.97 per cent, pentosans 15.13 per cent, lignin 24.13 per cent, cellulose 62.25 per cent (Bhargava, 1945). Analysis of hemicellulose showed 17.3 per cent yield with the following sugars, pentosans 79.8 per cent, methoxyl 0.8 per cent xylose 79.4 per cent, arabinose 79.4 per cent, rhamnose 0.2 per cent, glucose 16.2 per cent, glucuronic acid 2.1 per cent (Rita Dhawan and Singh, [illegible]). [illegible] characteristics of the species showed caustic soda 25 per cent; kappa [illegible] in bamboo 27 per cent, in pulp 4.1 per cent, pentosans in bamboo 19.6 per cent, [illegible] 15.5 per cent, pulp yield unscreened 43.9 per cent, screened 43.8 per cent ([illegible] et al., [illegible]). Spectral absorbance value of cellulose 0.275, lignin 0.255 (Sekar [illegible] [illegible]manian - personal communication).

2.8 PHYSICAL AND MECHANICAL PROPERTIES

Strength properties tested under air dry condition showed a moisture content of 12.8 per cent, specific gravity 0.751, fibre stress at elastic limit 43.4 N/mm2, modulus of rupture [illegible] N/mm2, modulus of elasticity 12.93 kN/mm2, compression strength parallel to grain 69.9 N/mm2.

2.9 USES

This species is used for building houses, for making woven ware and as an important source of superior paper pulp. Highly suitable for kraft paper making. The culms are strong, durable with inconspicuous nodes. 'Tabasheer' an ancient elixir of Manipur can be isolated from the culms and branches. Fruits are edible. The culms are used for making floats to transport wooden logs. Enormous logs can be transported by these floats.

2.10 Leptocanna chinensis

Leptocanna chinensis is a species of bamboo (tribe Bambuseae of the family Poaceae) and the sole in the genus Leptocanna. The genus name is formed from Greek ("thin-caned") and alludes to the bamboo's thin culm wall (as thin as 23 mm). The genus is endemic to Yunnan, found from 1,500 to 2,500 m.

2.11 Taxonomy

The species has been split from the genus Schizostachyum, where it was known as Schizostachyum chinense. Phylogenetically, Leptocanna is an intermediate genus between Melocanna and Schizostachyum.

2.12 Use

Natives of Southeast Yunnan use this species for the making of gao-sheng, a kind of native rocket used in festivals. The culm of this species is good for weaving.

Scientific classification

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Monocots

(unranked): Commelinids

Order: Poales

Family: Poaceae

Subfamily: Bambusoideae

Supertribe: Bambusodae

Tribe: Bambuseae

Subtribe: Melocanninae

Genus: Leptocanna L.C.Chia & H.L.Fung

Species: L. chinensis

Binomial name

Leptocanna chinensis

(Rendle) L.C.Chia et H.L.Fung

2.13 Schizostachyum chinense

Schizostachyum

Schizostachyum is a tall or shrub-like tropical genus of bamboo. The genus name comes from Greek schistos ("cleft") and stachys ("spike"), referring to the spacing of spikelets.These are clumping, and sometimes climbing bamboos. The genus has about 35 species *found in East Asia.*

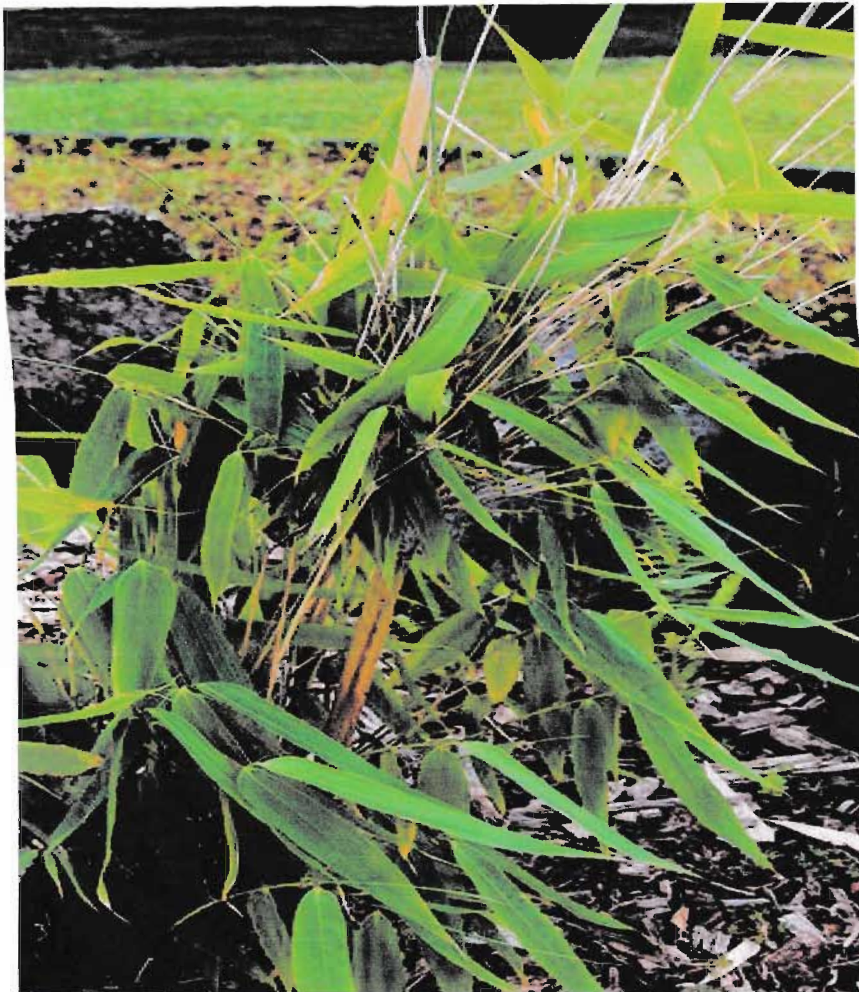


Figure 2.3 Leaves of Schizostachyum

Scientific classification

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Monocots
(unranked):	Commelinids
Order:	Poales
Family:	Poaceae
Subfamily:	Bambusoideae
Supertribe:	Bambusodae
Tribe:	Bambuseae
Subtribe:	Melocanninae
Genus:	Schizostachyum

Species

1. Schizostachyum aciculare
2. Schizostachyum glaucifolium
3. Schizostachyum hantu
4. Schizostachyum jaculans
5. Schizostachyum lima
6. Schizostachyum pilosum
7. Schizostachyum zollingeri

Chapter 3

Materials & Methods

3.1. Plant Materials

3.1.1. Identification of the Plant

Leaves of Melocanna baccifera (Roxb) Kurz.were collected for identification and were identified by Bangladesh National Herbarium, Mirpur, Dhaka. An accession number was given from there and the number is given below (Table). Date of investigation by the herbarium was 22/1/2011

Accession code of the plant given in the following table-

Table 3.1: Accession Code of the Plant

Local name	Botanical name	Family	Accession code
Bamboo leaf	*Melocanna baccifera (Roxb) Kurz*	Poaceae	35,402

Figure 3.1: Accession code of *Melocanna baccifera* on herbarium sheet

3.2. Collection, Drying and Pulverization of Plant Parts

3.2.1. Plant Collection

Leaves of *Melocanna baccifera (Roxb) Kurz* . were collected from Gazipur, Bangladesh. The time of collection was January, 07 at the daytime.

3.2.2. Drying

Leaves of *Melocanna baccifera (Roxb) Kurz* were dried by shade drying for 15 days.

3.2.3. Grinding

After drying, the plant parts were grinded by Blender Machine (NOWAKE, JAPAN). Fine powder was obtained after grinding.

3.3. Extraction of Leaves

Leaves of *Melocanna baccifera* were extracted by hot extraction method. Around 150gm powder was obtained from the dried leaves. From this powder 100.5gm leaves powder was soaked in 500ml of 80% of ethanol in a glass containers for three days and extraction was performed by Soxhlet extractor. The extract was concentrated by evaporation and dried to solid in an oven.

Figure 3.2: Grinding by Using a Blender

Figure 3.3: Hot extraction leaves of *Melocanna baccifera* by using soxhlte extractor.

Figure 3.4: Leaves extract of *Melocanna baccifera (Roxb) Kurz.*

3.3.1. Overall Extraction Process

The Flow Chart of the extraction process is shown below-

Identification of plants/plants part

Collection of plants at suitable time and session

Drying of leaves in a suitable size

Grinding

Powders were collected and stored in a cool and dry place

Preparation of extracts with ethanol by hot extraction

Evaporation and drying of ethanolic extract

Tests were performed on mice to observe the pharmacological effects of the extract.

Flow Chart **1.1** Over all extraction process

3.4. Materials Used in the Study

For the accomplishment of this study several materials are used. The materials which are used in this study are arranged in their category-

3.4.1. List of Apparatus Used

Different types of apparatus are used for the extraction and experimentation which are listed below-

Table 1.1: List of Apparatus Used in the Experiment

Serial No.	Apparatus Name	Source
1	Rotary vaccume evaporator	China
2	LC Oven	LAB-LINE USA
3	Syringe	Opsosaline Ltd. Bangladesh
4	Feeding Needle	Local made
5	Digital Weighing Balance	Denver Instrument Company USA
6	Cotton	India
7	Blender	NOWAKE, Japan
8	Aluminium Foil	DIAMOND, USA
9	Filter Paper	11cm DIA, China
10	Marker Pen	RED LEAF, Japan
11	Thermostatic Water Bath	Shanghai, China
12	Refrigerator	
14	Aluminum foil	
15	Spatula	
16	Lamp	
17	Table-top UV detector (254 & 366 nm)	CAMAG

3.4.2. List of Glassware Used

Different types of glassware are used during experimentation, major wares are listed below-

Table-1.2: List of Glassware Used

Serial no.	Apparatus Name	Source
1	Volumetric Flask (10ml & 50ml)	Changdu, China
2	Test tube	Changdu, China
3	Beaker	Changdu, China
4	Funnel	Changdu, China
5	Measuring Cylinder	Changdu, China
6	Glass Rod	Changdu, China
7	Watch glass	Changdu, China

3.4.3 List of Reagents Used

Many chemicals are used as solvent which are listed in Table-4.4.

Table-1.3: List of Reagents Used for the Experiment

Serial No.	Name of Reagents	Source
1	Methanol	Merck, Germany
2	Acetic Acid	Merck, Germany
3	Tween 80	India
4	Distilled Water	Laboratory Prepared
5	Diclofenac-Na	Opsonin pharma
6	DMSO (as suspending & solubilizing agent)	Merck, Germany
7	Normal saline solution (0.9% NaCl)	Beximco Infusion Ltd.
8	Diazepam	Square pharma, BD
9		

3.4.4 Animal Used

A special type of mice has been used for the experiment. Details of the mice are given in the following table.

Table-1.4.: Detail information of the mice used for the Experiment

Name of the Animal	Source
Swiss *albino* mice Weight: 20-25 gm.	ICDDRB Animal House, Mohakhali, Dhaka Bangladesh

3.4.5. Animal Feed Used

The mice were given special type of chocolate food which was supplied by the ICDDRB.

Table-1.5: Type of Food Used for the Mice

Name of the Animal Feed	Source
Mice Pellets (Chocolate Food)	ICDDRB Animal House, Mohakhali Dhaka, Bangladesh

Figure 3.5: Swiss albino mice

3.4.6. Materials Used for Animal House

The materials were used for the mice house are listed in Table-4.7

Table-1.6: Materials Used for Animal House

Serial No.	Apparatus Name	Source
1	Polyvinyl cages	ICDDRB Animal House, Mohakhali Dhaka, Bangladesh
2	Soft wood for bedding of animals	ICDDRB Animal House, Mohakhali Dhaka, Bangladesh

Table- 1.7 Material for Brine Shrimp Lethality Bioassy

	Materials
1	Artemia saline leach (brine shrimp eggs)
2	Small tank with perforated. Dividing dam to hatch the shrimp
3	Sea salt (NaCl)
4	Lamp to attract shrimps
5	Pipettes
6	Micropipette and Glass vials
7	Magnifying glass
8	Test samples of experimental plants

Table- 1.8 Material for Antioxidant Activity test

Reagent and apparatus for Antioxidant Activity test

DPPH(1,1-diphenyl 1-2picrylhydrazyl)	UV Spectrometer
Ethanol	Vials
Distilled water	test tubes
Vortex machine	Micropipette(50-200
Cotton	Pipette

Chapter-4

Methods

Cytotoxicity test

4.1 Brine Shrimp Lethality Bioassay

The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds, which can lead to the development of novel and safe medicinal agents. The *in- vivo* lethality in a simple zoologic organism can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural products. Meyer *et al.*, 1982 focused on *Artemia salina* Leach as a test organism and developed a protocol for Brine shrimp lethality bioassay to monitor cytotoxicity of a compound. Brine is closely correlated with 9KB (human nasopharyngeal carcinoma) cytotoxicity (ρ= 0.036 and κ=0.56). ED_{50} values for cytotoxicities are generally about one-tenth of the LC_{50} values found in the Brine Shrimp test. Thus it is possible to detect and monitor the fraction of cytotoxic as well as 3PS (P_{388}) (in vivo murine leukaemia) active extracts using the brine lethality bioassay.

The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the microwell scale. In the present study, chloroform, carbon tetra-chloride, hexane & aqueous soluble fraction of the ethanolic leaves extract of *Melocanna baccifera*(Roxb)Kurz. was screened for their cytotoxicity using brine shrimp lethality test.

4.1.1 Principle

Brine shrimp eggs are hatched in stimulated sea water to get nauplii. Test samples are prepared by dissolving in DMSO, desired concentration of test sample is prepared. The nauplii are counted by visible inspection are taken in vials containing 5 ml of stimulated sea water. Then the sample of different concentration are added to the remarked vials through micropipette. The vials are left for 24 hours and then the nauplii are counted again to find out the cytotoxicity of test samples.

4.1.2 Materials

- Artemia salina leaches (Brine shrimp eggs)
- Sea salt (NaCl)
- Small tank with electric air bubbler
- Lamp to attract shrimp
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test Samples of experimental plant

4.1.3 Preparation of stimulated water (Brine water)

Since the lethality test involves the culture of brine shrimp nauplii, that is the nauplii should be grown in a sea water. Sea water is needed for this purpose. Accordingly 3.8% of sodium chloride solution was made by dissolving sodium chloride (38 mg) in distilled water (1000 ml) & was filtered.

4.1.4 Hatching of Shrimps

Sea water was kept in a small tank & shrimps eggs were taken into the divided tank, constant oxygen supply was carried out & constant temperature (37C) was maintained.

Two days were allowed for the shrimp to hatch and mature as nauplii. These nauplii were taken for bioassay.

Figure 4.1: Hatching of brine shrimps in laboratory

4.1.5 Preparation of test solutions

Measured amount of each sample was dissolved in 60 µl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 30 µl were added to pre-marked glass vials/test tubes containing 5 ml of seawater and 10 Shrimp nauplii. So, the final concentration of samples in the vials/test tubes were 1000 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, dilutions.

4.1.6 Counting of Nauplii and Analysis of Data

After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors was counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Microsoft Excel. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

Anti-inflammatory

5.1 Introduction

Inflammation (Latin, inflammare, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of the inflammation.
[http://www.ualberta.ca/~csps/JPPS8(1)/H.Farsam/satureja.htm]

When a pathogen invades a tissue, it almost always elicits an inflammatory response which is basically changes in local blood vessels that cause a response characterized by pain, redness, heat, and swelling at the site of infection (physicians have recognized these four signs of inflammation, in Latin dolor, rubor, calor, and turgor, for thousands of years). The blood vessels dilate and become permeable to fluid and proteins, leading to local swelling and an accumulation of blood proteins that aid in defense, including components of the complement cascade. At the same time, the endothelial cells lining the local blood vessels are stimulated to express cell adhesion proteins that facilitate the attachment and extravasion of white blood cells, initially neutrophils, followed later by lymphocytes and monocytes (the blood-borne precursors of macrophages). These *blood cells and their* secretory molecules mediate the inflammatory response (Abbas, A.K. , Litchman A.H. & Baker, D.L. ,2006).

Various signaling molecules mediate the inflammatory response at the site of an infection. Activation of TLRs (Toll Like Receptors) results in the production of both lipid signaling molecules, such as prostaglandins, and protein (or peptide) signaling molecules, such as cytokines, all of which contribute to the inflammatory response, as do the complement fragments released during complement activation. Some of the cytokines produced by activated macrophages are chemoattractants (called *chemokines*) (Abbas, A.K. , Litchman A.H. & Baker, D.L. ,2006). Some of these attract neutrophils, which are the first cells recruited in large numbers to the site of a new infection. Other cytokines trigger *fever* (a result of inflammation), a rise in body temperature. On balance, fever helps fighting infection, since most bacterial and viral pathogens proliferate better at lower temperatures, whereas adaptive immune responses are more potent at higher temperatures (Abbas, A.K. , Litchman A.H. & Baker, D.L. ,2006).

Still other proinflammatory signaling molecules stimulate endothelial cells to express proteins that trigger blood clotting in local small vessels. By occluding the vessels and cutting off blood flow, this response can help prevent the pathogen from entering the bloodstream and spreading the infection to other parts of the body. The same inflammatory responses that help control local infections, however, can have disastrous consequences when they occur in response to a disseminated infection in the bloodstream, a condition called *sepsis* (Abbas, A.K. , Litchman A.H. & Baker, D.L. 2006). The systemic release of proinflammatory signaling molecules into the blood causes dilation of blood vessels, and loss of plasma volume, which, together, cause a large fall in blood pressure, or *shock*; in addition, there is widespread blood clotting. The end result, known as *septic shock,* is often fatal. Inappropriate or overzealous local inflammatory responses can also contribute to chronic diseases, such as *asthma* and arthritis (Abbas, A.K. , Litchman A.H. & Baker, D.L. ,2006).

5.2 Carrageenan-induced paw oedema

The mices were divided into three groups each containing 5 mices. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan into plantar surface of mice hind paw (Winter et al., 1962). The plant extracts, normal saline and Diclofenac as reference agent were administered 30 min before carrageenan injection. The paw volume was measured at 0, 1, 2 and 3h using a vernier caliper to determine the diameter of oedema. The difference between the readings at time 0 h and different time interval was taken as the thickness of oedema.

[http://www.ijppronline.com/index.php?option=com_content&view=article&id=179&Itemid=155]

Neuropharmacological screening

6.1.Neuropharmacological Screening of *Melocanna baccifera* (Roxb)

Drugs acting on the central nervous system (CNS) were first discovered by primitive humans and are still the most widely used group of pharmacologic agents CNS Action (Katzung, 1998). The effects of drugs on the central nervous system CNS with reference to the neurotransmitters for specific circuits, attenuation should be developed to general organizational principles of neurons. The view that synapses represent drug-modifiable control points within neuronal networks. It requires explicit delineation of the sites at which given neurotransmitters may operate and the degree of specificity with which such site that may be affected (Bloom, 1996).

6.1.1. Objectives

The purpose of this study was to examine neuropharmacological effect of ethanolic extract of bark of *Melocanna baccifera* on mice in a peripheral model of neuropharmacological screening test.

6.1.2. Study Design

Experimental animals were randomly selected and divided into three groups denoted as group-I, group-II, and group-III consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the three doses of the extract. The doses were administered orally in each group of mice. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

6.2. HOLE CROSS TEST

The experiment was carried out as described by Takagi et al. (1971). The most consistent behavioral change is a hyperemotional response to novel environmental stimuli. The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The number of head-dips in the hole-board test in single-housed mice was significantly greater. A steel partition was fixed in the middle of a cage having a size of 30 x 20 x 14 cm. A hole of 3cm diameter was made at a height of 7.5 cm in the center of the cage. Movement of the animals through the hole from one chamber to the other was counted for 3 minuets in this test. The observations were made on 0, 30, 60, 90 and 120 minutes after oral administration of the test drugs.

Figure 6.1: Hole cross test for neuropharmacological activity on mice

armacology and neuroscience. Despite the simplicity of the apparatus, however, pen field behaviour is complex. Consequently, it has been used to study a variety of ehavioural traits, including general motor function, exploratory activity and anxiety-related behaviours. Open-field behavioral assays are commonly used to test both locomotor activity and emotionality in rodents. The test group received leaves extract of *Melocanna baccifera* at the dose of 50, 100 and 200mg/kg body weight orally whereas the control group received vehicle (1% tween 80 in water). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 minuets at on 0, 30, 60, 90 and 120 minutes after oral administration of the test drugs.

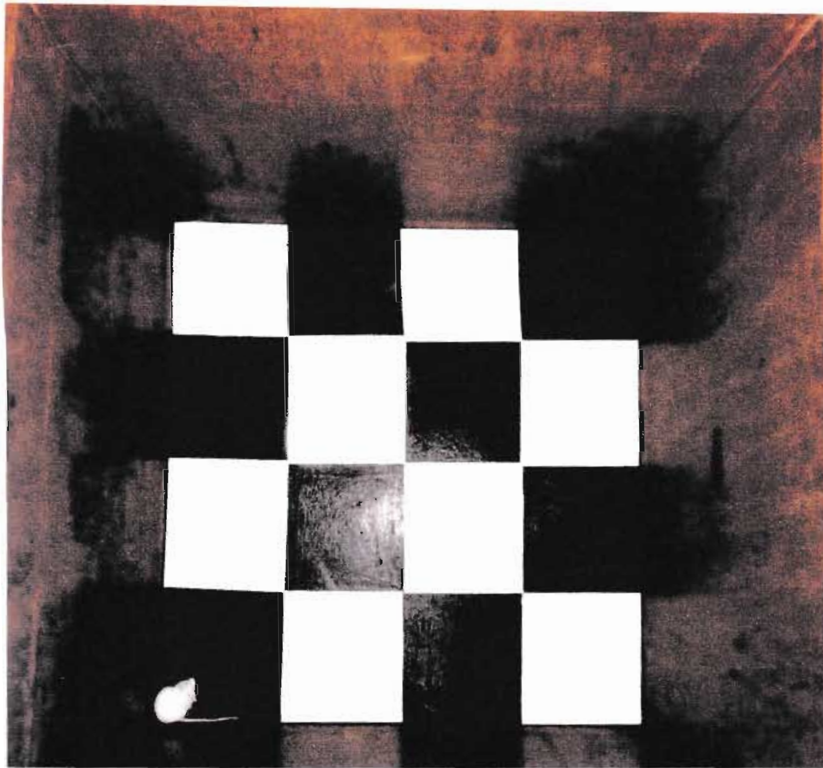


Figure 6.2: Open field test for neuropharmacological activity on Mice

Analgesic test

7.1. Analgesic Activity Test of Leaves Extract of *Melocanna baccifera*

Analgesic drugs which are currently in use are either narcotics or nonnarcotics which have proven side and toxic effects. To develop new synthetic compounds in this category is an expensive venture and again may have problems of side effects. On the contrary, many medicines of plant origin had been used and are in use successfully since long time without any serious effects (Ikram, 1983).

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function (Tripathi, 1999).

Analgesic means a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So, analgesic activity means capacity of a substance to neutralize the pain sensation. (Rang, 1993).

The lack of potent analgesic and anti-inflammatory drugs now actually in use prompted the present study, in which ethanolic leaves extract of *Melocanna baccifera (Roxb).* had been selected for its reported biological activities in indigenous system of medicine.

7.2. ANALGESIC ACTIVITY TEST BY ACETIC ACID INDUCED WRITHING TEST

7.2.1 Objectives

The purpose of this study was to examine analgesic effect of ethanolic leaves extract of *Melocanna baccifera* on mice in a peripheral model of analgesic activity test.

7.2.2. Principle

Acetic acid is a pain stimulus. Intraperitoneal administration of acetic acid (0.7%) causes localized inflammation. Such pain stimulus causes the release of free arachidonic acid from tissue phospholipids by the action of phospholipase A_2 and other acyl hydrolases.

There are three major pathways in the synthesis of the eicosanoids from arachidonic acid. All the eicosanoids with ring structures, which is the prostaglandins, thromboxanes and prostacyclines, are synthesized via the cyclooxygenase pathway. The released prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin-E have been reported to be responsible for pain sensation by exciting the Aδ-fibers. Activity in the A δ-fibers cause a sensation of sharp well localized pain (Rang *et al*, 1993).

Diclofenac used as the positive control in this method, acts by inhibition of prostaglandin synthesis. Any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis.

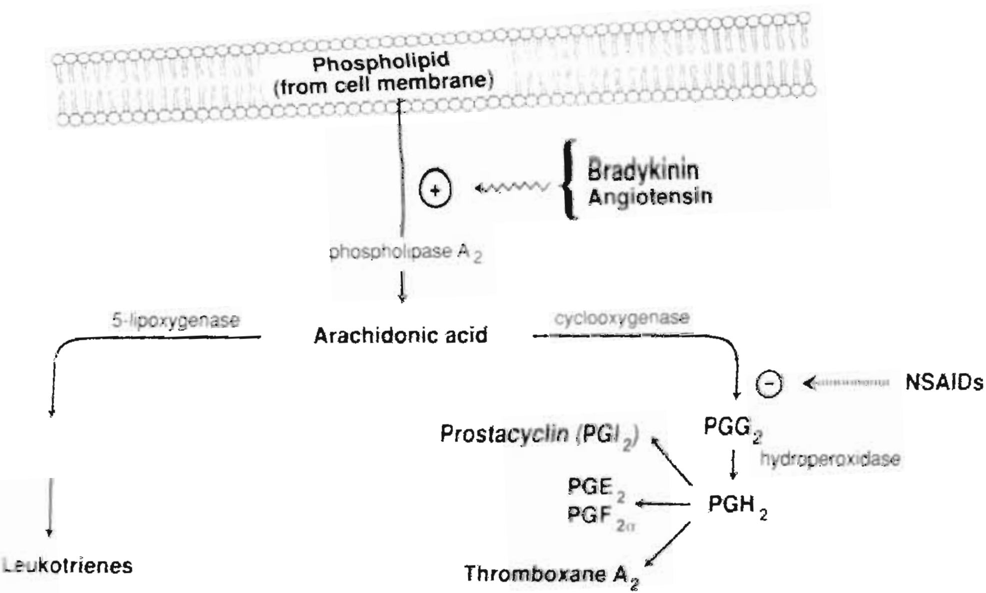


Figure 7.1: Synthesis of prostaglandins and leukotrienes.

East West University
LIBRARY
43, Mohakhali, C/A

7.2.3. Experimental Animal

Young *Swiss albino* mice aged 3-4 weeks, average weight 20-25 gm. were used for the experiment. For this experiment, three groups (I, II, and III) of mice was used and each group contains 5 mice.

7.2.4. Experimental Design

The method described by MAB Howlader et al. (2006) was adopted to study the effect of the *Melocanna baccifera* leaves extract on acetic acid induced writing test. Test samples and control were given orally by means of a feeding needle. A thirty (30) minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was injected intraperitoneally to each of the animals of a group. After an interval of forty five (45) minutes, this was given for absorption and no of squirms (writhing) was counted for 10 minutes.

7.2.5. Preparation of the Test Materials

To prepare solution of the plant extract at a doses of 50mg/kg, and 100mg/kg body weight respectively, 0.02gm extract was measured and added with it 2.5 ml of distilled water and mixing with the help of vortex apparatus. From this solution 0.3ml was taken for 50mg/kg and 100mg/kg dose.

Figure 7.2: Injecting mice intraperitoneally with acetic acid

7.2.6. Mechanism of Writhing Test

The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (diclofenac), control and test sample given orally 30 minutes prior to acetic acid injection.

7.2.7. Study Design

Experimental animals were randomly selected and divided into three groups denoted as group I, group II, and group III, consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

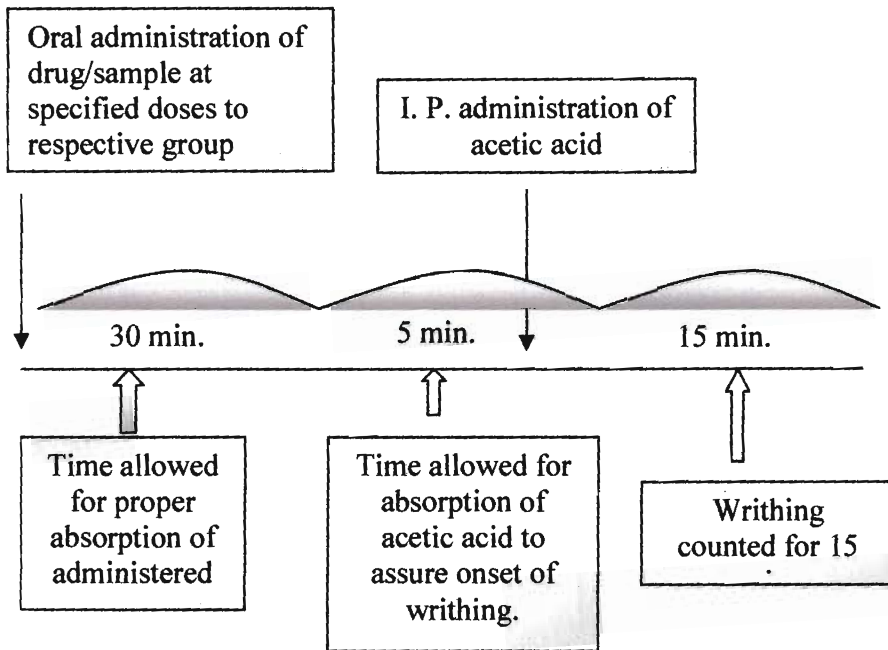


Figure 7.3: Schematic representation of acetic acid induced writhing of mice for investigation of analgesic activity.

Table-7.1: Experiment profile to assess the effect of crude leaves extract of *Melocanna baccifera (Roxb)* on acetic acid induced writhing of mice

Animal Group	Treatment	No. of Animals	Dose	Route of Administration
Control	Placebo (Water and Tween 80)	5	0.50ml	Oral
Animal Group	**Treatment**	**No. of Animals**	**Dose**	**Route of Administration**
Positive Control	Diclofenac-Na	5	50 mg/kg	Oral
Group-I	leaves extract of *Melocanna baccifera*(50 mg/Kg dose)	5	100 mg/kg	Oral
Group-II	leaves extract of *Melocanna baccifera*(1000mg /Kg dose)	5	200 mg/kg	Oral

[illegible] Counting of Writhing

After inducing pain, every mice of all groups was observed carefully to count the number of writhing which made within 10 minutes.

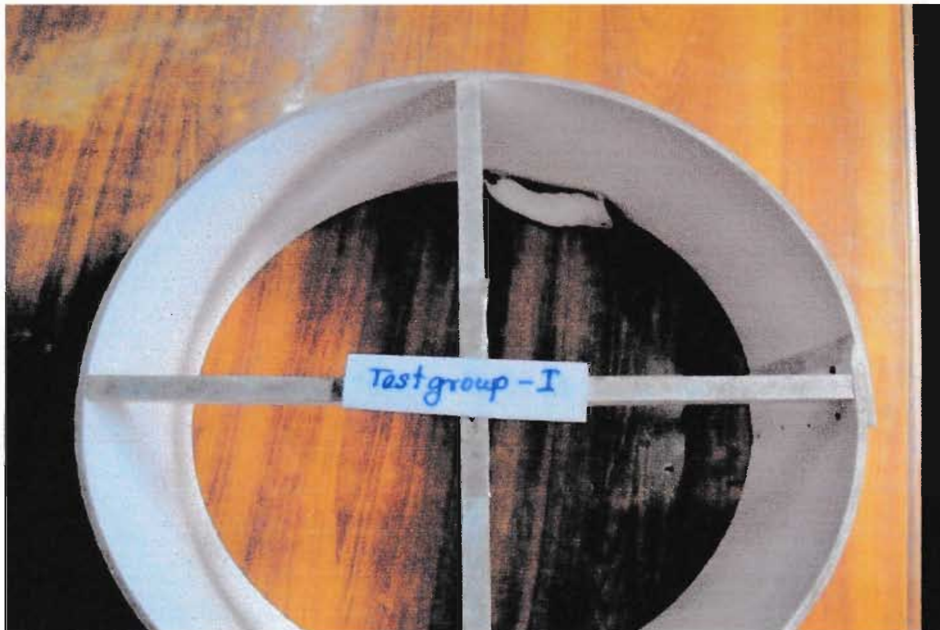


Figure 7.4: Full writhing given by mice

Figure 7.5: Half writhing given by mice

East West University
EAST WEST
LIBRARY
43, Mohakhali, C/A

Antioxidant activity

8.1 Antioxidant activity : DPPH assay

8.1.1 Introduction

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effect of free radicals in the human body and to prevent the deteriroration of fats and other constituents of foodstuffs. (Abdalla and Roozen, 1999)

8.1.2 Principle

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1,1- diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand- Williams et.al. 1995. 2.0 ml of a ethanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of ascorbic acid by UV spectrophotometer at 517nm.

8.1.3 Materials & Method

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medical plants (Choi et al ., 2000 Desmarchelier et al., 1997).

8.1.4 Materials

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants (Choi et al ., 2000 Desmarchelier et al., 2005).So, DPPH is used as reagents. DPPH has a strong absorption band at 517nm. The absorption was taken by UV- Spectrophotometer and methanol was taken for extraction and as a solvent. Ascorbic acid was a standard.

8.1.5 Method

1. DPPH solution (0.04%) was prepared in 95% methanol. The crude extract of *Melocanna baccifera* was mixed with 95% methanol to prepare the stock solution (4 mg/ 40 ml).
2. The concentration of the sample solution was 200microgm/ml. from stock solution 100 micro l solution were taken in 1st test tubes & 100micro l ethanol was added to the stock solution.
3. Then 2ml methanol as added to the test tube.then freshly prepared DPPH solution (3ml) was added in test tubes containinh extract and after 20 minutes incubation, the absorbance was taken at 517nm using a spectrophotometer.
4. Ascorbic acid was used as a positive control
5. The DPPH solution without sample solution was used as control. 955 methanol was used as blank.
6. Percent of the DPPH free radical was measured using the following equation-

   % DPPH radical scavenging (%) = [1-(As/Ac] x 100s

   Here , Ac = absorbance of control

   As = absorbance of sample solution

   Then % inhibitions were plotted against respective concentration used and from the graph IC50 was calculated.

CHAPTER 5

RESULTS

9.1. Results of Brine Shrimp Bioassay

Leaves extract of Melocanna baccifera produced concentration dependent increment in percent mortality of Brine Shrimp nauplii. Results are given in the following table 6.1

Table 9.1.1: Cytotoxic potential of ethanolic leaves extract of *Melocanna baccifera*

Test Solution	Conc. (µg/ml)	Log conc.	% Mortality	LC50 (µg/ml)
Ethanolic leave extract of *Melocanna baccifera*	50	3	33.333	177.86
	200	2.69897	53.333	
	500	2.30103	83.333	
	1000	1.69897	93.333	

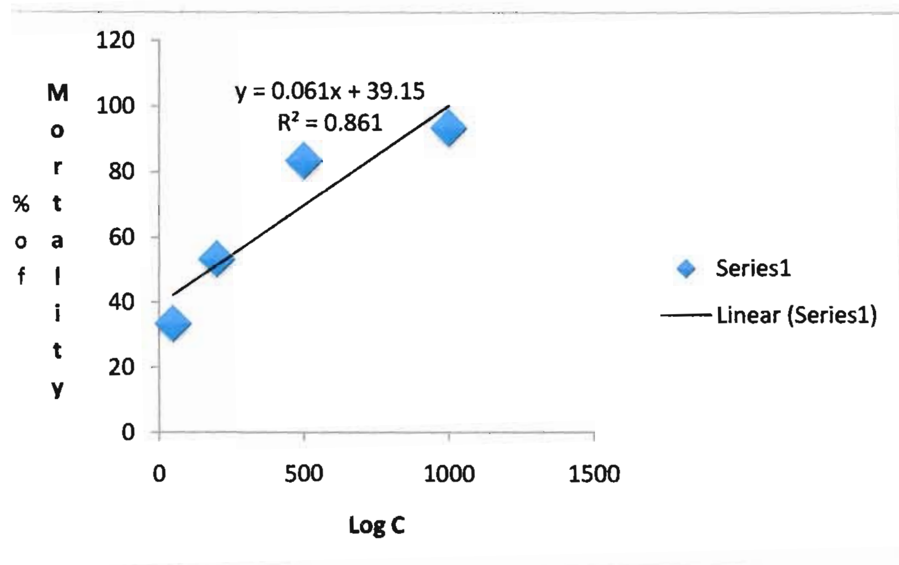
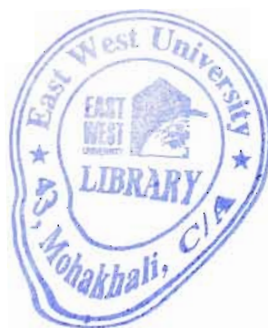


Figure 9.1: Graphical presentation of the cytotoxicity of ethanolic leaves extract of *Melocanna baccifera* toward Brine Shrimp nauplii.

9.2. Discussion:

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. Out of the several plants screened for toxicity against the brine shrimp, some species showed LC 50 values is 177.86 µg/ml and these interesting results lend further support to their traditional use. The ethanolic extract of *Melocanna baccifera (Roxb)* showed a strong lethality against brine shrimp nauplii with a LC50 value of 177.86 µg/ml at concentration 1000 µg/ml.



10.1. RESULTS OF CNS DEPRESSANT FOR HOLE CROSS

The acquired results for the test of leaves extract of *Melocanna baccifera* are presented both in the tabular and graphical form in the following discussion.

Control: Tween-80 + Water,

Group I: extract (50mg/kg), group II: extract (100mg/kg) and

Group III: extract (200mg/kg).

10.1.1. Data of Hole Cross Test

The hole cross test results of leaves extract of M. baccifera are given below in the following table no 10.1

Group	Time	Number of Hole Crossed					Mean	SD	SE
		M-1	M-2	M-3	M-4	M-5			
Control	0 min	18	25	20	27	22	22.4	3.64	1.82
	30 min	12	10	14	12	11	11.8	1.48	0.742
	60 min	11	14	10	12	10	11.4	1.67	0.837
	90 min	7	9	5	8	10	7.8	1.92	0.962
	120 min	11	9	10	11	10	10.2	0.83	0.418
Positive control	0 min	12	12	18	18	15	15	3	1.5
	30 min	5	6	9	9	6	7	1.87	0.935
	60 min	3	2	6	7	5	4.6	2.07	1.037
	90 min	3	1	3	6	5	3.6	1.94	0.975
	120 min	2	0	0	4	1	1.4	1.67	0.837
Group I	0 min	13	10	11	8	9	10.20	1.923	0.962
	30	5	5	4	4	5	4.60	0.547	0.274
	60	2	2	1	1	2	1.60	0.547	0.274
	90	2	4	4	6	2	3.60	1.673	0.837
	120	6	1	3	8	7	5.00	2.915	1.458
Group II	0	11	15	6	9	10	10.20	3.271	1.636
	30	6	9	2	5	6	5.60	2.509	1.255
	60	3	0	0	1	2	1.20	1.303	0.652
	90	0	0	0	2	1	0.60	0.894	0.447
	120	4	0	0	2	3	1.80	1.788	0.894
Group III	0	10	8	11	14	9	10.40	2.302	1.151
	30	7	5	7	6	7	6.40	0.894	0.447
	60	0	0	0	0	0	0.00	0.00	0.00
	90	2	2	2	0	1	1.40	0.894	0.447
	120	4	5	3	2	4	3.60	1.140	0.570

Table 10.1: Primary data table for hole cross test of leaf extract of *Melocanna baccifera* (Roxb)

Table 10.1.2: Effect of leaves extract of ***Melocanna baccifera*** on hole cross test

Group	Route of Admini-stration	Observation				
		0 min	30 min	60 min	90 min	120 min
Control	Oral	22.4± 1.82	11.8± 0.74	11.4± 0.837	7.8± 0.96	10.2± 0.418
Positive control	Oral	15± 1.5	7± 0.93	4.6± 1.03	3.6± 0.97	1.4± 0.83
Group I	Oral	10.20± 0.96	4.60± 0.27	1.60± 0.27	3.60± 0.837	5± 1.45
Group II	Oral	10.20± 1.63	5.60± 1.25	1.20± 0.65	0.60± 0.44	1.80± 0.89
Group III	Oral	10.40± 1.51	6.40± 0.44	0.00± 0.00	1.40± 0.44	3.60± 0.57

Control: Tween-80 + Water,

Group I: extract (50mg/kg), group II: extract (100mg/kg) and

Group III: extract (200mg/kg).

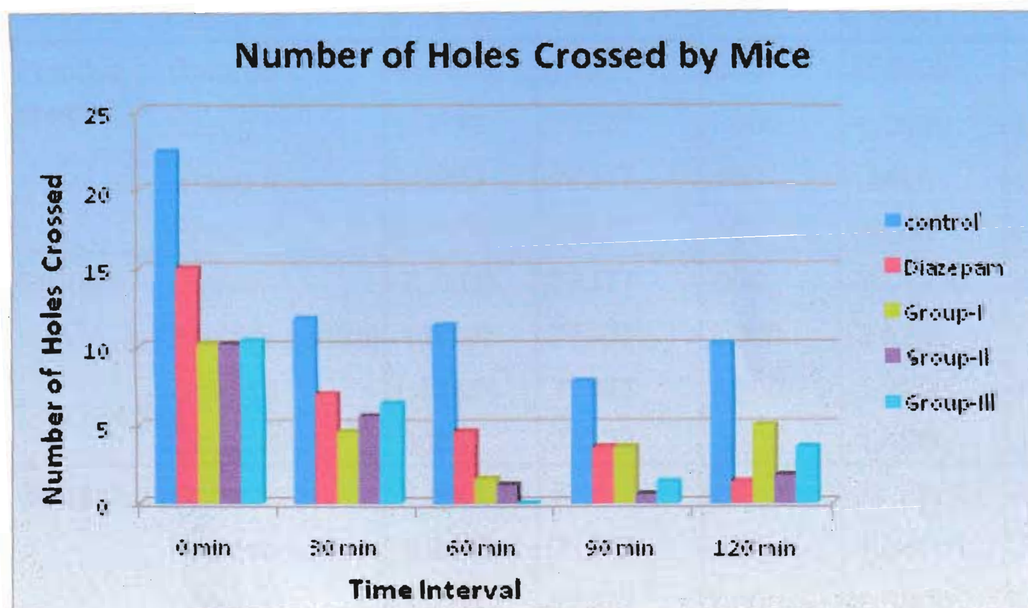


Figure 10.1: Bar diagram presentation of the results of Hole Cross Test of leaves extract of *Melocanna baccifera*

10.1.2. STASTICAL ANALYSIS:

Stastical analysis for hole cross is given below

iple Comparisons (Post Hoc Tests)

re:cns depressant acivity

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
roni	Control	Positive control	6.6400*	.77377	.000	4.2000	9.0800
		Group I	7.7200*	.77377	.000	5.2800	10.1600
		Group II	8.8400*	.77377	.000	6.4000	11.2800
		Group III	8.6800*	.77377	.000	6.2400	11.1200
	Positive control	Control	-6.6400*	.77377	.000	-9.0800	-4.2000
		Group I	1.0800	.77377	1.000	-1.3600	3.5200
		Group II	2.2000	.77377	.100	-.2400	4.6400
		Group III	2.0400	.77377	.158	-.4000	4.4800
	Group I	Control	-7.7200*	.77377	.000	-10.1600	-5.2800
		Positive control	-1.0800	.77377	1.000	-3.5200	1.3600
		Group II	1.1200	.77377	1.000	-1.3200	3.5600
		Group III	.9600	.77377	1.000	-1.4800	3.4000
	Group II	Control	-8.8400*	.77377	.000	-11.2800	-6.4000
		Positive control	-2.2000	.77377	.100	-4.6400	.2400
		Group I	-1.1200	.77377	1.000	-3.5600	1.3200
		Group III	-.1600	.77377	1.000	-2.6000	2.2800
	Group III	Control	-8.6800*	.77377	.000	-11.1200	-6.2400
		Positive control	-2.0400	.77377	.158	-4.4800	.4000
		Group I	-.9600	.77377	1.000	-3.4000	1.4800
		Group II	.1600	.77377	1.000	-2.2800	2.6000
t (2-	Positive control	Control	-6.6400*	.77377	.000	-8.6913	-4.5887
	Group I	Control	-7.7200*	.77377	.000	-9.7713	-5.6687
	Group II	Control	-8.8400*	.77377	.000	-10.8913	-6.7887
	Group III	Control	-8.6800*	.77377	.000	-10.7313	-6.6287

10.3 Discussion

The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS (Mansur, et al., 1980) and this decrease may be closely related to sedation resulting from depression of the central nervous system.The Multiple comparsion table shows the same result obtained by both Bonferroni and Dunnett. Dunnett t test treat one group as a control and compare all other groups against it.The paired comparison of the four groups with control group give P value = .0001. Whereas the comparison between groups gives P value = 1.0

11.1 Data of Open Field Test

The Open Field Test results of leaves extract of *Melocanna baccifera* are given in the following table

Group	Time	Number of Field Crossed					Mean	SD	SE
		M-1	M-2	M-3	M-4	M-5			
Control	0 min	115	110	102	118	120	113	7.211	3.605
	30 min	105	101	107	110	110	106.6	3.781	1.890
	60 min	93	90	96	87	90	91.2	3.420	1.710
	90 min	89	83	84	91	90	87.4	3.646	1.823
	120 min	105	100	97	98	90	98	5.431	2.715
Positive control	0 min	73	95	85	89	85	85.4	8.049	4.024
	30 min	37	29	68	65	53	50.4	17.082	8.541
	60 min	27	25	64	39	30	37	16.015	8.007
	90 min	22	23	29	37	21	26.4	6.693	3.346
	120 min	14	2	2	31	16	13	12	6
Group I	0 min	56	62	67	50	45	56	8.860	4.430
	30	40	46	38	34	28	37.20	6.723	3.362
	60	25	13	22	16	12	17.60	5.683	2.842
	90	22	16	24	19	13	18.80	4.438	2.219
	120	30	26	34	22	16	25.60	6.985	3.493
Group II	0	71	55	68	52	59	61	8.215	4.108
	30	32	26	39	28	22	29.40	6.465	3.233
	60	5	3	22	12	10	10.40	7.436	3.718
	90	6	6	11	9	18	10	4.949	2.475
	120	9	16	6	6	10	9.40	4.098	2.049
Group III	0	30	65	26	39	29	31.80	5.167	2.584
	30	17	27	16	15	15	18	5.099	2.550
	60	4	7	2	8	9	6	2.915	1.458
	90	3	3	19	10	11	9.20	6.648	3.324
	120	19	24	13	13	20	17.80	4.764	2.382

Table 11.1: Primary data table for open filed test of leaves extract of *M. baccifera*

Control: Tween-80 + Water,

Group I: extract (50mg/kg),group II: extract (100mg/kg) and

Group III: extract (200mg/kg)

Table 11.2: Effect of leaves extract of *Melocanna baccifera R.*on open field test

Group	Route of Admini-stration	Observation				
		0 min	30 min	60 min	90 min	120 min
Control	Oral	113± 3.60	106.6± 1.89	91.2± 1.71	87.4± 1.82	98± 2.71
Positive control	Oral	85.4± 4.02	50.4± 8.54	37±8	26.4± 3.34	13±6
Group I	Oral	56± 4.43	37.20± 3.36	17.60± 2.84	18.80± 2.21	25.60±3.49
Group II	Oral	61± 4.10	29.40± 3.23	10.40± 3.71	10± 2.47	9.40± 2.04
Group III	Oral	31.80± 2.58	18± 2.55	6± 1.45	9.20± 3.32	17.80± 2.38

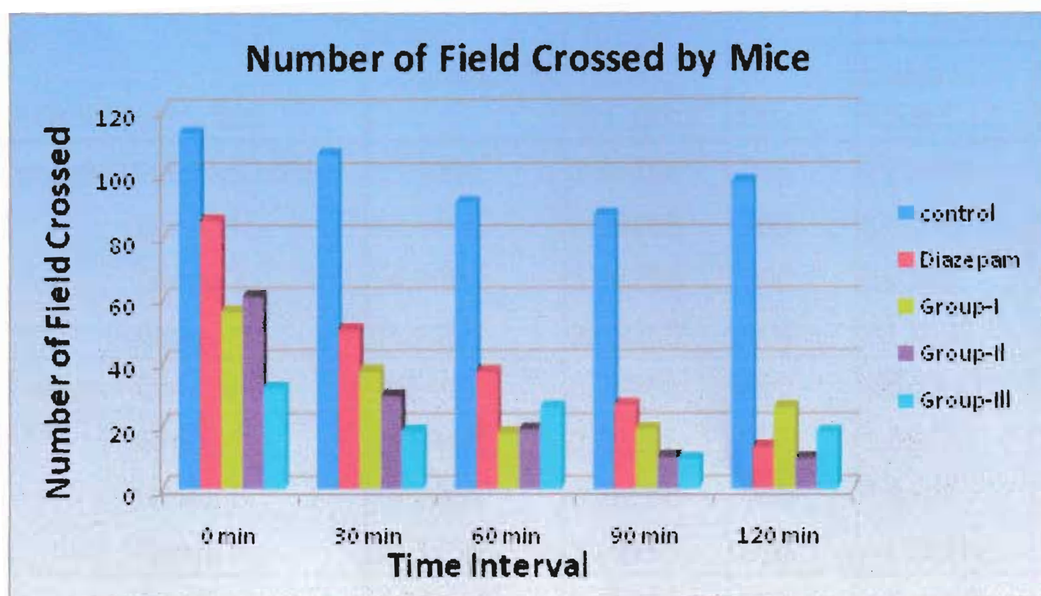


Figure 11.1: Bar diagram presentation of the results of Open Field Test of leaves extract of *Melocanna baccifera.*

11.1.1 STASTICAL ANALYSIS FOR OPEN FIELD

Stastical Analysis of *Melocanna Baccifera (Roxb)* on Open field is given below

ple Comparisons (Post Hoc Tests)

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95%Confidence Interval	
						Lower Bound	Upper Bound
roni	Control	Positive control	56.4000*	3.28258	.000	46.0487	66.7513
		Group I	67.8400*	3.28258	.000	57.4887	78.1913
		Group II	74.6000*	3.28258	.000	64.2487	84.9513
		Group III	81.1200*	3.28258	.000	70.7687	91.4713
	Positive control	Control	-56.4000*	3.28258	.000	-66.7513	-46.0487
		Group I	11.4400*	3.28258	.023	1.0887	21.7913
		Group II	18.2000*	3.28258	.000	7.8487	28.5513
		Group III	24.7200*	3.28258	.000	14.3687	35.0713
	Group I	Control	-67.8400*	3.28258	.000	-78.1913	-57.4887
		Positive control	-11.4400*	3.28258	.023	-21.7913	-1.0887
		Group II	6.7600	3.28258	.527	-3.5913	17.1113
		Group III	13.2800*	3.28258	.006	2.9287	23.6313
	Group II	Control	-74.6000*	3.28258	.000	-84.9513	-64.2487
		Positive control	-18.2000*	3.28258	.000	-28.5513	-7.8487
		Group I	-6.7600	3.28258	.527	-17.1113	3.5913
		Group III	6.5200	3.28258	.609	-3.8313	16.8713
	Group III	Control	-81.1200*	3.28258	.000	-91.4713	-70.7687
		Positive control	-24.7200*	3.28258	.000	-35.0713	-14.3687
		Group I	-13.2800*	3.28258	.006	-23.6313	-2.9287
		Group II	-6.5200	3.28258	.609	-16.8713	3.8313

Dunnett t (2-sided)[a]	Positive control	Control	-56.4000*	3.28258	.000	-65.1023	-47.6977
	Group I	Control	-67.8400*	3.28258	.000	-76.5423	-59.1377
	Group II	Control	-74.6000*	3.28258	.000	-83.3023	-65.8977
	Group III	Control	-81.1200*	3.28258	.000	-89.8223	-72.4177

11.2. Discussion

Based on observed means.The error term is Mean Square(Error) = 26.938.The mean difference is Significant at the .05 level.Dunnett t-test treat one group as a control , and compare all other groups Against it.

11.3 Results of Analgesic Activity

11.3.1. Data of Analgesic Activity of leaves extract of M. baccifera by Writhing Test

All the experimental data of leaves extract of *Melocanna baccifera* on analgesic activity by writhing test are presented in table 11.3.

Table 11.3: Data table of Acetic acid induced writhing test of leaves extract of *M. baccifera*

No. of mice	Control	Positive Control	Group 1	Group 2
m1	39	11.5	33.5	29
m2	38	10	32	25
m3	42	11.5	35	28
m4	45.5	10	34	30.5
m5	42	12	32.5	24
mean	41.3	11	33.4	27.3
SD	2.949576	0.935414	1.193734	2.729469
SE	1.474788	0.467707	0.596867	1.364734
No. of mice	Control	Positive Control	Group 1	Group 2
% of Writhing	100	26.63438	80.87167	66.10169
% of Inhibition	0	73.36562	19.12833	33.89831

Control: Tween-80 + Water

Positive Control: Diclofenac-Na (50 mg/kg)

Group 1: Extract (50mg/kg), Group2: Extract (100mg/kg) and Group 3: Extract (400mg/kg)

Table 11.4: Results of Acetic acid induced writhing test

Administered Substance	SE	Mean ± SE	% of Inhibition
Control	100	26.63438	80.87167
Positive control	0	73.36562	19.12833
Group-1	100	26.63438	80.87167
Group-2	0	73.36562	19.12833
Group 3	100	26.63438	80.87167

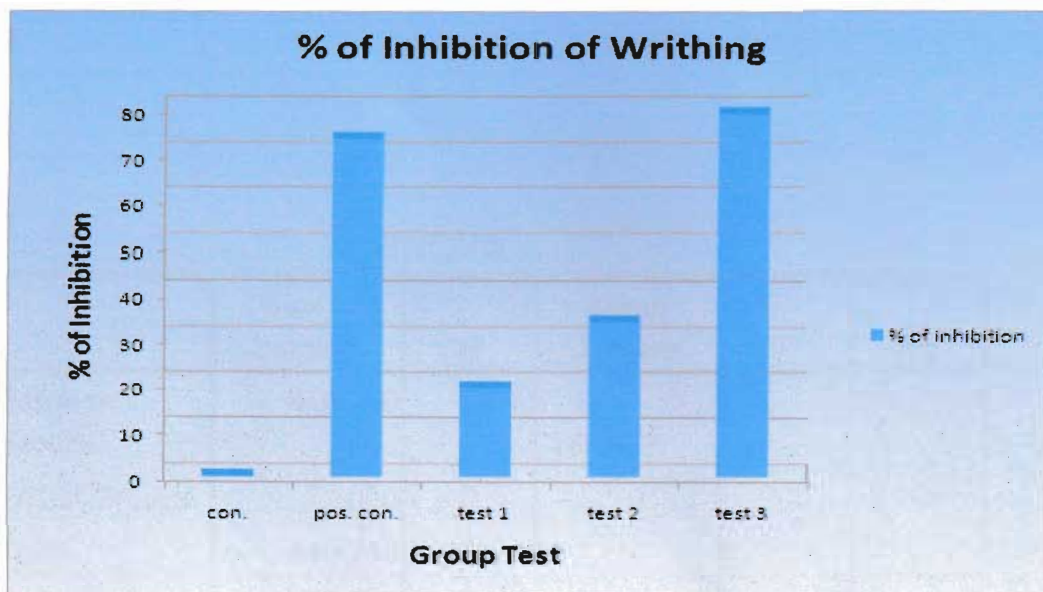


Figure 11.2: Bar diagram showing the result of writhing test by leaves extract of *M. baccifera.*

11.3.2 Stastical analysis

Stastical analysis for analgesis activity of writhing test by leaves extract of Melocanna *backfire was done by SPSS software.*

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
m1	Between Groups	448.250	3	149.417		
	Within Groups	.000	0		000	000
	Total	448.250	3			
m2	Between Groups	436.750	3	145.583	0000	0000
	Within Groups	.000	0			
	Total	436.750	3			
m3	Between Groups	512.188	3	170.729		
	Within Groups	.000	0		0000	0000
	Total	512.188	3			
m4	Between Groups	656.500	3	218.833		
	Within Groups	.000	0		000	000
	Total	656.500	3			
m5	Between Groups	487.688	3	162.563	000	000
	Within Groups	.000	0			
	Total	487.688	3			

11.4. Discussion

In this study, we attempt to use scientific methods to elucidate the anti-nociceptive properties of leaves extract *of Melocanna baccifera* . The data obtained clearly indicated that the leaves extract *of Melocanna baccifera* has no anti-nociceptive activity by the highly significant responses..

12.1 Result of Anti Inflammatory Test

Times and Groups of anti inflammatory test on mice given below.

Table 12.1 Data of anti inflammatory test

Group	Min_0	Min_60	Min_120	Min_180
1.00	3.50	3.00	2.90	2.50
1.00	3.40	3.00	2.60	2.40
1.00	3.40	2.90	2.60	2.50
1.00	3.40	2.80	2.50	2.30
1.00	3.50	3.00	2.80	2.60
2.00	3.40	3.00	2.60	2.50
2.00	3.50	3.10	2.80	2.60
2.00	3.50	3.00	2.90	2.50
2.00	3.00	2.80	2.60	2.30
2.00	3.40	3.00	2.60	2.30
3.00	3.60	3.00	2.90	2.60
3.00	3.50	3.00	2.80	2.50
3.00	3.00	2.70	2.50	2.20
3.00	3.50	3.20	2.80	2.50
3.00	3.60	3.00	2.60	2.40

12.2 STASTICAL ANALYSIS

This stastical analysis was done in SPSS software. Both ANOVA and Post hoc test in Multiple Comparisons is given below

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Min_0	Between Groups	.021	2	.011	.294	.751
	Within Groups	.436	12	.036		
	Total	.457	14			
Min_60	Between Groups	.005	2	.003	.154	.859
	Within Groups	.208	12	.017		
	Total	.213	14			
Min_120	Between Groups	.004	2	.002	.081	.923
	Within Groups	.296	12	.025		
	Total	.300	14			
Min_180	Between Groups	.001	2	.001	.037	.964
	Within Groups	.216	12	.018		
	Total	.217	14			

Multiple Comparisons (Post Hoc Test)

Bonferroni

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Min_0	Control	Standard	.08000	.12055	1.000	-.2551	.4151
		M.B.Extr.	.00000	.12055	1.000	-.3351	.3351
	Standard	Control	-.08000	.12055	1.000	-.4151	.2551
		M.B.Extr.	-.08000	.12055	1.000	-.4151	.2551
	M.B.Extr.	Control	.00000	.12055	1.000	-.3351	.3351
		Standard	.08000	.12055	1.000	-.2551	.4151
Min_60	Control	Standard	-.04000	.08327	1.000	-.2714	.1914
		M.B.Extr.	-.04000	.08327	1.000	-.2714	.1914
	Standard	Control	.04000	.08327	1.000	-.1914	.2714
		M.B.Extr.	.00000	.08327	1.000	-.2314	.2314
	M.B.Extr.	Control	.04000	.08327	1.000	-.1914	.2714
		Standard	.00000	.08327	1.000	-.2314	.2314
Min_120	Control	Standard	-.02000	.09933	1.000	-.2961	.2561
		M.B.Extr.	-.04000	.09933	1.000	-.3161	.2361
	Standard	Control	.02000	.09933	1.000	-.2561	.2961
		M.B.Extr.	-.02000	.09933	1.000	-.2961	.2561
	M.B.Extr.	Control	.04000	.09933	1.000	-.2361	.3161
		Standard	.02000	.09933	1.000	-.2561	.2961
Min_180	Control	Standard	.02000	.08485	1.000	-.2158	.2558
		M.B.Extr.	.02000	.08485	1.000	-.2158	.2558
	Standard	Control	-.02000	.08485	1.000	-.2558	.2158
		M.B.Extr.	.00000	.08485	1.000	-.2358	.2358
	M.B.Extr.	Control	-.02000	.08485	1.000	-.2558	.2158
		Standard	.00000	.08485	1.000	-.2358	.2358

12.3 DISCUSSION

The F test provides different F values with different P value. Here p value is higher than .05. Therefore the test is significant at 1% level of significance.

Chapter 6

Conclusion

6.1 CONCLUSION

In light of the results of the present study, it can be concluded that the leaves extract possess has no cytotoxicity, analgesic , anti inflammatory, cns depressant activity. In CNS depressant test no cns depression has been showed. In Brine Shrimp Lethality Bioassay 93% mortality has been showed at concentration 1000 (µg/ml). The LC50 value was 177.86 (µg/ml). In Anti-Oxidant test it was seen that this leaves extract no anti-oxidant property. So this plant can not use in medicinal purpose.

Chapter 7

References

Bertram G. Katzung, Basic & clinical pharmacology. 7TH Edition.

Fayyaz Ahmad, Rafeeq A. Khan, Shahid Rasheed, (1992). Anti- inflammatory activity from plants extracts of *Lactuca scariola* and *Artemisia absinthium*. *Journal of Islami Academy of Sciences*, Volume 5, Issue2, pp.111-114.

Ghani A (2005). Text Book of Pharmacognosy. 2nd Edition, pp.197-205.

Ghani A (2005). Practical Phytochemistry, pp. 149-152. Parash Publishers.

Ghani A (1998). Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd edition, pp. 4-19. Asiatic Society of Bangladesh,Dhaka.

Gupta BD, Dandiya PC, Gupta ML. (1971). A psychopharmacological analysis of behavior in rat. *Jpn J Pharmacol, Volume* 21, pp.293.

Ikram M, (1983). Hamdard Medicus, Volume26, pp.16-17.

Kirtikar KR, Basu BD (1981). Indian medicinal Plants. 2nd edition. India, Allahabad: Lalit M. and Basu, pp.53

Kirtikar K P and Basu B D (1953). Indian medicinal plants I. 2nd edition. (Allahabad: Basu & Co.)

M.A.B. Howlader, S.C. Bachar, F. Begum and A.S.S. Rouf(2006), Diuretic and *analgesic effect of the* methanol extract of *Phoenix sylvestris* root, *Pak. J. Pharm. Sci.* Volume 19,Issue 4, pp. 330-332.

Mansur RM, Martz W, Carlini E.A. (1980). Effects of acute and chronic administration of *Cannabis satis* and (-) 9-trans tetrahydro cannabinaol on the behavior of rats in open field arena. *Psychopharmacol,* Volume 2, pp.5-7.

Meyer BN, ferringni NR, puam JE, Lacobsen LB, Nichols DE and McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active constituents. *Planta Med,* Volume 45, pp.31-32.

Most. Nazma Parvin, Mohammad S. Rahman2, Mohammad S. Islam and Mohammad A. Rashid (2009). Chemical and biological investigations of *Melocanna baccifera (Roxb). Bangladesh J Pharmacol*, Volume 4, pp.122-125.

Ramesh C. Thapliyal A, Shyam S. Phartyal BE, Jerry M. Baskin C and Carol C. Baskin CD (2008), Role of mucilage in germination of *Melocanna baccifera*, *Australian Journal of Botany,* Volume 56, Issue 7, pp.583–589.

Rang, H.P., Dale, M.M., (1993). Pharmacology, 2nd edition, pp.706-711 Leptocanna chinensis Churchill Livingstone publisher, UK,

Rita Arbianti, Tania Surya Utami, Aji Kurmana, Andre Sinaga(2007). Comparison of antioxidant activity and total phenolic content of *Melocanna baccifera* leaves extracts obtained using various techniques. *14th Regional Symposium on Chemical Engineering.*

SB Yeshwante, AR Juvekar, DM Nagmoti, SS Wankhede, AS Shah, RB Pimprikar, DS Saindane (2009). Anti-inflammatory activity of methanolic extracts of *Melocanna baccifera* leaves, *J Young Pharm,* Volume1,Issue 1, pp.63.

Shome U, Khanna RK, Sharma HP (1980). Pharmacognostic studies on Proc. *Indian Acad Sci (Plant Sci)*
Volume 89, pp.91-104.

Sofowora A (1982). Medicinal Plants and Traditional Medicine in Africa, John Willey & Sons Ltd., Chichester. New York. Toronto. Singapore. pp. 6.

Takagi, K., Watanabe, M. and Saito, H. (1971). Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethan. Its acylesters on the central nervous system. *Jpn. J. Pharmacol.* Volume 21, pp.797.

Toma W, Graciosa JS, Hiruma-Lima CA, Andrane FDP, vilegas W, Souza Brita (2003). Evaluation of the Analgesic and Antiedemetogenic activities of Quassia amara bark extract. *J ethnopharmacol*, Volume 85, pp.19-23.

Chapter 8

Appendix

1. % mortality of Brine Shrimp nauplii =

$$\frac{\text{No. of live nauplii- no. of dead nauplii}}{\text{No. of live nauplii}} \times 100$$

2. Arithmetic Mean ($\overline{X}$) = $\frac{\Sigma X}{n}$

Where, ΣX = Summation of Observed Value

n = No. of Observation

3. Standard Deviation (SD) = $\sqrt{\frac{\Sigma(X-\overline{X})^2}{n}}$

Where, X = individual Value

$\overline{X}$ = Mean Value

n = No. of Observation

4. Standard Error (SE) = $\frac{SD}{\sqrt{(n-1)}}$

Where, SD = Std. Deviation

n = No. of Observation