Pharmacological Investigation of CNS Depressant and Analgesic Activity of Methanolic Extract of *Ixora coccinea*

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 Author

I, **Samira Shafi**, hereby declare that the dissertation entitled **"Pharmacological Investigation of CNS Depressant and Analgesic Activity of Methanolic Extract of *Ixora coccinea*"** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University. The thesis paper 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

This is to certify that the dissertation entitled **"Pharmacological Investigation of CNS Depressant and Analgesic Activity of Methanolic Extract of *Ixora coccinea*"** submitted to the Department of Pharmacy, East West University, Dhaka, in partial fulfilment of the requirements for the Degree of Bachelor of Pharmacy, was carried out by **Samira Shafi** (Student ID: 2013-1-70-054) under my supervision and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/ Diploma.

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Certificate by the Chairperson

This is to certify that the dissertation entitled **"Pharmacological Investigation of CNS Depressant and Analgesic Activity of Methanolic Extract of *Ixora coccinea*"** is a genuine research work carried out by **Samira Shafi** (Student ID: 2013-1-70-054), under the supervision of **Meena Afroze Shanta** (Senior Lecturer, Department of Pharmacy, in partial fulfilment of the requirements for the Degree of Bachelor of Pharmacy, East West University). I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in this connection are duly acknowledged.

_____

Shamsun Nahar Khan, Ph.D

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This Thesis Paper is dedicated to
MY FAMILY

Acknowledgement

All praises to Allah, the almighty, for the successful completion of my thesis soundly and orderly.

At first, I am specially gratefull to my parents for their support, help, sacrifices, cooperation and inspirations in my work.

I would like to express my respect, cordial gratitude and obligation to my thesis supervisor, **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University. I am thankfull and gratefull to her for his valuable instructions, continuous guidance, supervision and necessary advice related to the research work and completion of the thesis paper from the very beginning to the end.

I am thankfull to the Laboratory officers of the Department of Pharmacy of East West University, for their assistance in the laboratory.

I would like to thank **Didarul Islam**, **Suraya Yasmine Juthee**, **Lia Rose Merry**, **Mohammed Ali**, **Noosrat Imrose**, **Sabera Rahman**, **Saiful Arif**, **Fatema Shiraj** and all of my friends, who gave me support for my research work and for their extended cooperation for my study.

It is my great pleasure and privilege to acknowledge my deepest regards and gratitude to **Dr. Shamsun Nahar Khan**, Chairperson and Associate Professor of the department of Pharmacy, East West University for her inspiration in my study.

Moreover I am grateful to my administration for providing me with the excellent lab facilities for research work.

Abstract

Ixora coccinea is an ornamental plant from the family Rubiaceae containing around 500 species. *Ixora* plants have been used by various ethnic groups of Africa, Asia and Europe. The leaves of these plants are reported to have spasmolytic, antiinflammatory, anti-diarrheal, antiasthmatic, hypotensive, antiulcer, antinociceptic, antiviral, antigestagenic and anticancer activity. The aim of the present study was to evaluate CNS depressant and analgesic activity of methanolic extract of *I. coccinea*. The CNS depressant activity was evaluated by open field method and hole cross method, and the analgesic activity of was evaluated by acetic acid induced writhing test where the Swiss albino mice were treated with positive control test with Diazepam (in open field and hole cross) and Indomethacin (in writhing test), and negative control test with water. In CNS depressant test, central locomotion and peripheral locomotion was counted at 30 min interval and in writhing test, no of writhing was counted. The result showed that both the 200 mg/kg and 400 mg/kg of ICM leaves give depressant effect which is supported by reduction in spontaneous motor activity. ICM at 200 mg/kg caused significant reduction in the peripheral locomotion and central locomotion from 147.5± 21.45 at 0 minute to 32.17 ± 23.09 (P<0.001) at 120 minutes and for 400 mg/kg doses the peripheral locomotion was started from 133 ± 21.09 at 0 minute and ended in 49.5 ± 17.4 (P<0.001) at 120 min. In hole cross test, at both 200 mg/kg and 400 mg/kg body weight doses, it produced significant result (p<0.05) and (p<0.001), respectively. At 30 min, number of movement was 40.83 ± 10.06 and 27 ± 10.55 at the dose of 200 mg/kg and 400 mg/kg body weight, respectively; whereas it was 42.67 ± 40.83 and 29.5 ± 7.87 at 0 min at the dose of 200 mg/kg and 400 mg/kg body weight. In writhing test, % inhibition was 27.09% and 33.11% (p<0.05) at the dose of 200mg/kg and 400mg/kg body weight, respectively. From the finding of the result it can be concluded that ICM possesses strong CNS depressant activity in open field test and considerable pain inhibition activity in writhing test.

Key word: *Ixora coccinea,* CNS depressant, Hole Cross, Open Field, Analgesic, Writhing.

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LIST OF ABBREVIATIONS

CMC	Carboxy Methyl Cellulose
CNS	Central nervous system
COX	Cyclooxygenase
EC	Effective-dose Concentration
GABA	Gamma aminobutyric acid
GABAA	Gamma aminobutyric acid Type A
gm	Gram
ICM	Methanolic Extract of Iora coccinnea
i.p.	Intraperitoneal
IC_{50}	Inhibitory Concentration with 50% scavenging
LC_{50}	Lethal Concentration with 50% mortality
mg	Milligram
min	Minute
ml	Millilitre
PG	Prostaglandin
p.o.	Per oral
WHO	World Health Organisation

Chapter 01

Introduction

1.1 Rationale and Objective of the Work

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products. Because of plants role in curing disease our ancestors used various plants as ailments. In recent years, the interest in plants has been renewed in the western world. This interest is channeled into the discovery of new biologically-active molecules by the pharmaceutical industry and into the adoption of crude extracts of plants for self-medication by the general public. In both of these areas some attention is being paid to the investigation and use of ethnopharmacology, the traditional use of plants for medicinal purposes by particular cultural groups. Ethnopharmacologic leads have resulted in the introduction of new single molecule drugs but have a greater role to play if crude extracts are accepted for clinical use in the West. Plants used in traditional medicine therefore have an important role to play in the maintenance of health in all parts of the world and in the introduction of new treatments. Success in natural products research is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, ethnomedical information, field observations or even random collection. (Houghton, 2007)

The objective of this study was to biologically evaluate the effect of methanolic extract of *Ixora coccinea* on Central Nervous System, as well as to evaluate its analgesic property. There is medical flow about this plant and that is possesses various medicinal properties due to which it was extensively used as traditional medicine. Therefore, the objective of this work is to explore the possibility of developing new drug candidates from this plant for the treatment of various diseases.

1.2 Medicinal Plants and Its Importance in Drug Discovery

1.2.1 Definition of Medicinal Plants

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes. (Ghani, 1998)

1.2.2 Importance of Medicinal Plant in Drug Discovery

Some synthetic chemicals may alter body's normal functioning if those are exogeneous. They may even cause mutation. Another problem is less structural diversity. Synthesis of complex structures may be time consuming and also is a costly process. Again, the selectivity of synthetic compounds may be less than the natural compounds as the natural compounds have more complex structures. Certain synthetic drugs can create addiction. In contrast, there are many natural drugs that are very important for use. Often synthetic drugs are made in their comparison but they lack in therapeutic effects than then the natural drugs.

As herbs are not without disadvantages, herbal medicine is not appropriate in all situations. For example, crude medicine can't be used in severe trauma, sudden illness and accidents. Moreover, the appropriate dose can't be determined. Herbs can be poisonous, especially when it is collected from wild sources. At the same time, chances of interaction are higher as no pure constituent is isolated.

So, the most appropriate approach would be chemical isolation of the lead compounds from the crude drugs and modification of the according to decrease the toxic risk. As the

source would remain natural disadvantages of wholly synthetic agents could be overcome. Also, the problem of dose regimen and drug-interaction could be solved as the active principle would be isolated. (Galatea, 2016)

Medicinal plants play a very important role in the identification and synthesis of new chemical entities (NCEs). Between 1981 and 2002 approximately 28% of new entities were found from medicinal plants. Again 20% of synthetic compounds were also produced as mimicking agents of natural products. Natural products serve as the precursor for the synthesis of new synthetic compounds where the compounds have diverse structures and complex stereocenters, which would be challenging to produce without the help of natural products. There are many structural features that are common to natural products (for example chiral centers, aromatic ring, degree of saturation) that are very relevant to drug discovery efforts.

Newer methods of drug discovery include combinatorial chemistry that also has the limitation of less diversity. To overcome this problem medicinal chemists are exploring with the creation of natural product libraries. These libraries combine the structural characteristics of natural products with new synthetic products that are generated by the combinatorial chemistry. With the help of medicinal plants drug optimization is also possible. If during drug discovery from medicinal plants, new chemical structure is not found then important drug lead is also possible with the help of the older compounds that may show new biological activities. Medicinal plants may show promising and selectivity in activity when high-throughput screening is done. Newly validated molecular targets are also being occupied by several compounds that are isolated from traditional medicinal plants.

Some examples of such isolates include indirubin and kamebakaurin that selectively inhibits cyclin dependent kinases and NFnB respectively. Not only these certain compounds are also useful to fight cancer. For example betulinic acid, an isolated compound from medicinal plant is useful against myeloma.

(Balunas and Kinghorn, 2005)

1.3 Current Therapeutic Drugs Isolated from Medicinal Plants

To make prescriptions easily understandable by the patients, Paracelsus (1493 AD) started to use German instead of traditional Latin language used in medicine. His book On Diseases of Miners was very important at that time. Nuremburg Pharmacopoeia was published in 1546. First London Pharmacopoeia published in 1618. Later on its name became the British Pharmacopoeia. Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally isolated from plants. (Hosseinzadeh *et al*., 2015)

Table 1.1 Drugs of current therapeutic use and their plant source

Drug/Chemical	Action/Clinical Use	Plant Source
Allantoin	Vulnerary	*Several plants*
Atropine	Anticholinergic	*Atropa belladonna*
Benzyl benzoate	Scabicide	*Several plants*
Caffeine	CNS stimulant	*Camellia sinensis*

Drug/Chemical	Action/Clinical Use	Plant Source
Camphor	Rubefacient	*Cinnamomum camphora*
Camptothecin	Anticancerous	*Camptotheca acuminata*
Cocaine	Local anaesthetic	*Erythroxylum coca*
Codeine	Analgesic, antitussive	*Papaver somniferum*
L-Dopa	Anti-parkinsonism	*Mucuna sp.*
Digitalin	Cardiotonic	*Digitalis purpurea*
Emetine	Amoebicide, emetic	*Cephaelis ipecacuanha*
Ephedrine	Sympathomimetic, antihistamine	*Ephedra sinica*
Etoposide	Antitumor agent	*Podophyllum peltatum*
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*
Irinotecan	Anticancer, antitumor agent	*Camptotheca acuminate*
Morphine	Analgesic	*Papaver somniferum*
Noscapine	Antitussive	*Papaver somniferum*

Drug/Chemical	Action/Clinical Use	Plant Source
Papavarine	Smooth muscle relaxant	*Papaver somniferum*
Quinidine	Antiarrhythmic	*Cinchona ledgeriana*
Reserpine	Antihypertensive, tranquillizer	*Rauvolfia serpentina*
Taxol	Antitumor agent	*Taxus brevifolia*
Theophylline	Diuretic, brochodilator	*Theobroma cacao and others*
Vinblastine	Antitumor, Antileukemic agent	*Catharanthus roseus*
Vincristine	Antitumor, Antileukemic agent	*Catharanthus roseus*

(Taylor, 2000)

1.4 Global Trend of Using Medicinal Plants

During the past decades, western people have increased their interest in herbal medicines for a safer and natural health care. At the same time, western scientists have intensified their research on the medicinal properties of the plants of developing and third world countries so as to develop more effective and safer drugs for diseases of greater concern. In fact, more than 60% of the new anti-cancer drugs approved since 1983 were derived

from plants. Hence, the countries in Asia, Africa and Latin America see greater scope in earning valuable foreign exchange through export of their plant wealth to the western countries. Here is a list of all the major herbal products and its business in US:

Table 1.2 Major herbal products of US business and their market situation

Common Name	Scientific Name`	Use	Business in million US$
Psyllium	*Plantago ovate*	Laxative	250
Ginkgo	*Ginkgo biloba*	Memory enhancer	138
St. John Worts	*Hypericum perforatum*	Anti depression	121
Garlic	*Allium sativum*	Hypolipidemic	84
Aloe	*Aloe spp.*	Stimulant, Laxative, cosmetic	52
Peppermint	*Mentha piperita*	Anti tussive	40
Ginseng	*Panax spp.*	Adoptogenic	12
Saw-palmetta	*Serenao repens*	Prostate hyperplasia	30

The global exports of medicinal plants was US$ 759 million in the year 2001. China stood as the world's No.1 exporter of medicinal herbs with an export value of US$ 200 million in the same year. In terms of the value of export-import, Hong Kong (17%) plus mainland China (4%) had the largest share (21%) in the import market followed by US (14%) and Japan (10%) in 2001. Leading markets for herbal products in Europe are Germany followed by France, UK and Italy. Germany has the largest herbal extraction

industry in Europe. US is the major market for essential oils and herbal tea. While 80% of the world population still uses traditional medicines, in the developed countries the interest in alternative medicines has increased by 60% since 1989. In the US, consumer use of herbal products was less than 5% in 1991 which increased to about 50% in 2004. WHO estimated the world market for herbal medicines and herbal products is worth US$62 billion and would hit US$ 5 trillion by 2050. The market is growing 7% per annum.

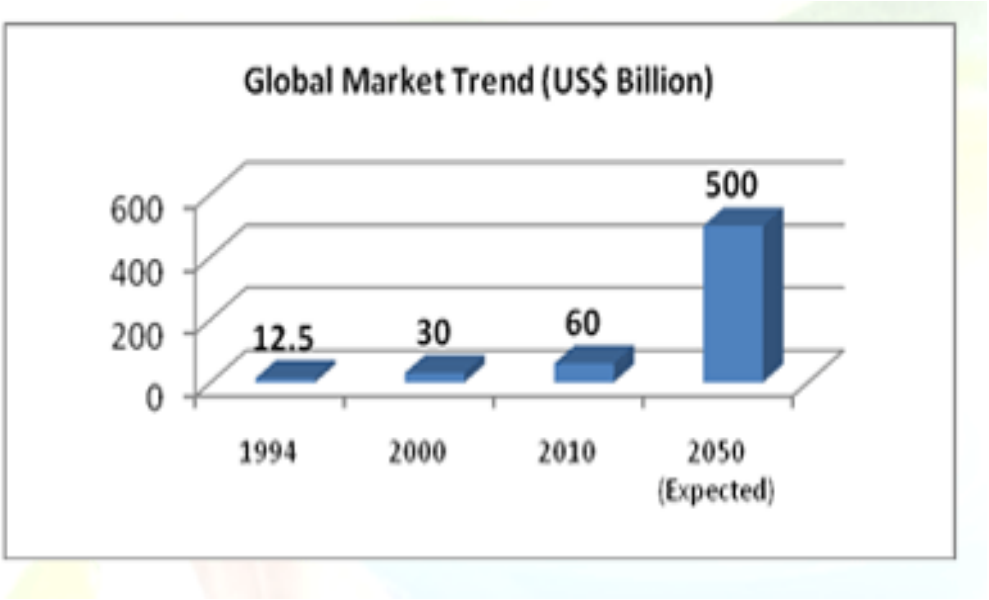

Figure 1.1 Past, present and expected future global market trend

(Rath, 2005)

1.5 History of the Use of Medicinal Plants

History of medicine practically dates back to existence of human civilization. Fossil records date back the use of plants, at least, to the middle Paleolithic age some 60,000 years ago.

The basis of development of modern medicine is rooted in traditional medicine and therapies. The Greek physician Galen (AD 129-200) devised a pharmacopoeia describing the appearance, properties and use of many plants of his time. Natural products chemistry actually began with the work of Seturner, who first isolated morphine from opium in

1805. This, in turn, was obtained from opium poppy (*Papaver somniferum* L.) by process that have been used for over 5000 years.

Use of plants as medicines is a very older tradition of Chinese, Egyptians, Babylonians, Indians and Native Americans. The oldest list of those medicinal plants was found in *Shennong Ben Cao Jing* (c. 3000 B.C.) by Shen Nung.

Materia Medica, compiled by Dioscorides (c. 40-c. 90) and Galen (131-200 A.D.) is another evidence of the use of medicinal plants in Greek and Rome. The book is almost 1500 years old. It also gives a proof that the knowledge of medicinal plants and its use were also spread in Spain, France, Germany and England.

The knowledge of the use of medicinal plants, however, those were preserved in the monasteries of Britain and mainland Europe, in the middle ages. At that time they served as the medical school.

In the seventh and eighth centuries the herbal medical texts of Greek and Roman were acquired by the Arabic scholars. Iranian physician Ibn Sina, (980-1037 A.D.) is known for his contribution as he combined the work of both Galen and Dioscorides. The work is combined in *'The Canon of Medicine'*. Within the eleventh and twelfth century this work spread in many countries of Europe.

In the mid-fifteenth century, mass production of plant extract followed the guidance from the work of Galen, Dioscorides and Ibn Sina and those extracts were available outside monastery and medical schools. General people just had to collect the medicines and use the, as per the prescribed manner and dosage.

Though the uses of medicinal plants have always been proved to be very effective but inappropriate use of them may also cause health issues. This fact was emphasized by Paracelcus (1493-1541). According to him each herb has a characteristic 'sign' which could be observed by its color, scent and the environment where it grows and thus lead to the direction of its proper use. Marigold and dandelion and some other plants with yellow

flowers were used for jaundice and flowers with heart shaped petals and white pansies ere used for heart diseases. (University of Virginia, N.D)

1.6. Traditional Medicine Practice in Bangladesh

Traditional Medicine is the medicine or treatment based on traditional uses of plants, animals or their products, other natural substances (including some inorganic chemicals). Bangladesh possesses a rich flora of medicinal plants. Most probably 5000 species of different plants growing in this country in which more than a thousand are regarded as having medicinal properties. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts. (Jahan, 2016).There is two distinct forms of Traditional medicine practice in Bangladesh:

- **One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:**

- **Folk medicine,** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting, bone-setting, , hot and cold baths, therapeutic fasting and cauterization.(Barre, 1942)
- **Religious medicine**, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and Gods, etc. (Chattopadhyay, 2007)

- **Spiritual medicine,** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient

along with incantations to drive away the imaginary evil spirits and other similar methods. (Chattopadhyay, 2007)

- **The other is the improved and modified form based on the following two main traditional systems:**

- **Unani-Tibb or Graeco-Arab system,** which has been developed by the Arab and Muslim scholars from the ancient Greek system, and

- **Ayurvedic system,** which is the old Indian system, based on the *Vedas* the oldest scriptures of the Hindu saints of the Aryan age.

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people, but practically a good proportion of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them. (Jahan, 2016)

1.7 Use of Medicinal Plants in Bangladesh

Popularity of herbal medicine in Bangladesh is high. There are many people, who have chosen to take alternative medicine over allopathic medicine as ailment. There are lots of people who still believes in natural treatment. That is why, the market share by herbal medicine is high. The market value for Bangladeshi medicinal plants are approximately Tk. 3,300 million at trade prices. The yearly turnover for the Ayurvedic sector is around Tk. 1,000 million, Unani around Tk. 1,800 million and homeopathy around Tk. 500 million.

Table 1.3 The estimated total value of medicinal plant in Bangladesh

Sector	Local		Imported		Total	
	Tk million	US dollar million	Tk million	US dollar million	Tk million	US dollar million
Unani	127	2.2	127	2.20	254	4.40
Ayurvedic	82	1.4	100	1.75	182	3.15
Herbal doctor	45	0.8	54	0.95	99	1.75
Self treatment	76	1.3	200	3.50	276	4.08
Subtotal	330	5.8	481	8.40	811	14.2
Tones	12,500		5,000		17,500	
Total value	810					14
Total quantity	17,500 tones					

(Sharmin, 2004)

1.8 Description of *Ixora:*

1.8.1 Morphology

Ixora is a genus of flowering plants which belongs to Rubiaceae family. It consists of tropical evergreen trees and shrubs and holds around 500 species. *Ixora* also grows commonly in subtropical climates in the United States, such as Florida. Although there are around 500 species in the genus *Ixora*, only a very few is commonly cultivated, There are numerous named cultivars differing in flower colour (red, yellow, pink, orange) and plant size. Several popular cultivars are dwarfs, usually staying under 3 ft (1 m) in height. Leaves are coriaceous, up to 10 cm long, sessile or subsessile, oblong, obtuse. Flowers are numerous and found to grow in clusters. They are bright scarlet, odorous, in sessile, corymbiform, dense-flowered cymes. Fruits are globose, fleshy, size of a pea and have two seeded berry seeds plano- convex. The plant flowers usually grow in April- May and fruits in May-June. (Dontha, Kamurthy and Mantripragada, 2015)

1.8.2 Classification of *Ixora*

Kingdom: Plantae– Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Asteridae

Order: Rubiales

Family: Rubiaceae – Madder family

Genus: *Ixora* L.

Some important species are given below:

Species: *Ixora coccinea*

Species: *Ixora acuminate*

Species: *Ixora casei*

Species: *Ixora chinensis*

Species: *Ixora ferrea*

Species: *Ixora finlaysoniana*

Species: *Ixora grandiflora*

Species: *Ixora macrothyrsa*

Species: *Ixora pavetta*

Species: *Ixora thwaitesii*

Species: *Ixora triantha*

(Dontha *et al*., 2015)

1.8.3 Traditional Uses

The genus Ixora is widely distributed in tropical and subtropical regions of Asia. The leaves, flowers, roots, stem and fruits are used for different purpose by ethnic groups of different region of Asia, Africa and Europe.

1.8.3.1 Leaves

- The leaves of *I. coccinea* were found to have anti-inflammatory, anti-diarrheal, antiasthmatic, antiulcer and antinociceptic activity. They are also used to pacify vitiated pitta, skin diseases, colic, flatulence, diarrhea, indigestion, ulcers, wounds, and used as antiseptic.
- The leaves of *I. grandiflora* are used as poultice in fresh form for treatment of sprain, eczema, boils and concussions. The decoction of leaves was used in treatment of wounds and skin ulcer.
- The leaves of *I. chinensis* have been used to treat headache and stomachache and as a remedy for incipient tuberculosis. *I. finlaysoniana* leaves showed antigestagenic activity.

- The leaves of *I.javanica* are used in treatment of cancer. The leaf extract of *I. parviflora* showed antiviral, hypotensive and spasmolytic activity.

1.8.3.2 Flowers

- The flowers of *I. coccinea* were used for the treatment of cancer, leucorrhoea, dysentery,

dysmenorrhoea, haemoptysis and hypertension.

- The flowers of *I.javanica* contains antitumor principal and are locally used as vegetable.
- In the Philippines, infusion of fresh flowers of *I. chinensis* is drunk *ad libitum* as it is said to be

good for incipient tuberculosis and for hemorrhage and headache. Flower decoction used for amenorrhea and hypertension.

- *I. finlaysoniana* flowers has been scientifically documented to posses estrogenic, abortifacient and anti-implantation properties.
- *parviflora* flowers are used in treatment of whooping cough and in treatment of ulcers.
- The flowers of *I. macrothyrsa* are used to impart color to herbal preparation.

1.8.3.3 Roots

- The roots of *I. coccinea* showed wound healing and anti microbial activity. It is also used as astringent and antiseptic against scabies and other skin diseases.
- The roots of *I. parviflora* are used in treatment of menorrhagia.
- The roots of *I. chinensis* are used in urinary trouble. The decoction is also given after parturition. In Indonesia, decoction of roots is used for bronchial disorders.
- The roots of *I. macrothyrsa* are used in burning sensations, eczema, ringworm other skin diseases menorrhagia, leucorrhea and general weakness.
- The roots of *I. grandiflora* are used in delivery and stomachache.

1.9 Description of *Ixora coccinea*

Four to six-inch globular clusters of bright red, orange, yellow, pink, or white tube-shaped flowers bloom continuously under ideal conditions in full sun. The two to three inch-long leaves are bronzy when young, later turning to a glistening dark green. The much-branched, compact habit of Ixora makes it ideal for hedges, borders, screens, or as a specimen planting, and it may be pruned at any time. Shearing to maintain a hedge will reduce the flower display.

1.9.1 Common Names of *Ixora coccinea* in Various Languages

Language	Name
English	Ixora, Jungle geranium, West Indian jasmine
Bengali	Rangan, Rookmini, Kangan
Hindi	Kangan, Rajana, Rangan, Rookmini, Rugmini
Burmese	Pan sayeik, Pundarik
Dutch	Faja lobi, Faya lobi
Indo Chaina	Bong trang do, Don do, Mau don
Malay	Pechah periuk, Todong periuk
Portuguese	Ixora, siderodendro, Flor de coral
Spanish	Cruz de Malta, Ixora guillermina, Santa rita
Tagalog	Santan

(Baliga and Kurian, 2012)

1.9.2. Availablity

Generally, *I. coccinea* is available at various places all over the world, mainly in Asia and North America.

1.9.3. Morphological Characteristics

- **Height:** 10 to 15 feet
- **Spread:** 4 to 10 feet
- **Plant habit**: upright; oval
- **Plant density:** dense
- **Growth rate:** slow
- **Texture:** medium

❖ **Foliage**

- **Leaf arrangement:** whorled
- **Leaf type:** simple
- **Leaf margin:** entire
- **Leaf shape:** ovate
- **Leaf venation**: pinnate
- **Leaf type and persistence:** evergreen
- **Leaf blade length:** 2 to 4 inches
- **Leaf color:** green
- **Fall color:** no fall color change
- **Fall characteristic:** not showy

❖ **Flower**

- **Flower color:** red; yellow; pink; white; orange
- **Flower characteristic:** year-round flowering

❖ **Fruit**

- **Fruit shape:** round
- **Fruit length:** less than 0.5 inch
- **Fruit cover:** fleshy
- **Fruit color:** purple
- **Fruit characteristic:** inconspicuous and not showy

❖ **Trunk and Branches**

Figure 1.2 Photograph of *Ixora coccinea*: leave, flower and fruit.

- **Trunk/bark/branches:** not particularly showy; typically multitrunked or clumping stems
- **Current year stem/twig color:** reddish
- **Current year stem/twig thickness:** thin

1.9.4. Culture

- **Light requirement:** plant grows in part shade/part sun
- **Soil tolerances:** clay; sand; acidic; loam
- **Drought tolerance:** moderate
- **Soil salt tolerances:** poor
- **Plant spacing:** 36 to 60 inches

1.9.5. Others

- **Winter interest:** Plant has winter interest due to unusual form, nice persistent fruits, showy winter trunk, or winter flowers
- **Feature:** Plant has outstanding ornamental features and could be planted more
- **Invasive potential:** Not known to be invasive
- **Pest resistance:** Very sensitive to one or more pests or diseases which can affect plants health or aesthetics

(Gilman, 1999)

1.10 Traditional Uses of *Ixora coccinea*

Ixora coccinea has been traditionally used in Ayurveda and in the various folk systems of medicine to treat diverse ailments. In the Ayurvedic system of medicine,

- The flowers are used to treat luecorrhoea, dysentery, dysmenorrhoea, haemoptysis, hypertension, menstrual irregularities, sprains, bronchitis fever, sores, chronic ulcers, scabies, and skin diseases. The concoction is prepared by boiling its flowers with the leaves of Coldenia procumbens, Centella asiatica, and the stem bark of Madhuca longifolia, mixed with coconut oil and then used as a wound healing agent. The

flowers are used to treat catarrhal bronchitis and dysentery. The shade-dried flowers are heated in coconut oil and the resultant decoction is externally applied to reduce eczema. The decoction prepared from the cleaned root is supposed to be effective against nausea, hiccups and anorexia.

- The finely powered roots are believed to be effective in healing sores and chronic ulcers. The decoction is supposed to be useful in clarifying the urine.
- The poultice prepared from fresh leaves and stems are believed to be useful in sprains, eczema, boils, and contusions.

(Dontha *et al*., 2015)

1.11 Study Design

Modern medicines are intimately related to chemistry and detailed examinations of active principles of plants and other products from an essential part of it. The healing properties of the plants are due to the presence of physiologically active chemical compounds inside the plant materials. The phytochemical investigation or screening is an evaluatory process for the detection of plant constituents through chemical analysis; phytochemical screening is co-related with phytochemical study. The compounds isolated through phytochemical study are applied on treated animal to find out the pharmacological effect either beneficial or toxic and thus toxic plants are separated.

In this research work three solvents methanolic extracts of *Ixora coccinea* were evaluated for central nervous system depressant activity and analgesic activity. It is found that chemical constituent such as epicatechin, ixoratannin A-2, procyanidin A2, cinnamtannin B-1 and the flavon-3-ol rhamnosides is present in the leaves of Ixora coccineaa which can be extracted out by methanol. Those constituents are rich in polar groups, therefore, these are soluble in methanol.

(Lim, 2014)

Table 1.4 Study Protocol

Extraction	
Plant part	**Solvent**
Leaves	Methanol
In vivo pharmacological activity	
Activity	**Method**
CNS Depressant Activity	1. Open Field Technique 2. Hole Cross Technique
Analgesic Activity	Acetic Acid Induced Writhing test

Chapter 02

Literature Review

2.1 Phytochemistry of *Ixora coccinea*

Phytochemical studies have shown that the major compounds present in Ixora coccinea are lupeol, ursolic acid, oleanolic acid, stearic acid, oleic acid, linoleic acids, and sitosterol.

- The flowers are reported to contain rutin, lecocyanadin glycoside, cyanadin-3-rutinoside, and delphinidin monoglycoside.
- The root bark contains octadecadienoic acid while the root oil has been shown to possess methyl esters of palmitic, stearic, oleic and linoleic acids.
- The leaves are reported to contain A-type trimeric proanthocyanidin epicatechin-(2β→O→7, 4β→8) -epicatechin-(5→O→2β, 6→4β)-epicatechin named ixoratannin A-2, epicatechin, procyanidin A2, cinnamtannin B-1 and flavon-3-ol rhamnosides, kaempferol-7-O-α-l-rhamnnoside, kaempferol-3-O-α-l-rhamnoside, quercetin-3-O-α-l-rhamnopyranoside, and kaempferol-3,7-O-α-l-dirhamnoside.

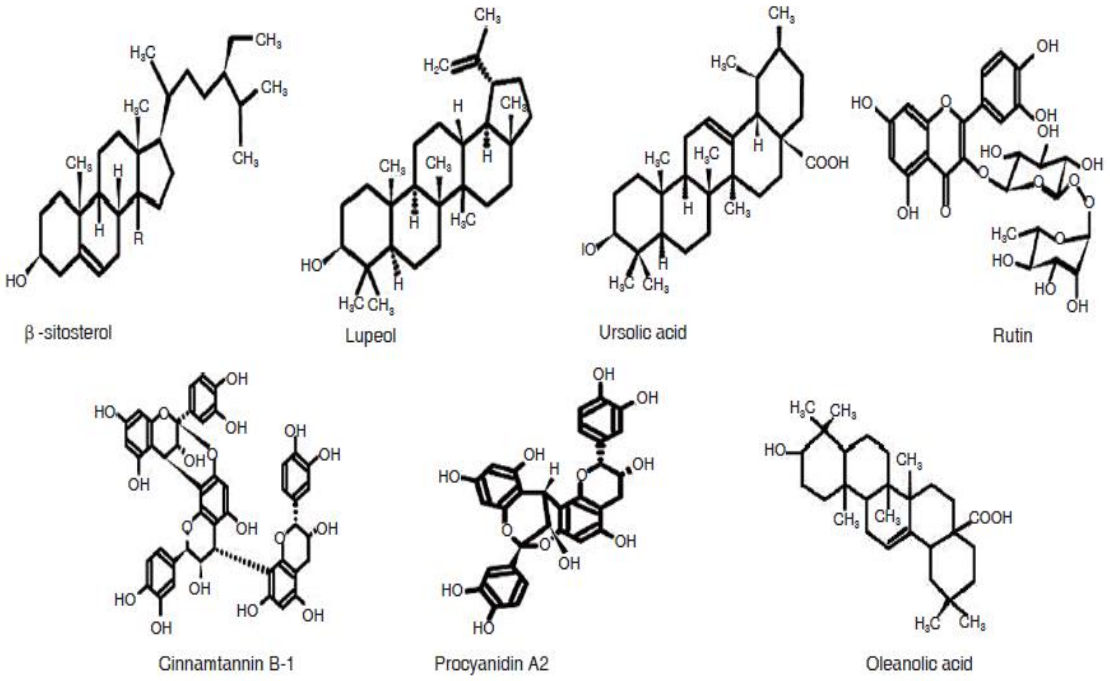

Figure 2.1 Important phytochemicals reported to be present in *Ixora coccinea* Linn. (Dontha *et al*., 2015)

2.2 *In vitro* Free Radical Scavenging Activity of *Ixora coccinea*

Antioxidant activity of the methanol extract of *Ixora coccinea L.* was determined by DPPH free radical scavenging assay, reducing power and total antioxidant capacity using phosphomolybdenum method. Preliminary phytochemical screening revealed that the extract of the flower of *I. coccinea* possesses flavonoids, steroids and tannin materials. The extract showed significant activities in all antioxidant assays compared to the standard antioxidant in a dose dependent manner and remarkable activities to scavenge reactive oxygen species (ROS) may be attributed to the high amount of hydrophilic phenolics. In DPPH radical scavenging assay the IC50 value of the extract was found to be 100.53 µg/mL while ascorbic acid had the IC50 value 58.92 µg/mL. Moreover, *I. coccinea* extract showed strong reducing power and total antioxidant capacity.

(Saha *et al.*, 2008)

2.3 Antioxidant Activity and Total Phenolic Content of Methanol Extracts of *Ixora coccinea*

I. coccinea flowers revealed the best antioxidant property, presenting much lower IC_{50} value (6.6 mg/mL for DPPH assay). The flower extract showed a significantly higher antioxidant capacity compared to the other extracts. Furthermore, the highest phenolic content (polyphenols) was found in the flower extract (210.55 ± 6.31 µg GAE/mg extract). Moreover, *I. coccinea* extracts scavenged the superoxide radical generated by the xanthine/xanthine oxidase system. The xanthine oxidase inhibition activity was in the order of allopurinol > leaf > flower > stem with the percentage of inhibition ranged from 39.7% to 77.3% for the plant parts investigated. The highest phenolic contents (polyphenols) were found in the flower extracts (210.55 ± 6.31 µg GAE/mg extract).

(Torey *et al.*, 2010)

2.4 Antimicrobial Activity and Phytochemical Screening of Various Part of *Ixora coccinea*

In this study, antimicrobial effect of methanolic extracts of various parts of *Ixora coccinea* was studied and the chemical groups of the active constituent were determined. The study was performed by using agar disc diffusion, microdiluation and thin layer chromatography (TLC) bioautography assays. Inhibition zone of methanolic extract of leaf, flower and stem of *I. coccinea* was 6.7 to 11.3 mm and minimum inhibitory concentration was 0.78 to 3.125 mg/mL for all these three extract. Leaf and stem extracts of *I. coccinea* have been proven to show broad spectrum activity. The MIC value of stem extracts against *Staphylococcus aureus* was 62.4 times less potent than vancomycin. Leaf and stem extracts shows 62.4 and 31.2 times, respectively lesser than gentamycin against *Shigella flexneri*. Active extract showed minimum bacteriostatic concentration (MBC) value was ranged from 0.78 to 6.25 mg/mL. After performing TLC and phytochemical screening it has been seen that the antimicrobial property of *I. coccinea* may be due to its active constituents such as terpenoid, flavanoid, coumarin, alkaloid and phenolic groups.

(Marimuthu *et al*., 2011)

2.5 Antimicrobial Activities of Different Species of *Ixora*

The leaves of four species of *Ixora* such as *Ixora chinensis, Ixora lutea, Ixora coccinea* and *Ixora parviflora* were investigated to evaluate the *in vitro* antimicrobial activity by agar well diffusion method against nine bacterial strains of which three are gram positive bacteria (*Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus*) and six are gram negative bacteria (*Escherichia coli, Acinetobacter, Salmonella paratyphi, Salmonella typhi, Klebsiella, Proteus* and *Pseudomonas*). The methanol extracts of the leaves of *I. chinensis, I. lutea* and *I. coccinea* did not show any antimicrobial activity, whereas the extract of *I. parviflora* showed significant antimicrobial activity against *Salmonella paratyphi* (19 mm), *Bacillus subtilis* (18 mm), *Salmonella typhi* (16 mm) and

Acinetobacter (13 mm). Gentamicin (10 µg/disc) was used as standard drug. The results of this study found only *I. parviflora* effective against some organisms.

(Akter *et al*., 2015)

2.6 Biosynthesis of Gold Nanoparticles of *Ixora coccinea* Flower Extract & Their Antimicrobial Activities

The synthesis of gold nanoparticles in aqueous medium using flower extracts of *Ixora coccinea* as reducing and stabilizing agent is done by treating chloroauric acid solution with extract. Rapid reduction of chloroaurate ions was observed leading to the formation of the highly stable gold nanoparticles in solution. The synthesized nanoparticles are confirmed by color changes and it has been characterized by UV-visible spectroscopy. The UV- visible spectra indicate a strong Plasmon resonance that is located at ~550 nm. Presence of this strong broad plasmon peak has been well documented for various Me-NPs, with sizes ranging all the way from 2 to 100 nm. The morphology and size of the biologically synthesized gold nanoparticles were determined using TEM. The images clearly show that the average size of the nanotriangles is about 200 nm, while, the spherical like particles show very small size about 5-10 nm. The study also shows that gold nanoparticles with antibiotic show more inhibitory zones than compared to the standard antibiotics.

(Nagaraj *et al*., 2011)

2.7 Efficacy of *Ixora coccinea* Against Common Fish Pathogens

This study was conducted to evaluate the antibacterial activity of methanol and aqueous extracts of flower of *Ixora coccinea* against six fish virulence strains of bacterial isolates such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Vibrio cholera, Aeromonas hydrophila* and *pseudomonas aeruginosa*. The antibacterial potential of *Ixora coccinea* methanol extract was tested by using Agar well diffused method. The methanolic extract of *Ixora coccinea* showed minimum zone of inhibition when compared to aqueous extract. Phytochemical test was performed for both extracts and showed that the antibacterial activity was due to the presence of phytochemical

compound like alkaloids, tripenoids, saponins, flavonoids, phenols, tannins, protein, carbohydrates and glycosides. The flower extracts were analyzed by using HPTLC.

(Nithiyasoundari *et al*., 2015)

2.8 Wound Healing and Antimicrobial Potentials of *Ixora coccinea* Root Extract

The ethanolic extract of *Ixora coccinea* root showed significant (P<0.001) wound healing activity when compared to standard drug Nitrofurazone (NFZ) ointment (0.2% w/v) with respect to normal control group. The ethanolic extract also showed highly significant antibacterial activity against bacterian strains such as *Staphylococcus aureus*, *Bacillus pumilius, Enterococcus faecalis, Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* and fungi *Candida albicans* and *Aspergillus niger* when compared to standard drug ciprofloxacin and chloramphenicol for antibacterial and griseofulvin for antifungal screening. The aqueous extract showed moderate significant inhibition against all bacterial strains used in this study compared to standard drug.

(Selvaraj *et al.*, 2011)

2.9 Wound Healing Activity of Various Extract of *Ixora coccinea* Leaves

I. coccinea was subjected to in vitro and in vivo wound healing investigation. Petroleum ether, chloroform, methanol, and water extracts of *I. coccinea* leaves were evaluated for in vitro antioxidant, antimicrobial, and fibroblast proliferation activities. The promising *I. coccinea* methanol extract (IxME) was screened for in vivo wound healing activity in Wistar rat using circular excision model. Wound contraction measurement, hydroxyproline quantification, and western blot for collagen type III (COL3A1), basic fibroblast growth factor (bFGF), and Smad-2, -3, -4, and -7 was performed with 7-day postoperative wound granulation tissue. Gentamicin sulfate (0.01% w/w) hydrogel was used as reference standard. IxME showed the potent antimicrobial, antioxidant activities, with significant fibroblast proliferation inducing activity, as compared to all other extracts. In vivo study confirmed the wound healing accelerating potential of IxME, as evidenced by faster wound contraction, higher hydroxyproline content, and improved

histopathology of granulation tissue. Western blot analysis revealed that the topical application of *I. coccinea* methanol extract stimulates the fibroblast growth factor and Smad mediated collagen production in wound tissue.

(Upadhyay *et al*., 2013)

2.10 Antidiarrheal Activity of Flowers of *Ixora Coccinea* Linn. In Rats

A study was performed to evaluate the effect of aqueous extract of *I. coccinea* for its antidiarrheal potential against several experimental models of diarrhea in Albino Wistar rats. The effects of aqueous extracts of flowers of *I. coccinea* evaluated in the castor oil induced diarrhea model. The gastrointestinal transit rate was expressed as the percentage of the longest distance traversed by charcoal divided by the total length of the small intestine. Weight and volume of intestinal content induced by castor oil were studied by the enteropooling method. Loperamide was used as a positive control. The plant-extract showed significant (P<0.001) inhibitor activity against castor oil induced diarrhea and castor oil induced enteropooling in rats at the dose of 400 mg/kg. There was also significant reduction in gastrointestinal motility in the charcoal meal test.

(Maniyar *et al*., 2010)

2.11 CNS Depressant Activity of the Flavonoid Fractions from the Fresh Leaves and Flowers of *Ixora coccinea*

The flavonoid fractions of leaves and flowers which was prepared from ethanolic extracts were tested for CNS depressant activity using Actophotometer where both the leaf at 500 mg/kg and flower extracts at 400 mg/kg showed marked CNS depression up to 87.36% and 95.40%, respectively.

The LD_{50} of the leaf extract was found to be 925.68 mg/kg body weight whereas that of flower extract was to be 1623.77mg/kg bodyweight. The ED_{50} (Leaf, 220.20 mg/kg body weight) is greater than ED_{50} (Flower, 38.85 mg/kg body weight). Thus, it can be inferred that the flower extract is more potent in causing CNS depression than the leaf extract. Therapeutic indexes of the leaf and flower extracts were found to be 4.20 and 41.20,

respectively. It can be concluded that the flower extract has greater Central Nervous System (CNS) depression activity in comparison to the leaf extract. The Therapeutic index of leaf extract (4.20) is lesser than the therapeutic index of the flower extract (41.80). So, it is evident that the flower extract is safer than the leaf extract.

(Sen *et al*, 2011)

2.12 Antiinflammatory and Analgesic Activity of *Ixora coccinea* Flower Extract

The anti-inflammatory and analgesic activity of methanolic flower extract of *Ixora coccinea* Linn. was investigated. The effect of methanolic flower extract of *Ixora coccinea* was studied using carrageenan induced paw edema, acetic acid induced writhing response and hot plate method for studying antiinflammatory and analgesic activity. The extract at the dose levels of 200 and 400 mg/kg body weight significantly reduces ($P<0.05$) carrageenan induced inflammation in rats and shows analgesic activity, as determined by acetic acid induced writhing response and hot plate method. The effect of methanolic flower extract showed dose dependent reduction in the number of writhing as compared to control drug, which was highly significant. The percentage inflammation protection of methanolic flower extract at 400 and 200 mg/kg was found to be 80.14 and 68.26, which were very close to the standard drug (83.86).

(Bhattyacharya *et al*., 2010)

2.13 Anxiolytic Activity of Ethanolic Extract of *Ixora coccinea* in Swiss Albino Mice

Elevated plus maze paradigm test and Hole board test were used to evaluate anxiolytic activity. The ethanolic extract of *Ixora coccinea* was found to be non-toxic up to the dose of 2000 mg/kg and did not cause any death, therefore it is considered as safe. Hence $1/10^{th}$ and $1/5^{th}$ of this dose i.e., 200 mg/kg and 400 mg/kg body weight was used for the activity. The quantitative phytochemical analysis of *Ixora coccinea* ethanolic extract indicates the presence of some active phytoconstituents like alkaloids, carbohydrates,

glycosides, saponins, tannins, flavonoids and reducing sugars. In elevated plus maze apparatus, the total duration in open arm and total number of arm entries during 5 minute period are statistically compared. When compared with control, the time of standard group in open arm was significantly increased (P<0.01). ICEE 200 is not significant and ICEE 400 is significant (P<0.01) when compared with control. The standard drug (diazepam 2 mg/kg, i.p) showed a significant increase in time spent in open arm and a significant decrease in number of arm entries. The standard group (P<0.01) and ICEE 400 (P<0.01) group showed significant reduction in total number of arm entries, when compared with control. In hole board test, the number of nose poking after treatment is statistically compared. When compared with the control group, Standard drug (diazepam 2 mg/kg, i.p) and ICEE 200 and 400 mg/kg, p.o, both significantly decrease the number of nose poking.

(Mohammed *et al*. 2014)

2.14 Anthelmintic Activity of *Ixora coccinea*

The earthworm *Pheretima posthuma* is one of the most important soil invertebrate which was used to investigate the activity of different extracts of *Ixora coccinea* root. The result of this study indicates that the extracts obtained from the roots of *Ixora coccinea* are active against the earthworm. Chloroform soluble fraction shows good anthelmintic activity than Ethyl acetate soluble, Methanolic and petroleum ether extract. Albendazole was used as standard in this study.

(Surana *et al.*, 2011)

Chapter 03

Materials and Methods

3.1 Collection and Preparation of *Ixora coccinea*

3.1.1 Collection and Identification of the Plant material

The leaves of Ixora coccinea were collected from Narayanganj, in March, 2016. The sample was collected and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a Voucher specimen has been deposited for future reference. In the local area this plant is known as 'Rangan'.

The accession number is: *Ixora coccinea* L. - 43416

The specimen samples are kept in the Bangladesh National Herbarium.

3.1.2 Preparation of *Ixora coccinea* Extract

3.1.2.1 Drying of the Fresh Leaves of *I. coccinea*

The plant was thoroughly washed with water. All the unwanted materials were discarded and spread in thin layers on poly bag and placed for shadow drying for 1 week.

3.1.2.2 Grinding and Storage of the Dried Samples

The dried leaves were ground to coarse powder with a mechanical grinder (Grinding Mill). This process breaks the plant parts to smaller pieces thus exposing internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The weight of the total dry powder was 945 gm.

3.1.2.3 Extraction of Dried Powdered Sample

Total amount of dried powder of sample was 945 gm. From the total amount, 315 gm of powder was soaked in Methanol for extraction.

The powder is soaked in 1000 ml of Methanol in amber color glass container for 7 days. When the powder became exhausted of its chemical constituents and to facilitate the process regular shaking was continued for those 7 days. After the completion of

extraction process, the liquid was filtered in three steps. At first, it was done by using sterilized cotton cloth, then by using sterilized cotton filter and finally by No. 1 Whatman filter paper. Then solvent was completely evaporated by rotary evaporator at 60 degree celsius. The yield is preserved in a petridish in refrigerator.

3.2 Drugs

Diazepam and Indomethacin were used for current study which was supplied from Square Pharmaceuticals Ltd, Bangladesh. SANDOZ respectively.

3.3 Experimental Animal

For the pharmacological investigation 40 mice were collected from ICDDRB, Bangladesh. The average weights of the mice were 20 to 25 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 ± 2°C temperature. Also sufficient amount of food and water was supplied all the time.

3.4 Ethnical Approval

Institutional animal ethical committee accepted the guidelines which were followed for animal test.

3.5 Pharmacological Investigation of Plant Extract

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 Methanol Extract

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 *Ixora coccinea* 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 test
- Hole cross test

3.5.1.1 The Design of the CNS Depressant Experiments

In both methods 24 mice were chosen randomly and then divided into 4 groups. They were group 1 to group 4 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 1 - Methanol 200 mg/kg
Group 2 - Methanol 400 mg/kg
Group 3 - Standard (Diazepam)
Group 4 - Control (Distilled Water)

3.5.1.2 Preparation of Standard Drug and Crude Drug

In order to administer the crude extract of methanol at dose 200 & 400 mg/kg body weight of mice. The extract was collected by calculating of mice weight & was sonicated in unidirectional way by the addition of 3 ml of distilled water. For proper mixing, small amount of suspending agent CMC was slowly added. The final volume of the suspension was made up to 4.5 ml. To stabilize the suspension it was stirred well. For the preparation of positive control group (1 mg/kg) Diazepam is taken & a suspension of 4.5 ml is made.

Table 3.1 Test samples used in the estimation of CNS Depressant activity of *Ixora coccinea* plant

Group	Treatment	Dose	Route of Administration
Group 1 (Extract)	ICM	200 mg/kg	Orally
Group 2 (Extract)	ICM	400 mg/kg	Orally
Group 3 (Standard)	Diazepam	1 mg/kg	Orally
Group 1 (Control)	Distilled Water	10 ml/kg	Orally

3.5.1.3 Open Field Test

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

Figure 3.1 Open field test

The flow chart of procedure for evaluation of CNS depressant effect of *Ixora coccinea* plant by open field test is shown below:

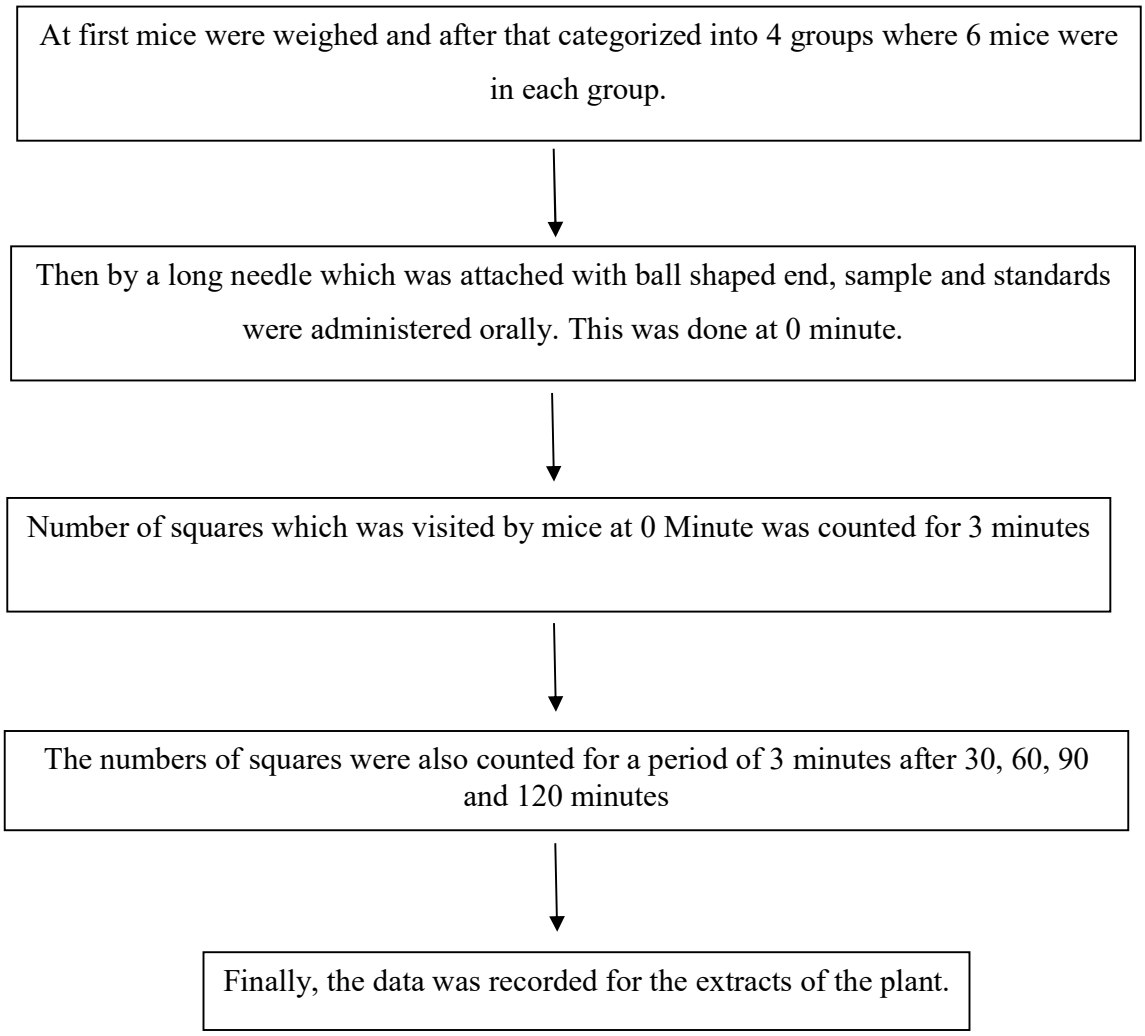

Figure 3.2 Flow chart of process for CNS depressant activity on mice by open field test

3.5.1.5 Hole Cross Test

The main purpose of hole cross test to analyze the locomotor and exploratory effects of the extract by using the hole-board on mice. Takagi's method (Takagi *et al.*, 1971) was followed to examine the test. The box where the hole-board test was tested, a size of 30 x 20 x 14 cm was measured.

The flow chart of procedure for evaluation of CNS depressant effect of *Ixora coccinea* plant by Hole Cross test is shown below:

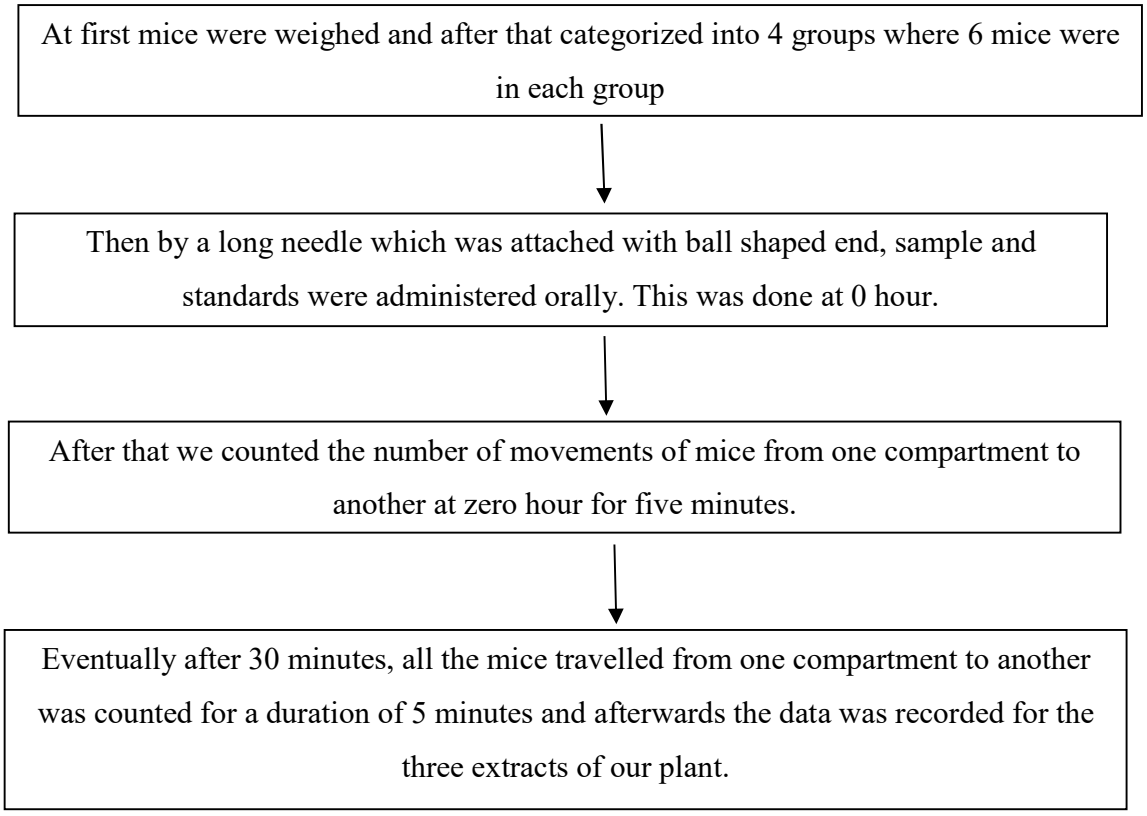

Figure 3.3 Flow chart of process for CNS depressant activity on mice by Hole Cross method

Figure 3.4 Hole cross test

3.5.2 Analgesic Activity of *Ixora coccinea* 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 acetic acid induced writhing technique.

3.5.2.1 Design of the Analgesic Experiments

24 mice were chosen anyway and divided into 4 groups where the groups were from group 1 to group 4 as well as 6 mice were in each group. Each group got a specific treatment. Before the treatment, each mouse were weighed properly as well as marked. Then the dosage of the test sample and control materials was also settled according to body weight.

Group 1 - Methanol 200 mg/kg
Group 2 - Methanol 400 mg/kg
Group 3 - Standard (Indomethacin)
Group 4 - Control (Distilled Water)

3.5.2.2 Acetic Acid-Induced Writhing Technique

Acetic acid induced writhing test is a technique where analgesic behaviour 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 distilled water, 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.

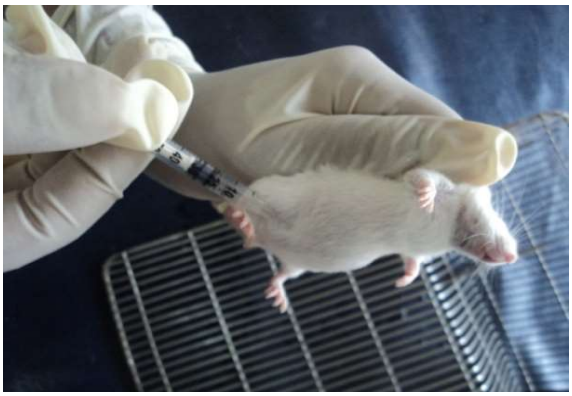

Figure 3.5 Peritonial acetic acid administration

Figure 3.6 Writhing test

3.5.2.2.1 Preparation of Standard Drug and Crude Drug

Standard drug, Indomethacin at dose of 10 mg/kg body weight was prepared by adding 10 mg indomethacin in 10 mL distilled water and the solution was triturated in a unidirectional way.

Crude extract solution was prepared at a dose of 200 mg/kg and 400 mg/kg body weight. For that, extracts were mixed with distilled water by sonication. CMC was added as suspending agent for proper mixing.

Table 3.2 Test samples used in the estimation of analgesic activity of *Ixora Coccinea* by acetic acid induced writhing technique.

Group	Treatment	Dose	Route of Administration
Group 1 (Extract)	ICM	200 mg/kg	Orally
Group 2 (Extract)	ICM	400 mg/kg	Orally
Group 3 (Standard)	Indomethacin	1 mg/kg	Orally
Group 1 (Control)	Distilled Water	10 ml/kg	Orally

3.5.2.2.2 Procedure of analgesic activity of *Ixora coccinea* extract by acetic acid induced writhing technique

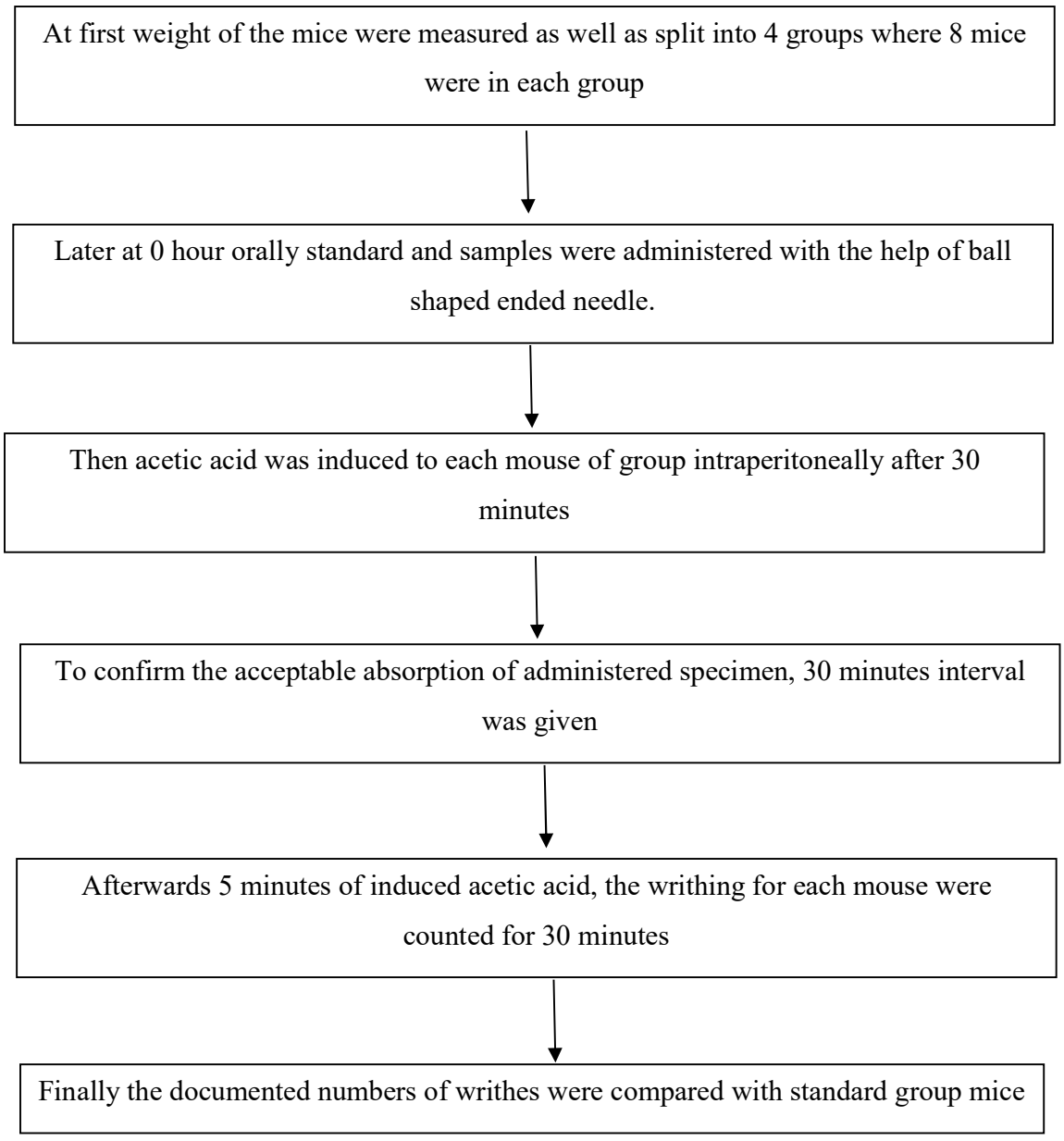

Figure 3.7 Flow chart of process for analgesic activity on mice by acetic acid induced

Chapter 04

Result and Discussion

4. 1 Open Field Test

4.1.1 Result

The CNS depressant effect of ICM in mice by Open-field method is represented in **Table 4.1.** The number of movement on central periphery of the open field board was counted for 3 min, from 0 to 120 min at a 30 min in interval. From this study it was found that ICM gives CNS depressant activity which is statistically significant (P<0.001) at 6o min, 90 min and 120 min for both 200 mg/kg body weight and 400 mg/kg body weight. On the other hand, standard drug gave statistically significant (P<0.001) result at 30 min, 6o min, 90 min and 120 min at 1mg/kg body weight. ICM at 200 mg/kg caused significant reduction in the peripheral locomotion and central locomotion from 147.5± 21.45 at 0 minute to 32.17 ± 23.09 (P<0.001) at 120 minutes and for 400 mg/kg doses the peripheral locomotion was started from 133 ± 21.09 at 0 minute and ended in 49.5 ± 17.4 (P<0.001) at 120 min.

Table 4.1 Effect of methanolic extract of *I. coccinea.*on the locomatory acitivity

Group	Treatment	Dose	Number of Movement				
			0 Minute	**30 Minute**	**60 Minute**	**90 Minute**	**120 Minute**
Group - 1 (Extract)	ICM	200 mg/ kg	147.5 ±21.45	128.33 ±14.23	77.67 ± 27.51***	41.17 ± 16.92***	32.17 ± 23.09***
Group - 2 (Extract)	ICM	400 mg/ kg	133 ± 21.09	113 ± 17.92	81 ± 23.59***	55 ± 21.98***	49.5 ± 17.49***
Group - 3 (Standard)	Diazepam	1 mg/ kg	137.5 ± 44.64	36.17 ± 26.09***	68.33 ± 39.40***	35.67 ± 26.64***	30.33 ± 39.07***
Group - 4 (Control)	Water	10 ml/ kg	137.5 ± 44.64	126 ± 32.79	159.67 ± 15.47	162.5 ± 25.33	153 ± 23.04***

Here, ICM means *Ixora coccinea* in Methanol. Number of writhing values are mean ± S.E.M., (n=6). *P< 0.05, **P<0.01, ***P<0.001 significantly different from control; done by Dunnet test using SPSS:17.00 software.

4.1.2 Discussion:

The most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The locomotor activity is a measure of the level of excitability of the CNS, and decreased activity results from CNS depression (Uma *et al.*, 2011). The result showed that the 200 mg and 400 mg of ICM causes sedative effect, reduction in spontaneous motor activity, exploratory behavior and motor coordination. Decreasing central locomotion count and peripheral locomotion count, supports the evidence of reduction of motor activity in mice. The standard drug (Diazepam) exerts CNS depressant action by stimulating GABAA, ligand-gated chloride-selective ion channels that are activated by GABA to inhibit the release of neurotransmitter. (Camposoria *et al.*, 2006) Ixora coccinea leaves are rich in flavonoids and tannin contents such as epicatechin, ixoratannin A-2, procyanidin A2, cinnamtannin B-1 and the flavon-3-ol rhamnosides (Donthan *et al*., 2015). Flavonoids can produce sedation followed by depression effect by stimulating GABA. (Hernandez et al., 2016). Therefore, the result of the experiment and reports of having high flavonoids strongly suggest that the mechanism of action of ICM may be linked to GABA stimulation and neurotransmitter release inhibition.

4.2 Hole Cross Test

4.2.1 Result

CNS depressant activity of ICM was determined by hole cross method. The movement was counted for 5 min at 0 min and at 30 in. The result of the CNS depressant effect of ICM in mice by hole cross method is represented in **Table 4.2.** From this study it was found ICM gives CNS depressant activity in a dose dependent manner. ICM at both 200 mg/kg and 400 mg/kg body weight doses, produced significant ($p<0.05$) and ($p<0.001$), respectively. At the 2nd observation (30 min), the locomotor activity was found to be lowered than the first observation (0 min). Maximum suppression of locomotor activity

was for reference drug diazepam at 1 mg/kg body weight which was significant (p <0.001).

Table 4.2 Effect methanolic extract of *I. coccinea.* on the locomatory acitivity of mice evaluated by hole-cross test

Group	Treatment	Dose	Number of Movement	
			0 Minute	30 Minute
Group - 1 (Extract)	ICM	200 mg/ kg	42.67 ±40.83*	40.83 ±10.06*
Group - 2 (Extract)	ICM	400 mg/ kg	29.5 ±7.87***	27 ± 10.55***
Group - 3 (Standard)	Diazepam	1 mg/ kg	19 ± 5.22***	9.83 ±6.31***
Group - 4 (Control)	Water	10 ml/ kg	58.5 ± 4.93	56.33 ±4.50

Here, ICM means *Ixora coccinea* in Methanol. Number of writhing values are mean ± S.E.M., (n=6).

*P< 0.05, **P<0.01, ***P<0.001 significantly different from control; done by Dunnet test using SPSS:17.00 software.

4.2.2 Discussion

The study shows decrease in locomotion of mice from its initial value during the period of experiment by the hole cross method. Since locomotor activity is a measure of the level of excitability of the CNS, this decrease in spontaneous motor activity could be attributed to the CNS depressant effect of the plant extracts. The activity may be due to the flavonoids present in the extracts (Hasan, 2009) that may interact with the gamma aminobutyric acid (GABA) type A receptors in the brain.

4.3. Acetic Acid-Induced Writhing Test

4.3.1 Result

Analgesic activity of ICM was determined by acetic acid induced writhing test. The result of the pain inhibitory effect of ICM in mice by hole cross method is represented in **Table 4.3.** From this study it was found that ICM inhibited writhes in a dose dependent manner. (%) inhibition was 27.09% for 200 mg/kg body weight and 33.11mg/kg (p<0.05) for 400mg/kg body weight, whereas for standard drug (Indomethacin) the percent inhibition is 86.96% (p<0.001) for 10mg/kg body weight. So, it can be concluded that the acetic acid induced pain inhibitory activity of 400 mg/kg body weight of methanolic extract of *I. coccinea* is statistically significant (p<0.05).

Table 4.3 No of writhing observed in different groups in Acetic Acid induced Writhing test and Inhibitory effect of methanolic extract of *I. coccinea*

Group	**Treatment**	**Dose**	**No. of Writhing (Average ± S.E.M)**	**% Inhibition**
Group - 1 (Extract)	ICM	200 mg/ kg	36.33 ±10.93	27.09
Group - 2 (Extract)	ICM	400 mg/ kg	33.33 ±14.36*	33.11
Group - 3 (Standard)	Indomethacin	10 mg/ kg	6.50 ± 4.59***	86.96
Group - 4 (Control)	Water	10 ml/ kg	49.83 ± 12.48	---

Here, ICM means *Ixora coccinea* in Methanol. Number of writhing values are mean ±S.E.M., (n=6).

*P< 0.05, **P<0.01, ***P<0.001 significantly different from control; done by Dunnet test using SPSS:17.00 software.

4.3.2 Discussion

Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids due to increase in prostanoinds (e.g. PGE2, PGF2α and lipoxygenase) in peritoneal fluid (Ahmed *et al*., 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate analgesic property. The response is thought to be mediated by peritoneal mast cells (Ronaldo *et al*., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al.*, 2006). In general, flavonoids act as potential inhibitors of cyclooxygenase, lipoxygenase, and nitric oxide synthase as well as being antioxidants. Therefore, the results of the acetic acid induced writhing and report of having high flavonoid content strongly suggest that the mechanism of action of ICM may be linked partly to lipoxygenase or cyclooxygenase pathways.

Chapter 05

Conclusion

Conclusion

Ixora coccinea is a well known medicinal plant and widely distributed in various part of the world. In this study, methanolic extracts *Ixora coccinea* Linn. Leaves (Fam. Rubiaceae) were subjected to pharmacological investigations to validate the traditional use and to find out any other therapeutic activities. The crude extract was evaluated for CNS depressant activity and analgesic activity on Swiss albino mice. The plant extract showed to have activity in a dose dependent manner and the activity was found to be significant. All the conducted experiments in the present study are based on crude extract and are considered to be preliminary. Therefore, more sophisticate research is necessary to reach a concrete conclusion about the findings of the present study. Elaborate phytochemical investigation must be arranged that might lead to isolation and characterization of chemical constituents present in the crude extracts. Subsequently, isolated phytoconstituents must be subjected to all the present plus some additional more advanced pharmacological, both in vivo and in vitro, tests to claim a particular chemical constituent to be responsible for a specific biological activity. In this study, only in vivo pharmacological activity for CNS depression and analgesic was conducted. No phytochemical screening was performed. As the result came out to be significant, chemical isolation would be a great step in an approach of new drug discovery.

Chapter 05

Reference

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