# Determination of Analgesic Activity of Two Medicinal Plants of Bangladesh

A Dissertation Submitted to the Department of Pharmacy, East West University,

in The Partial Fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

**Submitted by**

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# Declaration by the Research Candidate

I, **MD. Saiful Islam Arif,** hereby declare that the dissertation entitled **"An Analgesic Activity of two medicinal plants of Bangladesh"** submitted by myself to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2017, under the supervision and guidance of **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University and the thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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# Certificate by the Supervisor

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The thesis 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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# Abstract

Methanol extract of bark and leaves of *Stereospermum chelonoides* (SCBM & SCLM) and chloroform and pet ether extract from *Ixora coccinea* (ICC & ICPE) was assessed for analgesic properties on animal (mice) models. Administration of SCBM, SCLM and ICC, ICPE produced significant ($p$ <.05), dose-dependent analgesic effect in formalin-induced the biting and licking in mice, suggesting the involvement of both central and peripheral mechanisms in alleviating the pain response. In the current study, Ibuprofen was used as the standard analgesic drug. From the result it was found that, the SCBM inhibition 47.95 % and 47.62 % at doses of 250 mg/kg and 500 mg/kg respectively, SCLM inhibition 23.20 % and 31.05 % at doses of 250 mg/kg and 500 mg/kg respectively. On other hand extract of ICC inhibition 10.09% and 31.05% at the doses of 200 mg/kg and 400 mg/kg respectively and extract of ICPE gave 23.20% and 26.27% inhibition at the doses of 200 mg/kg and 400 mg/kg respectively. The standard group gives 58.19% of inhibition ($p<0.05$).

**Key Words:** *Stereospermum chelonoides*, *Ixora coccnea*, Formalin test, Analgesic activity, Flavonoids, Narcotic and NSAIDs

# Dedication

DEDICATED TO MY PARENTS

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# LIST OF ABBREVIATIONS

**CMC = Carboxy Methyl Cellulose**

**COX = Cyclooxygenase**

**SCBM = Methanol extract of *S. chelonoides***

**SCLM = Methanol extract of *S. chelonoides***

**ICC = Chloroform extract of *I. coccinea***

**ICPE = Pet ether extract of *I. coccinea***

**mg = Milligram**

**min = Minute**

**ml = Millilitre**

**p.o. = per oral**

**WHO = World Health Organisation**

# Chapter 1: Introduction

## 1.1 Overview

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences (Ahvazi *et al*., 2017). *Ixora coccinea* Linn. (Rubiaceae) commonly known as Rangan and Ranjan in Bangla is one of these plants. It is a shrub with small obvate to oval-oblong, rounded to subcordate base leaves on branched hard heavy twigs. Different plant parts of *I. coccinea* are used for treatment of various disease conditions some of which are associated with inflammation. A decoction of the flowers is given for haemophytis, acute bronchitis and dysmenorrhoea (Hundunette *et al*., 2009).

## 1.2 Medicinal Plants

World Health Organisation (WHO) has defined medicinal plants as plants that contain properties or compounds that can be use for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO 2008). Medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness. Typically, pharmaceutical drugs are modeled after compounds found in medicinal plants.

The plants that have restorative properties or apply gainful pharmacological impacts on the creature body are for the most part assigned as "Therapeutic Plants". In spite of the fact that there are no obvious morphological attributes in the therapeutic plants developing with them, yet they have some uncommon qualities that make them therapeutically imperative. It has now been built up that the plants which are naturally synthesized and contain some auxiliary metabolites, similar

to alkaloids, glycosides, tannins, unstable oils and contain minerals and vitamins, have medicinal properties (Sivastava *et al.,* 1996).

Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are considered to play a beneficial role in health care. Despite their importance, medicinal plants are seldom handled within an organized, regulated sector; most are still exploited with little or no regard to the future. The paper outlines the importance and usage of medicinal plants in health care and national conservation activities in selected countries. The final section focuses on developing country strategy needs for implementing policies covering medicinal plant conservation, cultivation, processing and marketing. Medicinal plants are viewed as a possible bridge between sustainable economic development, affordable health care and conservation of vital biodiversity (Sivastava *et al*., 1996).

## 1.3 History and the earliest known medicines to mankind

The use of plants as medicines has a long history in the treatment of various diseases. For thousands of year's natural products have played a very important role in healthcare and Prevention of diseases. Early medical traditions include of Babylon, China, Egypt and India. The Greeks introduced the concepts of medical diagnosis, prognosis, and advanced medical ethics. Traces of an opium poppy capsule were found on the teeth of a male skeleton buried at cave near Albuñol, Granada that dates back to around 4,000 BC.Writing in the journal Time and Mind, Professor Guerra-Doce said: 'Apart from its use as a food plant, there is also uncontested evidence for the exploitation of its narcotic properties. 'Early humans also used hallucinogenic plants, according to Professor Guerra-Doce. The is evidence dating back to between 8600BC and 5600BC that ancient inhabitants of caves in Peru's Callejon de Huaylas Valley were using *Echinopsis pachanoi* a cactus that contains the psychedelic substance mescaline. Archaeologists have found traces of the cactus and pollen in the Guitarrero cave in the area. Researchers have also found reddish stains on 13,000 year old human teeth found in a burial pit in Duyong Cave on Palawan Island in the southern Philippines, which are thought to be caused by chewing the leaves of the betel plant. It is still chewed throughout much of Asia as a mild stimulant. Marijuana and the opium poppy have also been reported in Bronze Age ceremonial sites located in the Kara Kurum desert of Turkmenistan. Charred cannabis seeds have also been found in

bowls that date from the Bronze age Pit-Grave culture that appeared in Romania around 2000BC. Professor Guerra-Doce also claims that tobacco was also used by many ancient human cultures with pipes for smoking being discovered in North West Argentina that date to 2100BC (Grey, 2015).

In 3rd century A Chinese text, the *Nei Ching* or 'Book of Medicine', describes the practice of acupuncture. The document is written in about the 1st century BC, by which time acupuncture is already a long-established tradition. In 2nd century known Galen is able to demonstrate that living arteries contain blood. His error, which will become the established medical orthodoxy for centuries, is to assume that the blood goes back and forth from the heart in an ebb-and-flow motion (Grey, 2015).

In late 19th and early 20th centuries, there was a great danger of elimination of medicinal plants from therapy. Many authors wrote that drugs obtained from them had many shortcomings due to the destructive action of enzymes, which cause fundamental changes during the process of medicinal plants drying, i.e. medicinal plants' healing action depends on the mode of drying. In 19th century, therapeutics, alkaloids, and glycosides isolated in pure form were increasingly supplanting the drugs from which they had been isolated. Nevertheless, it was soon ascertained that although the action of pure alkaloids was faster, the action of alkaloid drugs was full and long-lasting. In early 20th century, stabilization methods for fresh medicinal plants were proposed, especially the ones with labile medicinal components. Besides, much effort was invested in study of the conditions of manufacturing and cultivation of medicinal plants (Petrovska, 2012).

## 1.4 Natural products as medicines

The biosynthesis and breakdown of proteins, fats, nucleic acids and carbohydrates, which are essential to all living organisms, is known as primary metabolism with the compounds involved in the pathways known as "*primary metabolites"*. The mechanism by which an organism biosynthesizes compounds called *'secondary metabolites'* (natural products) is often found to be unique to an organism or is an expression of the individuality of a species and is referred to as "*secondary metabolism*" (Dias *et al.*, 2012).

The medicinal use of natural products compounds that are derived from natural sources such as plants, animals or micro-organisms precedes recorded human history probably by thousands of years. Palaeoanthropological studies at the cave site of Shanidar, located in the Zagros Mountains of Kurdistan in Iraq, have suggested that more than 60,000 years ago. ubsequently, a large number of well-known natural compounds were identified, analysed and synthesized: salicin from *Salix alba* (white willow), emetine from *Cephaelis ipecacuanha* (ipecacuanha), strychnine and brucine from *Strychnos nux-vomica* (strychnos), quinine from *Cinchona ledgeriana* (cinchona bark), colchicine fromm *Colchicum autumale* (colchicum), caffeine from *Coffea arabica*, nicotine from *Nicotiana tabacum*, atropine from *Atropa belladonna* and cocaine from *Erythroxylum coca.* Many of these compounds are still widely used as drugs. The twentieth century saw the discovery of the antibacterial properties of penicillin, derived from the mould *Penicillium notatum*, which was soon followed by various other antibacterials that gave physicians an enormously powerful weapon in their battle against infectious diseases.

The structural analysis of natural compounds and the ability to synthesize them allowed chemists to modify them in order to suppress or enhance certain characteristics such as solubility, efficiency or stability in the human body. Newman (2008) estimates that about 60% of the drugs that are now available—including household names such as artemisinin, camptothecin, lovastatin, maytansine, paclitaxel, penicillin, reserpine and silibinin—were either directly or indirectly derived from natural products. Moreover, natural products have also been an invaluable source of inspiration for organic chemists to synthesize novel drug candidates ( Koehn & Carter, 2005). Some have even claimed that the switch away from natural products to combinatorial chemistry during the 1990s might have led to the current paucity of new drug candidates in the development pipeline (Desai & Chackalamannil, 2008). It is therefore a matter of great scientific, economic and medical interest to analysis and understand why so many natural products are beneficial to human health (Ji *et al.,* 2009).

## 1.5 Status of plant research in various countries

Medicinal plants have long played important roles in the treatment of diseases all over the world. World health organization (WHO) recently has published a strategic plan for the development and promotion of traditional medicine in 4 areas, including; identification of traditional

medicine, presentation of a proper policy and plan. Development of research and education, especially in the university level, Establishment of unity and cooperation between the employees of traditional and modern medicine. And development of cultivation of the needed herbs to prevent destruction of natural resources. The release of this strategic plan shows the importance of this reliable source for the treatment and prevention of diseases. Nowadays there is revival of interest in the consumption of herbal medicines in the form of standardized extracts, partly due to their multiple side effects, and high cost of patentable chemical drugs. In most of countries in Europe, herbal medicines are either fully licensed as medicines with efficacy proven by clinical trials. However, in Iran and in the United States, most herbal products are considered as dietary supplements and thus are not required to meet the standards for drugs (Rafieian-Kopaei, 2012).

The activities carried out in this field by some of the countries are given below:

**Table1.1: Name of various plants and their activities.**

| Plants | **Activities** |
|---|---|
| 1. *Camellia sinensis* | In India decoctions of the dried and fresh buds and leaves are taken orally for headache and fever. Powder or decoction of the dried leaf is applied to teeth to prevent tooth decay.<br><br>In China hot water extract of the dried leaf is taken orally as a sedative, an antihypertensive, and anti-inflammatory.<br><br>In Turkey leaves are taken orally to treat diarrhea. |
| 2. *Coffea arabica* | In Brazil decoction of the seed is taken orally for Influenza.<br>In Haiti decoction of the grilled fruit and leaf is taken orally for anemia, edema, asthenia, and |

| | |
|---|---|
| | rage. The fruit is taken orally for hepatitis and liver troubles.<br>In Peru, hot water extract of the dried fruit is taken orally as a stimulant for sleepiness and drunkenness. Infusion of the leaf is taken orally to induce labor, and the hot water extract is taken orally as an antitussive in flu and lung ailments. |
| 3. *Ferula assafoetida* | In Afghanistan, hot water extract of the dried gum is taken orally for hysteria and whooping cough and to treat ulcers.<br>In Egypt, dried gum is applied vaginally as a contraceptive before or after coitus. Fifty two percent of the women interviewed practiced this method, and 48% of them depended on indigenous method and prolonged lactation.<br>In Saudi Arabia, dried gum is used medicinally for whooping cough, asthma and bronchitis.<br>In Malaysia gum is chewed by females for amenorrhea.<br>In Morocco gum is chewed as an antiepileptic. |
| 4. *Hordeum vulgare* | In Argentina decoction of the dried fruit taken orally for diarrhea and to treat respiratory and urinary tract infections.<br>In China decoction of the dried fruit is taken orally for diabetes.<br>In India powdered flowers of *Calotropis* |

| | |
|---|---|
| | *procera*, fruits of *Piper nigrum*, seed ash of *Hordeum vulgare*, and rose water are taken orally for cholera.<br>In Guatemala hot water extract of the dried seed is taken orally for renal inflammation and kidney disease. Hot water extract of the dried seed is used externally for dermatitis inflammations, erysipelas, and skin eruptions. |
| 5. *Larrea tridentata* | In Mexico decoction of the bark and dried branches is taken orally as an abortive and for diabetes. Decoction of the dried root is taken orally by pregnant humans as an abortive and for diabetes. Infusion of the shade-dried entire plant is taken orally treat infectious diseases. Decoction of the dried leaf is taken orally for treatment of diabetes. Hot water extract of the dried leaf is taken orally as a blood purifier; to treat kidney problems, urinary tract infections, and frigidity; for gallstones, rheumatism and arthritis, diabetes, wounds, and skin injuries, displacement of the womb, and paralysis; and to dissolve tumors. |
| 6. *Olea europaea* | In Arabic countries nn Unani medicine, dried plant is taken by fumigation as an abortifacient.<br><br>In Greece hot water extract of the leaf is taken orally for high blood pressure.<br><br>In Jordan seed oil is taken orally as a laxative and |

| | |
|---|---|
| | applied externally as an emollient and pectoral.<br><br>Japan hot water extract of the dried bark is taken orally as an antipyretic, for rheumatism, as a tonic, and for scrofula. |
| 7. *Serenoa repens* | In Germany the fruit is taken orally as a source of estrogen.<br>North America hot water extract of the fruit is taken orally as a sedative.<br>United States hot water extract of the fruit is taken orally for prostate inflammation and for benign prostatic hyperplasia. |
| 8. *Sesamum indicum* | In Iran oil of dried seeds is taken orally for its laxative effect.<br>In Ivory Coast the juice of new leaves is drunk to expel placenta.<br>In Jordan seed oil is taken orally to induce lactation and as an antitussive. |

(Ross, 2005)

## 1.6 Medicinal plants in India

Medicinal plants, as a group, comprise approximately 8000 species and account for about 50% of all the higher flowering plant species in India. A large number of the country's rural population depend on medicinal plants for treating various illnesses. About 1.5 million practitioners of the Indian Systems of Medicine and Homeopathy (ISM&H) use medicinal plants for preventive, promotive and curative applications. Furthermore, there are 7843 registered ISM pharmacies and 851 of homoeopathy as well as a number of unlicensed small-scale units. Besides meeting national demands, India caters to 12% of the global herbal trade (Batugal *et al*., 2004)

Some source and activities of Indian plants are given below

**Table1.2: Name of Indian Plants and their uses**

| Species | Common Name | Family | Use |
|---|---|---|---|
| *Acorus calamus* | Vacha | Araceae | Sedative |
| *Alpinia galanga* | Khulanjan | Zingiberaceae | Drug |
| *Commiphora wightii* | Guggal | Burseraceae | Drug |
| *Dendrobium pauciflorum* | Picotee dendrobium | Orchidaceae | Alkaloid |
| *Diplomeris hirsuta* | Snow orchid | Orchidaceae | Alkaloid |
| *Gentiana kurroo* | Kadu | Gentianaceae | Drug |
| *Rauvolfia serpentine* | Sarpagandha | Apocynaceae | Drug |

**(**Batugal *et al*., 2004)

## 1.7 Medicinal plants in Bangladesh

South Asian countries have a large number of valuable medicinal plants naturally growing mostly in fragile ecosystems that are predominantly inhabited by rural poor and indigenous community. In Bangladesh 5,000 specie s of angiosperm are reported to occur. The number of medicinal plants included in the 'materia medica' of traditional medicine in this subcontinent at present stands at about 2,000.More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh Dhaka, Rajshahi, Shylet and Chittagong division is rich in medicinal plants (Sharmin, 2004)

Ghritakumari is the most cultivated medicinal plants in the study villages. The ghritakumari sarbat keeps the stomach cool and also helps in mitigating constipation. Misridana (root), cultivated largely in the study villages, is used to control gastric. They take one root and eat it like a fruit, then drinks lot of water. Rajkantha and Nilkantha is also cultivated widely by the villagers. One of the cultivators stated that, one of the neighbour was running to her by grabbing her stomach for pain (gastric pain) and asked her to give her some nilkantha leaves. After taking the leaves with salt the pain immediately stopped. Simul (root of simul tree) also cultivated in the study villages. Simul root is also used in the treatment of constipation and piles. Lazzaboti plant and arjun bark are also used for the treatment of piles. Though not cultivated, these are available naturally. Some of them keep the lazzaboti (sada) in their nursery (Sharmin, 2004).

Rajkantha, Nilkantha, Simul and Ghritakumari are used widely in treating body and teeth pain. Rajkantha and Nilkantha are popular for treating teeth pain. Simul suppress weakness and are used in arthritis (Sharmin, 2004).

Ghritakumari is also used to control burning of hand and feet. Kalomegh and Misridana are used to treat Jaundice in the study area (Sharmin, 2004)

Misridana is used to control high blood pressure and arjun bark is used for heart problems, though very few arjun trees were found in that locality. So, it is assumed that common people except ‘kabiraj’ do not use arjun bark very frequently, but they know the use of arjun bark. Saktibindu, sankhamul, Hastipalash, bhaichandal and sometimes simul root were used to increase sexual power. One of the cultivators said that he used to take one ‘sankhamul’ with betel leaf and that act immediately to increase his sexual power. In the study area, sotomuli and sometimes talamuli are used to treat white discharge of women (Sharmin, 2004).

Sotomul, one of the most cultivated medicinal plants used to treat urinary infection and diabetes (Sharmin, 2004).

Another herbal plant i.e. Ulatkambal was used there to increase delivery pain and sometimes applied in the hair for louse killing. Many villagers told about treating snake-bite by Ishwarmul plant. One of the villagers informed that, he knows the use of bhaichandal / for treating evil spirit (Sharmin, 2004).

## 1.8 Plant review

### 1.8.1 Botanical Name

*Ixora coccinea* L. the species name coccinea is a latin derivative which means scarlet coloured. And *Steriospermum chelonoides* common name is (pulila) in Sanskrit, and Parul in Bengali.

### 1.8.2 Synonym of *Ixora coccinea* and *Stereospermum chelonoides*

*Ixora grandiflora* Bot, *Ixora bandhuca* Roxbg

*Stereospermum suaveolens* Roxbg

## 1.8.3 Classification

#### 1.8.3.1 Taxonomic Hierarchy of the Plant ( *Ixora coccinea*)

**Kingdom:** Plantae

**Order:** Gentianales

**Family:** Rubiaceae

**Subfamily:** Ixoroideae

**Tribe:** Ixoreae

**Genus:** *Ixora*

**Species:** *Ixora coccinea*

### 1.8.3.2 Taxonomic Hierarchy of the Plant (*Steriospermum chelonoides*)

**Kingdom:** Plantae

**Phylum:** Tracheophyta

**Class:** Magnoliopsida

**Order:** Scrophulariales

**Familly:** Bignoniaceae

**Genus:** *Stereospermum*

**Species:** *Stereospermum chelonoides*

### 1.8.4 Vernacular Names:

### 1.8.4.1 Vernacular name of *Ixora coccinea*

**Table 1.3: Vernacular names of *Ixora coccinea***

| | |
|---|---|
| Bengali | Rangan, Ranjan |
| Tribal | Kaya Machaoi (Marma) |
| English | Jungle-flame lxora, Flame of the Woods, Jungle Geranium |

### 1.8.4.2 Vernacular name of *Steriospermum cholonoides*

**Table 1.2: Vernacular names of *Stereospermum chelonoides***

| | |
|---|---|
| Bangla | Barul-jata, Atkapali, Dharmara (Chittagong), Pahari Awal (Sylhet), Paruljata, Dharomara, parul. |
| Tribal Name | Hamarang gaas (Chakma), Chain-cha (Marma), Sekwai (Chakma), Goda-kamarang (Mogh), Batsil (Garo), Bol-sal (Garo). |
| English | Trumpet flower tree, Yellow snake tree. |

## 1.8.5 Habitat and Distribution

### 1.8.5.1 *Ixora coccinea*

*Ixora coccinea* is cultivated in gardens throughout Bangladesh. It is also a common flowering shrub native to Southern India and Sri Lanka and widely cultivated in Indonesia, Malaysia, the Philippines, Vietnam, Cambodia, Laos and Thailand. It has become one of the most popular flowering shrubs in South Florida –USA gardens and landscapes. It grows in tropical areas with in medium annual rainfall in well drained soils.

### 1.8.5.2 *Stereospermum chelonoides*

*Steriospermum chelonoides* is found in North circus and Deccan, in deciduous forests; Western Ghats and also in deciduous forests, in the hills of Mysore, Malabar and Travancore. In Bangladesh this is tree found in Chittagong, bashkhali and bandorban area.

## 1.8.6 Description

### 1.8.6.1 *Ixora coccinea*

*Ixora coccinea*, commonly called flame of the woods or jungle geranium, is a rounded evergreen shrub that that typically grows to 4-6' (less frequently to 10') tall. It is native to India, Sri Lanka and Southeast Asia, but is now widely grown in tropical areas around the world. It has become a very popular flowering shrub in southern Florida. Woody stems are clad with opposite, leathery, and elliptic to oblong, glossy, dark green leaves (each to 4" long). Tubular, 4-petaled, bright red flowers bloom in coyrmbose cymes (each to 5" wide). Primary bloom is in summer, but sporadic bloom occurs throughout the year. Flowers are followed by round dark purple/black fruits.

**Figure 1.1: Picture of *Ixora coccinea***

### 1.8.6.2 *Steriospermum Chelonoides*

Fragrant Padri Tree is a large deciduous tree, 10-20 m tall, with velvet-hairy branches. Leaves are compound, 1-2 ft long, with 3-4 pairs of leaflets. Leaflets are 7-15 cm long, broadly elliptic, long-pointed. Velvety on the underside, rounded and unequal at base, with 6-8 nerves, short stalked. Fragrant flowers are borne in large lax panicles. They are 10-20 cm long, pinkish. Sepal cup is bell-shaped, 1 cm long, hairy, 3-5 lobed. Stamens are 4, remaining inside the flower-tube. Seed-pod is 1-2 ft long, cylindric, ribbed, rough. Fragrant Padri Tree is globally distributed in Indo-Malesia. Within India, it is found in tropical Himalayas, Assam, and Meghalaya and in moist deciduous forests of Western Ghats.

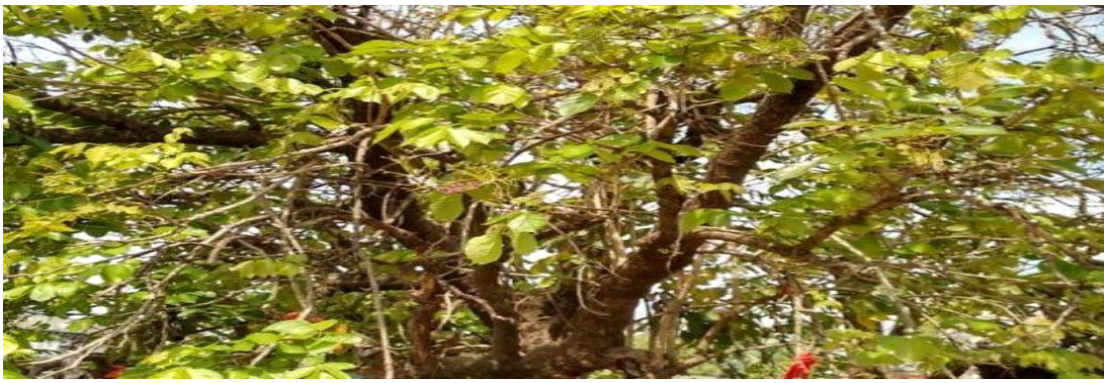

**Figure 1.2: Picture of *Steriospermum chelonoides***

### 1.8.7 Flower

#### 1.8.7.1 *Ixora coccinea*

Flowers are red, brownish purple in color, yellow within,

Inflorescence lax terminal panicles, petals wooly.

**Figure 1.3: Flower *of Ixora cocinea***

#### 1.8.7.2 *Steriospermum chelonoides*

Fragrant flowers are borne in large lax panicles. They are 10-20 cm long, pinkish. Sepal cup is bell-shaped, 1 cm long, hairy, 3-5 lobed. Stamens are 4, remaining inside the flower-tube.Seed-pod is 1-2 ft long, cylindric, ribbed, rough (Prema *et al*., 2013).

Flower color: pink or white

Flower characteristics: Year-round flowering


**Figure 1.4: Flower of *Steriospermum chelonoides***

## 1.8.8 Fruits and seeds

#### 1.8.8.1 *Ixora coccinea*

Fruits Shape: round

Fruits length: less than .5 inch

Fruits cover: fleshy

Fruit color:  purple

**Figure 1.5: Fruits of *Ixora coccinea***

#### 1.8.8.2 *Steriospermum chelonoides*

Capsule, 4-angled, contorted, to 40 cm long; seeds many winged (Prema *et al*., 2013).

**Figure 1.6: Fruits of *Steriospermum chelonoides***

## 1.8.9 Leaves

#### 1.8.9.1 *Ixora coccinea*

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

**Figure 1.7: leaves of *Ixora coccinea***

### 1.8.9.2 *Steriospermum chelonoides*

Leaves compound, imparipinnate, opposite, decussate, to 60 cm long; rachis 6-16.5 cm long, canaliculate, glabrous; leaflets 3-5 pairs, opposite with odd terminal one; petiolule 0.8-1.5 cm long, canaliculate; lamina 5-15 x 2.5-b 7.5 cm, elliptic, apex caudate (acumen 1.5-4 cm long) base cuneate to asymmetric, margin entire, chartaceous, glabrous; midrib flat above; secondary nerves 8-10 pairs gradually curved; tertiary nerves weakly percurrent (Prema *et al*., 2013).

**Figure 1.8: Leaves of *Steriospermum chelonoides***

### 1.8.10 Traditional Use of *Ixora coccinea* and *Steriospermum chelonoides:*

#### 1.8.10.1 *Ixora coccinea*

*I.coccinea* Linn. is a small shrub cultivated throughout India. Roots and flowers are used in dysentery, dysmenorrhea, leucorrhoea, hemoptysis, and catarrhal bronchitis. Leaves are used in diarrhea. Roots are also used in hiccup, nausea, loss of appetite and externally for the treatment of sores, eczema, chronic ulcers. Roots ground into pulp, mixed with water and as tincter are used for diarrhea and dysentery. However, scientific evidence to verify these claims is limited (Maniyar *et al*., 2010)

#### 1.8.10.2 *Steriospermum chelonoides*

*Stereospermum chelonoides*, DC. is a large sized tree, deciduous, branches and usually 9 to 10 m tall and distributed in sub Himalayan tract, central parts of India. It is commonly called as "Patla and "Padri" and belongs to the "Bignoniacea" family (Troup 1986, Masoumeh & Deokule 2013).The decoction of the root is antipyretic and it is useful in asthma, cough and excessive thirst. The bark and all parts contain a napthaquinone and lepachol (Sandermann & Dietrichs 1957, Joshi *et al*. 1977). Flowers are used in bleeding disease, sore throat and diarrhoea; fruits are useful in blood diseases. The root-bark is an ingredient of Dashmoola (Tomar *et al.* 2013). It is regarded as cooling, astringent cardio tonic, bitter, diuretic and generally used in combination with other medicine; the ashes of this plant are used in the preparation of alkaline water and caustic pastes. Fruits are useful in hic cough and blood diseases (Tomar A., 2015).

### 1.11 Objective

In order to achieve these aims, the following research objectives have been identified to determine the analgesic activity by formalin test.

# Chapter 2: Literature Review

## 2.1 Chemical Constituents

### 2.1.1 Chemical constituents of *I. coccinea*

The genus *Ixora* has been reported to possess different classes of compounds mainly triterpenoids (lupeol, urosilic acid, oleanolic acid betunolic acid, amyrins, etc.), aromatic acrid oils, tannins, saponins, carbohydrate, fatty acids, flavanoids (rutin, formononetin, β-sitosterol, quercetin and kaempferol) and sterols. Out of many species of Ixora much research was done on *I. coccinea* and some part of the work on *I. chinensis*, *I. javanica*, *I. finlaysoniana*, *I. parviflora* and *I. macrothyrsa*. The main aim is to provide a comprehensive review on the phytochemical and pharmacological aspects of various species of Ixora. In the present review, efforts are made in addressing its ethnomedicinal uses, chemical constituents and reported pharmacological activities (Dontha *et al.*, 2015). Roots contain aromatic acrid oil, tannin, fatty acids. Leaves yield flavonols, kaemferol, quercetin, proanthrocyanidines, phenolic acids, and ferulic acids. Flowers yield cyanidins, flaconboides, and cooling material related to quercitin (Maniyar *et al*., 2010).

### 2.1.2 Antioxidant Activity of *Ixora coccinea*

I. coccinea flowers revealed the best antioxidant property, presenting much lower IC50 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 ($210.55 \pm 6.31$ 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 ($210.55 \pm 6.31$ μg GAE/mg extract) (Torey et al., 2010).

### 2.1.3 Antimicrobial Activity 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).

### 2.1.4 Anti-diarrheal Activity of Flowers of *Ixora Coccinea* Linn.

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).

### 2.1.5 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 LD50 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 ED50 (Leaf, 220.20 mg/kg body weight) is greater than ED50 (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).

### 2.1.6 Anti-inflammatory 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).

### 2.2.1 Chemical constituents of *S. chelonoides*

It was found from a research work with the leaves of Stereospermum chelonoides contain flavones glycoside 6-O-glucosylscutellarein, dinatin, dinatin- 7glucuroniside,dinatin 7-glucuronide, quinones, stereochenols A and B, naphthoquinones, sterekunthal B and sterequinone C,stereolensin, p-coumaric acid, palmitic, stearic and oleic acids. previously been reported from this plant. Fig 2.1 Basic Structure of Flavonoides It was also been reported that plants of the genus sterepspermum contains naphthaquinone, lapachol, root bark contains β-sitosterol, n-triacontanol, root heart wood contains lapachol, dehydro-α-lapachone and dehydrotectol. (Mohammmad et al, 2006) In another study fresh and market roots of drug Stereospermum chelonoides DC. were analyzed for study in changes of chemical constituents under storage. Root samples were stored under different 30, 50, 75, 96 and 100 % relative

humidity and different incubation days 15, 30, 45 and 60 days. Quantitative estimation of carbohydrates, proteins and phenols in fresh and market roots was done. The results indicated that biodeterioration of selected chemical constituents were observed under high relative humidities 75, 96 and 100% RH and with increased incubation days (45 and 60). More deterioration of chemical constituents recorded in case of market samples as compared to fresh samples. Analysis of variance also showed that the effect of relative humidity and incubation days on biodeterioration of chemical constituents amount were significant (Masoumeh, 2013).

### 2.2.2 Antimicrobial and Cytotoxic activities

This research reports the antimicrobial and cytotoxic activities of the extracts of S. chelonoides. Extraction of dried powdered stem bark of S. chelonoides with methanol and subsequent Kupchan partitioning gave n-hexane and chloroform soluble fractions which showed significant cytotoxic activity against brine shrimp nauplii and the LC50 values for them were found to be 0.98 and 1.00 µg/ml, respectively. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted on the graph paper and the values of LC50 were calculated using Microsoft Excel 2000. All the values were compared with vincristine sulphate whose LC50 was found to be 0.33 µg/ml (Mohammad *et al*, 2006).

### 2.2.3 Antioxidant and Anti-Cancer Activity

It was found from a research study that Stereospermum chelonoides contains phytochemicals comprising of phenols and flavonoids have cancer prevention agent properties, in the long run renders a lucrative apparatus to search receptive oxygen species (ROS). Along these lines, different in vitro measure methodologies were executed to assess cancer prevention agent capability of Stereospermum chelonoides, utilizing DPPH (1,1-diphenyl-2-picrylhydrazyl) searching test, ferric decreasing cell reinforcement control (FRAP), add up to cell reinforcement limit, assurance of aggregate phenol and flavonoid substance. The IC50 estimation of the rough methanol concentrate of bark and leaf was 53.99±3.25 µg/mL and 84.73±4.02 µg/mL, individually, while IC50 esteem for the reference ascorbic corrosive was 14.56±0.24 µg/mL. Additionally, significant aggregate cancer prevention agent movement was watched for bark (309.88±1.03 mg/g proportionate to ascorbic corrosive) and leaf (147.09±1.79 mg/g identical to

ascorbic corrosive) at 200 µg/mL remove focus. Moreover, extricate indicated great lessening power capacity in both bark and leaf division. Add up to phenol content for the bark was 574.82 mg/g identical to gallic corrosive and for leaf was 189.86 mg/g. For bark, the aggregate flavonoid substance was discovered 55.82 mg/g comparable to quercetin and for leaf it was 49.44 mg/g (Meena *et al.*, 2013).

### 2.2.4 Hepatoprotective activity

The present study intends to assess the hepatoprotective action on via carbon tetrachloride (CCl4)- instigated liver harm in pale skinned person rats. Biochemical parameters, for example, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), antacid phosphatase (ALP), add up to bilirubin, LDL-cholesterol and SOD, CAT, GSH, add up to thiols, NO, and lipid peroxidation in liver tissue homogenate were used.. The outcomes propose that the methanol stem bark concentrate of the plant at the dosages 125, 250, and 500 mg/kg and reference standard Liv-52 treated gathering created huge ($p < 0.001$) hepatoprotection against CCl4-initiated liver harm by diminishing the exercises of serum proteins, bilirubin and lipid peroxidation. The concentrate fundamentally ($p < 0.001$) expanded levels of SOD, CAT, GSH and add up to thiols, when contrasted with control amass (V.M. CHandrashekhar *et al*, 2010).

# Chapter 3: Materials and Methods

## 3.1 Method and materials

### 3.1.1 Plant material

*Ixora coccinea* and *Stereospermum chelonoides* are collected form botanical garden in Dhaka.

### 3.1.2 Preparation of Plant extract

First of all we dried the leaf and bark of both plants *S. chelonoide* and *I.cocinea*. After drying we grind them into powder. The powder of bark and leaf of S. *chelonoides* was submerged in methanol solvent and *I. coccinea* submerged in both chloroform and pet ether for 1 week. Then stored solvents in a cool dry place in an air tight container, with occasional shaking and stirring.

### 3.1.3 Extraction Procedure

After 1 week the major portion of the extractable compounds of the plants materials were dissolved in the solvent. Then we extracted the plants components by Rotary machine within 2-3 hours.

### 3.1.4 Filtration of the Extract

a. After the extraction process the plant extracts was filtered with soft and thin square piece of cloth.

b. Then filtered with sterilized cotton filter fitted in the funnel.

c. Then again it was filtered What man's filter paper, used for getting more clear extract.

d. Then the filtrate was taken into a volumetric flask and covered with aluminium foil paper.

e. Finally the filtrate was prepared for rotary evaporator.

#### 3.1.4.1 Procedure

- After the filtration process two parts were obtained namely 'residual part' and filtered part or filtrate".

- The filtered part, which contains the substance soluble in methanol, was putted into a 1000 ml round bottom flask and then the flask was place in a rotary evaporator.
- The evaporation was done at 50℃ temperatures for methanol/chloroform/pet ether.
- The number of rotation per minute was selected as 60 rpm. The pressure of the vacuum pumper machine was 6 bars.
- The water flow through the distillation chamber was also provided in a satisfactory flow rate.
- When the evaporation seemed to be satisfactory, then the methanol extract was collected in a 50 ml beaker.
- The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask.
- The evaporator flask was rinsed by methanol in case of the extract of methanol/Chloroform/Pet ether extract.
- Then the beaker was covered with aluminium foil paper and kept for 60 minutes.
- Finally the concentrated plant extract was found and stored in the laboratory refrigerator from which the extract was used for many chemical investigations.

The extracts of methanol of *Stereospermum chelonoides* and extraction of chloroform and pet ether of *Ixora coccinea* was chosen for investigation and was labelled as-SCLM (the extract of methanol of *Stereospermumchelonoids* leaves),SCBM (the extract of methanol of *Stereospermumchelonoids* bark), ICC (the extract of chloroform of *Ixora coccinea*)and ICPE (the extract of pet ether of *Ixora coccinea)*

**3.2 Drugs**

Ibuprofen (Flamex) was used for current study which was supplied from ACI Pharmaceuticals Ltd, Bangladesh.

### 3.2.1 Phytochemical analysis

By using standard procedure, the plant extract was introduced to phytochemical screening (Ghani,2005).

### 3.2.2 Experimental animal

**Figure 3.1: Swiss Albino Mice**

We purchased mice from ICDDR, B Dhaka. They were 16-18gm. We kept them in animal house in plastic cages having a dimension of (28×22×13) cm. We kept mice in a temperature controlled environment (23°C) with 12hours light-dark cycle , relative humidity 40-70%, with food and water *ad libitium* and fasted overnight (18 hours) before days of the experiment. For food we gave them Mouse–pellets supplied by ICDDR,B Dhaka. After one week mice are became prepared for experiment where they were 25-30gm of body weight.

#### 3.2.2.1 Identification of Animals during Experiment

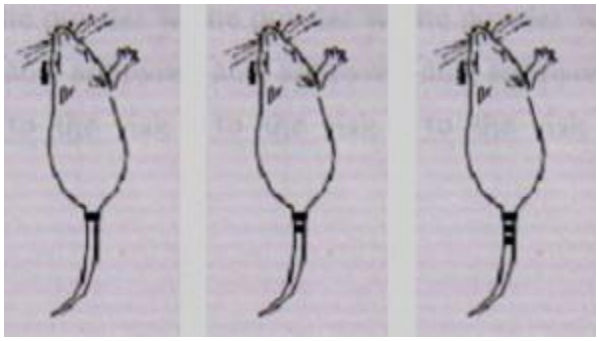

**Figure3.2: Identification of test animals for analgesic property screening**

Each group consists of six mice and hence it is difficult to identify and observe at a time six mice receiving same treatment. Thus, it was important to identify individual animal of a group during the treatment. The animals were individualized by marking: marked as M1=mice 1, M2=mice 2, M3=mice 3, M4=mice4 and so on.

### 3.2.3 Formalin- induced biting & licking

Sixty mice about (25–30 g) are separated into 10 groups and in each group contain 3 males and 3 females. One group of mice were treated (dose volume 10 ml/kg, p.o.) with water, other group of mice were treated by Ibuprofen (100 mg/kg), and other eight groups of SCBM (250-500 mg/kg), SCLM (250-500 mg/kg), ICC (200–400 mg/kg) and ICPE (200–400 mg/kg) 1 h before formalin injection. The procedure was similar to that described previously (Santos et al., 1994). Briefly, 20 µl 2.5% formalin (37% formaldehyde) made up in distilled water was injected under the surface of the right hind paw. Two mice (control and treated) were observed simultaneously from 0 to 30 min after formalin injection. The initial nociceptive scores normally peaked 5 min (first phase) and 15–30 min after formalin injection (second phase), representing the tonic and inflammatory pain responses, respectively. After intraplantar injection of formalin, the animals were immediately placed into a glass cylinder 20 cm in diameter, and the amount of time spent licking the injected paw was time with a chronometer and was considered as indicative of nociception.

### 3.2.4 Statistical analysis.

The results are presented as mean±SD and the statistical significance between the groups was determined by means of analysis of variance (ANOVA) followed by Dunnett test, where P values less than (**p < .01), (***p < .001) and (*p < 0.05) were considered as indicative of significance. And we used Microsoft Exel 2007 for calculation.

### 3.2.5 The Design of the Formaline Experiments

In these methods 60 mice were chosen randomly and then divided into 10 groups. They were group 1 to group 10 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 - SCBM 250 mg/kg

Group 2 - SCBM 500 mg/kg

Group 3 - SCLM 250 mg/kg

Group 4 – SCLM 500 mg/kg

Group 5 – ICC 200 mg/kg

Group 6 – ICC 400 mg/kg

Group 7 – ICPE 200 mg/kg

Group 8 – ICPE 400 mg/kg

Group 9 - Standard (Ibuprofen)

Group 10 - Control (Distilled Water).

### 3.2.6 Preparation of drug and chemical solution

In order to administer the crude SCBM and SCLM at dose 250 & 500 mg/kg body weight and ICC and ICPE 200 and 400 mg/kg body weight of mice. First we take 5 ml of distilled water mixed with 5% CMC for proper mixing, small amount of suspending agent. Then added the extract of SCBM/SCLM of 250mg/kg and 500 mg/kg or ICC/ICPE 20mg/kg and 400 mg/kg. After proper mixer total volume of the suspension was made up to 5 ml. To stabilize the suspension it was stirred well or sonicated for 15 minutes. For the preparation of positive control group (100 mg/kg) Ibuprofen is taken & a suspension of 5 ml is made.

**Table 3.1: Test samples used in the estimation of Analgesic activity of *S. chelonoides* plant**

| Group | Treatment | Dose | Route of Administration |
|---|---|---|---|
| **Group1 (Extract)** | SCLM | 250mg/kg | Orally |
| **Group2 (Extract)** | SCLM | 500mg/kg | Orally |
| **Group3(Extract)** | SCBM | 250mg/kg | Orally |
| **Group4 (Extract)** | SCBM | 500mg/kg | Orally |
| **Group 5(Standard)** | Ibuprofen | 100 mg/kg | Orally |
| **Group6(Control)** | Distilled Water | 10 ml/kg | Orally |

T**able 3.2: Test samples used in the estimation of Analgesic activity of *I. coccinea* plant**

| Group | Treatment | Dose | Route of Administration |
|---|---|---|---|
| **Group1 (Extract)** | ICC | 200mg/kg | Orally |
| **Group2 (Extract)** | ICC | 400mg/kg | Orally |
| **Group3(Extract)** | ICPE | 200mg/kg | Orally |
| **Group4 (Extract)** | ICPE | 400mg/kg | Orally |
| **Group 5(Standard)** | Ibuprofen | 100 mg/kg | Orally |
| **Group6(Control)** | Distilled Water | 10 ml/kg | Orally |

# Chapter 4: Results and Discussion

# 4.1 Results

## 4.1.1 Result for ICC and ICPE

From the Table 4.1 it is found that, licking and biting count for ICC and ICPE for both 200 & 400 mg/kg decrease the pain which is induced by formalin much better then control group. Significant ($P < 0.05$) result was found at early and late phase for both the strengths. ICC showed low inhibition about 18.98% in early phase and 10.92% in late phase in 200 mg/kg dose. But In 400 mg/kg dose showed inhibition about 19.19% in early phase and 31.56% in late phase. On other hand ICPE showed inhibition about 11.51% in early phase and 23.20% in late phase in 200 mg/kg dose. But In 400 mg/kg dose showed inhibition about 10.70% in early phase and 26.27% in late phase

**Table 4.1: Result of extracts of ICC and ICPE in mice by Formalin test.**

| Group | Treatment | Dose (mg/kg) | Early phase | Late phase | Inhibition %(Early phase) | Inhibition %(Delayed phase) |
|---|---|---|---|---|---|---|
| Group-1 (Extract) | ICC | 200 mg/kg | 66.83±29.93 | 87±25.82 | 18.98% | 10.92% |
| Group-2 (Extract) | ICC | 400 mg/kg | 66.66±15.39 | 66.833±22.10 | 19.19% | 31.56% |
| Group-3 (Extract) | ICPE | 200 mg/kg | 73±14.38 | 75±13.47 | 11.51% | 23.20% |
| Group-4 (Extract) | ICPE | 400 mg/kg | 73.66±18.26 | 72±15.54 | 10.70% | 26.27% |
| Group-5 (Standard) | Ibuprofen | 100 mg/kg | 28.33*±10.34 | 40*±18.24 | 65.65% | 58.19% |
| Group-6 (Control) | Water | 10 ml/kg | 82.5±24.52 | 97.667±30.40 | - | - |

Here, ICC means *Ixora coccinea* in chloroform and ICPE means *Ixora coccinea* in pet ether
Number biting and licking mean ± S.D.; (n=6). *P < 0.05, **P < 0.01, ***P < 0.001 significantly different from control.

## 4.1.2 Result for SCBM and SCLM

From the Table 4.2 it is found that, licking and biting count of SCBM and SCLM for both 250 & 500 mg/kg decrease the pain which is induced by formalin much better then control group.their value are closer to the standard drug. Significant ($P < 0.05$) result was found at early and late phase for both the strengths. SCBM showed great inhibition about 40.8% in early phase and 47.95% in late phase in 250 mg/kg dose. In 500 mg/kg dose showed great inhibition about 42.62% in early phase and 47.26% in late phase. On other hand SCLM showed low inhibition about 1.41% in early phase and 23.20% in late phase in 250 mg/kg dose. In 500 mg/kg dose showed mild potency and inhibition about 12.72% in early phase and 31.57% in late phase

**Table 4.2: Result of extracts of SCBM and SCLM in mice by Formalin test.**

| Group | Treatment | Dose (mg/kg) | Early phase | Late phase | Inhibition %(Early phase) | Inhibition %(Delayed phase) |
|---|---|---|---|---|---|---|
| Group-1 (Extract) | SCBM | 250 mg/kg | 48.833*±12.22 | 50.833*±34.14 | 40.80% | 47.95% |
| Group-2 (Extract) | SCBM | 500 mg/kg | 47.333*±14.20 | 51.5*±26.61 | 42.62% | 47.26% |
| Group-3 (Extract) | SCLM | 250 mg/kg | 81.33±28.80 | 75*±27.93 | 1.41% | 23.20% |
| Group-4 (Extract) | SCLM | 500 mg/kg | 72±15.68 | 67.33±27.33 | 12.72% | 31.05% |
| Group-5 (Standard) | Ibuprofen | 100 mg/kg | 28.33*±10.34 | 40*±18.24 | 65.65% | 58.19% |
| Group-6 (Control) | Water | 10 ml/kg | 82.5±24.52 | 97.667±30.40 | - | - |

Here, SCBM means *Steriospermum chelonoides* bark in methanol and SCLM means *Steriospermum chelonoides* leaves in methanol. Number biting and licking mean ± S.D.; (n=6). *P < 0.05, **P < 0.01, ***P < 0.001 significantly different from control.

## 4.2 Discussion

Narcotics or non-narcotics (NSAIDs) drugs are employed for the pain management. For the production of commercial drugs or in the improvement of lead compounds, it has been built that medicinal plants are good reservoir of it. However these drugs have toxic effects also (Park *et al*., 2004). From the analgesic activity results and the reduction in the number of biting and licking compared to the control groups was considered as evidence of analgesic effect in *Stereospermum chelonoides* and *Ixora coccinea*. Pain is an unpleasant and emotional experience associated with tissue damage. Analgesics are the drugs used to relieve pain. Inflammation causes trigger in inflammatory mediators such as TNF-α, interleukins and prostaglandins. Antiinflammatory agents are capable of inhibiting the cyclooxygenase COX-1 and COX- 2 pathway of arachidonic acid metabolism, which produces prostaglandins thus reduce the pain sensation (Tasleem *et al*., 2014). Classical analgesics of natural origin include opiates and non-steroidal anti-inflammatory drugs but they are associated with side effects such as gastric lesions and tolerance and dependence (Ezeja *et al*., 2011). So, there is a need to explore natural available alternative sources to NSAIDs and opiates. Formalin-induced test is the most commonly used test to assess peripherally acting analgesics. Licking and biting generated by parenteral administration of formalin in mice, are due to profound pain of endogenous nature which recur for a prolonged period of time. Biting and licking is an explicit response to the intense pain induced by irritant principles via nociceptors characterized by episodes of biting and licking of mice's paw. The signals transmitted to central nervous system in response to pain due to irritation, because release of mediators such as prostaglandins which contributes to the increased sensitivity to nociceptors (Shivaji, 2012).The increase in prostaglandin production further enhances the vascular permeability. The decrease in the number of biting and licking assumes decrease of prostaglandins synthesis which results in significant analgesic activity. Formalin induced abdominal constriction is a standard, simple, and sensitive test for measuring analgesia induced by both opioids and peripherally acting analgesics. Table 4.1 and 4.2 show that extracts reduced the number of biting and licking induced by the i.p. administration of formalin solution. The methanolic bark extract of *Stereospermum chelonoides* shows significant result in ANOVA test ($p$ value $< 0.5$) and the standard is highest significant comparing all other groups. Here *Stereospermum chelonoides* bark extraction showing potential analgesic activity then its leaf

extraction and Ixrora coccinea extraction. Preliminary phytochemical screening revealed the presence of sterols, coumarins, higher fatty acids and the absence of flavones aglycone and alkaloids in the leave extract (Aliyu et al., 2010). These data also suggest that the extract can produce analgesic action through inhibition of COX (Inhibition of the enzyme cyclo-oxygenase) and consequently prostaglandin synthesis. But this inhibition of pain may also occur due to phytochemical constituents which is present in the extract of the experimented plant. Phytochemical screening of SCBM gives evidence of containing some secondary metabolites, such as polyphenols, flavonoids, alkaloids, terpenoids and glycosides which have gained importance due to their diverse pharmacological activities such as anti-inflammatory, analgesic and antipyretic, etc. (Mohammad et al.,2006) Significant analgesic activity was shown by plants that contain organic acids and flavonoids (Sasikala *et al*., 2011). Flavonoids may attribute to a number of pharmacological activities. Some flavonoids are reported to possess significant analgesic and anti-inflammatory activity. Some flavonoids can significantly interfere with inflammatory mediators (Ullah *et al*., 2014). Certain members of flavonoids significantly affect the function of the immune system and inflammatory cells. A number of flavonoids such as hesperidin, apigenin, luteolin, and quercetin are reported to possess anti-inflammatory and analgesic effects. Flavonoids may affect specifically the function of enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases. The inhibition of kinases is due to the competitive binding of flavonoids with ATP at catalytic sites on the enzymes. These enzymes are involved in signal transduction and cell activation processes involving cells of the immune system. It has been reported that flavonoids are able to inhibit expression of isoforms of inducible nitric oxide synthase, cyclooxygenase, and lipooxygenase, which are responsible for the production of a great amount of nitric oxide, prostanoids, leukotrienes, and other mediators of the inflammatory process such as cytokines, chemokines, or adhesion molecules Flavonoids also inhibit phosphodiesterases involved in cell activation. Much of the anti-inflammatory effect of flavonoid is on the biosynthesis of protein cytokines that mediate adhesion of circulating leukocytes to sites of injury. Certain flavonoids are potent inhibitors of the production of prostaglandins, a group of powerful pro inflammatory signaling molecules (Shashank and Abhay, 2013).

# Chapter 5 : Conclusion

## 5.1 Conclusion

Natural products, especially those of plant origin, have been a promising source of new lead compound for drug discovery for ages. Bangladesh is blessed with rich floristic resources, where a large number of plants still remain unexplored. So well designed, systematic and objective research in this area might benefit our people who have been deluged with superfluity of diseases, and who lack technological and economic resources to cope up with them with orthodox medicine.

Based on the results of the present study, it can be proposed that the leaf and bark part of *Stereospermum chelonoides* in general methanol soluble fractions in particular, has less strong CNS depressant property. These results also may lend support to the relevant phytochemical and pharmacological works carried out so far on this plant. Even Based on the findings, it may be concluded that the *Ixora coccinea* leaves possessed analgesic, anti-inflammatory, and antipyretic activities. Phytochemical constituents of *Ixora coccinea* leaves such as flavonoids, tannins, and triterpenes in methanol extract could be correlated with its observed biological activities.

However, further studies are suggested to be undertaken to understand the underlying mechanism of the observed activities and to isolate, purify and characterize active phytochemical ingredient(s) responsible for these bioactivities in animal models.

The future goal of this study is to identify effective, cheap and available modalities to cope up with the upsurge of the dangers of diseases of different etiology in Bangladesh. Approaches may be developed to prevent and/or treat illness easily and effectively with readily available and cheaper resources. This research may be a platform for further investigation in this area. It is likely to show directions for the researchers to find ways out to save our lay people from the curse of diseases. Future endeavors in this area may open up exciting new therapeutic avenues.

# Chapter 6: References

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