"A Study of Analgesic Activity of Methanol and Pet ether Extracts of Barks of *Stereospermum chelonoides*"

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, **Lia Rose Merry D. Cruze** hereby declare that the dissertation entitled **"A Study of Analgesic Activity of Methanol and Pet ether Extracts of Barks of *Stereospermum chelonoides*"** 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 is a complete record of original research work carried out by me during 2016, 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

This is to certify that the thesis entitled **"A Study of Analgesic Activities of Methanol and Pet ether Extracts of Barks of *Stereospermum chelonoides*"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by **Lia Rose Merry D. Cruze**, ID# 2012-1-70-005 in 2016, under the supervision and guidance of **Meena Afroze Shanta**.

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

The plant *Stereospermum chelonoides* has been used for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases pain, fever, inflammations, asthma, liver disorders etc. The aim of the present study was to evaluate the analgesic activity of pet-ether extract and methanol extract of *Stereospermum chelonoides*. The powdered barks of *Stereospermum chelonoides* were extracted with methanol and pet ether. The fraction was used to evaluate analgesic activities. The analgesic activity was measured by acetic acid induced writhing method. Methanol fraction and pet ether fraction showed analgesic activity respectively with 59.53 % inhibition of pain and 55.85% in the $P<0.001$. The standard (Indomethacin) showed pain of inhibition by 86.96 %. The results of study clearly indicate the presence of analgesic properties of methanol and pet-ether extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Dedication

DEDICATED TO MY

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ABBREVIATION:

Meaning of abbreviated form	Abbreviated form
Carboxy Methyl Cellulose	CMC
Gram	g or gm
Hour	hr
Microgram	µg
Micro liter	µl
Milligram	mg
Milliliter	ml
Minutes	mins.
Stereospermum chelonoides Bark Extracts of Mathanol	SCBM
Stereospermum chelonoides Bark Extracts of Pet ether	SCBPE
World Health Organization	WHO

CHAPTER 1

INTRODUCTION

1. Introduction

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 semisynthesis." When a plant is designated as medicinal it is implied that the said plant is used as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorousmammals.

In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants (such as myrrh and opium). In 1500 B.C., the Ancient Egyptians wrote the Ebers Papyrus, which contains information on over 850 plant medicines, including garlic, juniper, and cannabis.

Ethnobotany, the scientific study of the relationships that exist between humans and plants, is a recognized way to discover new effective medicines for future and further use. In ancient Greece, plants were classified and descriptions of them were given by scholars. It aids in the identification process. Researchers identified in 2001, 122 compounds that were isolated and identified from "ethno medical" plant sources, are used in modern medicine. The current use of the active elements of the plants is 80% similar to those of ethno medical use (Balunas, 2005).

1.1 Medicinal Plant

A 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. The therapeutic properties of medicinal plants are conditioned by the presence in their organs of active substances, such as alkaloids, flavonoids, glycosides,

vitamins, tannins, and coumarin compounds, which physiologically affect the bodies of humans and animals or which are biologically active in relation to the causative agents of various diseases. A special group of medicinal plants are antibiotics (Verma *et al*., 2008).

There are a huge number of medicinal plants. In the US, almost 1800 medicinal plant species are commercially available. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of the world's flowering plant species have been used medicinally. Sometimes the figure of 70,000 medicinal plant species is cited, but this includes many algae, fungi, and micro-organisms that are not really plants as the word is understood by botanists. In any event, there is no other category of plants useful to man (with the possible exception of ornamental plants) that includes so many species, and the question naturally arises why such a staggering number of plants have useful medicinal properties. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Verma *et al*., 2008).

1.2 History of Medicinal plant

The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.

The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: *Rhei rhisoma*, camphor, *Theae folium, Podophyllum*, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra (Verghese *et al*., 1998).

The Indian holy books Vedas mention treatment with plants, which are abundant in that country. The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc. (Verghese *et al*., 1998).

Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time.Among others. Circa 77 AD he wrote the work "De Materia Medica." This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic *materia medica* until the late Middle Ages and the Renaissance. Of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic.The most distinguished Roman physician (concurrently a pharmacist), Galen (131 AD–200), compiled the first list of drugs with similar or identical action (parallel drugs), which are interchangeable—"De succedanus" (Verghese *et al*., 1998).

In the middle ages, the skills of healing, cultivation of medicinal plants, and preparation of drugs moved to monasteries. Therapy was based on 16 medicinal plants, which the physicians-monks commonly grew within the monasteries as follows: sage, anise, mint, Greek seed, savory, tansy, etc. The Arabs introduced numerous new plants in pharmacotherapy, mostly from India, a country they used to have trade relations with, whereas the majority of the plants were with real medicinal value, and they have persisted in all pharmacopoeias in the world till today. Throughout the Middle Ages European physicians consulted the Arab works "De Re Medica" by John Mesue (850 AD), "Canon Medicinae" by Avicenna (980-1037), and "Liber Magnae Collectionis Simplicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197-1248), in which over 1000 medicinal plants were described (Verghese *et al*., 1998).

In 18th century, in his work *Species Plantarium* (1753), Linnaeus (1707-1788) provided a brief description and classification of the species described until then. Early 19th century

was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipecacuanha (1817), strychnos (1817), quinine (1820), pomegranate (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy. With the upgrading of the chemical methods, other active substances from medicinal plants were also discovered such as tannins, saponosides, etheric oils, vitamins, hormones, etc (Verghese *et al*., 1998).

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 (Verghese *et al*., 1998).

1.3 Classification of Medicinal Plants

Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification (Verghese *et al*., 1998).

1.3.1 Based on part used

i) Whole plant: Boerhaavia diffusa, Phyllanthus neruri

ii) Root: Dasamula

iii) Stem:Tinospora cordifolia, Acorus calamus

iv) Bark: Saraca asoca

v) Leaf: Indigofera tinctoria, Lawsonia inermis, Aloe vera

vi) Flower:Biophytum sensityvum, Mimusops elenji

vii) Fruit: Solanum species

viii) Seed: Datura stramonium

1.3.2 Based on Habit

i) Grasses: Cynodon dactylon

ii) Sedges: Cyperus rotundus

iii) Herbs : Vernonia cineria

iv) Shrubs: Solanum species

v) Climbers: Asparagus racemosus

vi) Trees: Azadirachta indica

1.3.3 Based on Habitat

i) Tropical: Andrographis paniculata

ii) Sub-tropical: Mentha arvensis

iii) Temperate: Atropa belladonna

1.3.4 Based on Therapeutic Value

Antimalarial : Cinchona officinalis, Artemisia annua

Anticancer : Catharanthus roseus, Taxus baccata

Antiulcer : Azadirachta indica, Glycyrrhiza glabra

Antidiabetic : Catharanthus roseus, Momordica charantia

Anticholesterol : Allium sativum

Antiinflammatory : Curcuma domestica, Desmodium gangeticum

Antiviral : Acacia catechu

Antibacterial : Plumbago indica

Antifungal : Allium sativum

Antiprotozoal : Ailanthus sp., Cephaelis ipecacuanha

Antidiarrhoeal : Psidium gujava, Curcuma domestica

Hypotensive : Coleus forskohlii, Alium sativum

Tranquilizing : Rauvolfia serpentina

Anaesthetic : Erythroxylum coca

Spasmolytic : Atropa belladona, Hyoscyamus niger

Diuretic : Phyllanthus niruri, Centella asiatica

Astringent : Piper betle, Abrus precatorius

Anthelmentic : Quisqualis indica, Punica granatum

Cardiotonic : Digitalis sp., Thevetia sp.

Antiallergic : Nandina domestica, Scutellaria baicalensis

Hepatoprotective : Silybum marianum, Andrographis paniculata

1.3.5 Based on Ayurvedic formulations

a) The ten roots of the Dasamoola (*Dasamoolam*)

i) Desmodium gangeticum (Orila)

ii) Uraria lagopoides (Cheria orila)

iii) Solanum jacquinii (Kantakari)

iv) Solanum indicum (Cheruchunda)

v) Tribulus terrestris (Njerinjil)

vi) Aegle marmelos (Koovalam)

vii) Oroxylum indicum (Palakapayyani)

viii) Gmelina arborea (Kumizhu)

ix) Steriospermum suaveolens (Pathiri)

x) Premna spinosus (Munja)

b) The ten flowers of the Dasapushpa (*Dasapushpam*)

i) Biophytum sensitivum (Mukkutti)

ii) Ipomea maxima (Thiruthali)

iii) Eclipta prostrata (Kayyuniam)

iv) Vernonia cineria (Poovamkurunnil)

v) Evolvulus alsinoides (Vishnukranthi)

vi) Cynodon dactylon (Karuka)

vii) Emelia sonchifolia (Muyalcheviyan)

viii) Curculigo orchioides (Nilappana)

ix) Cardiospermum halicacabum (Uzhinja)

x) Aerva lanata (Cherula)

c) The four trees of the Nalpamara (*Nalpamaram*)

i) Ficus racemosa (Athi)

ii) Ficus microcarpa (Ithi)

iii) Ficus relegiosa (Arayal)

iv) Ficus benghalensis (Peral)

d) The three fruits of the Triphala (*Thriphalam*)

i) Phyllanthus emblica (Nellikka)

ii) Terminalia bellerica (Thannikka)

iii) Terminalia chebula (Kadukka)

(Varghese *et al*., 1998)

1.4 Bangladesi Plants

Bangladesh is blessed with lots of medicinal plants. These are normally used by the rural people of our country.

Table: 1.1 Medicinal Plants of Bangladesh

Name	Family	Bengali name	Parts used	Medicinal uses
Abroma augusta (L.).L.f.	Sterculiaceae	Ulatkambal	Leaves, stems, roots, barks, leaf stalks	Gonorrhea, amenorrhoea, dysmenorrhoea, menstrual disorders, dysentery, urinary problems
Aloe vera (L.) Burn.f.	Aloaceeae	Ghrita kumari	Whole part	Asthama, cirorhosis, constipation, dehydration, dullness of skin, duodenal ulcer, eczema, facial paralysis, flatulence, gout, impotence, rheumatism, spermatorrhoea
Andrographis paniculata	Acathaceae	Kalomegh, kalmegh, mohatita	Leaves, roots	Stomachic, anthelmintic, constipation, cholera,dysentery, diarrhea, diabetes, dyspepsia, influenza, bronchitis, malaria, hepatitis
Argemone maxicana L.	Papaveraceae	Shial kanta	Root, Stems, Seed, Seed oil, flowers	Anthelmic, antileprotic, tonic, diuretic, skin diseases, cough, jaundice, laxative

Asparagus racemosus Wild.	Liliaceae	Sastamuli	Leaves, fruits, barks	Anticancer, antibacterial, antifungal, diuretic, anti-dysentric, diabetes, jaundice
Azadirachta indica A. Juss.	Meliaceae	Neem	Leaves, Barks, Gums, Seeds	Gingivitis, sores, fever, tumors, smallpox, cholera, ulcers, eczema,antiviral, antineoplastic, antifungal
Boerhaavia diffusa L.	Nyctaginaceae	Gondhapurna	Roots, leaves	Jaundice, anemia, opthalmia, gonorrhea, inflammatory agents, abdominal cancer
Bombax ceiba L.	Bombacaceae	Shimul	Root, bark, stem, flower	Fever, smallpox, rheumatism, leprosy, dirrhoea, dysentery, diuretic ulceration of bladder and kidneys
Bryophyllum pinnnatum (Lamk.) Oken	Crassulaceae	Patharkuchi	Leaves, saps	Diabetes, applied to wounds, asthma, dysures, epilepsy, gout, tuberculosis
Calotropis gigantean (L.) R. Br.	Asclepiadaceae	Akand	Leaves, stems, roots	Abdominal pain, tumors, cancers, leprosy, skin diseases, dysentery
Centella asiatica	Apiaceae	Thankuni	Leaf, root	Diabetes, menorrhagia, dysentery, bronchitis,

(L.) Urban				small pox, flatulence, mental disorder
Cinnamomum tamala Nees & Eberm.	Lauraceae	Tejapata	Leaves	Abdominal pain, anorexia, body pain, cardiac weakness, chicken pox, sexual weakness
Datura metel L.	Solanaceae	Dhutura	leaves, fruits, seeds	Narcotic, antispasmodic, tumors, skin diseases, diarrhea
Gmelina arborea Roxb.	Verbenaceae	Gameri	Leaves, barks, roots	Demulcent, diuretic, anemia, laxative, fever, piles
Polyalthia longifolia (Sonn.) Thw	Annonaceae	Debdaru	Leaves, barks	Hysteria, influenza, oedema, fever, skin diseases, hypertension
Santalum album L.	Santalaceae	Sheto chandan	Wood, stem, bark, oil	Fever, itching, vomiting, skin diseases
Saraca asoca (Roxb.) de Wild.	Caesalpiniaceae	Ashok	Bark	Astringent, menorrhagia, bleeding, dyspepsia, dysentery, uterine tonic
Spondias pinnata (L.f) Kurz	Anacardiaceae	amrah	Fruit, bark	Antiscorbutic, astringent, dyspepsia, dysentery, vomiting, anemia, asthma

Tamarindus indica L.	Caesalpiniaceae	Tentul	Bark	Cancer, heart diseases, hypertension, astringent, diabetes, menstrual problems
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	Bohera	Fruit, bark, heartwood	anorexia, cardiac weakness, constipation, dyspepsia, fever, hyper acisity, sight weakness
Terminalia chebula Retz	Combretaceae	Horitoki	Fruits, barks	Indigestion, constipation, dysentery, jaundice, piles, menstruation, flatulence, urinary diseases
Vitex negundo L.	Verbenaceae	Nishinda	Leaves, flowers, fruits	Antiparasitic, rhematic attack, headache, astringent,

(Motaleb, 2011)

1.5 Scope of Development

The medicinal plants are closely connected with the traditional knowledge of its use. During the early periods, the knowledge of the medicinal properties of the plants was transferred from one generation to another generation orally and no documentation of the medicinal plants have been maintained. Even though, the herbal formulations are regaining their momentum, the major problem behind the herbal medicine is that there is a lack of standard protocol for their standardization and problem behind carrying out clinical trials. It is essential to evaluate the herbal plant scientifically and documents

should be made to know their medicinal properties. To revitalize Indian medicinal heritage, through creative application of the traditional health sciences for the enhancement the quality of health care in rural and urban India, extensive research on plants for natural leads is very essential. Free radicals have found a place in etiology of many diseases and there is a great deal of enthusiasm regarding the role played by the free radicals in many diseases, like in asthma, rheumatoid, hypertension, liver cell injury and carcinogenesis. Extensive scientific research has been carried out all over the world to use the medicinal plants and their extracts/lead molecules from herb as anti-oxidants. So there is a great demand of herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin when compared with synthetic drugs. As an herbal medicine is the first level of contact for rural people when they require medicine care, it is imperative for governments to take immediate steps to introduce the use of traditional medicine (Katiyar *et al*., 2012).

1.6 Metabolites

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (Irchhaiya *et al.,* 2014):

a) Primary metabolites such as sugars and fats, which are found in all plants; and

b) Secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination.

It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs (Irchhaiya *et al.,* 2014) :

- Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine (Irchhaiya *et al.,* 2014).

- Polyphenols (also known as phenolics) are compounds contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics (Irchhaiya *et al.,* 2014).

- Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis (Irchhaiya *et al.,* 2014).

- Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpenesqualene. When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes (Irchhaiya *et al.,* 2014).

1.7 Cultivation of Medicinal Plants

Cultivation of medicinal plants requires intensive care and management. The conditions and duration of cultivation required vary depending on the quality of medicinal plant materials required. If no scientific published or documented cultivation data are available, traditional methods of cultivation should be followed, where feasible.

1.7.1 Site selection

Medicinal plant materials derived from the same species can show significant differences in quality when cultivated at different sites, owing to the influence of soil, climate and other factors. These differences may relate to physical appearance or to variations in their constituents, the biosynthesis of which may be affected by extrinsic environmental conditions, including ecological and geographical variables, and should be taken into consideration (WHO, 2003).

1.7.2 Ecological Environment and Social Impact

The introduction of non-indigenous medicinal plant species into cultivation may have a detrimental impact on the biological and ecological balance of the region. The ecological impact of cultivation activities should be monitored over time, where practical. The social impact of cultivation on local communities should be examined to ensure that negative impacts on local livelihood are avoided (WHO, 2003).

1.7.3 Climate

Climatic conditions, for example, length of day, rainfall (water supply) and field temperature, significantly influence the physical, chemical and biological qualities of medicinal plants. The duration of sunlight, average rainfall, average temperature, including daytime and night-time temperature differences, also influence the physiological and biochemical activities of plants, and prior knowledge should be considered (WHO, 2003).

1.7.4 Soil

The soil should contain appropriate amounts of nutrients, organic matter and other elements to ensure optimal medicinal plant growth and quality. Optimal soil conditions, including soil type, drainage, moisture retention, fertility and pH, will be dictated by the selected medicinal plant species and/or target medicinal plant part. The use of fertilizers is often indispensable in order to obtain large yields of medicinal plants (WHO, 2003).

1.7.5 Irrigation and Drainage

Irrigation and drainage should be controlled and carried out in accordance with the needs of the individual medicinal plant species during its various stages of growth. Water used for irrigation purposes should comply with local, regional and national quality standards. Care should be exercised to ensure that the plants under cultivation are neither over- nor under-watered (WHO, 2003).

1.7.6 Plant Maintenance and Protection

Any agrochemicals used to promote the growth of or to protect medicinal plants should be kept to a minimum, and applied only when no alternative measures are available. Integrated pest management should be followed where appropriate. When necessary, only approved pesticides and herbicides should be applied at the minimum effective level, in accordance with the labelling and/or package insert instructions of the individual product and the regulatory requirements that apply for the grower and the end-user countries. Only qualified staff using approved equipment should carry out pesticide and herbicide applications. All applications should be documented. Growers and producers should comply with maximum pesticide and herbicide residue limits, as stipulated by local, regional and/or national regulatory authorities of both the growers' and the end-users' countries and/or regions (WHO, 2003).

1.8 Phytochemistry

Phytochemistry can be defined as the biochemical study of plants which is concerned with the identification, biosynthesis, and metabolism of chemical constituents of plants, especially used in regard to natural products. Phytochemistry is considered as one of the early subdivisions of organic chemistry. It has been of great importance in the identification of plant substances of medicinal importance. Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants. Effect of extracted plant phytochemicals depends on:

- ✓ The nature of the plant materials
- ✓ Its origin
- ✓ Degree of processing
- ✓ Moisture content (Das *et al*., 2010).

1.8.1 Procedure of Drug Development

Since drug development is an expensive practice, careful phytochemical analysis and pharmacological screening and if promising clinical tests are required. The way of developing drugs from plants involves several stages (Ghani, 1998), which include:

a) Selection and correct identification of the proper medicinal plant.

b) Extraction with suitable solvent(s).

c) Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.

d) Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.

e) Repeated fractionation of active fractions to isolate pure compound(s).

f) Elucidation of chemical structure of pure compound(s) using spectroscopic methods.

g) Evaluation of biological activity of pure compound(s)

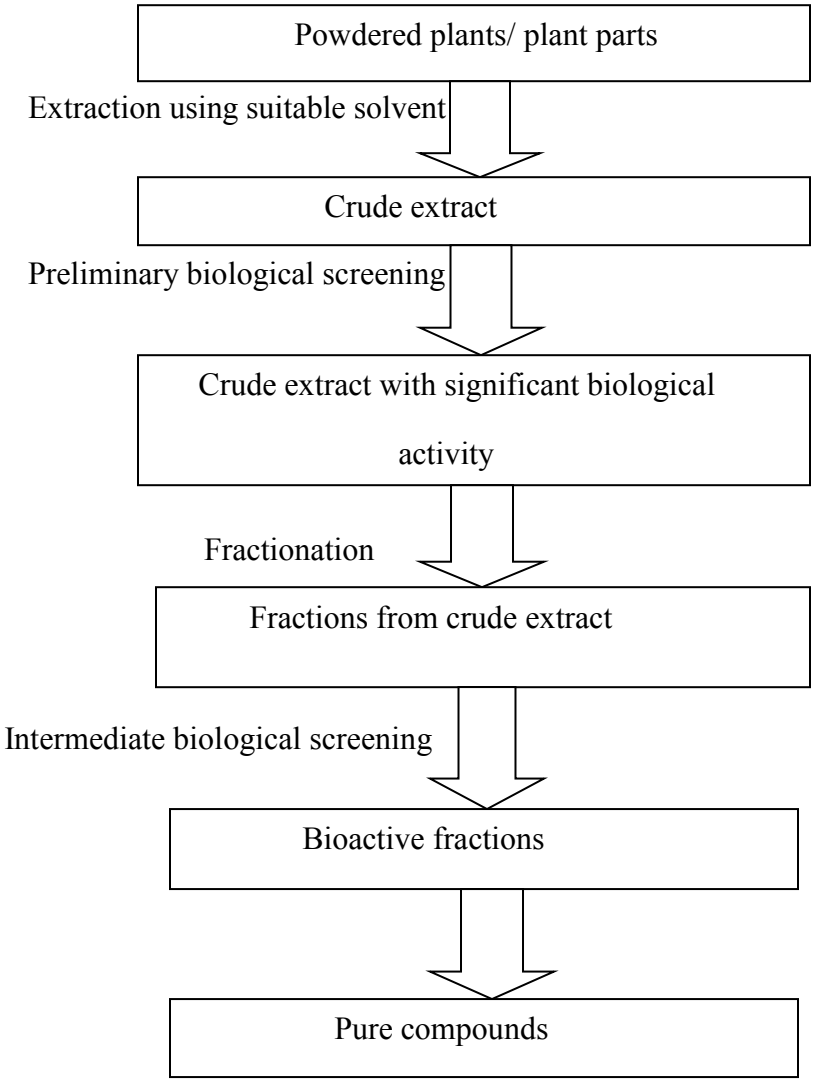

Figure 1.1: Schematic diagram of Developing Bioactive Compounds Medicinal Plants

1.9 Selection of Medicinal Plants for Collection

Where applicable, the species or botanical variety selected for collection should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country, as the source for the herbal medicines concerned. In the absence of such national documents, the selection of species or botanical varieties specified in the pharmacopoeia or other authoritative documents of other countries should be considered (WHO, 2003).

1.9.1 Collection

The population density of the target species at the collection site(s) should be determined and species that are rare or scarce should not be collected. To encourage the regeneration of source medicinal plant materials, a sound demographic structure of the population has to be ensured. Management plans should refer to the species and the plant parts (roots, leaves, fruits, etc.) to be collected and should specify collection levels and collection practices. It is incumbent on the government or environmental authority to ensure that buyers of collected plant material do not place the collected species at risk. Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of both source materials and finished products. In the course of collection, efforts should be made to remove parts of the plant that are not required and foreign matter, in particular toxic weeds. Decomposed medicinal plant materials should be discarded. The collected medicinal plant materials should be protected from insects, rodents, birds and other pests, and from livestock and domestic animals. If more than one medicinal plant part is to be collected, the different plant species or plant materials should be gathered separately and transported in separate containers. Cross-contamination should be avoided at all times (WHO, 2003).

1.9.2 Drying

When medicinal plant materials are prepared for use in dry form, the moisture content of the material should be kept as low as possible in order to reduce damage from mould and other microbial infestation. Information on the appropriate moisture content for particular

medicinal plant materials may be available from pharmacopoeias or other authoritative monographs. Medicinal plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms or buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials (WHO, 2003).

1.10 Methods of Extraction of Medicinal Plants

1.10.1 Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing (Handa, 2008; Chapman, 2004).

1.10.2 Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs (Handa, 2008; Chapman, 2004).

1.10.3 Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased (Handa, 2008; Chapman, 2004).

1.10.4 Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-

soluble, heatstable constituents. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further (Handa, 2008; Chapman, 2004).

1.10.5 Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting (Handa, 2008; Chapman, 2004).

1.10.6 Hot Continuous Extraction (Soxhlet)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber of the Soxhlet apparatus. The extracting solvent in flask is heated, and its vapors condense in condenser . The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube , the liquid contents of chamber siphon into flask . This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated (Handa, 2008; Chapman, 2004).

1.10.7 Aqueous Alcoholic Extraction by Fermentation

The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (*kasaya*), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel (Handa, 2008; Chapman, 2004).

1.10.8 Counter-current Extraction

In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce a fine slurry. In this process, the material to be extracted is moved in one direction (generally in the form of a fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. The further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end (Handa, 2008; Chapman, 2004).

1.10.9 Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfia root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules (Handa, 2008; Chapman, 2004).

1.10.10 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt. The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step, leading the analyst to believe that the actual efficiency was poor. There are many advantages to the use of CO2 as the extracting fluid (Handa, 2008; Chapman, 2004).

1.11 Parameters for Selecting an Appropriate Extraction Method

1) Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated.

2) Use the right plant part and, for quality control purposes, record the age of plant and the time, season and place of collection.

3) Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Hot or cold blowing air flow for drying is generally preferred. If a crude drug with high moisture content is to be used for extraction, suitable weight corrections should be incorporated.

4) Grinding methods should be specified and techniques that generate heat should be avoided as much as possible.

5) Powdered plant material should be passed through suitable sieves to get the required particles of uniform size.

6) Nature of constituents:

a) If the therapeutic value lies in non-polar constituents, a non-polar solvent may be used. For example, lupeol is the active constituent of *Crataeva nurvala* and, for its extraction, hexane is generally used. Likewise, for plants like *Bacopa monnieri* and *Centella asiatica*, the active constituents are glycosides and hence a polar solvent like aqueous methanol may be used.

b) If the constituents are thermolabile, extraction methods like cold maceration, percolation and CCE are preferred. For thermostable constituents, Soxhlet extraction (if nonaqueous solvents are used) and decoction (if water is the menstruum) are useful.

c) Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g. flavonoids and phenyl propanoids.

d) In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to break upon continuous exposure to higher temperature.

e) Standardization of time of extraction is important

7) Insufficient time means incomplete extraction.

8) If the extraction time is longer, unwanted constituents may also be extracted. For example, if tea is boiled for too long, tannins are extracted which impart astringency to the final preparation.The number of extractions required for complete extraction is as important as the duration of each extraction.

9) Concentration and drying procedures should ensure the safety and stability of the active constituents. Drying under reduced pressure (e.g. using a Rotavapor) is widely used. Lyophilization, although expensive, is increasingly employed.

10) The design and material of fabrication of the extractor are also to be taken into consideration.

11) Analytical parameters of the final extract, such as TLC and HPLC fingerprints, should be documeted to monitor the quality of different batches of the extracts (Handa, 2008; Chapman, 2004).

1.12 Separation of these components from the medicinal plants

Methods of separation includes

- column chromatography
- preparative thin layer chromatography
- preparative high performance liquid chromatography
- droplet counter current chromatography
- centrifugal thin layer chromatography, etc. (Verma et al., 2008).

1.13 Plants Profile

Plant Name- *Stereospernum chelonoides*

Figure- 2.1: *Stereospermum chelonoides*

1.14 Taxonomy-

KINGDOM Plantae

PHYLUM Magnoliophyta

CLASS Magnoliopsida

ORDER Lamiales

FAMILY Bignoniaceae

GENUS *Stereospermum*

SPECIES *Stereospermum chelonoides*

(Taxonomy details, 2012)

1.15 Vernacular names

Region	**Vernacular names**
Assamese	Parhori, paroli, ser phang
Chakma	Hamarang gaas
Chittagong	Barul-jata, Atkapali, Dharmara
English	Fragant padri tree
Garo	Bolsel
Hindi	Padeli
Kannada	Adri, bili paadri, giri, hadari
Khasi	dieng sir
Malayalam	kacasthali, karannavu, karanyavu
Nepali	Kuber bacha, jinghal, parhori

Sanskrit	kastapatala, patala

(Awale, 2008; Fern and Morris, 2016)

1.16 Bignoniaceae Family

This is a family of around 650 species in 120 genera, found mainly in the tropics, particularly South America. Most species are woody, often climbing. They are grown for their timber and as ornamental plants, and include many plants frequently seen by travellers in tropical regions, as many species are used as street trees or in hotel gardens..

Leaves, Stem & Roots ~ Nearly all members of this Family have woody stems. The leaves are opposite, with no stipules, and are usually compound. There are often tendrils used for climbing. The calyx has five lobes, and is sometimes 2-lipped (*BIGNONIACEAE*, no date).

Flowers ~ The flowers are bell or funnel shaped, also with five lobes. They grow in clusters and arusually large and brightly-coloured. There are four stamens.

Seeds ~ The seedpod forms inside the flower (a superior ovary). The seeds are usually flat with papery wings, although sometimes there is an indehiscent fleshy fruit containing unwinged seeds (*BIGNONIACEAE*, no date).

1.17 Phytochemical analysis of the family Bignoniaceae:

Several phytochemical studies revealed that the extracts from many species of Bignoniaceae contained secondary metabolites such as saponins,tannins, flavonoids, quinones, alkaloids, anthralene derivatives, reducing sugars, glycosides, carbohydrates, querletin,kaempferol, â-sitosterol, terpenes, steroids, coumarins etc. secondary metabolites and their derivatives (Choudhury *et al.,* 2011).

1.18 Other Plants Of Stereospermum

1. *Stereospermum acuminatissimum* K.Schum.
2. *Stereospermum angustifolium* Haines
3. *Stereospermum annamense* Dop
4. *Stereospermum arcuatum* H.Perrier
5. *Stereospermum boivini* (Baill.) H.Perrier
6. *Stereospermum cylindricum* Pierre ex Dop
7. *Stereospermum euphorioides* DC.
8. *Stereospermum fimbriatum* (Wall. ex G.Don) DC.
9. *Stereospermum harmsianum* K.Schum.
10. *Stereospermum hildebrandtii* (Baill.) H.Perrier
11. *Stereospermum kunthianum* Cham.
12. *Stereospermum leonense* Sprague
13. *Stereospermum longiflorum* Capuron
14. *Stereospermum nematocarpum* (Bojer) DC.
15. *Stereospermum neuranthum* Kurz
16. *Stereospermum rhoifolium* (Baill.) H.Perrier
17. *Stereospermum strigillosum* C.Y. Wu & W.C. Yin
18. *Stereospermum strigilosum* C.Y.Wu
19. *Stereospermum tetragonum* DC.
20. *Stereospermum tomentosum* H.Perrier
21. *Stereospermum undatum* H.Perrier
22. *Stereospermum zenkeri* K.Schum. ex De Wild. (The plant list, 2010)

1.19 Range

E. Asia - India, Sri Lanka, Nepal, Bangladesh, Myanmar, Thailand, Cambodia, Laos, Malaysia (Fern and Morris 2016).

1.20 Cultivation Details

A plant of the moist subtropics and tropics, where it is found at elevations up to 1,300 metres. It grows best in areas where annual daytime temperatures are within the range 24- 32°c, but can tolerate 5 - 47°c. When dormant, the plant can survive temperatures down to about -5°c, but young growth can be severely damaged at -1°c. It prefers a mean annual rainfall in the range 1,200 - 2,500mm, but tolerates 750 - 3,800mm. It grows best in a sunny position, tolerating light shade. Succeeds in a wide range of well-drained soils. Prefers a pH in the range 6 - 7, tolerating 5.5 - 7.5. The tree sometimes suckers very freely.Trees can survive forest fires. It is one of the commonest trees to be seen in the savannah lands of India, apparently able to shoot up yearly in spite of fire, and to grow on into a tree if only a short period of immunity from fire can be obtained. The tree regenerates very freely from seed in the wild (Fern and Morris 2016).

1.21 USES:

1.21.1 Edible Uses

Tender young fruit - cooked and eaten as a vegetable

Flowers - cooked and used as a vegetable (Fern and Morris 2016).

1.21.2 Traditional uses:

- Traditionally, the decoction of bark and root is used for the treatment of pain, fever, inflammations, asthma, liver disorders, acidity and as a diuretic.
- The flowers are mixed with honey and given orally, for the control of hiccup .

- In southern India, the bark is used traditionally for the treatment of diabetes.
- The fruit is useful for the treatment of leprosy. Fruits are useful in hic cough and blood diseases.
- The root has an anticancer activity and also used in preparation of Ayurvedic formulation.
- The root-bark is an ingredient of Dashmoola and it is regarded as cooling, astringent cardio tonic, bitter, diuretic and tonic and generally used in combination with other medicine the ashes of this plant are used in the preparation of alkaline water and caustic paste**s.** (Fern and Morris 2016)

1.21.3 Other Uses

The grey wood is hard, elastic, moderately durable, easy to work. Usually there is no heartwood. It is used for making furniture, construction, tea boxes, canoes etc. An excellent fuel, the wood also makes a good charcoal (Fern and Morris 2016).

CHAPTER 2

LITERATURE REVIEW

2. Chemical Compounds of *Stereospermum Chelonoides*

A research work has found that S. *chelonoides* have 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. 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).

2.1 Antimicrobial and cytotoxic activities of *Stereospermum chelonoides*

This research reported that 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 LC_{50} 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 (Rashed *et al.,* 2007).

2.2 Phytochemical screening and *InVitro* determination of antioxidant potential of methanolic extract of *Streospermum chelonoides*

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 49.44mg/g (Shanta *et al*., 2013).

CHAPTER 3

METHODOLOGY

3. Analgesic activity study of *Stereospermum chelonoides*

The acetic acid induced writhing method is an analgesic behavioral observation assessment that demonstrates a noxious stimulation in mice . The test consists of injecting 0.7% acetic acid solution intraperitoneally and then, observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing is made test sample given 30 min prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample, will give lower number of writhing than the control, *i.e.* the sample having analgesic activity will inhibit writhing.

3.1 Material and method

3.1.1 Experimental animal

Mice (Age: 4-5 weeks, Avg. weight: 18-25 g) were used for the experiment. The mice were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research (ICDDR), Bangladesh. They were kept under standard environmental condition for one month for adaptation after their purchase and fed ICDDRB formulated 'Mouse-pellets' and water.

Animal feed: 'Mouse-pellets' supplied by ICDDRB Dhaka.
Material used for animal housing: Plastic cages having a dimension of (28×22×13) cm; Soft wood Shaving.

3.1.2 Collection of plant material:

At first with the help of a comprehensive literature review *Stereospermum chelonoides* was selected for this investigation. The leaves were collected from Jahangirnagar University, Dhaka, Bangladesh during the month of June. The plant is identified by Mr. Rahim and a specimen of this is submitted for further investigation. The Accession no. was DACB43467.

3.1.3 Plant Material Preparation

The leaves of the plants were collected in fresh condition. It was sun-dried to make suitable for grinding purpose. The coarse powders were then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation. Total amount of the dried powder was approximately 530gm.

3.1.4 Extraction of the plant material:

The dried, coarsely powdered plant material was successively extracted by maceration over 1 week period with petroleum ether and finally with methanol at room temperature. Then the extracts were filtered and concentrated with a rotary evaporator at low temperature (40-50°C) and reduced pressure and were subsequently defatted to get the dried petroleum ether (SCPE) and methanol (SCME) extracts. Filtration was done using Whatman No.1 filter paper and the filtrate collected in a beaker and then covered using aluminum foil. The filtrate was then freeze dried. The extracts were placed in airtight bottle and stored in a refrigerator at 4ºC.

3.1.5 Study design

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV, group-V, group-VI consisting of 6 mice in each group. Each group received a particular treatment *i.e.* control, standard and the two doses of the extract. Each mouse was weighed properly and dose of samples and control were adjusted accordingly.

3.1.6 Acetic acid induced writhing method

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice (Ahmed *et al.*, 2004). In this method, acetic acid is administered intra-peritoneally to the experimental animals to create pain sensation. As a positive control,

any standard NSAID drug can be used. In the present study Indomethacine was used to serve the purpose. The plant extract was administered orally in two different doses (250and 500 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Indomethacine was administered 15 minutes prior to acetic acid injection.

Figure: 4.1- Mice giving writhing due to pain induced by acetic acid

Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Indomethacin(10 mg/kg) was used as a reference substance (positive control).

3.2 Statistical Analysis

Total values which were obtained from the experiments are represented as mean ± standard error of the mean (SEM). Statistically obtained data was estimated by using ANOVA (Analysis of variance) followed by post-hoc Dunnett's test which was associated with SPSS program (SPSS 17.0, USA). The results obtained were compared with the vehicle control group. p values < 0.05, 0.01 and 0.001 were considered to be statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

4. Result

Table-4.2.1 Analgesic activity test of SCBM by acetic acid induced writhing method

Group	**Dose**	**Vehicle**	**Average ±SEM WRITHING**	**% of inhibition**
Group 1	10ml/kg	WATER (control)	49.83±12.48	
Group 2	10mg/kg	INDOMETHACIN (Standard)	6.60±4.59***	86.96
Group 3	250mg	SCBM	36.17±13.41	27.42
Group 4	500mg	SCBM	20.17±6.62***	59.53

Values are mean ±SEM (n = 6); One-way ANOVA followed by Dunett's test; ****P<0.001***P*<0.01, **P*<0.05 compared to control.

SCBM- *Stereospermum chelonoides* bark extracts of Methanol

Table-4.2.2 Analgesic activity test of SCBPE by acetic acid induced writhing method

Group	**Dose**	**Vehicle**	**Average ±SEM WRITHING**	**% inhibition**
Group 1	10ml/kg	WATER (control)	49.83±12.48	
Group 2	10mg/kg	INDOMETHACIN (Standard)	6.60±4.59***	86.96
Group 3	250mg	SCBPE	42.50±10.39	14.72
Group 4	500mg	SCBPE	22.00±40.00	55.85

Values are mean ±SEM (n = 6); One-way ANOVA followed by Dunett's test; ****P<0.001***P*<0.01, **P*<0.05 compared to control.

SCBPE- *Stereospermum chelonoides* bark extracts of Pet ether

Analgesic activity of *Stereospermum Chelonoides* was investigated by giving dose in 250mg/kg and 500 mg/kg. From the study it has been found that, SCBM and SCBPE inhibited pain at both doses in dose dependent manner and the result was significant. SCBM and SCBPE produce significant analgesic effect (*P<0.001)* at the dose of 500 mg/kg in a dose depending manner. The inhibition of SCBM, SCBPE at the doses of 250 mg/kg and 500 mg/kg were 27.42%, 59.53%, 14.72 % and 55.85 % respectively. For the standard (Indomethacin) the inhibition was 86.96%.

4.2 Discussion

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs The crude extracts of both the plants showed significant analgesic action compared to the reference drug Indomethacin . Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed *et al*., 2006) via cyclooxygenase (COX), and prostaglandin biosynthesis In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The significant pain reduction of both the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways (Zulfiker *et al*., 2010).

Phytochemical screening of SCB 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). Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins. Besides alkaloids are well known for their ability to inhibit pain perception.

These above data also suggest that the extract can produce analgesic action through inhibition of COX (Inhibition of the enzyme cyclo-oxygenase) and consequently prostaglandin synthesis.

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. 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 proinflammatory signaling molecules (Zulfiker *et al.,* 2010).

CHAPTER 5

CONCLUSION

5. Conclusion

Among all the natural sources surrounding *us Stereospermum chelonoides* is also a nature's gift to us which contains pharmacological activity to give analgesic activity. From the acetic acid induced analgesic test we have found that, the SCBM and SCBPE increase the inhibition percentage at the dose 500 mg/kg compared to 250 mg/kg. These indicated that this plant could be a potential source for discovery of newer analgesic and anti-inflammatory "leads" for drug development. So it is clear that our experimental plant on which we worked is helpful plant but our work was only preliminary effort which will require further comprehensive exploration as well as depiction of active compounds to elucidate the mechanism of action of producing the analgesic effects of the extract and necessitates preformulation studies for expansion of a potential dosage form.

CHAPTER 6

REFERENCE

6. Reference

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