A Study of CNS Depressant Activity of Methanol Extract of *Stereospermum chelonoides* Leaves

This dissertation is submitted for the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy



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July, 2017

Declaration by the Research Candidate

I, FatemaShiraj, hereby declare that the dissertation entitled "A Study of CNS Depressant Activity of Methanol Extract of *Stereospermum chelonoides* Leaves" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree. All of the research works are carried out by me during 2017, under the supervision and guidance of MeenaAfroze 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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Acknowledgement

First, all praise and glory are to Allah for all the bounties granted to me and only with His guidance and help this achievement has become possible.

I would like to express my deepest gratitude to my supervisor and respected teacher, **Meena Afroze Shanta**, Lecturer, Department of Pharmacy, East West University for her constant supervision, intense support, enthusiastic encouragements and constructive criticism during the research work. Her timely advice and encouragement have made it possible for me to accomplish the task as per schedule.

I put forward my most sincere regards and profound gratitude to respected teacher, **Dr. Chowdhury Faiz Hossain**, Professor & Chairperson, Department of Pharmacy, East West University. I also have great respect to our previous Chairperson, **Dr, Shamsun Nahar khan Phd**, of this department.

I would like to convey deepest love and obedience to my caring parents for their support and guiding me all through my life until today, which keeps me strong and honest to do the things I needed to do.

Cordial thanks to my friends and research mates, Suraya Yasmin Juthee, Lia Rose Merry D, Cruze, Saiful Islam Arif, RefatUz Zaman, Rezaul Karim Rabbi, Nasrin Jahan Billal Sonia, who gave me support in my research work and for their extended cooperation for my study. Special thanks to all my well-wishers for their whole hearted inspiration throughout the period of the research work.

Dedication DEDICATED TO MY PARENTS

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List of Abbreviation

СМС	Carboxy Methyl Cellulose
CNS	Central Nervous System
S. chelonoids	Stereospermum chelonoides
WHO	World Health Organisation
m	Meter
cm	Centimetre
kg	Kilogram
mg	Milligram
Min	Minute
SD	Standard Deviation
GABA	Gamma aminobutyric acid
GABAA	Gamma aminobutyric acid Type A

Abstract

Recently worldwide different researches on medicinal plants are holding attention. A number of experiments were found where medicinal plants have been used for different complementary and traditional systems to promote newer and safer drugs. Keep this in mind our current study was designed to find out CNS depressant activity from methanol extracts of leaves of Stereospermumchelonoides. In our present study, we have checked above mentioned pharmacological activities of our experimental plant extracts in mice. By the open-field and hole-board method, CNS depressant activity was inspected with the decline of locomotor activity on mice. Here crude extracts were administered to mice at a dose of 250mg/kg and 500mg/kg. All the results of our experiments were statistically significant (p<0.05, p<0.01, p<0.001). In CNS depressant activity tests, the movement of mice decreased in a dose depending manner comparing to the standard diazepam. In conclusion we can say that our present findings suggest that methanol extracts of Stereospermumchelonoides leaves contain potent CNS depressant principles. It can also be said that the obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Chapter- 01 Introduction

1.1 Overview

Plants are very important element of whole world. Human has been using plants as medicine from ancient times. Plants were identified as source of medicine after different type of observations and experiments. Hence in the early stage of human civilization, treatment with various plants began (Malik, 2001).

The study of disease and their treatment have been existing since the beginning of human civilization. Norman R. Farnsworth of the University of Illinois declared that, for every disease that affect mankind there is a treatment and cure occurring naturally on the earth. Plant kingdom is one of the major search areas for effective works of recent days. The importance of plants in search of new drugs is increasing with the advancements of medical sciences (Malik, 2001).

Many higher plants produce economically important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavours and fragrances, pharmaceuticals, and pesticides. However, most species of higher plants have never been described, much less surveyed for chemical or biologically active constituents, and new sources of commercially valuable materials remain to be discovered. Advances in biotechnology, particularly methods for culturing plant cells and tissues, shouldprovide new means for the commercial processing of even rare plants and the chemicals they produce. These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals(Ghani, 1998; Khan*et al.*, 2001).

In the future, biologically active plant-derived chemicals can be expected to play an increasingly significant role in the commercial development of new products for regulating plant growth and for insect and weed control. Recently, dramatic changes have taken place in the primary health care system of world population through the development of science, technology and medical science, but till to day 400 cores ofpeople of the world are totally dependent on herbal medicine. It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources and herbal medicines are used by about 75.80% of the world's population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects (Balandrin, 2016).

1.2 Medicinal plants

WHO defines medicinal plants in the following way:

"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 is a precursor for synthesis of useful drugs".

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. (Srivastava, 1996).

1.3 Herbal medicines

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations (WHO, 2017).

1.3.1 Herbs

Crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered (WHO, 2017).

1.3.2 Herbal materials

In addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting, or stir-baking with honey, alcoholic beverages or other materials(WHO, 2017).

1.3.3 Herbal preparations

The basis for finished herbal products and may include fine or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials (WHO, 2017).

1.3.4 Finished herbal products

Herbal preparations made from one or more herbs. If more than one herb is used, the term mixture herbal product can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal (WHO, 2017).

The proper and judicious use of herbs is often successful in the treatment of illness when other, more conventional medicines and methods fail. Herbs can be used to cleanse the bowels, open congested sinuses, help mend broken bones, stimulate the brain, increase libido, ease pain, aid digestion, and a thousand other purposes. Topically, herbs can repair damaged skin, soothe a wound, improve complexion, heal bruises and relieve aching muscles. Herbs demonstrate great versatility for the treatment of a broad variety of health needs (Medicinehunter, 2014).

1.4 History of using medicinal plants

1.4.1 Use of Medicinal plant in ancient time

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 (Medknow Publications. 2012).

The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (CentauriumumbellatumGilib) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents (Medknow Publications, 2012).

Theophrastus (371-287 BC) founded botanical science with his books "De CausisPlantarium"— Plant etiology and "De HistoriaPlantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the

time.Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants (Medknow Publications, 2012).

In his work "De re medica" the renowned medical writer Celsus (25 BC–50 AD) quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc (Medknow Publications, 2012).

1.4.2 Medicinal plant in 21st century

For centuries people have used plants for healing. Plant products - as parts of foods or botanical potions and powders - have been used with varying success to cure and prevent diseases throughout history. Written records about medicinal plants date back at least 5000 years to the Sumerians (Swerdlow, 2000) and archeological records suggest even earlier use of medicinal plants. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers (Verpoorte and Alfermann, 2000). The twentieth century became a triumph for the synthetic-chemistrydominated pharmaceutical industry, which replaced natural extracts with synthetic molecules that often had no connection to natural products. The spectacular rise of the pharmaceutical industry had a tremendous impact on disease treatment and prevention, saved countless lives and became one of the outstanding achievements of the twentieth century. However, the benefits of modern drugs are felt primarily in developed countries, and developing countries continue to rely on ethnobotanical remedies as their primary medicines, leaving almost 75% of the world population without access to the modern healthcare products taken for granted in the West (Raskin, 2002).

1.4.3 Modern drug from medicinal plant

In present days, almost all pharmacopoeias in the world—PhEur 6, USP XXXI, BP 2007 proscribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany) that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine). For the sake of adequate and successfully applied therapy, knowledge of the precise diagnosis of the illness as well as of medicinal plants, i.e. the pharmacological effect of their components is essential. Germany employed the rational phyto-therapy, based on applications of preparations, whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs. With the new Law on Drugs and Medical Devices dated September 2007 and enacted in the Republic of Macedonia, dry or sometimes fresh parts of medicinal plants (herbal substances) may be used for preparation of herbal drugs, herbal processed products, and traditional herbal drugs (Medknow Publications, 2012).

Drug/Chemical	Action/Clinical Use	Plant Source
Acetyldigoxin	Cardiotonic	Digitalis lanata
Adoniside	Cardiotonic	Adonis vernalis
Allyl isothiocyanate	Rubefacient	Brassica nigra
Atropine	Anticholinergic	Atropa belladonna
Caffeine	CNS stimulant	Camellia sinensis
Camphor	Rubefacient	Cinnamomumcamphora
Digitoxin	Cardiotonic	Digitalis purpurea
Ephedrine	Antihistamine	Ephedra sinica
Quinidine	Antiarrhythmic	Cinchona ledgeriana

 Table 1.1- Some commonly used drugs derived from plant sources

Reserpine	Tranquillizer	Rauvolfiaserpentina
Taxol	Antitumor agent	Taxusbrevifolia

(Islam, 2000)

1.5 Future of medicinal chemistry

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. As part of our National Cooperative Drug Discovery Group (NCDDG) research project, numerous compounds from tropical rainforest plant species with potential anticancer activity have been identified. Our group has also isolated several compounds, mainly from edible plant species or plants used as dietary supplements that may act as chemo-preventive agents. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate highthroughput screening bioassays, and the scale-up of active compounds (Balunas, and Kinghorn, 2005).

1.6Medicinal plants and their uses around the world

From marijuana to catnip, there are hundreds of remarkably common herbs, flowers, berries and plants that serve all kinds of important medicinal and health purposes that might surprise you: anti-inflammatory, anti-fungal, insect repellent, antiseptic, expectorant, antibacterial, detoxification, fever reduction, antihistamine and pain relief.

Plant Names	Description	Uses
1. Marijuana	It is a remarkable and renewable plant, offering all kinds of foodstuff and product uses that surpass cotton and plastic. It is legal in 12 states for	 Depression and anxiety relief. Reduce blood pressure. Used in pain alleviation & glaucoma treatment.

Table 1.2 Some of the potent medicinal plants

	medicinal purposes but is still illegal in the United States.	
2. Blood Flower	Also called Mexican butterfly weed; is a type of tropical milkweed with toxic milky sap that is emetic.	 Heart stimulant and worm expellant. Pretty useful for a number of potential hiking disasters.
3. Tansy	It is an old-world aster and remedy, used for flavouring beer and stews as well as repelling insects.	• Effective bug repellent, treat worms.
4. Korean Mint	Most of the various types of "mint "or menthe spearmint,Korean mint, apple mint, regular old mint – offer reported health benefits and medicinal properties.	 Calming the stomach. Reducing headache and nervousness. Useful for fighting colds and the flu.
5. Sage	It grows in the Northern Hemisphere.	 The stems and leaves for healing. Effective treatment against dysentery and diarrhoea as well as serving usefulness as

		 an anti-inflammatory and astringent. Ideal for treating cuts and inflammation in the mouth.
6.Wild Quinine	It is a potent herb. Found in eastern native America	• Traditionally been used in alternative medicine to treat debility, fatigue, respiratory infection, gastrointestinal infection, and venereal disease.
7. Blackberries	Found widely in native America.	 Used as antioxidant and for vitamins. Effective treatment against dysentery and diarrhoea. Used in treating cuts and inflammation in the mouth.
8. Winter savory	Originally from Europe and Mediterranean.	• It is a saviour against insect bites and stings. It's also used for flavouring meats and stews.

(Islam, 2000)

1.7 Medicinal plants inindia

India is rich in culture. We have had different human advancements that have added to making this land flourish. There have been numerous who have come, bestowed intelligence identified with different points and have helped us as a human race to develop and advance. Without our current mechanical progressions, we have checked and cured various diseases

and sicknesses with the assistance of therapeutic plants and home grown cures (Mpbd.info, 2014).

It is very important to have medicinal plants around the house because you never know when you might need them. So here a list of medicinal plants that have the highest medicinal value compared to the other million species around the world worth planting around the house (Mpbd.info, 2014).

Plant Name	 Uses Taken as the herbal tea. Tulsi oil is also used as the ear drop. Tulsi helps in curing malaria. It is very effective against indigestion, headache, hysteria, insomnia and cholera. TheTulsi is the effective remedy for the severe acute Respiratory Syndrome. 	
1.Tulsi		
2. Aloe Vera	 Speed up the healing. Reduce the risk of infections of various things like wounds, cuts, burns and even reduces, Inflammation, Ulcerative colitis, Chronic constipation, Poor appetite, Digestive problems. 	
3. Basil 4. Rosemary	Used in, • Cuts • Lack of appetite • Stomach gas • Scrapes. • Rosemary is the great reviver.	
	• This perennial woody herb stimulates energy and optimism and sharpens memory and concentration by bringing more oxygen to the brain.	

	• It's a wonderfully stimulating alternative to caffeine.	
5. Lavender	 It's also used in creams to treat sunburns and acne. Woody lavender plants prefer hot, sunny, and dry environments. The fresh flowers are tasty in small doses when added to salads, honey, butter, lemonade, and even shortbread cookies. 	
6)Fenugreek, Methi	 Fenugreek seeds are nourishing and taken to encourage weight gain. Inhibit cancer of the liver, lower blood cholesterol levels. Treat inflammation and ulcers of the stomach and intestines. Drain off sweat ducts, for late onset diabetes. Helps poor digestion, painful menstruation, labour pains. 	
	 Freshen bad breath and restores a dull sense of taste. 	
7. Peppermint	 Crushed leaves rubbed on the skin help soothe and relax the muscles. Reduce irritable bower syndrome. Against upset stomachs. Inhibit bacterial growth. Treat fevers, flatulence and spastic colon. 	

(Ayushakti, 2017)

1.8 Medicinal plants in Bangladesh

Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. In recent years, the growing demand for herbal product has led to a quantum jumping in volume of plants materials trade within and across the country. In Bangladesh there are no systematic cultivation process or conservation strategies about medicinal plants. The local people conserve traditional knowledge through their experience and practice, which is handed down orally without any documentation (Mpbd.info, 2014).

Scenario of Medicinal plants in Bangladesh." conducted by the DEBTEC researchers at Chakbazar, Dhaka, Bangladesh, found that there is worth of 11 million US dollars medicinal plant market in Bangladesh, which have been imported but not in the name of medicinal plants rather in the name of spices and other products. Another research aimed at documenting the 'Present Status and Market Scenario of Medicinal Plants' in Bangladesh shows that 84.1% of the respondent use medicinal plants in health care. 18.3% of the villagers use Kabirazi in the disease in medium category. 55.0% of respondent's source of knowledge of using medicinal plant is family where 34.7% gained knowledge from neighbor. Only 14.3% of the respondents are involved with trading of medicinal plant. About 10.4% of the villagers are involved in cultivation, collection or business of medicinal plant. From the survey report it has been found that 46.6% industries are using above 60% of imported medicinal plants as their raw materials and 53.3% of the industries are using below 40%. The study revealed that 86.7% industries are importing Indian raw materials, 53.3% are importing the Pakistani one and very few of them are importing the raw materials from Nepal, Iran and Korea. According to the response of shop owners, the local raw materials of their products are mostly coming from 5 different areas of the country. Among those 90% are coming from Chittagong and again 76.6% from Tangail, 30% from Gazipur and another 30% from Khulna (Mpbdinfo, 2014).

 Table 1.4- Medicinal plant species listed by WHO which can be grown in Bangladesh commercially

Scientific name	Bengali name	Used parts
Winthaniasomnifera	Ashwagandha	Root, Leaf, fruit, seed, whole plant
Aloe vera	Ghritokumari	Leaf
Andrographispanniculata	Satomuli	Root, leaf, flower, fruit
Plumbagozeylanica	Vasak	Leaf, stem, bark, root, flower
Rauvolfia serpentine	Swarpagandha	Root
Glycyrrhizaglabra	Jastimodhu	Root, stem
		(Islam <i>et al</i> , 20

1.9 Synthetic medicine

A synthetic medicine is a medicine with properties and effects similar to a known hallucinogen or narcotic but having slightly altered chemical structure, especially such a drug created in order to evade restrictions against illegal substances.

Examples of synthetic drugs are synthetic phenethylamines, including synthetic cathinones or synthetic hallucinogens and, more commonly known as "bath salts". Synthetic cannabinoids, also known as synthetic marijuana, are another example of designer drugs and these are often found in herbal incense products that mimic the effects of delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marijuana. (New York State Department of Health, 2012).

1.10 Studying herbal medicine

Herbal medicine remains largely an unproven, inexact science. Although the history of herbal medicine provides decades, sometimes centuries, of anecdotal information, scientific study of herbal medicine is relatively new. The U.S. Department of Health and Human Service's National Center for Complementary and Alternative Medicine (NCCAM) has only been in operation since 1992. Compared to the Federal Food and Drug Administration (FDA), which was founded over 100 years ago, NCCAM has only begun to scratch the surface of scientific research (Jeanne, 2014).

Despite the criticism of herbal medicine among mainstream medical professionals, it is wise to remember that many common drugs we use today were derived from plant-based sources. For example, scientists originally derived aspirin from willow bark; herbalists prescribe white willow for headaches and pain control. Digitalis, a drug prescribed for certain heart conditions, comes from an extract of potentially toxic foxglove flowers(Jeanne G., 2014). These are just a few examples of why it's important studying herbal medicine but without significant scientific study it is not safe of using herbal medicine as treatment of any disease. Because, herbs are not without disadvantages, and herbal medicine is not appropriate in all situations. These are a few of the disadvantages to consider:

1.11 Inappropriate for many conditions- would not be able to treat serious trauma, such as a broken leg, nor would he be able to heal appendicitis or a heart attack as effectively as a conventional doctor using modern diagnostic tests, surgery, and drugs (Jeanne, 2014 and MedicinePlus, 2017).

1.12 Lack of dosage instructions- may cause accidentally overdosing on cold remedies, many herbs do not come with instructions or package inserts. There's a very real risk of overdose (Jeanne, 2014 and MedicinePlus, 2017).

1.13 Poison risk associated with wild herbs- Harvesting herbs in the wild is risky, if not foolhardy, yet some people try to identify and pick wild herbs (Jeanne, 2014 and MedicinePlus, 2017).

1.14 Medication interactions- Herbal treatments can interact with medications. Nearly all herbs come with some warning, and many, like the herbs used for anxiety such as Valerian and St. John's Wort, can interact with prescription medication like antidepressants (Jeanne, 2014 and MedicinePlus, 2017).

1.15 Lack of regulation- Because herbal products are not tightly regulated, the quality of herbal products may vary among batches, brands or manufacturers. This can make it much more difficult to prescribe the proper dose of an herb (Jeanne, 2014 and MedicinePlus, 2017).

The bottom line is that herbs are medicines, and like other medications, though they have some disadvantages. With proper scientific investigation we can make the best use of our natural products (Jeanne, 2014 and MedicinePlus, 2017).

1.16 Reasons behind physicochemical investigations

The importance of medicinal plants, are extremely useful for us on the one hand they provide us with the oxygen we need to be able to breathe for edible landscaping. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes. Traditional knowledge of medicinal plants has always guided the search for new-cures. In spite of the advance of modern high throughout drug discovery and screening techniques traditional knowledge systems have given clues to the discovery of valuable drugs (Belay and Sesay, 2014).

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstruum. The extract thus obtained, after standardization, may be used as a medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain a complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans(Ahmad, 2013).

1.17 Significances of medicinal plants to human being

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.
- Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.
- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological.
- Diversity, for example metabolic engineering of plants.

(Cals.ncsu, 2017)

1.18 Importance of medicinal plant

Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, atisine, lobeline, nicotine, strychnine, digoxin, atropine, morphine are some common examples.

While medicinal plants are the actual plants themselves, plant medicines are preparations made from those plants. Plant medicines are the most widely used medicines in the world today. An estimated eighty percent (80%) of the world's population employs herbs as primary medicines. And while drugstore shelves in the US are stocked mostly with synthetic remedies, in other parts of the world the situation is quite different. In parts of Europe, for example, pharmacies dispense herbs prescribed by physicians (Medicinehunter, 2014; Worldpress, 2015).

1.19 Plant Review:



1.19.1 Plant Name: *Stereospermumchelonoides*(*S. chelonoides*)

Figure 1.1Stereospermumchelonoides

1.19.2 Description of this plant: *S. chelonoides* Tree is a large deciduous tree, 10-20 meter tall, with velvet-hairy branches. Leaves are compound, 1-2 feet 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 12 feet long, cylindrical, ribbed, rough. The 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 (Shrikant, 2017).

1.19.3 Classification

Kingdom- Plantae

Phylum;Tracheophyta

Class - Magnoliopsida

Order - Lamiales

Family - Bignoniaceae

Genus -Stereospermum

Species -Stereospermumchelonoides (L. fil.) DC.

1.19.4 Synonyms of the plant: *Bignonia chelonoides*, *Bignonia suaveolens*, *Stereospermumsuaveolens* (Biodiversity, 2017).

Table 1.5 Vernacular Names of Plant

Bangla/Vernacular	Dharmara(Chittagong),Barul-jata,Atkapali,Pahari	
	Awal (Sylhet)	
English	Rose Flower Fragrant, Bignonia suaveolens	
Assamese	Parhori, paroli, serphang	
Garo	Bolsel	
Hindi	Padeli	
Kannada	Adri,bilipaadri, giri, hadar	
Khasi	Dieng sir	
Malayalam	kacasthali, karannavu, karanyavu	
Mizo	Zinghal	
Naga	Ing-nge-ching	
Nepali	Kuber bacha, jinghal, parhori	
Sanskrit	kastapatala, patala	
Tamil	ambuvagina, padiri, pathiriver, pumbadir	
Telugu	ambuvaasini,gallugudu, goddalipukusu, isakarasi.	
	(ShrIkant 2012	

(ShrIkant, 2017)

1.19.5 Tribal Name:Hamaranggaas (Chakma), Chain-cha (Marma), Sekwai(Chakma), Goda-kamarang (Mogh), Batsil (Garo), Bol-sal (Garo) (ShrIkant, 2017).

Numerous	
Fleshy	
Absent	
1, 2, and 3	
Taproot system	
Reticulate or net Veined	
Bisexual	
Imbricate or valvate (Corolla)	
2- locular, rarely 1 - or 4 –locular	
Axile or parietal	
Bracts, bractlets, flowers	
Capsule, Loculicidal or septicidal, rarely indehiscent	
tendrils modified somtimes into hooks or suckers (in	
Climbers) Opposite, alternate, or whorled	
4(fertile stamens)	
Numerous	
Absent	
2 – lobed	

Table 1.6 Profile of Stereospermumchelonoides

(Species Directory, 2017)

1.19.6 Leaf anatomy

1.19.6.1 Physical anatomy



Figure 1.2 Leaves of Stereospermumchelonoides

The leaf lamina dorsiventral (usually), or bifacial (isobilateral recorded in Kigelia). Stomata present; nearly always mainly confined to one surface (abaxial); anomocytic, or paracytic, or diacytic (Kigelia). Adaxial hypodermis present (rarely, e.g. in Pandorea), or absent. The mesophyll with sclerenchymatousidioblasts (spicular cells), or without sclerenchymatousidioblasts; containing crystals. The crystals mostly solitaryprismatic (in the form of small octahedra, prisms or needles: large solitary crystals and druses rare). Minor leaf veins without phloem transfer cells (Catalpa, Jacaranda, Tecomella) (Watson &Dallwitz, 1992).

1.19.6.2 Axial (stem, wood) anatomy: Cork cambium present; initially deepseated, or initially superficial. Nodes unilacunar (3 to several traces). Primary vascular tissues in a cylinder, without separate bundles. Internal phloem absent. Cortical bundles absent. Medullary bundles present (these inversely orientated, e.g. in Campsis, Tecoma), or absent. Secondary thickening developing from a conventional cambial ring, or anomalous (represented by assorted variations, featured in Solereder's generic key). The anomalous secondary thickening via concentric cambia (e.g. Campsis, Clytostoma, Tecomaria, where a second series of bundles forms internally to the primary cylinder, in the pith; or in the secondary cortex), or from a single cambial ring. Primary medullary rays wide (in lianes), or mixed wide and narrow (Watson &Dallwitz, 1992).

1.19.7 Inflorescence, floral, fruit and seed morphology: Flowers solitary, or aggregated in 'inflorescences'; when aggregated, in cymes, or in racemes. The ultimate inflorescence units cymose, or racemose. Inflorescences terminal, or axillary; usually dichasial, tending to cincinnial. Flowers somewhat irregular to very irregular. The floral irregularity involving the perianth and involving the androecium (K irregular, C sometimes more or less regular). Flowers more or less 5 merous; cyclic; tetracyclic. Hypogynous disk usually present (Watson &Dallwitz, 1992).

1.19.8Perianth with distinct calyx and corolla: 10; 2 whorled; isomerous. Calyx 5; 1 whorled; gamosepalous; entire, or toothed; campanulate; unequal but not bilabiate, or bilabiate; often open in bud. Corolla 5; 1 whorled; gamopetalous. Corolla lobes markedly shorter than the tube, or about the same length as the tube. Corolla imbricate, or valvate (rarely); usually campanulate, or funnel-shaped; bilabiate (often, the upper lip with two lobes, the lower with three), or regular (rarely) (Watson &Dallwitz, 1992).

1.19.9 Gynoecium 2 carpelled: The pistil 1 celled, or 2 celled, or 4 celled. Gynoecium syncarpous; synovarious to synstylovarious; superior. Ovary 1 locular, or 2 locular, or 4 locular. Locules secondarily divided by 'false septa', or without 'false septa'. Gynoecium median; stylate. Styles 1; attenuate from the ovary; apical. Stigmas 1; 2 lobed; wet type; papillate; Group III type. Placentation when unilocular parietal; when 2 or 4 locularaxile. Ovules in the single cavity when unilocular, 6–100 (to 'many'); 6–50 per locule ('many'); ascending; orthotropous, or hemianatropous; unitegmic; tenuinucellate. Endothelium differentiated. Embryo-sac development Polygonum-type. Polar nuclei fusing prior to fertilization. Antipodal cells formed; 3; not proliferating; ephemeral to persistent. Synergids hooked (large). Hypostase usually present (but weak). Endosperm formation cellular. Endosperm haustoria present; chalazal, or chalazal and micropylar (Watson &Dallwitz, 1992).

1.19.10 Fruit: non-fleshy (usually), or fleshy (rarely); dehiscent (usually), or indehiscent (rarely); a capsule (usually), or a berry (rarely). Capsules septicidal, or loculicidal. Seeds nonendospermic (with Schlegelieae excluded); winged (usually), or wingless. Cotyledons 2; flat (enlarged, foliaceous). Embryo achlorophyllous (4/4); straight. Seedling. Germination phanerocotylar, or cryptocotylar(Watson &Dallwitz, 1992).

1.19.11 Physiology, phytochemistry: C3. C3 physiology recorded directly in Catalpa, Chilopsis. Sugars transported as oligosaccharides + sucrose (predominantly), or as sugar alcohols + oligosaccharides + sucrose. Not cyanogenic. Alkaloids present, or absent. Anthraquinones detected (4 genera); derived from shikimic acid. Verbascosides detected (8 genera). Cornoside detected (Eccremocarpus). Arbutin absent. Iridoids detected (very commonly); 'Route I' type (normal, doubtfully), or

'Route II' type (normal and decarb.). Saponins/sapogenins present (rarely), or absent. Proanthocyanidins absent. Flavonols present, or absent; when present, quercetin. Ellagic acid absent (8 species, 7 genera). Ursolic acid present. Aluminium accumulation not found (Watson &Dallwitz, 1992).

1.19.12 Special distinguishing feature

Thefunicles not as in Acanthaceae(Watson &Dallwitz, 1992).

1.19.13 Geography, cytology

Temperate (a few), or sub-tropical and tropical (mainly). Widespread, with Catalpa common to the Old and New Worlds. N = 20 (mostly). Supposed basic chromosome number of family: 7 (Watson & Dallwitz, 1992).

1.19.14 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 $24 - 32^{\circ}$ c, but can tolerate $5 - 47^{\circ}$ 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. 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(Ken, 2014).

1.19.15 Uses

Edible Uses: Tender young fruit - cooked and eaten as a vegetable.

Flowers - cooked and used as a vegetable (Insadreams, 2017).

1.19.16 Traditional uses:

It has been found that the root and bark have medicinal beefits. Traditionally, the decoction of bark and root is used for the treatment of many diseases.

- The chemical lapachol present in it can prevent cancer.
- The decotion made from the roots given to the patients of rheumatoids,
- The ash got from the burning of the roots and barks mixed with water can be used to treat blockage in urinary tract,
- The flowers ontain carbohydrates and fats,
- 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,
- The root has an anticancer activity and also used in preparation of Ayurvedicformulation known as Dashmula (Insadreams, 2017).

It can be also used to treat:

- pain,
- fever,
- inflammations,
- asthma,
- liver disorders,
- acidity and
- as a diuretic (Insadreams, 2017).

1.19.17 Objective

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

• to determine the CNS depressant activity by open field and hole-board test (EncyclopediaBritanica, 2017).

Chapter- 02

Literature

2.1 Chemical Constituents

2.1.1 Metabolites that are found in this plant

It was found from a research work with the leaves of *S. chelonoides*contain flavones glycoside 6-O-glucosylscutellarein, dinatin, dinatin7glucuroniside,dinatin 7-glucuronide, quinones, stereochenols A and B, naphthoquinones, sterekunthal B and sterequinoneC,stereolensin, p-coumaric acid, palmitic, stearic and oleic acids, previously been reported from this plant. 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 (Mohammad, 2006).

In another study fresh and market roots of drug *S. chelonoides* DC. wereanalyzed 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 Antioxidant and Anti-Cancer Activity

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, phenol content and flavonoid substance are found in this plant (Shanta*et al*, 2013).

Chapter- 03 Materials & Methods

3.1 Preparation of the Plant Sample

3.1.1 Collection and Proper Identification of the Plant Sample

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

3.1.2 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 stored in air- tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

3.1.3 Extraction Procedure

All of the powdered plant material were submerged into its three times of methanol solvent in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent.

3.1.4 Filtration of the Extract

3.1.4.1 Procedure of Filtration

- a. After the extraction process the plant extract 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 Whitman'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.



Fig 3.1 Rotary Evaporator

3.1.4.2 Procedure of Evaporation in Rotary Evaporator

- After the filtration process two parts were obtained namely 'residual part' and filtered part or filtrate".
- The filtered part, which contains the substance soluble in methanol was putted into a 1000ml round bottom flask and then the flask was place in a rotary evaporator.
- > The evaporation was done at 50 degree temperatures for methanol.
- The number of rotation per minute was selected as 60 rpm. The pressure of the vacuum pumper machine was 6bar.
- The water flow through the distillation chamber was also provided in a satisfactory flow rate.
- When the evaporation seemed to be satisfactory, then the methanol extract was collected in a 50mL 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 extract.
- > Then the beaker was covered with aluminium foil paper and kept for 60 minutes.
- Finally the concentrated methanol plant extract was found and stored in the laboratory refrigerator from which the extract was used for many chemical investigations.

The extracts of methanol was chosen for investigation and was labelled as-SCLM (the methanol extract of *Stereospermumchelonoids* leaves).

3.1.5 Drugs

Diazepam was used for current study which was supplied from Square Pharmaceuticals Ltd, Bangladesh.

3.1.6 Experimental animal

3.1.6.1 About the testing animal



Figure 3.2Swiss albino Mice

We did our experiments on *Swiss albino* mice from ICDDR, B Dhaka. They were 16-18gm. We kept them in animal house in plastic cages having a dimension of $(28 \times 22 \times 13)$ cm. We kept mice in a temperature controlledenvironment $(23^{\circ}C)$ with 12hours light-dark cycle, relative humidity 40-70%, with food and water 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.

3.1.6.2Identification of Animals during Experiment

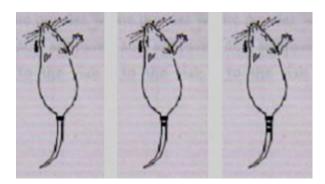


Figure 3.3: Identification of test animals

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 and so on with different colors.

3.2 Study of CNS Depressant Effect of Methanol Extract

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

The CNS depressant action of *S.chelonoides* plant extracts were observed by comparing with the standard diazepam in the experimented rodents. CNS depressant activity was determined by using two techniques. They are:

- Open field test
- Hole-board test.

3.2.1 Open field test

Principle:

The open field test was carried out to evaluate the effect of extract on locomotor activity of mice by the method described by Gupta et al. (1971). Here, micewere divided in to 4 groups of 6mice each. The control group received distilled water (10 ml/kg body weight), the standard group received Diazepam (1 mg/kg body weight) and the experimental groups

received crude extract at 250 and 500 mg/kg body weight. The floor of an open field was divided into a series of squares each alternatively coloured black and white. The apparatus had area of half square meter and a wall of 40 cm height. The number of squares visited by the animals was countedfor 5 minute, on 0, 30 and 60 during the study period.

3.2.2 Hole-board test

Principle:

The method described by Takagi et al. (1971) was implemented for this study. Again 24mice were equally divided into 4 groups. The control group received distilled water (10 ml/kg body weight), the standard group received Diazepam (1 mg/kg body weight) and the experimental groups received crude extract at 250 and 500 mg/kg body weight. 16 holes, each of 4cm in diameter, were made at a plane plate of a woody table at a height of 1 foot from the ground. The number of poking and deeping of mice through the hole was counted for a period of 5 minute after 0 and 30min of oral administration of the extract.

3.2.3 Statistical analysis

All the values in the test are expressed as mean \pm standard deviation (SD). The data were statistically analysed by ANOVA (Analysis of variance) and post-hoc Dennett's tests with the statistical package for social sciences (SPSS 16.0, USA) program. Dissimilarity between the means of the various groups were measured significantly at p < 0.05, p < 0.01 and p<0.001.

3.3 The Design of the CNS depressant Experiments

3.3.1 Overview of the Design

In both methods 24 mice were chosen randomly and then divided into 4 groups. They were group 1 to group 4 where 6 mice were in each group. A particular treatment was given to each group. Before this specific treatment, weight of every mouse was measured accurately and marked. Also the dosage of the sample and standard were settled according to body weight.

Group 1 -SCLM 250 mg/kg

Group 2 – SCLM 500 mg/kg

Group 4 - Control (Distilled Water).

3.3.2Preparation of drug and chemical solution: In order to administer the crude extract of methanol at dose 250 &500 mg/kg body weight of mice, the extract was collected by calculating of mice weight. For proper mixing, small amount of suspending agent CMC was slowly added in the distilled water. Then the extract was added to the mixture. The final volume of the suspension was made up to 5 ml. To stabilize the suspension it was stirred well &then was sonicated in unidirectional way. For the preparation of positive control group (1 mg/kg), Diazepam is taken & a suspension of 5 ml is made.

 Table 3.1Test samples used in the estimation of CNS Depressant activity of S.chelonoides

 plant

Group	Treatment	Dose	Route of Administration
Group1 (Extract)	SCLM	250mg/kg	Orally
Group2 (Extract)	SCLM	500mg/kg	Orally
Group3 (Standard)	Diazepam	1 mg/kg	Orally
Group4 (Control)	Distilled Water	10 ml/kg	Orally

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

At first mice were weighed and after that categorized into 4 groups where 6 mice were in each group

Then by a long needle which was attached with ball shaped end, sample and standards were administered orally. This was done at 0

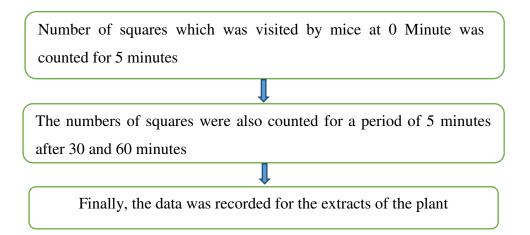


Figure 3.4: Flow chart of process for CNS depressant activity on mice by open field test.

The flow chart of procedure for evaluation of CNS depressant effect of *S. chelonoides* plant by Hole-board test is shown below:

At first mice were weighed and after that categorized into 4 groups				
where 6 mice were in each group				
Then by a long needle which was attached with ball shaped end,				
sample and standards were administered orally. This was done at 0				
After that we counted the number of movements of mice from one				
compartment to another at zero hour for five minutes				
Eventually after 30 minutes, all the mice travelled from one				
compartment to another was counted for a duration of 5 minutes and				
afterwards the data was recorded for the three extracts of our plant				

Figure 3.5: Flow chart of process for CNS depressant activity on mice by Hole-board method

Chapter-04

Results and Discussions

4.1.1 Results of Open Field method

At 250 mg/kg and 500 mg/kg dose, experimental leave extracts were administered to mice. As a result, the movements of mice were reduced in a dose depending manner. Also, it was comparable with diazepam (standard). This movement lowering effect of extract on mice was observed at 30 min interval from zero minute up to 60 minutes. The extracts caused reduction in movement and this may be connected to CNS depression, as reduction or depression of movement is common to most antipsychotics.

Table 4.1: Data of CNS Depressant activity test of *S. chelonoides* plant extracts by Open

 field method:

Group	Treatment	Dose	Number of movement		
			0 min	30 min	60 min
Group 1 (Extract)	SCLM	250mg/kg	141±44.6	114.2±21.6	47.3±20.9
Group 2 (Extract)	SCLM	500mg/kg	203.2±64.4	59.3±30.5***	30.7±19.5***
Group 3 (Standard)	Diazepam	1ml/kg	137.5±44.6	36.2±26.1***	68.8±39.4***
Group 4 (Control)	5% CMC in Distilled water	10ml/kg	137.5±44.6	126±32.8	159.7±15.5

Values are expressed as Mean \pm SD; n=6. One-Way Analysis of Variance (ANOVA) trailed by Dunnet's test. ***p<0.001; **p<0.01; *p<0.05 are considered to be significant.

From this table, we can see that the tested results were dose dependent and statistically significant.

4.1.2 Results of Hole-board

Group	Treatment	Dose	Number of movement	
			0 min	30 min
Group 1 (Extract)	SCLM	250mg/kg	10.3±7.6***	7.3±4.1***
Group 2 (Extract)	SCLM	500mg/kg	10.8±5.3***	8.2±2.6***
Group 3 (Standard)	Diazepam	1ml/kg	19±5.2***	9.8±6.3***
Group 4 (Control)	5% CMC in Distilled water	10ml/kg	58.5±4.9	56.3±4.5

Table 4.2: Data of CNS Depressant activity test of *S. chelonoides* plant extracts by Hole

 board method:

Values are expressed as Mean \pm SD; n=6. One-Way Analysis of Variance (ANOVA) trailed by Dunnet's test. ***p<0.001; **p<0.01; *p<0.05 are considered to be significant.

From this table, we can see that the tested results were dose dependent and statistically significant.

In most of the tests, the mice showed the depressant activity by having change in their movements. After proper dosing, most of the mice decreased or changed their movement as for being depressed and it was understood by counting the number of squares in open-field test and number of poking and deeping in hole-board test.

4.2 Discussions

The present study was conducted to elucidate CNS depressant activity of the methanol extract of *S. chelonoides* leaves. It contains some of the phytochemicals such as flavonoids, phenol (i.e.lapachol) etc.

Diazepam is in a group of drugs called benzodiazepines. Diazepam is used to treat anxiety disorders, it affects chemicals in the brain that may become unbalanced and cause anxiety. Diazepam works by acting on receptors in the brain called GABA receptors. This causes the release of a neurotransmitter called GABA in the brain. Neurotransmitters are chemicals that are stored in nerve cells in the brain and nervous system. They are involved in transmitting messages between the nerve cells. GABA is a neurotransmitter that acts as a natural 'nerve-calming' agent. It helps keep the nerve activity in the brain in balance, and is involved in inducing sleepiness, reducing anxiety and relaxing muscles. As diazepam increases the activity of GABA in the brain, it increases its calming effect and results in sleepiness, a decrease in anxiety and relaxation of muscles(Trofimiuk, 2005).

This metabolite, flavonoid, has been reported to have depressant activity (Sucher, 2005, 2006).Phytochemical analyses of different fraction of the methanol extract of leaf parts of *S. chelonoides* revealed the presence of carbohydrates, phenols, flavonoids, steroid etc. So the observed bioactivities may be attributed to flavonoid, phenolic or steroid compounds. However, many flavonoids were found to be ligands for the gamma aminobutyric acid type A (GABAA) receptors in the central nervous system (CNS); which led to the hypothesis that they act as benzodiazepine-like molecule. Thus the anxiolytic and sedative effects observed might be due to the interaction of flavonoids with the GABA/ benzodiazepine receptor complex in brain (Trofimiuk, 2005).

Chapter-05 Conclusion

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 part of *S*. *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. However, further studies are suggested to be undertaken to understand the underlying mechanism of the observed activities and to isolate, purify and characterise 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 endeavours in this area may open up exciting new therapeutic avenues.

Chapter-06

References

Afroze, M., Ahmed, T., Uddin, M., Majumdar, S., Hossain, S. and Rana, S. (2013); Phytochemical Screening and In-vitro Determination of Antioxidant Potential of Methanolic-Extract of StreospermumChelonoides, Journal of applied pharmaceutical science;3:117-121.

Ayushakti (2016a) List of medicinal plants archives - Ayushakti Blog; [Accessed: 4 July 2017].

Ayushakti (2016b) Medicinal plants and their uses and properties; [Accessed: 4 July 2017].

Ayushakti (2016c) Medicinal plants list archives - Ayushakti Blog; Accessed: 4 July 2017].

Balasubramanian, T., Lal, Sarkar, M. and Chatterjee, T. (2009) Antihyperglycemic and antioxidant activities of medicinal plant Stereospermumsuaveolens in streptozotocininduced diabetic rats, Journal of dietary supplements., 6(3), pp. 227–51.

Balunas, M. and Kinghorn, A. (2005). Drug discovery from medicinal plants. Life Sciences. Vol.78(5), pp. 431-441.

Baul, T.K., Mezbahuddin, M. and Mohiuddin, M. (2008) Vegetative propagation and initial growth performance of Stereospermumsuaveolens DC., a wild tropical tree species of medicinal value, New Forests, 37(3), pp. 275–283. doi: 10.1007/s11056-008- 9123-6.

BiodiversityIndia (2016) Stereospermumchelonoides (L. Fil.) DC (2017); Accessed: 4 July 2017].

Compaoré, M., Lamien-Meda, A., Mogoşan, C., Lamien, C.E., Kiendrebeogo, M., Voştinaru, O., Vlase, L., Ionescu, C. and Nacoulma, O.G. (2011) Antioxidant, diuretic activities and polyphenol content of Stereospermumkunthianum Cham. (Bignoniaceae), Natural Product Research, 25(19), pp. 1777–1788. doi: 10.1080/14786419.2010.488630; [Accessed: 4 July 2017].

DSc, J.V.R. and Botting, R.M. (1998) Mechanism of action of Nonsteroidal antiinflammatory drugs, The American Journal of Medicine, 104(3), pp. 2S–8S. doi: 10.1016/S0002-9343(97)00203-9; [Accessed: 4 July 2017]

Fern, K., Fern, A. and Morris, R. (2016) Stereospermumchelonoides - useful tropical plants; [Accessed: 4 July 2017]. Ghani, A. (1998a). Medicinal plants of Bangladesh : Chemical constituents and uses; [Accessed: 4 July 2017]

Ghani, A. (2003b). Medicinal plants of bangladesh with chemical constituents and uses; [Accessed: 4 July 2017].

Hoareau, L., UNESCO and DaSilva, E.J. (1999) Medicinal plants: A re-emerging health aid, Electronic Journal of Biotechnology, 2(2), pp. 3–4. doi: 10.4067/S0717-34581999000200002 [Accessed: 4 July 2017].

Haque, M.R., Rahman, K.M., Begum, B., Hasan, C.M. and Rashid, M.A. (2007) Secondary metabolites from Sterespermumchelonoides, Dhaka University Journal of Pharmaceutical Sciences, 4(1). doi: 10.3329/dujps.v4i1.201 [Accessed: 4 July 2017].

InsaDreams. (2016) what are the medicinal properties and growing methods of Stereopermumchelonoides?; [Accessed: 5 July 2017].

Diniz, T., Silva, J., Lima-Saraiva, S., Ribeiro, F., Pacheco, A., de Freitas, R., Quintans-Júnior, L., Quintans, J., Mendes, R. and Almeida, J.; 2015; The Role of Flavonoids on Oxidative Stress in Epilepsy;doi: 10.1155/2015/171756; [Accessed: 4 July 2017].

Jackson, D. and Bergeron, K. (2016) Wild Quinine; [Accessed: 5 July 2017]

Joshi, K.C., Bansal, R.K. and Patni, R. (1977) Chemical examination of the roots of Stereospermumsuaveolens DC, Journal. ISSN: 0019-4522.

Medicinehunter. (2014). About Plant Medicines | Medicine Hunter; [Accessed: 4 July 2017].

MedicinePlus (2016) Herbal medicine; [Accessed: 4 July 2017].

Mpbd.info, (2014). Medicinal Plants of Bangladesh; [Accessed: 4 July 2017].

Musa, Y.M., Haruna, A.K., Ilyas, M., Yaro, A.H., Ahmadu, A.A. and Usman, H. (2007) Phytochemical, analgesic and anti-inflammatory effects of the Ethylacetate extract of the leaves of PseudocedrellaKotschyii, 5(1): 92–96.

Taylor, L. (2000a) Plant Based Drugs and Medicines; [Accessed: 4 July 2017].

Taylor, L. (2005b) Differences and Similarities of Drugs and Medicinal Plants; [Accessed: 4 July 2017].

The Editors of Encyclopædia Britannica (2013) Analgesic | drug, in Encyclopædia Britannica; [Accessed: 4 July 2017].

Uddin, S. (2012) Stereospermumchelonoides (L.F.) DC. Available at: http://www.ebbd.info/stereospermum-chelonoides.html; [Accessed: 4 July 2017].

Verma, S. and Singh, S.P. (2008). Current and future status of herbal medicines. Veterinary World. Vol-1(11), pp. 347-350.

Worldpress. (2005) Importance of Medicinal plants; [Accessed: 4 July 2017].

WebMD (2005) STEREOSPERMUM: Uses, side effects, interactions and warnings; [Accessed: 4 July 2017].

WHO. (2015a) Essential Medicines and Health Products Information; [Accessed: 4 July 2017].

WHO (2015b) Traditional medicine: Definitions; [Accessed: 4 July 2017].

Yusuf, M., Wahab, M., Chowdhury, J. and Begum, J. (2007) Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. Bangladesh Journal of Plant Taxonomy. Vol.14(2), pp.15-28.