Determination of CNS Activity of Methanol Extract Of Stereospermum chelonoides's Bark.

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

Submitted By

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Declaration by the Candidate

I, **Md. Refat uz-zaman**, hereby declare that the dissertation entitled "**Determination of CNS Depressant Activity of methanol Extract of stereospermum chelonoides's bark**" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the dissertation entitled "**Determination of CNS Depressant Activity of methanol Extract of stereospermum chelonoides's bark**", submitted to the Department of Pharmacy, East West University, Dhaka, in partial fulfilment of the requirements for the Degree of Bachelor of Pharmacy, was carried out by **Md. Refat uz-zaman**, ID: 2012-3-70-006 under my supervision and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/ Diploma.

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This is to certify that the dissertation entitled "**Determination of CNS Depressant Activity of METHANOL Extract of Stereospermum chelonoides's bark"** is a genuine research work carried out by **Md. Refat uz-zaman**, under the supervision of **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University. I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in thus connection are duly acknowledged.

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Abstract

Recently worldwide different researches on medicinal plants have grabbed 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 the current study was designed to find out CNS depressant and analgesic principles from chloroform solvent extracts of stereospermum chelonoides. The traditional use of this plant is in the treatment of dysentery, leucorrhoea, dysmenorrhoea, haemoptysis and catarrhal bronchitis. It is also claimed that the plant is a good reservoir of such compounds which have the possibility to be a better source of CNS depressant agents in living organism. In this study, above mentioned pharmacological activities of the experimental plant extract was checked in swiss albino mice. By the open field and hole cross method, CNS depressant activity was inspected with the decline of locomotor activity on mice.. Here methanol extract of s.chelonoides were administered to mice at a dose of 250 mg/kg and 500 mg/kg. All the results of the experiments were statistically significant ($p<0.001$). In CNS depressant activity tests, the movement of mice decreased in a dose depending manner comparing to the standard diazepam. The experimental extracts have good cns effect comparing to control group.. In conclusion it can be said that methanol extract of s.chelonoidespossesses good CNS depressant as well as analgesic activity.

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1.1Overview:

Medicinal plants, medicinal herbs, or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defence against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex. A medicinal plants 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 chemopharmaceutical 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. (Newman, 2003)

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, cannabis. (Newman, 2003)

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. (Newman, 2003)

1.2 History of medicinal plant:

Medicinal plants, medicinal herbs, or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defence against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex. (WHO, 2002).

The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt describes over 850 plant medicines, while Dioscorides documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*, forming the basis of pharmacopoeias for some 1500 years. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes, the alkaloids, glycosides, polyphenols, and terpenes. (WHO, 2002).

1.2.1 Prehistoric times:

Plants, including many now used as culinary herbs and spices, have been used as medicines from prehistoric times. Spices have been used partly to counter food spoilage bacteria, especially in hot climates, and especially in meat dishes which spoil more readily. Angiosperms (flowering plants) were the original source of most plant medicines.[Human settlements are often surrounded by weeds useful as medicines, such as nettle, dandelion and chickweed Humans were not alone in using herbs as medicines: some animals such as non-human primates, monarch butterflies and sheep ingest medicinal plants to treat illness. Plant samples from prehistoric burial sites are among the lines of evidence that Paleolithic peoples had knowledge of herbal medicine. For instance, a 60 000-year-old Neanderthal burial site, "Shanidar IV", in northern Iraq has yielded large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies, A mushroom was found in the personal effects of *Ötzi the Iceman*, whose body was frozen in the Ötztal Alps for more than 5,000 years. The mushroom was probably used to treat whipworm. (Pichersky, 2000)

1.2.2 Ancient time:

In ancient Sumeria, hundreds of medicinal plants including myrrh and opium are listed on clay tablets. The ancient Egyptian Ebers Papyrus lists over 800 plant medicines such as aloe, cannabis, castor bean, garlic, juniper, and mandrake. From ancient times to the present, Ayurvedic medicine as documented in the Atharva Veda, the Rig Veda and the Sushruta Samhita has used hundreds of pharmacologically active herbs and spices such as turmeric, which contains curcumin. The Chinese pharmacopoeia, the *Shennong Ben Cao Jing* records plant medicines such as chaulmoogra for leprosy, ephedra, and hemp. This was expanded in the Tang Dynasty *Yaoxing Lun*. In the fourth century BC, Aristotle's pupil Theophrastus wrote the first systematic botany text, *Historia plantarum*. In the first century AD, the Greek physician Pedanius Dioscorides documented over 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*; it remained the authoritative reference on herbalism for over 1500 years, into the seventeenth century. (Williamson et al., 1996)

1.2.3 Middle ages:

In the Early Middle Ages, Benedictine monasteries preserved medical knowledge in Europe, translating and copying classical texts and maintaining herb gardens. Hildegard of Bingen wrote *Causae et Curae* ("Causes and Cures") on medicine. In the Islamic Golden Age, scholars translated many classical Greek texts including Dioscorides into Arabic, adding their own commentaries. Herbalism flourished in the Islamic world, particularly in Baghdad and in Al-Andalus. Among many works on medicinal plants, Abulcasis (936–1013) of Cordoba wrote *The Book of Simples*, and Ibn al-Baitar (1197–1248) recorded hundreds of medicinal herbs such as *Aconitum*, nux vomica, and tamarind in his *Corpus of* Simples. (Williamson et al., 1996)

1.2.4 Early modern age:

The Early Modern period saw the flourishing of illustrated herbals across Europe, starting with the 1526 *Grete Herball*. John Gerard wrote his famous *The Herball or General History of Plants* in 1597, based on Rembert Dodoens, and Nicholas Culpeper published his *The English Physician Enlarged*.[Many new plant medicines arrived in Europe as products of Early Modern exploration and the resulting Columbian Exchange, in which livestock, crops and technologies were transferred between the Old World and the Americas in the 15th and 16th centuries. Medicinal herbs arriving in the Americas included garlic, ginger, and turmeric; coffee, tobacco and coca travelled in the other direction. In Mexico, the sixteenth century *Badianus Manuscript* described medicinal plants available in Central America. (De Pasquale, 1984)

1.3 Cultivation of medicinal plant:

Medicinal plants demand intensive management. Different species each require their own distinct conditions of cultivation. The World Health Organization recommends the use of rotation to minimise problems with pests and plant diseases. Cultivation may be traditional or may make use of conservation agriculture practices to maintain organic matter in the soil and to conserve water, for example with no-till farming systems. In many medicinal and aromatic plants, plant characteristics vary widely with soil type and cropping strategy, so care is required to obtain satisfactory yields. (De Pasquale, 1984)

1.3.1 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. (Yue-Zhong Shu, 1998)

1.3.2 Ecological environment and social impact:

The cultivation of medicinal plants may affect the ecological balance and, in particular, the genetic diversity of the flora and fauna in surrounding habitats. The quality and growth of medicinal plants can also be affected by other plants, other living organisms and by human activities. 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. In terms of local income- earning opportunities, small-scale cultivation is often preferable to large-scale production, in particular if small-scale farmers are organized to market their products jointly. If large-scale medicinal plant cultivation is or has been established, care should be taken that local communities benefit directly from, for example, fair wages, equal employment opportunities and capital reinvestment. (Soldati, 1997)

1.3.3 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. (Gruenwald, 1997)

1.3.4 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. It is, however, necessary to ensure that correct types and quantities of fertilizers are used through agricultural research. In practice, organic and chemical fertilizers are used. (Brevoort, 1997)

1.3.5 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/or national quality standards. Care should be exercised to ensure that the plants under cultivation are neither over- nor under-watered. In the choice of irrigation, as a general rule, the health impact of the different types of irrigation (various forms of surface, sub-surface or overhead irrigation), particularly on the risks of increased vector-borne disease transmission, must be taken into account Medicinal plants should be harvested during the optimal season or time period to ensure the production of medicinal plant materials and finished herbal products of the best possible quality. The time of harvest depends on the plant part to be used. Detailed information concerning the appropriate timing of harvest is often available in national pharmacopoeias, published standards, official monographs and major reference books. (Brevoort, 1997)

1.4 Collection of medicinal plant:

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. It is well known that the quantitative concentration of biologically active constituents varies with the stage of plant growth and development. The best time for collection (quality peak season or time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts. In general, the collected raw medicinal plant materials should not come into direct contact with the soil. If underground parts (such as the roots) are used, any adhering soil should be removed from the plants as soon as they are collected. Collected material should be placed in clean baskets, mesh bags, other well aerated containers. (Elisabetsky, 1986; Calixto, 1996)

1.4.1 Time:

Therapeutic efficacy varies during different times or seasons of the year. The constituent and active principles vary quantitavely at different seasons of the year and the majority of plant materials are usually best collected during the dry season, when the herbs are at peak maturity and concentration. Dry as quickly as possible, away from bright sunlight, to preserve the ingredient..
Leaves: The most opportune time is when the plant is about to bloom.
Flowers: Buds are preferred, best collected in the morning after the morning dew has evaporated; flowers, just before or shortly after opening. Dry the herbal materials as quickly as possible.
Bark materials and stems: Generally, best gathered in summer time. When the climate is warm and humid, the bark of any plant usually contains richer nutritive substances including the medicinal metabolites. Preferably, barks and stems should be removed only from fully grown plants. Do not remove all the bark or a band of surrounding bark.
Fruits and seeds: Fully ripened fruits and mature seeds are preferred. Collection of pod fruits is done in the morning to avoid unnecessary opening up of the fruit wall to the detriment of losing the seeds. Turn the fleshy fruit frequently for even drying.
Whole plant: When the whole plant is desired, it is advisable to harvest the plant at the time when the flowers are all in bloom. Old and withering plants are less effective when used as a source of drugs. (Elisabetsky, 1986; Calixto, 1996)

1.4.2 Habitat:

Information about the whereabouts of the plants, especially the rare ones, can facilitate the search for them. It saves both time and energy. Low altitudes probably range from sea level to about 300 meters; medium altitudes from about 310 meters to about 1000 meters; and high altitude from about 1000 meters and up. (Hamburger and Hostettman, 1991)

1.4.3 Storage:

Many medicinal plants are seasonal, some not easily accessible, available only in deep forests or mountain peaks. Such restrictions necessitate ways and devices to store them

for future use. Dirt and other foreign substances should be removed. If washing is needed, it should be done quickly to minimize deterioration and loss of active substances. As a rule, all parts of the plant collected should be dried as soon as possible to avoid unnecessary waste of the drug materials through natural processes of denaturation, decay and fungal attacks. Some commonly used storage methods.
Sun-drying method: Spread the herbs over the dry beaches, patio or benches that are under the direct scorch of the sun until the materials turn dry and brownish.
Shade-drying method: Some plant materials are preferably dried under shade at room temperature by wind action- because of heat-labile substances that they contain. As such, free circulation of air is important. Drying processes should be shortened, if higher drug contents are to be sought for. Floral and fruit materials should be dried by this method.
Heat-drying method: Some materials may be placed over an oven and dried under the intense heat released or under regulated soft heat. Plants that contain high sugar and starch are best. .
Other Special Methods: Succulent materials are usually washed first in boiling water or steam-cooked in a container before actually drying it. For spiny and hairy materials, remove the unwanted appendages. Some plant materials (ex. succulent materials) may require cutting or sectioning before drying. In general, the moisture content of the dried plant materials should be less than 10% before storage. Moisture content higher than 10% usually leads to growth of microorganisms and pest infestation with consequent drug deterioration. (Hamburger and Hostettman, 1991)

The dried plant materials should be placed in plastic containers or tightly covered bottles; brown colored bottles are preferred as they minimize deterioration due to sunlight. Dry charcoal (separated from the medicinal plant) may be placed inside the bottles to absorb moisture. The storage place should be dry, well-ventilated, and spacious, lest fungi and insects may invade rampantly. Drug materials (dry ones) after proper processing can be kept in large open wooden shelves. The humidity of the storehouse should then be as low as possible. Materials rich in volatile oils are advised to be kept in airtight containers. Otherwise, their efficacy will decrease as time passes by. If all factors are favorable, the prepared drugs can be used even after years of storage. Complete depletion of all medicinal plants founds in an area should be avoided. Once collected, all the materials should be processed at once for long storage. Well planned activity in the collection of plant materials will always prove to be economical and advantageous in the long run. Cultivation of these medicinal plants should be tried in places where conditions favor because cultivated plants contain higher percentages of the medicinal principles desired. (Williamson et al., 1996)

1.5 Diversity of medicinal plant used worldwide:

More than one-tenth of plant species are used in drugs and health products, with more than 50,000 species being used. However, the distribution of medicinal plants is not uniform across the world. For example, China and India have the highest numbers of medicinal plants used, with 11,146 and 7500 species, respectively, followed by Colombia, South Africa, the United States, and another 16 countries with percentages of medicinal plants ranging from 7 % in Malaysia to 44 % in India versus their total numbers of plant species. Certain plant families not only have higher numbers of medicinal plants, but also have higher proportions of threatened species than others . Only a portion of medicinal plants that suffer from genetic erosion and resource destruction have been listed as threatened. (Williamson et al., 1996)

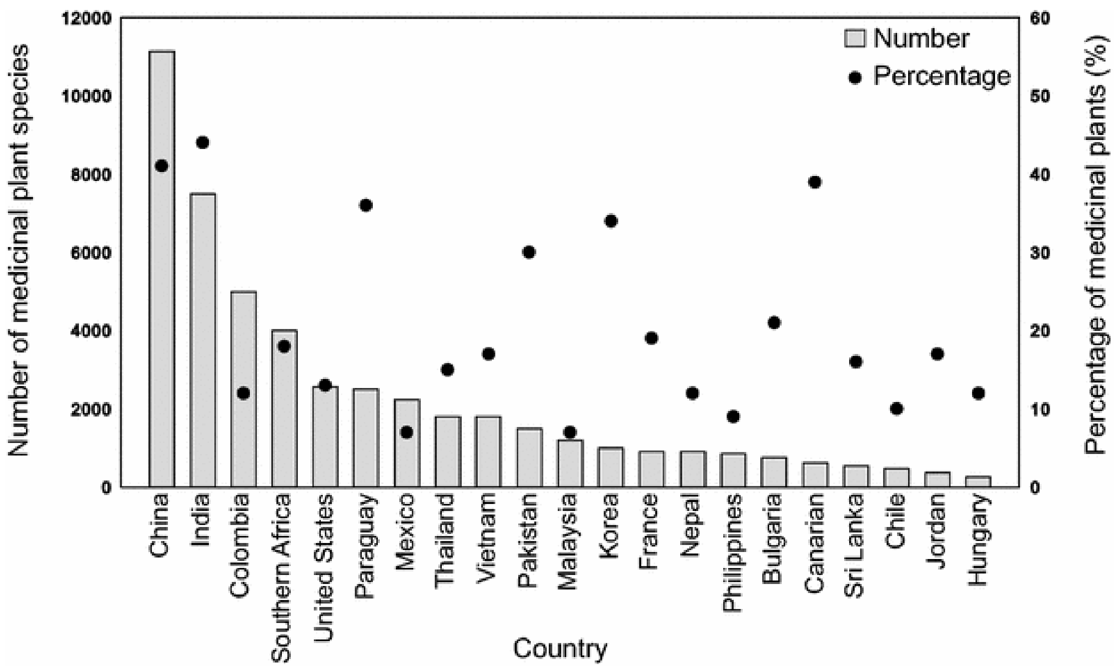

Figure: 1.1 Number and percentage of medicinal plant species in different countries. (Williamson et al., 1996)

1.5.1 Conservation of medicinal plant:

Medicinal plant resources are being harvested in increasing volumes, largely from wild populations. Indeed, demand for wild resources has increased by 8–15 % per year in Europe, North America, and Asia in recent decades. There is a threshold below which species reproductive capacity becomes irreversibly reduced. Various sets of recommendations relating to the conservation of medicinal plants have been developed, such as providing both in situ and ex situ conservation . Natural reserves and wild nurseries are typical examples to retain the medical efficacy of plants in their natural habitats, while botanic gardens and seed banks are important paradigms for ex situ conservation and future replanting. The geographic distribution and biological characteristics of medicinal plants must be known to guide conservation activities, e.g. to assess whether species conservation should take place in nature or in a nursery. (De Pasquale, 1984; Verpoorte, 1989)

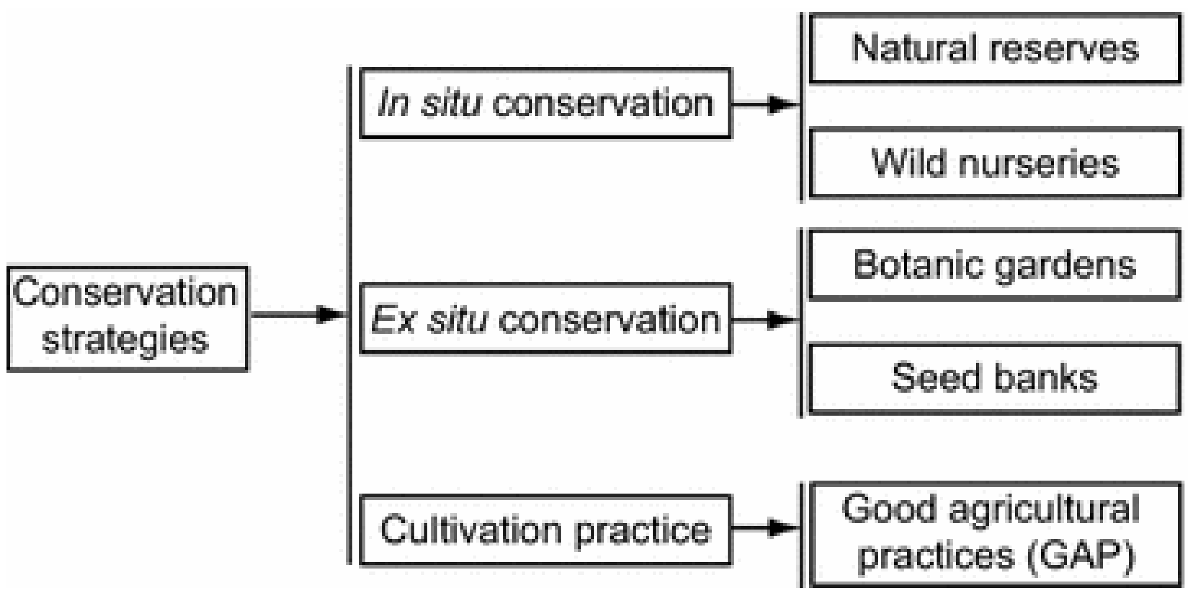

Figure: 1.2 Diagram of methodological systems involved in the conservation of medicinal plants. (De Pasquale, 1984; Verpoorte, 1989)

1.5.1.1In situ conservation:

Most medicinal plants are endemic species, and their medicinal properties are mainly because of the presence of secondary metabolites that respond to stimuli in natural environments, and that may not be expressed under culture conditions. In situ conservation of whole communities allows us to protect indigenous plants and maintain natural communities, along with their intricate network of relationships. Additionally, in situ conservation increases the amount of diversity that can be conserved, and strengthens the link between resource conservation and sustainable use. In situ conservation efforts worldwide have focused on establishing protected areas and taking an approach that is ecosystem-oriented, rather than species-oriented. Successful in situ conservation depends on rules, regulations, and potential compliance of medicinal plants within growth habitats. (Petrovick et al., 1997; Sharapin, 1997)

1.5.1.2 Natural reserves:

The degradation and destruction of habitats is a major cause of the loss of medicinal plant resources. Natural reserves are protected areas of important wild resources created to preserve and restore biodiversity. Around the world, more than 12,700 protected areas have been established, accounting for 13.2 million km^2, or 8.81 % of the Earth's land surface . Conserving medicinal plants by protecting key natural habitats requires assessing the contributions and ecosystem functions of individual habitats. (Petrovick et al., 1997; Sharapin, 1997)

1.5.1.3 Wild nurseries:

It is impossible to designate every natural wild plant habitat as a protected area, owing to cost considerations and competing land uses. A wild nursery is established for species-oriented cultivating and domesticating of endangered medicinal plants in a protected area, natural habitat, or a place that is only a short distance from where the plants naturally grow . Although the populations of many wild species are under heavy pressure because of overexploitation, habitat degradation and invasive species, wild nurseries can provide an effective approach for in situ conservation of medicinal plants that are endemic, endangered, and in-demand. (Elisabetsky and Posey, 1986)

1.5.2 Ex situ conservation:

Ex situ conservation is not always sharply separated from in situ conservation, but it is an effective complement to it, especially for those overexploited and endangered medicinal plants with slow growth, low abundance, and high susceptibility to replanting diseases. Ex situ conservation aims to cultivate and naturalize threatened species to ensure their continued survival and sometimes to produce large quantities of planting material used in the creation of drugs, and it is often an immediate action taken to sustain medicinal plant resources. Many species of previously wild medicinal plants can not only retain high potency when grown in gardens far away from the habitats where they naturally occur, but can have their reproductive materials selected and stored in seed banks for future replanting. (Gottlieb and Kaplan, 1993; Souza Brito, 1996)

1.5.2.1 Botanic garden:

Botanic gardens play an important role in ex situ conservation, and they can maintain the ecosystems to enhance the survival of rare and endangered plant species . Although living collections generally consist of only a few individuals of each species and so are of limited use in terms of genetic conservation, botanic gardens have multiple unique features. They involve a wide variety of plant species grown together under common conditions, and often contain taxonomically and ecologically diverse flora. Botanic gardens can play a further role in medicinal plant conservation through the development of propagation and cultivation protocols, as well as undertaking programs of domestication and variety breeding. (Hamburger and Hostettman, 1991)

1.5.2.2 Seed banks:

Seed banks offer a better way of storing the genetic diversity of many medicinal plants ex situ than through botanic gardens, and are recommended to help preserve the biological and genetic diversity of wild plant species. The most noteworthy seed bank is the Millennium Seed Bank Project at the Royal Botanic Gardens in Britain. Seed banks allow relatively rapid access to plant samples for the evaluation of their properties, providing helpful information for conserving the remaining natural populations , . The challenging tasks of seed banking are how to reintroduce the plant species back into the wild and how to actively assist in the restoration of wild populations. (Hamburger and Hostettman, 1991)

1.5.3 Cultivation practice:

Although wild-harvested resources of medicinal plants are widely considered more efficacious than those that are cultivated, domestic cultivation is a widely used and generally accepted practice. Cultivation provides the opportunity to use new techniques to solve problems encountered in the production of medicinal plants, such as toxic components, pesticide contamination, low contents of active ingredients, and the misidentification of botanical origin. Cultivation under controlled growth conditions can improve the yields of active compounds, which are almost invariably secondary metabolites, and ensures production stability. Cultivation practices are designed to provide optimal levels of water, nutrients, optional additives, and environmental factors including temperature, light and humidity to obtain improved yields of target products. Moreover, increased cultivation contributes to decreases in the harvest volume of medicinal plants, benefits the recovery of their wild resources, and decreases their prices to a more reasonable range.

1.6 Drug development from medicinal plant:

Today, approximately 80% of antimicrobial, cardiovascular, immunosuppressive, and anticancer drugs are of plant origin; their sales exceeded US$ 65 billion in 2003 It is widely accepted that more than 80% of drug substances are either directly derived from natural products or developed from a natural compound. And, in fact, around 50% of pharmaceuticals are derived from compounds first identified or isolated from herbs/plants, including organisms, animals, and insects, as active ingredients. Drug discovery from herbs may be divided into three stages, namely, predrug stage, quasidrug stage, and full-drug stage. Plants, which constitute a major component of foodstuffs in humans, have formed the basis of various traditional medicine systems and folk medicines that have been practiced for thousands of years during the course of human history. Until now, plants/herbs are still highly esteemed all over the world as a rich source of therapeutic agents for the treatment and prevention of diseases and ailments; at present, more than 35,000 plant species are used for medicinal purposes around the world . In conventional Western medicine, 50–60% of pharmaceutical commodities contains natural products or are synthesized from them; 10–25% of all prescription drugs contain one or more ingredients derived from plants. It is well known that the medicinal value of herbs/plants depends on the presence of biological active ingredient(s) with drug-like properties. Recent research has identified a lot of biologically active substances/ingredients from both terrestrial and marine botanicals. (Williamson et al., 1996)

1.6.1 Current approaches to drug discovery:

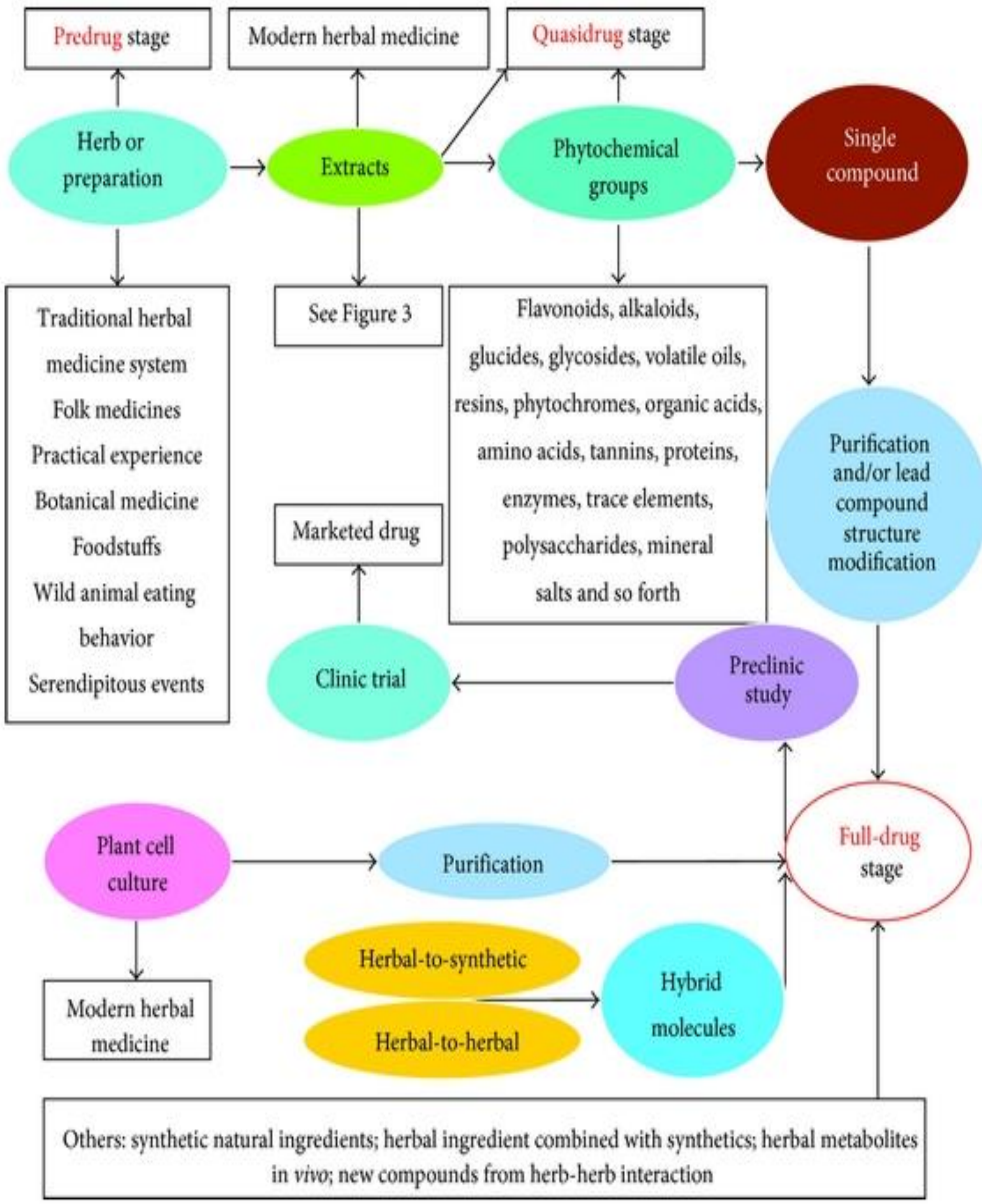

Fig:1.3 current approaches to drug discovery. (Williamson et al., 1996)

1.6.1.1 Predrug stage:

It has been estimated that approximately 420,000 plant species exist on earth, but for most of these only very limited knowledge is available. Three approaches, which are closely related to diet (foodstuffs), medical practice (folk and traditional medicines), and scientific research (phytochemical analysis), can be adopted to explore the value of herbal preparations. Based on the experience from random trials and observations in animals, ancient people acquired the knowledge of using herbs for treating illness. In this connection, Chinese herbal medicine (CHM) and Indian herbal medicine (IHM), which were highly developed in ancient China, Japan, Korea, and India, are still influencing the modern healthcare. The World Health Organization (WHO) estimates that herbal medicines provide primary healthcare for approximately 3.5 to 4 billion people worldwide, and about 85% of traditional medicine involves the use of plant extracts , which may be called “modern herbal medicine.” (Hamburger and Hostettman, 1991; Souza Brito, 1996).

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It is well known that the medicinal value of herbs/plants depends on the presence of biological active ingredient(s) with drug-like properties. Recent research has identified a lot of biologically active substances/ingredients from both terrestrial and marine botanicals. For example, by 2007, 3, 563 extracts and 5,000 single compounds from 3,000 THMs have been collected in China ; the United States has screened about 114,000 extracts from an estimated 35,000 plant samples against a number of tumor systems as early as before the 1990s . The search for new drug from plant/herb has been rapidly increasing in recent few decades, and it has led to the collection of a remarkably diverse array of over 139,000 natural products . All these compounds are potential candidates for drug development. During the period 1981 to 2006, 47.1% of a total of 155 clinically approved anticancer drugs were derived from nature in North America, Europe, and Japan market. (Verpoorte, 1989)

1.6.1.2 Quasidrug stage:

The “quasidrug” stage in drug discovery from herbal medicine includes the preparation of extracts and phytochemical groups from herbs, including the discovery of lead compounds by using modern and conventional research tools. Phytochemical study of extracts of herbal preparations or botanicals involves isolation, structure/composition elucidation, and bioactivity evaluation . Sometimes, the biological activity of an herb or herbal extract can be speculated on basis of the phytochemical composition. Plant polysaccharides, which are polymers consisting of either mono- or disaccharides joined together by glycosidic bonds, produce stimulating/suppressing effect on immune system , and the immunomodulatory polysaccharide-containing herbs include Ganoderma lucidum (Leyss.ex Fr.) Karst. , Cordyceps sinensis (Berk.) Sacc. , and Açaí fruit. Flavonoids, which are compounds with a heterocyclic ring structure consisting of an aromatic ring and a benzopyran ring with a phenyl substituent and include flavones, isoflavone, flavonols, flavonones, and xanthones, have been shown to possess strong antioxidant, anti-inflammatory, antiproliferative, and antiaging activities. They are found in almost all plants, and some of them may be used for ameliorating cardiovascular, mood problems, and cancer . Amino acids and proteins in herbs are usually regarded as natural nutritional supplements for patients recovering from diseases. (Elisabetsky, 1987).

Following harvesting, portions of the herb/plant that are used as herbs (leaves, bark, roots, flowers, seeds, or fruits) are dried either in air or using a specialized industrial drier. The dried herbs can be cut or ground into finer particles and then extracted with either water or organic solvents at an herb to solvent ratio of 1 : 4 (w/v). If fresh herbs are used, the herb to solvent ratio is 1 : 1. Physical (lipid soluble versus water soluble) and chemical (heat resistant versus heat labile) properties of the ingredients present in herbs should be taken into consideration when carrying out the extraction process. Water and organic solvent extraction is the standard technique in the pharmaceutical industry for the isolation of bioactive components from materials . Steam distillation is used in the manufacture and extraction of essential oils from botanical materials. The hot steam forces open the pockets in which the oils are kept in the plant material and then the volatile oils escape from them and evaporate into the steam. The applications of several new technologies—supercritical carbon dioxide extraction technology, membrane separation technology, semi bionic extraction method, molecular distillation technology, and enzyme method in extracting effective components of medicinal plants such as THM—have been induced in the pharmaceutical industry . (Elisabetsky, 1987).

1.6.1.3 Full drug stage:

Nowadays, drugs have become a daily necessity for many people, especially the elderly with multiple health problems. In China, for example, there are 187,518 kinds of home-manufactured drugs, 8,492 kinds of imported drugs, and 1,489 patent-protected products of THM (Chinese patent medicine, Zhong-Cheng-Yao in Chinese) in the pharmaceutical market, and 8,409 drug candidates are now undergoing clinical trials . In addition, in the 2005 Chinese Pharmacopoeia, 582 herbal medicines are officially recognized and described. In fact, there are about 13,000 herbs and over 130,000 prescriptions currently used in various traditional medicines in China . In USA, 2,900 chemical entities are currently under research and development, including agents to be used in the treatment of cancer (750), cardiovascular diseases (312), diabetes (150), AIDS (109), and Alzheimer's/senile dementia (91) . (Dold & Cocks, 2001)

Multinational pharmaceutical companies typically spend an annual amount of US$ 110 billion in an attempt to discover new drugs from herbal medicine. In 2003, the 10 largest pharmaceutical companies spent US$ 0.54 billion on research and development of THM, and the American AIDS Prevention Center conducted studies on screening more than 300 kinds of herb for anti-AIDS activity. In 2002, Novartis announced that 500 compounds derived from THMs would enter the patent application process . At present, the development of herbal medicines mainly remains at the quasidrug stage in China (i.e., to develop modern herbal medicine) while multinational pharmaceutical companies are keen to exploit the novel drug entities (therapeutic agents) from herbal or plant extracts. In this regard, current approaches in the development of herb-derived chemicals for therapeutic use are described in detail in our previous review articles. (Dold & Cocks, 2001)

In particular, pharmaceutical chemists are interested in hybrid molecules consisting of two distinct drug entities covalently linked in a single molecule , such as the hybrid of M1 muscarinic receptor agonist xanomeline and the cholinesterase inhibitor tacrine . The hybrid molecule may contain natural-to-natural, such as tanshinol-borneol ester , natural-to-synthetic, such as HA'(10)-tacrine , or synthetic-to-synthetic components. Protein-protein hybrid may also be possible in bioharmceuticals . Because of the high potential of naturally occurring compounds in producing pronounced biological activities, they have become a major source of components used for constructing hybrid molecules in the development of anticancer agents, antioxidants, and antimalarial drugs. (Schippmann et al., 2002)

1.7 Medicinal plant in banglaadesh:

Bangladesh has very rich in Bio-diversity. It has more than 500 medicinal plants species.

Table:1.1 a list of medicinal plant grow able in Bangladesh given by WHO.

Scientific name.	Bengali name	English name	Used parts
Winthania somnifera Duna.	Ashwagandha	Winter Cherry	Root, Leaf, Fruit, Seed, whole plant.
*Andrographis panniculata*Wall.ex Nees.	Kalomegh	creat	Leaf, Stem, whole plant
Asparagus racemosus Willd.	Satomuli	Aspargus	Tuberous root, Leaf, Flower, Fruit
Aloe vera Tour. ex Linn.	Ghritokumari	Aloe	leaf
Plumbago zeylanica Linn.	Chita	trash	Root
Adhatoda zeylanica Nees. (Syn. name- *A. vasica* Linn.)	Vasak	Vasaka	Leaf, Stem, Bark, Root, Flowe
Rauvolfia serpentine (Linn.) Benth.	Swarpagandha	Snake root	Root

Glycyrrhiza glabra Linn.	Jastimodhu	Liqourice root	Root, Stem

1.8 Plant profile:

Plant name:stereospermum chelonoides.

z

fig 1.4 :stereospermum chelonoides

1.8.1 Synonym:

pulila in Sanskrit, palol in Sinhala, parul in Bengali, Bignonia chelonoides L.f, Bignonia suaveolens ROXB, Hieranthes fragrans RAFIN.

1.8.2 Taxonomic Hierarchy of the Plant

Kingdom: Plantae

Phylum: Tracheop

class : Magnoli

Order: Lamiales.

Family: Bignoniaceae.

Genus: *Stereospermum*

Species*: Stereospermum chelonoides (L.fil.)DC.*

1.8.2 Natural distribution of stereospermum chelonoides:

Widely distributed in continental tropical SE. Asia, from Ceylon and the Deccan to Assam and Burma, not yet reported for Thailand and erroneously so for Indo-China (SANTISUK, l.c.); in Malesia: very locally found in East Java, but somewhat doubtful whether native.

BEUMEE l.c. recorded this tree for the first time for East Java, where it was found locally in some places in the (teak) forest districts S. Surabaya and E. Tuban He suggested that this occurrence would fit the theory of a number of forest officers of early import by Hindus of teak and some associated trees (Butea monosperma, Schleicher a oleosa, etc.) and several other plants. I certainly agree that in the Hindu period (roughly 800-1400 A.D.) plants have come from India, especially those favoured for sacred purposes; for example Cochlospermum religiosum (L.) ALSTON, and others went to India vice versa, as for example Santalum album L. (see). The first is still only found near Hindu temples in Bali and the latter is still spreading in India. The disjunction between the localities in East Java and India-Burma is in these cases certainly caused by intentional dispersal by man in historic time.

There are, however, a large number of other plants showing this same disjunction, and all bound to a seasonal climate, that is, subject to a distinct annual period of drought. In a succinct analysis I found these to belong to 4 classes (). Later I have further elaborated this problem and tried to solve it (). From this it appeared that the ecological disjunction of the seasonal climate between the colossal area it covers in SE. Asia (south as far as Tenasserim) and a similar ecology in Central & East Java and the Lesser Sunda Is. is shared by a homologous plant-geographical disjunction of many hundreds of plants which do not occur in everwet West Malesia, or only in very local seasonal spots in Celebes and the Philippine Islands. A fair number extend their range south-eastwards to Australia. This proves that such patterns are quite natural; I have assumed they originated during the Pleistocene Glacial Period, which created a temporary pathway for drought plants between SE. Asia and Australia, to vanish in the Late Holocene. It could thus well be that also S. chelonoides does occur in the native state in the East Javanese teak forest. As a matter of fact no fruit has yet been collected, although flowering was abundant. I cannot subscribe to the opinion of BEUMEE that its dispersal is here by vegetative means, because I cannot well see by what vegetative means and furthermore because it is difficult to see how it would have maintained itself vegetatively in this way for many centuries. On the other hand the existence of a Javanese vernacular name is no argument that it is native; experience tells us that such names are often invented quickly. If it is native, it remains curious that, though it is obviously of rare occurrence, it was only recently discovered. It cannot be disproved, however, that its seed was inadvertently introduced by the Forest Service with teak seed from India or Burma. (Vadivu R et al., 2009)

1.8.3 Description of the tree:

Deciduous tree, up to 30 m, 80 cm ø; Leaves opposite (rarely in whorls of 3), 30-50 by 15-25 cm; Flowers dull crimson to dull purple, yellow streaked within, very fragrant. Ovary 4-ribbed, sometimes sparsely glandular. Capsule smooth or valves obscurely 3-ribbed, to 45 by l½-l3/ 4cm; Seeds 3½ by 3/ 4 cm. .

Fig1.5:flower of stereospermum chelonoides. Fig:1.7 bark of stereospermum chelonoides

Fig1.6: leaf of stereospermum chelonoides.

1.8.4 Use of stereospermum chelonoides:

Stereospermum Chelonoides is used for Pain, Wounds, Dyspepsia, Excessive thirst, Cough, Asthma and other conditions. This salt may also be used for purposes not listed in this medication guide. the chemical lapachol present in this tree can prevent cancer. this plant can also cure the pain and inflammation of the body. the decoction made from the root of this tree is given to the patient with rheumatism. the ash of root of this tree taken with water can rectify blockage of urinary tract. (Vadivu R et al., 2009)

1.8.5 Usable parts:

Root, leaf, bark.

1.9 Study objective:

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

2.1 Chemical constituent:

Bark of stereospermum chelonoides contains iridoid glycoside,flavanoids, Plant contains naphthoquinone lapachol. Root bark contains 6-sitosterol, n-triacontanol. Root, heart-wood contains lapachol, dehydro-a-lapachone and dehydrotectol and Leaves contain flavone glycoside scutellarein, dinatin-7-glucuronide. Seeds contain non-drying oil.Bitter substances, sterols, glycosides and glyco alkaloids. Its Root bark contains bitter substance. Lapachol isolated which showed highly significant activity against walker 256 carcinoma. Lapachol, Scuttellarrein, Dehydrotectol, ceryl alcohol, oleic, Palmitic, Stearic acid. (Gloria and Gibson, 2011).

2.2 Pharmacological study:

2.2.1 Anti inflammatory activity:

The anti-inflammatory potential of an methanol bark extract (ALE) of stereospermum chelonoides in rats by carrageenan-induced paw edema (acute inflammatory model) and cotton pellet granuloma tests (chronic inflammatory model) at oral. In the former test, ALE significantly impaired both early and late phases of the inflammatory response and also the edema maintained between the two phases. In the latter test, it significantly suppressed granuloma formation (only highest dose tested). Collectively, these data show promising anti-inflammatory activity against both acute and chronic inflammation. (Ratnasooriya et al., 2005)

2.2.2 Wound healing activity:

The wound healing activity of methanol extract of the bark of stereospermum chelonoides by using a dead space wound model in rats. Significant increases in granuloma tissue. weight, tensile strength, hydroxyproline and glycosaminoglycan content were observed in extract treated rats. The prohealing actions seem to be due to increased collagen deposition as well as better alignment and maturation. The drug induced a hypertropic effect on the thymus gland but had no effect on the adrenals. (Nayak et al., 1999)

2.2.3 Cytotoxic and Anti tumor activity:

Study of the active fraction of stereospermum bark showed greater activity on ascitic tumors than solid tumors. It had no toxicity to normal lymphocytes but was toxic to lymphocytes from leukemic patient. (Latha and panikar, 1998)

2.2.4 Anti microbial activity:

The antimicrobial activity was performed on 50% methanol extract of bark of stereospermum chelonoides The effective inhibitory concentration of extract for both bacteria and fungus was found to be 125 µg/ml beyond which the inhibitory activity declined and organism started reviving from antimicrobial principle. (Latha PG et al., 1995)

2.2.5 Anti oxidant activity:

The anti-oxidant activity of the methanol extract of stereospermum chelonoides by DPPH free radical scavenging assay, reducing power and total antioxidant capacity using phosphor molybdenum method. Preliminary phytochemical screening revealed that the extract of the bark of stereospermum chelonoides possesses flavonoids, steroids and tannin materials. The methanolic extract showed significant activities in all antioxidant assays compared to the standard antioxidant in a dose dependent manner and remarkable activities to scavenge reactive oxygen species (ROS) may be attributed to the high amount of hydrophilic phenolic. (Moni Rani et al., 2008)

3.1 The Design of the CNS depressant Experiments:

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

Group 1 - SCBM 250 mg/kg

Group 2 - SCBM 500 mg/kg

Group 3 - Standard (Diazepam)

Group 4 - Control (Distilled Water)

SCBM= methanol extract of bark of stereospermum chelonoides.

3.1.1 Preparation 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 & was sonicated in unidirectional way by the addition of 3 ml of distilled water. For proper mixing, small amount of suspending agent CMC was slowly added. The final volume of the suspension was made up to 5 ml. To stabilize the suspension it was stirred well. For the preparation of positive control group (1 mg/kg) Diazepam is taken & a suspension of 5 ml is made.

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

Group	Treatment	Dose	Route of administration
Group 1 (Extract)	SCBM	250 mg/kg	Orally
Group 2 (Extract)	SCBM	500 mg/kg	Orally
Group 3 (Standard)	Diazepam	1 mg/kg	Orally
Group 1 (Control)	Distilled Water	10 ml/kg	Orally

3.1.2 Hole board test:

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

3.1.3 Method:

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

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

c) After that we counted the number of movements of mice from one compartment to another at zero hour for five minutes.

d) 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.

3.1.4 Openfield test:

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

3.1.4.1Method:

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

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

c) Number of squares which was visited by mice at 0 Minute was counted for 3 minutes.

d) The numbers of squares were also counted for a period of 3 minutes after 30, 60, 90 and 120 minutes.

e) Finally, the data was recorded for the extracts of the plant.

4.1 Openfield test:

4.1.1 Result:

From the Table 4.1 it is found that, central and peripheral locomotion count for methanol extract of stereospermum chelonoides for both 250 & 500 mg/kg body weight was increased as the time proceeded it was due to the sedative effect and reduction in spontaneous locomotion. Significant (P < 0.001) result was found at 30 min and 60 min for both the strengths. In contrast, for standard (Diazepam) at 1 mg/kg body weight the result was significant (P < 0.001) at 30 min, 60 min.

Openfield

Group	**Treatment**	**Dose**	Number of movement		
			0 Minute	**30 Minute**	**60 Minute**
Group - 1 (Extract)	SCBM	250 mg/kg	159.5± 79.05	43.83± 14.53***	38.16667± 23.72***
Group - 2 (Extract)	SCBM	500 mg/kg	159.83± 49.15	22.16± 14.82***	19.16± 16.38***
Group - 3 (Standard)	Diazepam	1 mg/kg	137.5± 44.64	36.16± 26.08***	68.83± 39.40***
Group - 4 (Control)	Water	10 ml/kg	137.5± 44.64	126± 32.78	159.66± 15.47

values are mean ± S.E.M., (n=6). *P < 0.05, **P < 0.01, ***P < 0.001 significantly different from control; done by Dunnet test using Excel 2007 and SPSS 17.00 software.

4.1.2 Discussion:

The most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The locomotor activity is a measure of the level of excitability of the CNS, and decreased activity results from CNS depression The result showed that the 250 mg and 500 mg of SCBM causes depressant effect. reduction in spontaneous motor activity, exploratory behavior and motor coordination. Decreasing central locomotion count and peripheral locomotion count, supports the evidence of reduction of motor activity in mice. The standard drug (Diazepam) exerts CNS depressant action by stimulating GABAA, ligand-gated chloride-selective ion channels that are activated by GABA to inhibit the release of neurotransmitter. (Camposoria et al., 2006) Stereospermum chelonoides's barks are rich in flavonoids and tannin contents such as epicatechin, , procyanidin A2, cinnamtannin B-1 and the flavon-3-ol rhamnosides (Donthan et al., 2015). Flavonoids can produce sedation followed by depression effect by stimulating GABA. (Hernandez et al., 2016). Therefore, the result of the experiment and reports of having high flavonoids strongly suggest that the mechanism of action of SCBM may be linked to GABA stimulation and neurotransmitter release inhibition.

4.2 Hole board test:

4.2.1 Result:

methanol extract of SCBM at both 250 mg/kg body weight dose, produced insignificant ($p<0.001$) at 0 min and 30 min, respectively, and at 500 mg/kg body weight dose the result is significant ($p<0.001$) at 30 min. This indicates decrease of locomotion from its initial value during the period of experiment by the Hole-cross method. Maximum suppression of locomotor activity was for reference drug diazepam at 1 mg/kg body weight which was significant ($p<0.001$).

Table 4.2: Effect of methanol extracts of SCBM in mice by Whole-cross method

Hole board cross

Group	**Treatment**	**Dose**	Number of movement	
			0 Minute	**30 Minute**
Group – 1 (Extract)	SCBM	250 mg/kg	11.83±6.02	10.33±4.81
Group – 2 (Extract)	SCBM	500 mg/kg	10.5±5.75	6.16±3.65***
Group - 3 (Standard)	Diazepam	1 mg/kg	19±5.22	9.83±6.31***
Group - 4 (Control)	water	10 ml/kg	58.5±4.93	56.33±4.50

values are mean ± S.E.M., (n=6). *P < 0.05, **P < 0.01, ***P < 0.001 significantly different from control; done by Dunnet test using Excel 2007 and SPSS 17.00 software.

4.2.2 Discussion:

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

Conclusion:

After observing the results of recent study, it can be said that methanol extract of the experimental plant at dose of 250 mg/kg and 500 mg/kg showed significant ($p<0.001$) CNS depressant activity compare to control group. The extract inhibited activity in a dose dependent manner in analgesic test. At dose of 500 mg/kg showed significant ($p < 0.001$) cns activity compare to control group. So it is clear that the experimental plant is helpful plant and the work was only preliminary effort which will require further comprehensive exploration as well as depiction of active compounds and necessitates preformulation studies for expansion of a potential dosage form.

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