In vivo Pharmacological Investigation of *n*-hexane fraction of *Geodorum densiflorum* (Lam.) Schltr.

This Thesis Paper is Submitted to The Department of Pharmacy, East West University in the partial fulfillment of the requirements for The Degree of Bachelor of Pharmacy

Submitted By:

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THIS THESIS PAPER IS DEDICATED

TO MY BELOVED PARENTS

CERTIFICATE

This research paper is submitted to the department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy (B.Pharm) was carried out by Milton Nath (2008-1-70-046) under my guidance and supervision and that no part of the thesis has been submitted for any other degree. I further certify that all the resources of the information in this research paper are duly acknowledged.

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CERTIFICATE

This is to certify that, the research work on "In *vivo* assay of Analgesic Activity, CNS Depressant Effect of the Methanolic Extract of *Geodorum densiflorum*) submitted to the department of pharmacy, East West University, Mohakhali, Dhaka, in partial fulfillment of the requirement for the degree of bachelor of pharmacy (B.Pharm) was carried out by Milton Nath, ID# 2008-1-70-046 under our guidance and supervision and that no part of the thesis has been submitted for any other degree. I further certify that all the resources of the information in this research paper are duly acknowledged.

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Abstract

Geodorum densiflorum is an endangered terrestrial orchid, which has long been used traditionally for various medicinal purposes in the Indian subcontinent. Therefore, the present study was designed to investigate analgesic and CNS activity *n*-hexane fraction of the roots of *Geodorum densiflorum.*

In Analgesic activities of *n*-hexane fraction of methanolic extract of the root parts of *Geodorum densiflorum* by Acetic Acid Induced Writhing Method in mice, showed significant analgesic activities compared to control. At the dose level of 400 mg/kg *n*-hexane fraction showed 75.73% ($p<0.001$) inhibiton of writhing which is statistically highly significant and at the dose level of 200 mg/kg *n*-hexane fraction showed 25.25% ($p<0.05$) inhibiton of writhing which is statistically significant. Further study is necessary to find out the active ingredient responsible for analgesic activities.

The sedative properties of n-hexane fraction of the methanolic extract of the root part of *G.* densiflorum was investigated using Hole Cross method and Open Field Method. The *n*-hexane fraction of methanolic extract of *G. densiflorum* on Hole Cross Test in mice, at the dose level 200 mg/kg & 400 mg/kg body weight showed dose dependent CNS depressant activity compared to control which is statistically significant ($p<0.001$ and $p<0.05$ respectively). Further study is necessary to find out the active ingredient responsible for CNS depressant activities.The *n*-hexane fraction of methanolic extract of *G. densiflorum* on Open Field Test in mice, 200 mg/kg & 400 mg/kg body weight showed CNS depressant activity ($F=29.835$, $p<0.001$ & $p<0.05$ respectively) which is statistically significant.

The anxiolytic properties of *n*-hexane fraction of the methanolic extract of the root part of G. densiflorum was investigated using Hole Board Method and Elevated Plus Maze test. The *n*-hexane fraction of methanolic extract of *G. densiflorum* on Hole Board Test in mice, at doses of 200mg/kg BW and 400mg/kg BW showed anxiolytic activity which is statistically highly significant ($p<0.001$). In EPM test, DCM fraction with 200mg/kg BWand 400mg/kg BW doses significantly ($p < 0.05$) showed increased exploration and time spent by the DCM fraction treated mice in EPM open arms in a way greater than that of the reference anxiolytic drug Diazepam $F=2.570$.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

CHAPTER-1

INTRODUCTION

1.1 Medicine in ancient time

Human civilization and health care or treatments are always together, because treatment fulfils one of the most basic needs. As man made his way through isolated times or places, he shielded himself against disease as best as he could, reaching out, often blindly, towards the property of nature but in the process gradually elaborating pharmaceutical theory, techniques and equipment.

In past, men's way of tackling diseases depended largely on how they defined its cause. In the sunrise of history, a patient was considered as a sufferer of evil forces or of god's anger, thus disease as punishment for crime. So diseases came in strange ways that called for supernatural as well as natural countermeasures. It was believed that the medicinal practitioner best knew how to command the spirit and what substances from the natural world to be used to drive away the evil power.

Through experiences over thousands of years, men came to know about herbs that were more powerful than others to heal disease. So people mainly started treatment with the use of substances from plant, animal and mineral sources. Today many plant-based and animal-based medicines are used. Minerals, including sulfur, arsenic, lead, copper sulfate and gold are also used in modern drugs. This type of treatment is called Ayurveda as it uses plant, animal and mineral source for treatment (Singh, et al. 2002).

1.2 History of Ayurveda

Ayurveda can be defined as a system, which uses the inherent principles of nature, to help maintain health in a person by keeping the individual's body, mind and spirit in perfect equilibrium with nature. Ayurveda is a Sanskrit term, made up of the words "*ayus*" and "*veda*." "*Ayus*" means life and "*Veda*" means knowledge or science. The term "*ayurveda*" thus means 'the knowledge of life' or 'the science of life'. According to the ancient Ayurvedic scholar Charaka, "ayu" comprises the mind, body, senses and the soul. Most popular and the oldest form of healthcare in the world, Ayurveda is a difficult medical system that originated in India thousands of years ago. The basics of Ayurveda can be found in Hindu scriptures called the *Vedas* the ancient Indian books of wisdom. The *Rig Veda*, which was written over 6,000 years ago,

contains a series of prescriptions that can help humans overcome various ailments (Hinduism, Ayurveda).

Other early works of ayurveda include the *Charaka Samhita*, attributed to Charaka. The earliest surviving excavated written material which contains references to the works of Sushruta is the *Bower Manuscript*, dated to the 6th century AD. The Bower manuscript is of special interest to historians due to the presence of Indian medicine and its concepts in Central Asia. Vagbhata, the son of a senior doctor by the name of Simhagupta, also compiled his works on traditional medicine. Early ayurveda had a school of physicians and a school of surgeons. Tradition holds that the text *Agnivesh tantra*, written by the sage Agnivesh, a student of the sage Bharadwaja, influenced the writings of ayurveda (Jayananda, 1989).

The Chinese pilgrim Fa Hsien (ca. 337–422 AD) wrote about the health care system of the Gupta empire (320–550) and described the institutional approach of Indian medicine, also visible in the works of Charaka. Madhava (fl. 700), Sarngadhara (fl. 1300), and Bhavamisra (fl. 1500) compiled works on Indian medicine. The medical works of both Sushruta and Charaka were translated into the Arabic language during the Abbasid Caliphate (ca. 750). These Arabic works made their way into Europe via intermediaries. In Italy, the Branca family of Sicily and Gaspare Tagliacozzi (Bologna) became familiar with the techniques of Sushruta. British physicians traveled to India to see rhinoplasty being performed by native methods. Reports on Indian rhinoplasty were published in the *Gentleman's Magazine* in 1794. Joseph Constantine Carpue spent 20 years in India studying local plastic surgery methods. Carpue was able to perform the first major surgery in the western world in 1815. Instruments described in the *Sushruta Samhita* were further modified in the Western World. Then this treatment system will widely spread in total world (Jayananda, 1989).

Ayurveda and Kabiraji (herbal medicine) are two important forms of alternative medicine that is widely available in India. Ayurvedic form of medicine is believed to be existent in India for thousands of years. It employs various techniques and things to provide healing or relief to the ailing patients. One of the things that ayurveda uses is medications of plant origin.

Many herbs and spices used in Indian cooking, such as onion, garlic, ginger, turmeric, clove, cardamom, cinnamon, cumin, coriander, fenugreek, fennel, ajowan (ajwain), anise, amchur, bay leaf, hing (asafoetida) etc., are known to have medicinal properties. Ayurvedic medicine uses all of these either in diet or as medicine. In India over 7,000 medicinal plant species are known to exist. Some of these medicinal plants have been featured on Indian postage stamps.

The first set of stamps showing medicinal plants came out in 1997. The set had four stamps showing four different medicinal plants Tulsi (Ocimum sanctum), Haridra (Curcuma longa), Sarpagandha (Rauvolfia serpentina), and Ghritkumari (Aloe barbadensis), Amla (Emblica officinallis), Ashwagandha (Withania somnifera), Brahmi (Bacopa monnieri), and Guggulu (Commiphora wightii) (Jayananda, 1989).

A very well known medicinal plant in Ayurveda is Neem or Margosa (Azadirachta indica). Neem is being used by Ayurvedic practioners in India for thousands of years for such a wide range of ailments that in Sanskrit it is often called *sarva roga nibarak* ("healer of all ailments"). In many tropical countries, Neem is often referred to as "the village pharmacy." Practically, every part of the Neem tree (seeds, leaves, flowers and bark) are used in Ayurvedic medicine. In Indian sub-continent, poor villagers use the chewed Neem twig to brush their teeth. The Neem oil is used to prepare cosmetics like soaps, shampoos, balms, creams, toothpastes etc. Ayurveda uses Neem in various forms to treat skin ailments to diabetes to cancer and everything in between. In fact, the medicinal properties of Neem is so powerful and so diversified that it is being researched by modern scientists not only in India but all over the world including USA. Interested people will find hundreds of references about Neem in the Internet. Neem is shown in the following Indian stamp issued in 1998 (Hinduism, Ayurveda).

1.3 Medicinal Plants

Medicinal plants are various plants used in herbals and considered to have medicinal properties. Few plants or their phytochemical constituents have been proven to have medicinal effects by rigorous science or have been approved by regulatory agencies. Pharmacognosy is the study of medicines derived from natural sources, including plants.

The practice of use of medicinal plants as natural medicines has existed since early times. There are three ways in which plants have been found useful in medicine. First, the plants used directly as teas or in other extracted forms for their natural chemical constituents. Second, it is used as agents in the synthesis of drugs. Finally, the organic molecules found in plants may be used as models for synthetic drugs. Traditionally, the medicinal value of plants was tested by trial and error. Modern approaches to determining the medicinal properties of plants involve shared efforts that can include ethno botanists, anthropologists, pharmaceutical chemists, and physicians. Many modern medicines had their origin in medicinal plants. Examples include aspirin from willow bark (Salix *spp.*), digitalis from foxglove (Digitalis *purpurea*), and vinblastine from Madagascar periwinkle (Vinca *rosea*) for the treatment of childhood leukemia .

According to WHO*"**A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs"*** (Sofowora, 1982,).

The term medical as applied to a pant indicates that it contains a substance or substances which modulate beneficially the physiology of sick mammals, and that it has been used by man for that purpose.

'All higher plants that have been alleged to have medicinal properties, effects that relate to health, or which have been proven to be useful as drugs by western standards or which contain constituents that are used as drugs'(Farnsworth, 1991).

Plants cannot run away from their enemies nor get free of difficult pests as humans or other animals do, so they must have some mechanism for protection. Whatever this protection is it must be successful, for the diversity and richness of green plants is extraordinary, and their dominance in most ecosystems of the world is unquestioned. Plant successes are closely intertwined with the evolution and production of highly diverse compounds known as secondary metabolites, compounds that are not essential for growth and reproduction, but rather, through interaction with their environment, enhance plant prospects of survival. These metabolites are therefore plant agents for chemical warfare, allowing plants to ward off microorganisms, insects, and other animals acting as predators and pathogens. Such compounds may also be valuable to humans for the same purposes, and therefore may be used as medicines (Balick, et al 1996).

At the present time, substances from plants are used in the following main ways in modern medical treatment, Directly as pharmaceuticals either as single purified drugs, for example, morphine (extracted from the opium poppy Papaver somniferum) and vincristine (extracted from the rosy periwinkle Catharanthus roseus) or in advanced extract from often in admixtures with other ingredients (M.B Burbage, 1985).

As building blocks or starting materials for the production of semi-synthetic drugs, for example plants saponins which can be extracted and altered chemically to produce sapogenins for the manufacture of steroidal drugs. An enormous number of products are made by the manufacture of steroidal drugs. An enormous number of products are made by chemical alteration of plants materials a problem (in terms of recognition of our reliance on the plant world) being that the end-production often appears, because of its name, quite unrelated to plants. An example of this is Trimethoprim (a urinary antibacterial drug) made from 3, 4, 5, trimethoxybenzaldehyde, itself produces by the chemical or enzymatic hydrolysis of gallic acid, taken from the South American tree Caesalpinia spinosa (M.B Burbage, 1985).

1.4 Medicinal Plants in Bangladesh

There are more than twenty thousand known secondary plant metabolites, all exhibiting a remarkable array of organic compounds that clearly provide a selective advantage to the producer, which outweighs their cost of production. The majority of which are secondary metabolites, such as alkaloids, glycosides, terpenes, steroids, and other classes grouped according to their physiological activity in humans or chemical structure. Medicinal plants products of the *Unani* and *Ayurvedic* systems in Bangladesh are now prepared by using both indigenous and modern pharmaceutical technology under strict quality control measures. These medicinal plant products are dispensed as broken pieces or coarse and fine powders, pills of different sizes, in the form of compressed tablets, as liquid preparations, as semi-solid masses and in the form of creams and ointments neatly packed in appropriate sachets, packets, aluminum foils, plastic or metallic containers and glass bottles. The containers are fully labeled with indications/contra-indications, doses and directions for use and storage.

Both the *Ayurvedic* and *Unani* systems of traditional health care have firm roots in Bangladesh and are widely practised all over the country. Traditional medicine plays a very important role in Bangladesh, particularly at the primary health care level, as an estimated 70 to 75% people of the country still use traditional medicine for management of their health problems (Abdul Ghani, 1987).

1.5 Contribution of Medicinal Plants to Modern Science

There are twenty thousand known secondary plant metabolites, all exhibiting a remarkable array of organic compounds that clearly provide a selective advantage to the producer, which outweighs their cost of production. Humans benefit from their production by using many of them for medicinal purposes to fight infections and diseases. To show the width of human reliance on medicinal plants, the accompanying table provides a list of the most significant plants, their uses in modern medicine, and the major secondary metabolites responsible for their activities. This list grows annually as new plants are found with desired activities and remedies to become pharmaceuticals for use in medicine (Nigg, et al, 1992).

Table 1.1: Common medicinal plants and their uses

Scientific Name	Common Name	Family	Compounds	Compound Class	Uses
Atropa belladonna, Duboisia myoporoides	Belladonna	Solanaceae	Atropine, scopolamine	Alkaloid	Anticholinergic motion sickness, mydriatic
Cassia/Senna species	Senna	Fabaceae	Sennoside	Glycoside, anthraquinone	Laxative
Catharanthus roseus	Madagascar periwinkle	Apocynaceae	Vincristine, vinblastine	Alkaloid	Anticancer (antileukemia)

Chondrodendron tomentosa, Curarea toxicofera	Curare	Menispermaceae	(+)-Tubocurarine	Alkaloid	Reversible muscle relaxant
Cinchona calisaya, Cinchona officinalis	Jesuits' bark	Rubiaceae	Quinine, quinidine	Alkaloid	Antimalaria (quinine), antiarrhythmia (quinidine)
Colchicum autumnale	Autumn crocus	Liliaceae	Colchicine	Alkaloid	Gout
Digitalis lanata, Digitalis purpurea	Foxglove	Scrophulariaceae	Digoxin, digitoxin, lanatosides	Cardiac glycoside (steroidal)	Heart failure and irregularity
Dioscorea species	Yam	Dioscoreaceae	Diosgenin, precursor of human hormones and cortisone	Saponin glycoside (steroidal)	Female oral contraceptives, topical creams
Ephedra sinica	Ephedra, Ma huang	Ephedraceae	Ephedrine	Alkaloid	Bronchodilator, stimulant
Pilocarpus species	Jaborandi	Rutaceae	Pilocarpine	Alkaloid	Glaucoma
Podophyllum peltatum	May-apple	Berberidaceae	Podophyllotoxin, etoposide	Resin	Anticancer

Rauwolfia serpentina		Apocynaceae	Reserpine	Alkaloid	Antihypertensive, tranquilizer
Taxus brevifolia	Pacific yew	Taxaceae	Taxol	Diterpene	Anticancer (ovarian, breast)

(Nigg, et al, 1992)

Table 1.2 Some drugs or chemicals which come from different plant source

Drug/Chemical	Action/Clinical Use	Plant Source
Acetyldigoxin	Cardiotonic	Digitalis lanata
Adoniside	Cardiotonic	Adonis vernalis
Allyl isothiocyanate	Rubefacient	Brassica nigra
Atropine	Anticholinergic	Atropa belladonna
Benzyl benzoate	Scabicide	Several plants
Berberine	Bacillary dysentery	Berberis vulgaris
Borneol	Antipyretic, analgesic,	Several plants
Caffeine	CNS stimulant	Camellia sinensis
Cocaine	Local anaesthetic	Erythroxylum coca
Codeine	Analgesic, antitussive	Papaver somniferum
Cynarin	Choleretic	Cynara scolymus
Digitoxin	Cardiotonic	Digitalis purpurea
Digoxin	Cardiotonic	Digitalis purpurea
Emetine	Amoebicide, emetic	Cephaelis ipecacuanha
Etoposide	Antitumor agent	Podophyllum peltatum
Galanthamine	Cholinesterase inhibitor	Lycoris squamigera
Gitalin	Cardiotonic	Digitalis purpurea
Gossypol	Male contraceptive	Gossypium species
Hydrastine	Hemostatic, astringent	Hydrastis canadensis
Hyoscyamine	Anticholinergic	Hyoscyamus niger

Irinotecan	Anticancer, antitumor agent	Camptotheca acuminata
Kawain	Tranquillizer	Piper methysticum
Kheltin	Bronchodilator	Ammi visaga
Lanatosides A, B, C	Cardiotonic	Digitalis lanata
Lapachol	Anticancer, antitumor	Tabebuia sp.
Menthol	Rubefacient	Mentha species
Monocrotaline	Antitumor agent (topical)	Crotalaria sessiliflora
Morphine	Analgesic	Papaver somniferum
Nicotine	Insecticide	Nicotiana tabacum
Noscapine	Antitussive	Papaver somniferum
Palmatine	Antipyretic, detoxicant	Coptis japonica
Papain	Proteolytic, mucolytic	Carica papaya
Podophyllotoxin	Antitumor anticancer agent	Podophyllum peltatum
Protoveratrines A, B	Antihypertensives	Veratrum album
Quinidine	Antiarrhythmic	Cinchona ledgeriana
Quinine	Antimalarial, antipyretic	Cinchona ledgeriana
Rhomitoxin	Antihypertensive, tranquillizer	Rhododendron molle
Rorifone	Antitussive	Rorippa indica
Sparteine	Oxytocic	Cytisus scoparius
Strychnine	CNS stimulant	Strychnos nux-vomica
Tetrahydrocannabinol(THC)	Antiemetic, decrease occular tension	Cannabis sativa
Tubocurarine	Skeletal muscle relaxant	Chondodendron tomentosum
Vincristine	Antitumor, Antileukemic agent	Catharanthus roseus
Yohimbine	Aphrodisiac	Pausinystalia yohimbe

(Taylor, 2000)

CHAPTER-2

PLANT DETAILS

2.1 About Orchids

Orchid family (Orchidaceae) is the second largest family of flowering plants with approximately 20,000 species with more than 850 genera. This diversity increases towards the tropic; where the epiphytic species predominate that almost constitute 73% of the family. Colombia is the country with greatest number of species in America (3000 spp.) followed by Ecuador and Brazil (2,500 spp. each one) (Dressler, 1981).

In contrast to the peaceful symbiotic associations between many other terrestrial plants and mycorrhizal fungi, this association is a life-and-death struggle. The fungi always try to invade the cytoplasm of orchid cells to obtain nutritional compounds. On the other hand, the orchid cells restrict the growth of the infecting hyphae and obtain nutrition by digesting them. It is likely that antifungal compounds are involved in the restriction of fungal growth (Shimura et al, 2007).

Orchid have numerous varieties of exquisitely beautiful blossoms which are sold commercially, the only economically important product in this great plant family is the delicious spice known as vanilla. Vanilla comes from several species of perennial vines of the genus *Vanilla* native to Mexico and tropical America. The Aztecs originally used vanilla as a flavoring for chocolate, and the Spanish conquerors carried it back to Europe where it was used for this same purpose. One of the most famous ornamental orchids is the black orchid, *Paphiopedilum wardii* (Dressler, 1981).

2.2 Introduction to the family Orchidaceae

The orchid family is a very large group of monocots. It is one of the most recently evolved plant families. Divisions among species and general are not distinct and many intergeneric hybrids exist. Orchids are found in nearly every climatic zone from arctic to tropic and on all continents except Antarctica. Many are native to higher elevations in the tropics and grow best at cool temperatures year round. There are both terrestrial and epiphytic types. Seeds of orchids are extremely small, about the size of particle of dust. Epiphytic orchids are divided into two groups, sympodial and monopodial, based on their stem structure. Monopodial orchids grow upright and develop side shoots, which also grow upright. They bear their leaves in two ranks, perpendicular to the stem and do not produce pseudobulbs. Sympodial orchids often have pseudobulbs, a thickened stem from which the leaves arise. Their growth is horizontal, the pseudobulbs being

attached to a common basal rhizome. Growth of the pseudobulbs and extension of the rhizomes develops out of the base of the old pseudobulbs. These pseudobulbs store food and water and enable the orchid plant to withstand periods of drought. Cattleya is the genus most people think of when they hear the word orchid. Although they come in a wide range of colors and color patterns, the color orchid is named for one of the flower colors found in cattleyas. Most cattleyas make the large, showy flowers common in corsages. They are tropical epiphytes with sympodial growth and pronounced pseudo bulbs (Aggie-horticulture).

Dendrobium is a sympodial epiphyte native from India to Japan and Australia. There are several subtypes with distinctly different cultural requirements. Many dendrobiums produce smaller flowers but in large sprays. Many need a dry period prior to bud development. Laelia is a genus of orchids closely related to the cattleyas. They are epiphytic, evergreen orchids with pseudobulbs. The flowers of laelias have a distinct resemblance to cattleya flowers. The genus Miltonia is native to Central and South America. These are the pansy orchids, so named because their flowers resemble the flat-faced pansy flowers. Oncidium is a sympodial, epiphytic orchid native from Mexico to Brazil. Like dendrobiums, many of the oncidiums produce large sprays of smaller flowers. Oncidiums have a range in temperature requirements depending on the species, but most do best in when given a lot of sunlight and a rest period of several weeks following the development of their new growth. Paphiopedilum is the lady slipper orchid. It is a terrestrial orchid. This genus is adapted to a wide range in temperature conditions, some being decidedly tropical and others almost temperate. Phalaenopsis is the genus of the moth orchids. They produce large, moth-like flowers in a plane along the two sides of a long, arching stem. They are monopodial, epiphytic orchids native to south Asia and Australia to West Africa (Aggie-horticulture).

2.3 History of Orchids

The Chinese were the first to write books about orchids. In 1233, Chao Shih-Keng wrote Chin Chan Lan P'u, and described 20 species and how to grow them (Berliocchi,2004). In 1247, Wang Kuei-hsueh wrote his Treatise on Chinese orchids, and described 37 species. The first Western volumes dedicated to orchids did not appear until Eberhard Rumphius (1628 - 1702) Herbarium Amboinense was eventually published in 1741 - 1755, two of 12 volumes being devoted to

orchids. There is no doubt that the Chinese were the first to cultivate and describe orchids, and they were almost certainly the first to describe orchids for medicinal use. Reinikka in 1995 reports a Chinese legend that Shên-nung described *Bletilla striata* and a *Dendrobium* species in his Materia Medica of the 28th century BC. The earliest Middle East report of plant remedies is in a 4000-year-old Sumerian clay tablet included some orchids (Kong et al, 2003). Dioscorides, who was a Greek working as a Roman military physician, wrote his De Materia Medica, including two terrestrial orchids (Dioscorides, 1543). He adopted and promoted the 'Doctrine of Signatures' whereby plants mainly on its material symbiosis with fungus and asepsis seedlings (Fan et al, 1997).

Genus *Bulbophyllum,* a member of the Orchidaceae family, consists of over 1000 species found in Africa and Asia is widely distributed in China, Nepal, Sikkim, Bhutan, India, Burma, Thailand, Laos and Vietnam. It is a rich source of aromatic compounds such as phenanthrenes and bibenzyls. *Bulbophyllum kwangtungense* Schlecht (Chinese name "Shi dou-Ian") has long been used in traditional Chinese medicine as a Yin tonic (Yi et al, 2005).

Genus *Cymbidium*s tend to grow more leaves than most orchids. Roughly eight long, green, narrow leaves originate from the sheath of each pseudo bulb. It is one of the most popular and desirable orchids in the world because of the beautiful flowers. These plants make great houseplants, and are also popular in floral arrangements and corsages. They have been cultivated for thousands of years, especially in China. *Cymbidium*s became popular in Europe during the Victorian era. One feature that makes the plant so popular is the fact that it can survive during cold temperatures (Yi et al, 2005).

2.4 Medicinal Uses of Orchids

A large number of orchids are frequently analyzed by perfumers (using headspace technology and gas-liquid chromatography) to identify potential fragrance chemicals. The other important use of orchids is their cultivation for the enjoyment of the flowers. Most cultivated orchids are tropical or subtropical, but quite a few which grow in colder climates can be found on the market. Temperate species available at nurseries include *Ophrys apifera* (bee orchid), *Gymnadenia conopsea* (fragrant orchid), *Anacamptis pyramidalis* (pyramidal orchid) and *Dactylorhiza fuchsii* (common spotted orchid).

More recent ethnopharmacological studies show that orchids are used in many parts of the world and in treatment of a number of diseases: skin, infectious diseases, and problems concerning the digestive, respiratory reproduction organs, the circulation, against tumours, for pain relief and for reducing fever (Table 2)

Table 2.1: Medicinal use of orchids

Specie	Country	Part(s) used	Ethnomedical uses	Preparation(s)	Reference(s)
Anoectochilus Formosanus	Taiwan	Whole plant	Chest and abdominal pain, diabetes, fever, nephritis, hypertension, impotence, liver spleen disorders, and pleurodynia, antiinflammatory agent	Decoction. Stem and leaves are one of the ingredients in certain medicinal oils	Satish et al. (2003)
Bletilla Formosana	China	Tuber	Is associated with the lung, stomach and liver meridians and has a bitter taste and cool properties	Tuber is peeled and dried in the sun, then cut into slices or ground into a powder	Lin et al. (2005)
Catasetum barbatum	Guianas, Japan, Paraguayan	Whole plant	Febrifuge, anti-inflammatory	Infusion or decoction	Shimizu et al. (1988)

Dendrobium Amoenum	China	Leaves	Skin diseases	Dried and ground	Venkateswarlu et al. (2002)
Epidendrum Mosenii	China, Corea	Stems	Analgesic activity	Infusion or decoction	Floriani et al. (1998)
Galeola foliate	Morobe, Papua New Guinea	Stems	Treatment of some Infections	Infusion or decoction	Khan and Omoloso (2004)
Pholidota chinensis	India, China	Pseudobulbs	Is taken for scrofula, feverish stomachache and toothache, chronic bronchitis, and duodenal ulcer	Tincture	Wang et al. (2006)
Spiranthes australis	Trinidad and Tobago, China	Whole plant	Used for urinary problems and diabetes Mellitus. Treatment of bacterial and inflammatory diseases, cancer, blood, andchest disorders	Infusion or decoction	Lans (2006), Peng et al. (2007)

2.5 Geographical Distribution of Orchids

Orchidaceae are found everywhere, occurring in almost every habitat apart from glaciers. The world's richest concentration of orchid varieties is found in the tropics, mostly Asia, South America and Central America, but they are also found above the Arctic Circle, in southern Patagonia, and even two species of *Nematoceras* on Macquarie Island, close to Antarctica.

The following list gives a rough overview of their distribution:

- tropical Asia: 260 to 300 genera
- tropical America: 212 to 250 genera
- tropical Africa: 230 to 270 genera
- Oceania: 50 to 70 genera
- Europe and temperate Asia: 40 to 60 genera
- North America: 20 to 26 genera

2.6 Characteristics of Orchids

Orchids can be too easily distinguished from other plants, as they share some very clear apomorphies. Among these are: bilateral symmetry (zygomorphism), many beautiful flowers, a nearly always highly modified petal (*labellum*), fused stamens and carpels, and extremely small seeds.

All orchids are perennial herbs, fixed woody structure, and can grow in two patterns, one is monopodial and another is sympodial.

- **Monopodial(one-footed):**The stem grows from a single bud, leaves are added from the apex each year and the stem grows longer accordingly. The stem of orchids with a monopodial growth can reach several metres in length, as in *Vanda* and *Vanilla*.
- **Sympodial(many-footed):** The plant produces a series of adjacent shoots which grow to a certain size, bloom and then stop growing, to be then replaced. Sympodial orchids grow laterally rather than vertically, following the surface of their support. The growth continues by development of new leads, with their own leaves and roots, sprouting from or next to those of the previous year, as in *Cattleya*. While a new lead is developing, the rhizome may start its growth again from a so-called 'eye', an undeveloped bud, thereby branching (Orchid, 2010).

2.7 Botanical features of Orchids

Leaf

Orchids generally have simple leaves with parallel veins; Leaves may be ovate, lanceolate, or orbiculate, and very variable in size. Their characteristics are often analytic. They are normally alternate on the stem, often plicate, and have no stipules. The structure of the leaves corresponds to the specific habitat of the plant. Species that typically bask in sunlight, or grow on sites which can be occasionally very dry, have thick, leathery leaves and the laminae are covered by a waxy cuticle to retain their necessary water supply. The leaves of most orchids live on, attached to their pseudobulbs, for several years. The leaves of some species can be most beautiful. (Ramirez, 2007).

Flowers

Orchidaceous are well known for the many structural variations in their flowers. Some orchids have single flowers sometimes with a large number of flowers. The orchid flower, like most flowers of monocots, has two whorls of sterile elements. The outer whorl has three sepals and the inner whorl has three petals. Orchid flowers with abnormal numbers of petals or lips are called peloric (Ramirez, 2007).

Seeds

The seeds are generally almost microscopic and very numerous, in some species over a million per capsule. After ripening, they blow off like dust particles or spores. They lack endosperm and must enter symbiotic relationships with various mycorrhizal basidiomyceteous fungi that provide them the necessary nutrients to germinate, so that all orchid species are mycoheterotrophic during germination and reliant upon fungi to complete their lifecycles. The main component for the sowing of orchids in artificial conditions is the agar (Ramirez, 2007).

Roots

All orchids are perennial herbs, lacking any permanent woody structure. Some orchids are terrestrial, growing rooted in the soil. Terrestrial orchids may be rhizomatous, forming corms or

tubers. These act as storage organs for food and water A great many orchids are epiphytes, which do not require soil and use trees for support. They occur in warmer regions. Epiphytic orchids have modified aerial roots and, in the older parts of the root, an epidermis modified into a spongy, water-absorbing velamen. The aerial roots of epiphytes that lack leaves have an additional function.They contain chlorophyll and take up carbon dioxide (Ramirez, 2007).

2.8 General Care of Orchids

Table 2.2: General care of the family of Orchidaceae

Temperature	**Most tropical orchids grow best at a uniformly warm, but not hot, temperature. Some orchids thrive at cooler temperatures.**
Medium	Epiphytic orchids require good drainage and aeration of their roots. Coarse media such as tree bark, osmunda fiber or moss mounted on tree bark are common. If potted in common media, such as the peatlite mix, orchids usually fail to thrive and eventually die. Terrestrial orchids thrive in a rich, organic medium that drains well.
Water	Most orchids grow best in a humid environment with good aeration. They should be watered to keep the medium moist. For epiphytic orchids potted in a well drained, airy medium this may be twice a day. Orchids cannot tolerate a wet medium.
Light	Orchids occur in many different environments and this affects their light requirements. Some grow near the tops of trees in rainforests. They need brighter light than others which may be found growing in dense forests and at lower levels in the tree canopy. Terrestrial orchids in cultivation are usually found on the forest floor and grow well in moderate light. Most orchids need protection from hot, midday sun in summer, but could be grown in direct light in other seasons.

Fertilization	Orchids need a steady supply of nutrients but cannot tolerate salts. Dilute solutions of inorganic fertilizers are common or organic sources of nutrients are used.
Pests and problems	Protecting the roots from rot is essential. This is done by using the proper medium for the type of orchid that is to be grown. Pests are relatively few on orchids but diseases of the leaves and stems may be a serious problem, disfiguring or killing them.
Propagation	Seed of orchids is extremely small and is usually planted on an aseptic medium such as agar. Seed is the only way to propagate some orchids, but others may be propagated by division, separation and stem cuttings. Asexual micropropagation, a process commonly called mericloning, is done by excising the meristem of a plant, culturing it aseptically to form a mass of cells and then triggering the development of many plantlets from this mass of cells.

(Aggie-horticulture)

2.9 Taxonomy of *Geodorum densiflorum* (Lam.) Schltr

Domain: *Eukaryota*

Kingdom: *Plantae*

Subkingdom: *Viridaeplantae*

Phylum: *Tracheophyta*

Subphylum: *Euphyllophytina*

Infraphylum: *Radiatopses*

Class: *Liliopsida*

Subclass: *Liliidae*

Superorder: *Lilianae*

Order: *Orchidales*

Family: *Orchidaceae*

Genus: *Geodorum*

Specific epithet: *densiflorum* - (Lam.) Schltr.

Botanical name: - *Geodorum densiflorum* (Lam.) Schltr.

Figure 2.1: Plant of *Geodorum densiflorum*

2.10 Members of the genus Geodorum

Table 2.3: Some examples of *Geodorum* genus

Name of the species	**Name of the species**
G. appendiculatum	*G. neocaledonicum*
G. attenuatum	*G. nutans*
G. bicolor	*G. pacificum*
G. bracteatum	*G. pallidum*
G. candidum	*G. parviflorum*
G. citrinum	*G. pictum (Painted Orchid)*
G. densiflorum (Ground Gem Orchid)	*G. plicatum*

(Dockrill, 1967)

2.11 Nodding Orchids, Shepherds Crook Orchids

These are deciduous terrestrials with flattish, subterranean or partly emergent pseudo bulbs and broad pleated leaves that are distinctly stalked. All of the leaf stalks on a pseudo bulb are enclosed together by several tubular bracts. Inflorescences are unbranched and characteristically nod in bud and flower, straightening and elongating as the capsules develop. The flowers are relatively small, crowded and in the native species remain semi-tubular with the segments not spreading widely. The labellum, which is stiffly but flexibly attached to the apex of the column foot, is 3-lobed with large lateral lobes, a short midlobe and a ridged or keeled callus. The column is short and broad with a short column foot (Garay, 1974).

2.12 Taxonomical identification of *Geodorum densiflorum*

Shankhyamul was collected from the local market of Savar which was cultivated in the garden of Pharmacy department of Jahangirnagar University campus.

(a)

(b)

Figure 2.2: Different parts of *G. densiflorum* (a) Aerial part with flower (b) Dried plant roots

2.13 Ecology

Geodorum densiflorum is widespread in a range of habitats including rainforest, especially monsoonal vine thickets, rainforest margins, open forest, heathland and grassland, usually in well-drained soil, sometimes in sites that are seasonally moist.

2.14 Reproduction

Reproduction in *Geodorum* is solely from seed. Seed dispersal takes 2-4 months from pollination and after fertilisation the peduncle straightens and the capsules develop in a pendant position. Apomixis is unknown in the genus (Kores, 1989).

2.15 Seasonal Growth

Plants of *Geodorum densiflorum* are deciduous and grow during the spring and summer months, becoming deciduous in autumn and winter (Kores, 1989).

2.16 Flowering

Geodorum densiflorum flowers in summer (wet season) (Kores, 1989).

2.17 Derivation

The name *Geodorum* is derived from the Greek *ge*, earth and *doron*, gift, apparently in reference to the terrestrial growth habit (Kores, 1989).

2.18 Botanical Description

In Perennial *Geodorum densiflorum* , there are geophytic herbs, deciduous, sympodial, Plants glabrous, Roots filamentous.Stems pseudo bulbous, subterranean or partially emergent, multinoded.Trichomes absent.Leaves 3-5 per shoot, deciduous annually, plicate, stalked, the petioles of all leaves forming a pseudostem and enclosed together in 2-4 common sheathing bracts.Flowering and non-flowering plants monomorphic. Inflorescence racemose, arising from a node on a developing new shoot, multiflowered. Peduncle longer than the rhachis, the distal part

nodding in flower, straightening in fruit, with few-several tubular or semi-tubular sterile bracts. Rhachis shorter than the peduncle. Floral bracts narrow, scarious, partially sheathing the base of the pedicel. Pedicel short, merging with the ovary. Ovary short, straight. Flowers resupinate, small, crowded, lasting 2-several days, opening sequentially, white, pink or reddish, pedicellate, scentless. Perianth segments free, thin to fleshy, Dorsal sepal free, similar to the lateral sepals. Lateral sepals free, similar to the dorsal sepal. Pseudospur formed by the column, column foot and large lateral lobes. Anther terminal, incumbent, 2-celled, persistent, smooth, with a short blunt rostrum. Pollinarium present. Pollinia 2, grooved, hard, waxy, sessile. Viscidium large. Rostellum small, entire. Stigma entire, large, concave. Capsules dehiscent, glabrous, pendant; peduncle elongated in fruit; pedicel not elongated in fruit. Seeds numerous, light coloured, winged (Kores, 1989).

2.19 Traditional use of *Geodorum densiflorum*

Geodorum densiflorum have been widely employed for centuries, and they remain one important source for the discovery of new bio-active compounds. Many orchids are used widely to prevention and treatment of disease.

This orchid is unmistakable when in flower and cannot be confused with any other orchid.

2.20 Literature Review of *Geodorum densiflorum*

A study conducted by Habib et al. (published in the Journal of Herbal Medicine and Toxicology) focused on the antioxidant, cytotoxic and antibacterial properties of the different extracts (methanol, ethyl acetate and petroleum ether) of the roots of *G. densiflorum*. Antioxidants offer health benefits in preventing various diseases by fighting cellular damage caused by free radicals in the body. Polyphenolic compounds (flavonoids, phenolic acids) found in plants have multiple biological effects, including antioxidant activity. The preliminary phytochemical screening revealed the presence of flavonoid steroid and alkaloid in the plant extracts, which indicate having antioxidant and anti-inflammatory activities (Habib, 2009).

An extensive research was carried out to find the potential antioxidant activity of the plant by carrying out in vitro tests using DPPH radical scavenging, nitric oxide (NO) scavenging,

reducing power, cupric reducing antioxidant capacity (CUPRAC), total antioxidant capacity, total phenol and total flavonoid content determination assays. The study is a good demonstration of how to carry out antioxidant tests in a standard format. Since the plant extract contained flavonoid steroid and alkaloid, the antioxidant activity of the plat extract was well expected. All the tests carried out showed positive results for antioxidant activity.

Brine shrimp lethality bioassay is used for probable cytotoxic action. It is considered to be a reliable and effective test. The cytotoxicity test carried out by Habib et al. was carried out using brine shrimps which were hatched in a tank at a temperature around 37°C. The percentage lethality of brine shrimp calculated revealed that EAGD had very good lethality, PEGD had moderate and MEGD had fair cytotoxic activity in terms of LC_{50} values.

Antimicrobial activity is another area of great interest. Plants having antimicrobial properties can prove to be of paramount importance in today's world of evergrowing bacterial resistance. One of the easy ways of carrying out antimicrobial assay is by using the Kirby-Bauer disc diffusion technique. Habib et l. used thirteen pathogenic bacteria as test organisms for antibacterial activity of dried sample extracts that were compared with the standard antibiotic Amoxicillin. It was done by measuring the diameter of the zone of inhibition. MEGD showed the highest zone of inhibition indicating this fraction had the best antimicrobial activity while EAGD and PEGD showed moderate antimicrobial activity (Habib, 2009).

Geodorum densiflorum, an orchid, the arboreal *Cymbidium madium*, has a starchy pseudobulb (edible when treated) but its Aboriginal application was more likely for the treatment of diahorrea. *Geodorum densiflorum* which is locally known as Kukurmuria in Jarapa, INDIA and root is used to regularize menstrual cycle in women Mode of administration: One gm of fresh root paste, 2 drops of ghee and 5 ml of honey taken orally twice a day for 15 days on an empty stomach (Datta et al, 1999).

Another orchid of this family *Geodorum recurvum* (Roxb.) which is locally known as Tejraj, collected from Kesarpada, INDIA and root paste is applied externally to suppress tumors. Mode of administration: A decoction made from 100 gm of dried tuber, 15 to 20 gm of black pepper, and 20 to 25 nos. of garlic and taken orally twice a day for 15 days to cure malaria fever (Datta et al, 1999).

Aims and objectives

The aim and objectives of the present study were to:

1. Assess the CNS activity of the plant *G.densiflorum* by open field study.
2. Hole cross test for determination of the CNS activity of the plant *G.densiflorum*.
3. Elevated maze test to investigate the anxiolytic activity of the plant *G.densiflorum*.
4. Analyze the anxiolytic activity of the plant *G.densiflorum* by Hole board test.
5. Examine the analgesic activity of the plant *G.densiflorum* by Writhing test.

Rationale of the study

Orchids have been used as a source of medicine to treat different diseases andailments including tuberculosis, paralysis, stomach disorders, chest pain, arthritis, syphilis, jaundice, cholera, acidity, eczema, tumour, piles, boils, inflammations, leucoderma, diahorrhea, muscular pain, blood dysentery, hepatitis, dyspepsia,bone fractures, rheumatism, asthma, malaria, earache, etc. And many orchidaceous preparations were used as emetic, purgative, aphrodisiac, bronchodilator, sex stimulator, and contraceptive, cooling agent and snake bite. Some of the preparations are supposed to have miraculous curative properties. High alkaloids and glycosides contents, on orchids were full of potential. Many novel compounds and drugs, both in phytochemical and pharmacological point of view have been reported from orchid's .So there were many species of orchid's plants, different species orchids has different pharmacological activities. So many investigations was done about Orchids, and got so many medicinal uses (Barrett *et al.*, 1999).

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (Farnsworth, N. R. and Soejarto, D. D. 1991).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people. Herbal medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. The potential of medicinal plants can be assessed by finding new chemical entities of wide structural diversity. These new chemical substances can also serve as templates

for producing more effective drugs through semi-synthetic and total synthetic procedure. According to World Health Organization (WHO), about 74% of 119 plant-derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate directly with their traditional uses (Barrett *et al.*, 1999).

In Bangladesh, ninety percent of the medicinal plants are wild sourced. Out of approximately 5,000 species of indigenous and naturalized phanerogamic and pteridophytic plants growing in the country, more than a thousand of them, including many food, vegetable, beverage, spice & ornamental plants, contain medicinally useful chemical substances (Mia, 1990). Growing in the forests, jungles, wastelands, and along roadsides the types of medicinal plants in Bangladesh are varied. A total of 546 medicinal plants that occur in the country have been counted thus far. However, this list is not exhaustive since it is believed that many other medicinal plants also grow there, but have not yet been discovered or identified (Yusuf *et al*., 1994).

Orchid constitutes an order of royalty in the world of ornamental plants. They are of immense horticultural importance and also play a very useful role to balance the forest ecosystems. In Bangladesh, 159 species and two varieties fewer than 63 genera are are distributed mainly in the hilly areas of greater Sylhet, Chittagong and Mymensingh districts (Huda *et al.* 1999). The *Orchidaceae*, by far the largest family of the plant kingdom, comprises more than 30,000 species in approximately 750 genera, and is one of the most widespread of all plant families; there are terrestrial, saprophytic and epiphytic species. The use of orchids in herbal medicine has a very long history. A total of 365 plants, including several orchids are listed in the earliest known Chinese Materia Medica. Orchids have great medicinal value. *Rig Veda* and *Atharva Veda* which are known to be the oldest books provide inquisitive information about medicinal value of orchids (Kaushik,1985).

Throughout the ages, several health-promoting benefits, including diuretic, anti-rheumatic, anti-inflammatory, anticarcinogenic, hypoglycemic activities, antimicrobial, anticonvulsive, relaxation, neuroprotective, and antivirus, activities have been attributed to the use of orchids extracts. In the following, scientific research supporting pharmacological properties of *Geodorum densiflorum.*

Geodorum densiflorum is a species of Orchids, and in past microbial assay, cytotoxic and antioxidant test were investigated, but analgesic, CNS activity was not investigate before or there was no reference for analgesic, CNS activity test.

So, there is a scope to find a new results or activity of *Geodorum densiflorum* and aim to-

- Find out the possible newer medicinal activities of the same plant
- Explore different pharmacological activities of the plant

CHAPTER-3

MATERIALS AND METHODS

3.1 Plants Extraction

3.1.1 Materials

Reagent

1. Methanol
2. *n*-hexane
3. Dichloromethane
4. Ethyl acetate
5. *n*-butanol

Equipments:

1. Beaker
2. Funnel
3. Glass rod
4. Grinding machine
5. Filter paper
6. Cotton

3.2 Preparation of plant extract for experiment

3.2.1 Collection and Identification of plants

The fresh roots of the plant *Geodorum densiflorum* was collected from the area of Savar, Dhaka district during the month October, 2011.

3.2.2 Drying and grinding of the root

About four kilogram of fresh root was collected from plant and it was dried under sunlight for about two weeks. After drying, the leaves were weighed in an electrical balance and the total weight was found to be 3.20 kg (three kilogram and two hundred gram). Then the dried roots were grinded using a grinding machine to get fine powder. After grinding, the weight of the grinded root was measured and the weight was about 330 gm. All grinded roots were preserved in an air tight container.

3.2.3 Maceration of plant material

Duration of extraction: 7 days

Solvent used:

1) Methanol

Volume of solvent used: 800 ml

Procedure:

- A glass made jar with plastic cover was taken and washed thoroughly The jar was rinsed with ethanol and dried
- Then the dried sample of *Geodorum densiflorum* was taken in the jar.
- After that Methanol (800 ml) was poured into the jar up to 1-inch height above the sample surface so that it can sufficiently cover the sample surface.
- This process was performed for 7 days. The jar was shaken several times during the process to get better extraction.

3.2.4 Filtration of the Extracts

Procedure:

- After the extraction process the *Geodorum densiflorum* plant extracts was filtered with sterilized cotton filter.
- The cotton was rinsed with ethanol and fitted in a funnel.
- The filtrate was collected in a beaker.

3.2.5 Drying of extract

The liquid extract was dried with rotary evaporator to achieve a dry extract. The temperature of the evaporator was at 45°C and the rotation was set to 120 rpm.

Figure3.1: Rotary Evaporator in EWU Laboratory (IKA ®RV05 Basic, Biometra)

3.2.6 Solvent –solvent partitioning of methanolic extracts

1. Partition with *n*-hexane

Slurry of concentrated methanolic extract of *Geodorum densiflorum* was made with water. The slurry was taken in a separating funnel and few ml of *n*-hexane (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The *n*-hexane fraction (upper fraction) was collected .The process was repeated three times. The *n*-hexane fraction of root parts of the plants was found to be concentrated.

2. Partition with Dichloromethane

Slurry of concentrated methanolic extract of Geodorum densiflorum was made with water. The slurry was taken in a separating funnel and few ml of Dichloro methane (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The Dichloro methane fraction (lower fraction) was collected .The process was repeated three times. The Dichloro methane fraction of root parts of the plants was found to be concentrated.

3. Partition with Ethyl acetate

Slurry of concentrated methanolic extract of Geodorum densiflorum was made with water. The slurry was taken in a separating funnel and few ml of Ethyl acetate (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The Ethyl acetate (lower fraction) was collected .The process was repeated three times. The Ethyl acetate fraction of root parts of the plants was found to be concentrated.

4. Partition with N-butanol

Slurry of concentrated methanolic extract of *Geodorum densiflorum* was made with water. The slurry was taken in a separating funnel and few ml of *n*-butanol (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The *n*-butanol (lower fraction) was collected .The process was repeated three times. The *n*-butanol fraction of root parts of the plants was found to be concentrated.

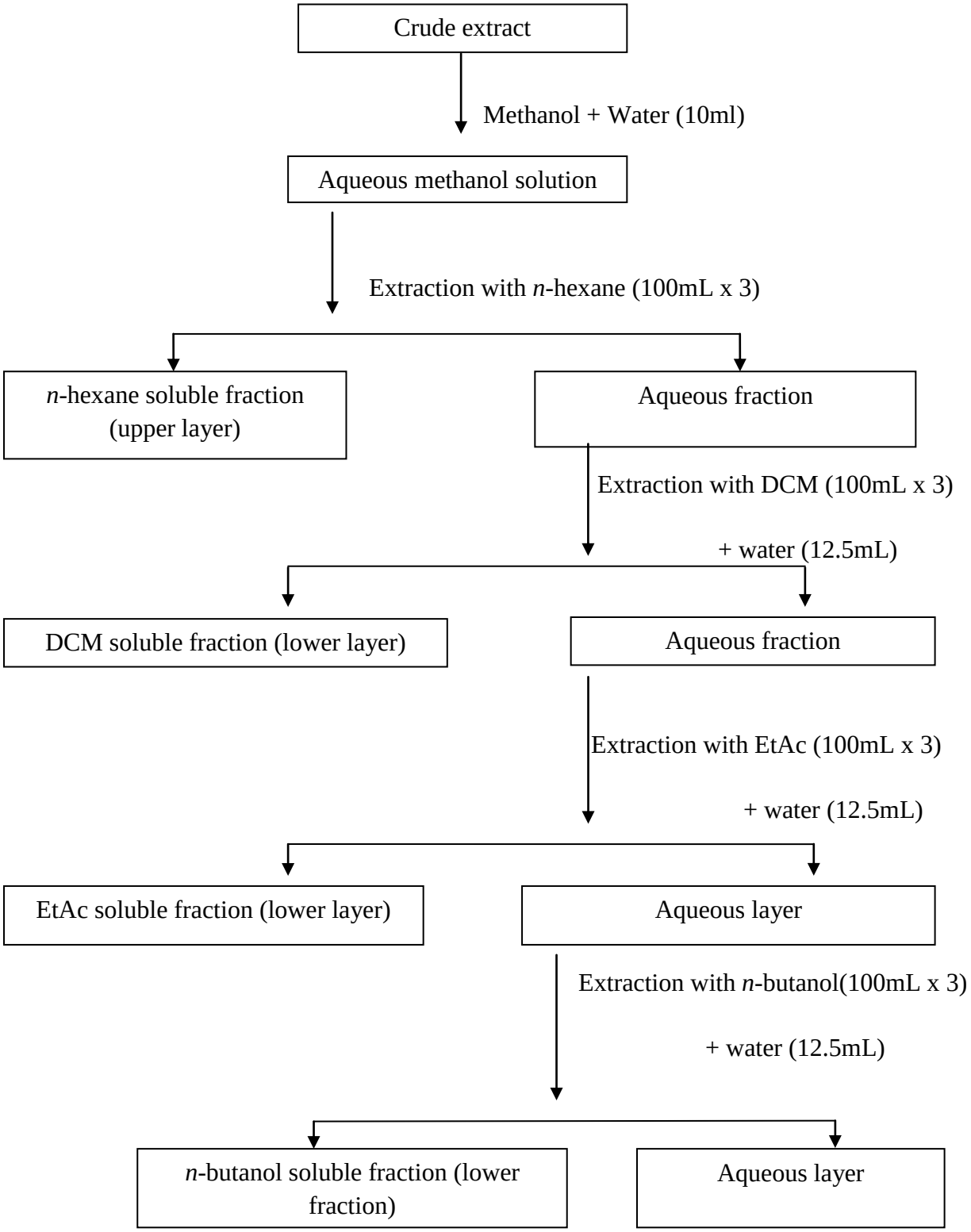


Figure3.2: Schematic representation of the modified Kupchan Partitioning of methanolic crude extract of *G. densiflorum*.

3.3 Pharmacological investigation of *G. densiflorum* of *n*-hexane portion

3.3.1 Test for analgesic activity

3.3.2 Animal

For the experiment male Swiss albino mice of 3 - 4 weeks of age, weighing between 20 - 25 gm, were collected from the animal research branch of the international center for diarrheal disease & research, Bangladesh (ICDDRB). Animals were kept in standard environmental conditions and had free access to feed and water which is ICDDRB formulated.

Figure 3.3: Swiss Albino Mice

3.3.3 Drugs and chemicals

1. Acetic acid
2. Tween-80
3. Normal saline solution
4. Diclofenac
5. *Geodorum densiflorum* (*n*-hexane and Dichloromethane part)

3.3.4 Experimental design

Thirty experimental animals were randomly selected and divided into six groups denoted as experimental group *Geodorum densiflorum n*-hexane part (200mg, 400mg) and Dichloromethane part(200mg, 400mg) positive control group & negative control group. Each group of mouse was weighed properly & dose of the test sample & control materials was adjusted accordingly.

3.3.5 Preparation of test material

In order to administer the crude extract of *n*-hexane and Dichloromethane at dose 200 & 400 mg/kg body weight of mice. The extract was collect by calculating of mice weight & was triturated in unidirectional way by the addition of 1.5 ml of distilled water. For proper mixing, small amount of suspending agent Tween-80 was slowly added. The final volume of the suspension was made 3 ml. To stabilize the suspension it was stirred well. For the preparation of positive control group (10 mg/kg) Diclofenac is taken & a suspension of 3 ml is made. Test sample and vehicle were administered orally 30 minute before intraperitoneal administration of 0.7% acetic acid, but diclofenac was administered 15 minutes before injection of acetic acid.

3.3.6 Method of identification of animals

Each group consisted of five animals. It was difficult to observe the biological response of five mice at a time receiving the same treatment. It was quite necessary to identify individual animal of groups during treatment. The animals were individualized by marking: marked as M1=mice 1, M2=mice 2, M3=mice3, M4=mice 4 & M5=mice 5.

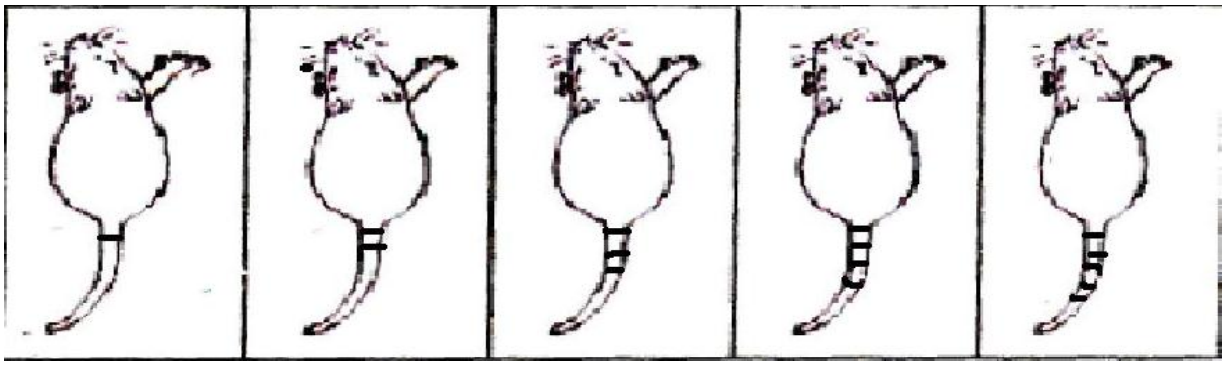


Figure 3.4: Identification of test animals for analgesic property screening

Table 3.1: Test sample used in evaluation of analgesic activity

Test sample	Group	Purpose	Dose	Root of administration
1% Tween-80 in saline	1	Negative control group	0.1 ml/10 gm of body weight	Oral
Diclofenac	2	Positive control group	10mg/kg	Intraperitoneal
***Geodorum densiflorum* *n*-hexane part**	3	Test sample	200 mg/kg	Oral
	4		400 mg/kg	
***Geodorum densiflorum* Dichloromethane part**	5	Test sample	200 mg/kg	Oral
	6		400 mg/kg	

3.3.7 Procedure of the experimental

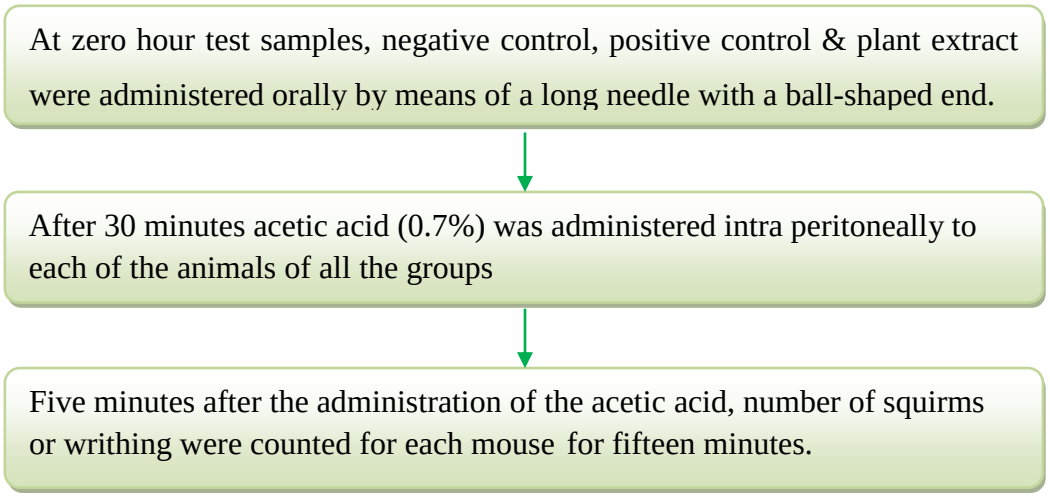


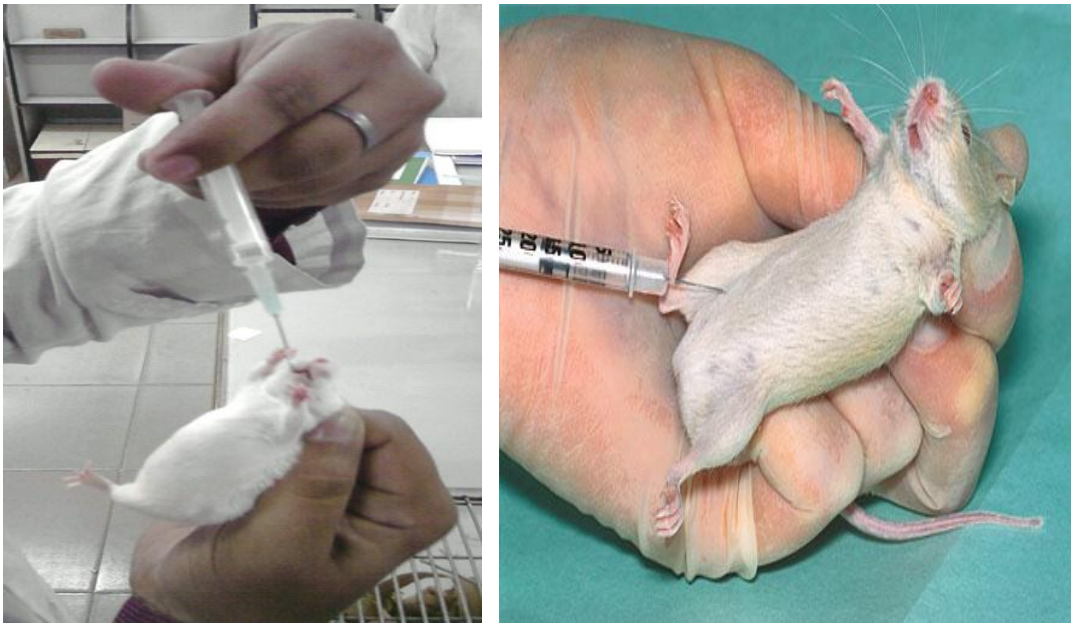
Figure 3.5: Schematic representation of procedure for screening of analgesic property on mice acetic acid induced method.

3.3.8 Counting of writhing

Each mouse of all groups were observed individually for counting the number of writhing they made in 20 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half writhing. Accordingly two half was writhing, then taken as one full writhing.

$$\% \text{ of writhing} = \frac{\text{(Mean writhing of the control group-Mean writhing of the test group)}}{\text{Mean writhing of the control group}} \times 100\%$$

% of writhing inhibition = 100 - % of writhing.



(A) **(B)**

Figure 3.6: (A) Oral administration, (B) Intraperitoneal administration of test sample

3.4 Test for CNS activity

3.4.1 Experimental design

Thirty experimental animals were randomly selected and divided into six groups denoted as experimental group *Geodorum densiflorum* n-hexane part (200mg, 400mg) and Dichloromethane part(200mg, 400mg) positive control group & negative control group. Each group of mouse was weighed properly & dose of the test sample & control materials was adjusted accordingly.

3.4.2 Drugs and chemicals

1. Tween-80
2. Normal saline solution
3. Diazepam
4. *Geodorum densiflorum* (n-hexane and Dichloromethane part)

3.4.3 Method of identification of animals

Each group consists of five animals. It was difficult to observe the biological response of five mice at a time receiving same treatment. It is quite necessary to identify individual animal of groups during treatment. The animals were individualized in the following way i.e. marked as M1=mice 1, M2=mice 2, M3=mice3, M4=mice 4 & M5=mice 5.

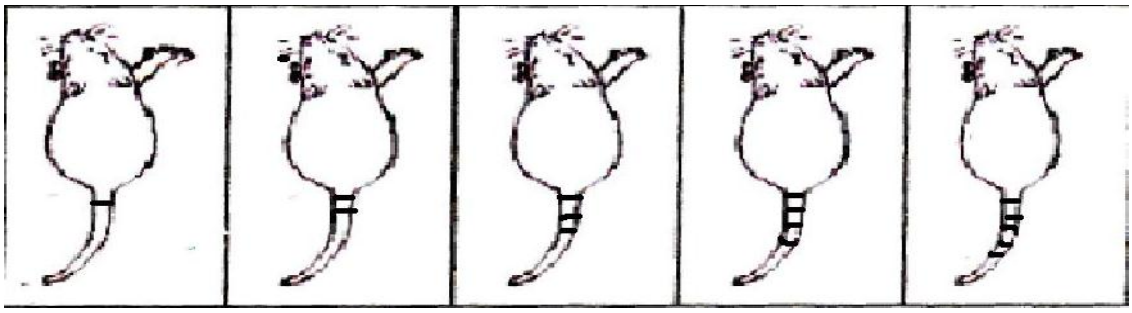


Figure 3.7: Identification of test animals for CNS depressant property screening

3.4.4. Animal

For the experiment male Swiss albino mice of 3 - 4 weeks of age, weighing between 20 - 25 gm, were collected from the animal research branch of the international center for diarrheal disease & research, Bangladesh (ICDDRB). Animals were kept in standard environmental conditions and had free access to feed and water which is ICDDRB formulated.

3.4.5. Preparation of test material

In order to administer the crude extract of N-hexane and Dichloromethane at dose 200 & 400 mg/kg body weight of mice. The extract was collect by calculating of mice weight & was triturated in unidirectional way by the addition of 3 ml of distilled water. For proper mixing, small amount of suspending agent Tween-80 was slowly added. The final volume of the suspension was made 3 ml. The final volume of the suspension was made 3 ml. To stabilize the suspension it was stirred well. For the preparation of positive control group (5 mg/kg) Diazepam is taken & a suspension of 3 ml is made.

Table 3.2: Test sample used in evaluation of CNS depressant activity

Test sample	Group	Purpose	Dose	Root Of Administration
1% Tween-80 in saline	1	Negative control group	0.1 ml/10 gm of body weight	Oral
Diazepam	2	Positive control group	5mg/kg	Oral
***Geodorum densiflorum n*-hexane part**	3	Test sample	200 mg/kg	Oral
	4		400 mg/kg	
Geodorum	5	Test sample	200 mg/kg	Oral

***densiflorum* Dichloromethane part**	6		400 mg/kg	

3.4.6. Procedure of Hole cross test

The method was adopted as described by Takagi (Takagi *et al*., 1971). A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The animals were divided into control and test groups containing 5 mice each. The test group received *Geodorum densiflorum* extract at the doses of 200 and 400 mg/kg body weight orally whereas the negative control group received vehicle (1% Tween- 80 in water) & positive control group received Diazepam (5mg/kg). The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60 & 90 minutes after oral administration of the extract.

At zero hour the plant extract (N-hexane 200/400 dose and DCM 200/400 dose), diazepam and 1% Tween solution were received orally by the test groups, positive group and control group respectively by a feeding needle with a ball shaped end .

↓

At zero hours the number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 minutes chemical interaction.

↓

After 30, 60 & 90 minutes the number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 minutes potentiality is present.

Figure 3.8: Schematic representation of procedure for screening of CNS depressant property on mice by hole cross method.

Figure 3.9: Crossing the hole of cross hole

3.4.7 Open field test

This experiment was carried out as described by Gupta (Gupta *et al*., 1971). The animals were divided into control and test groups containing 5 mice each. The test group received *Geodorum densiflorum* extract at the doses of 200 and 400 mg/kg body weight orally whereas the negative control group received vehicle (1% Tween- 80 in water) & positive control group received Diazepam (5mg/kg).The floor of an open field of half square meter was divided into a series of squares each alternatively coloured black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60 & 90 min after oral administration of the test drugs. Then measure the behaviors' Line crossing, Center square

entries, Center square duration, Rearing, Stretch attend postures, Grooming, Freezing, Urination, and Defecation

At zero hour negative control (1% Tween solution) & plant extract were administered orally by means of a long neddle with a ball shaped end.

↓

At zero hours the number of square visited by the animals was counted for a period of 3 minutes chemical interaction.

↓

After 30, 60 & 90 minutes the number of square visited by the animals was counted for a period of 3 minutes potentiality is present.

Figure3.10: Schematic representation of procedure for screening of CNS depressant property on mice by open field method.

Figure 3.11: Crossing the chamber of open field and rearing.

3.4.8 Elevated Maze test

The method first described *by Handley and Mithani (1984).*The standard elevated plus-maze test is commonly used to assess anxiety-like behavior .The maze is usually a cross shaped elevated maze, with two open arms and two closed arms. When anxious, the natural tendency of rodents is to prefer enclosed dark spaces to opened brightly lit spaces. The anxiety-related behavior is measured by the degree to which the rodent avoids the unenclosed arms of the maze. The test group received *Geodorum densiflorum* extract at the doses of 200 and 400 mg/kg body weight orally whereas the negative control group received vehicle (1% Tween- 80 in water) & positive control group received Diazepam (5mg/kg). Mice are placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm is recorded by a video-tracking system and observer simultaneously for 5 min. Then observe the open arm duration, open arm frequency, and close arm duration, close arm frequency, duration of center, center cross, rearing and grooming

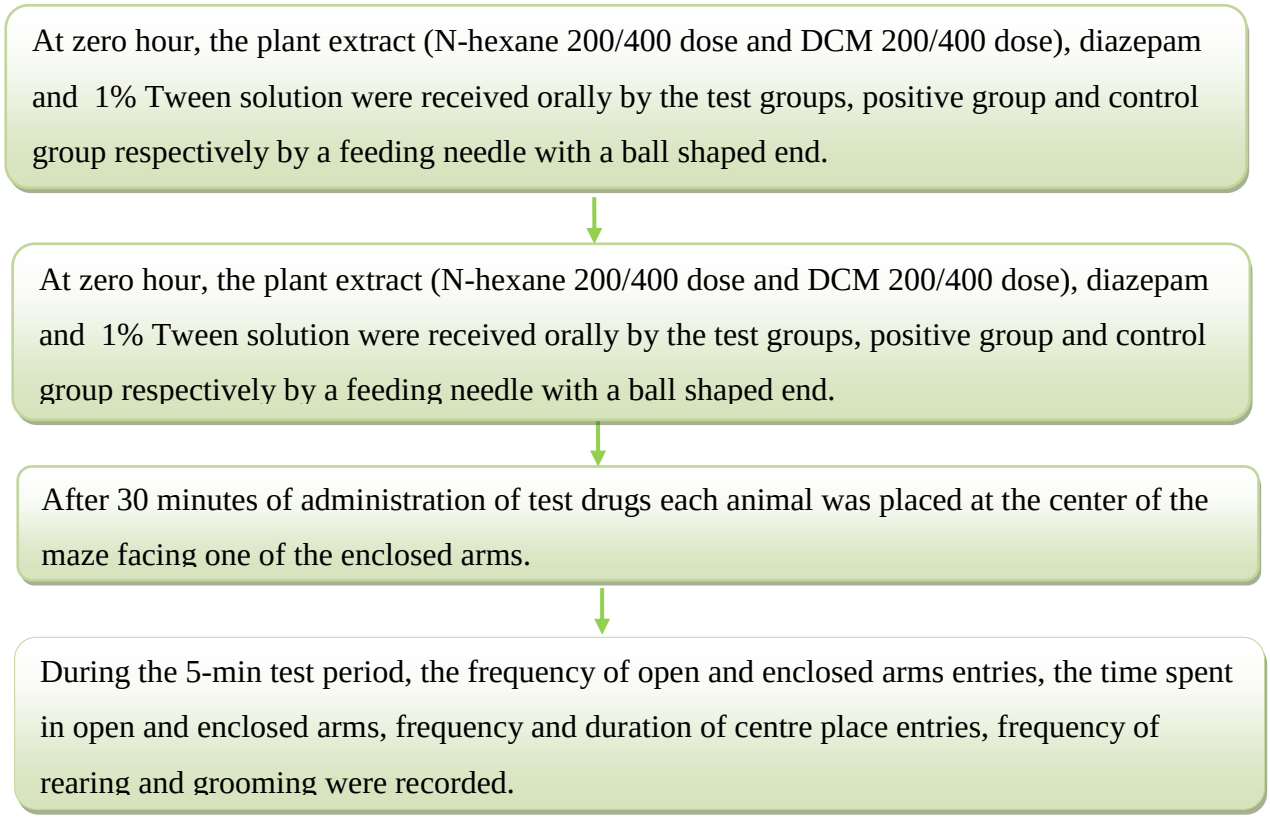


Figure3.12: Schematic representation of procedure for screening of CNS depressant property on mice by Elevated Maze Test method.

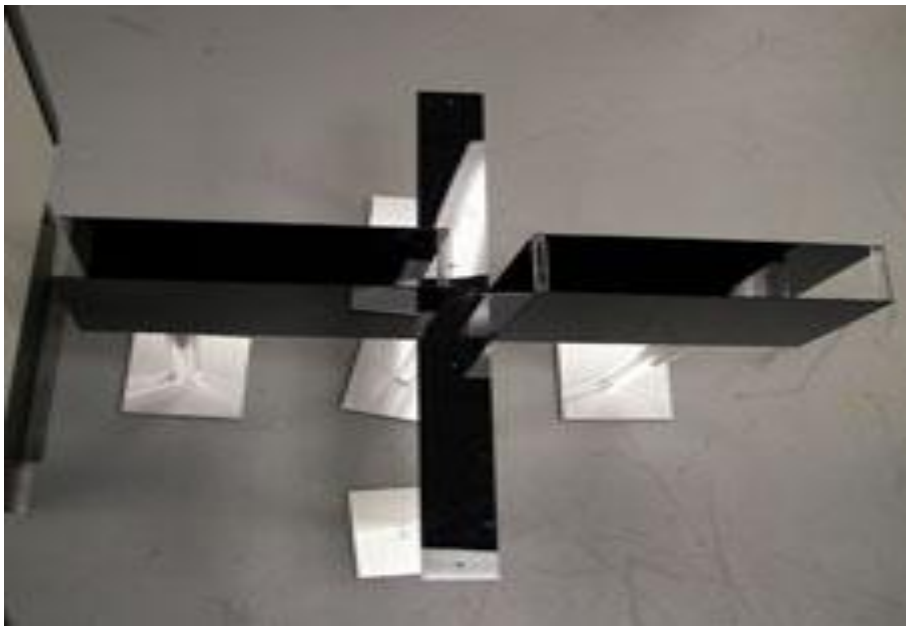


Figure 3.13: Elevated Plus Maze Board

3.4.9 Hole board test

Hole board test is a generally used method for screening the potential anxiolytic character of drugs. The test is based on the theory, that head-dipping activity of the animals is inversely proportional to their anxiety state. There are sixteen holes in the board, and was first described by Christchurch's David L Smith (1991). At first head-dip was measured; and found the proportion of animals with short latency was significantly increased in moderately and highly

aversive environments. It was fulfilled, that the inverse relation between anxiety state and head-dipping activity is true only in a certain range of anxiety level. In more aversive situations, then the anxiety level of the animals is high. Count the total number of dipping.

At zero hour, the plant extract (N-hexane 200/400 dose and DCM 200/400 dose), diazepam and 1% Tween solution were received orally by the test groups, positive group and control group respectively by a feeding needle with a ball shaped end.

↓

After 30 minutes of administration the mouse was placed at the middle of the hole board.

↓

The time of first head dipping and the total number of head dipping during the 5-min test period was counted.

Figure 3.14: Schematic representation of procedure for screening of CNS depressant property on mice by Hole Board Test method.

Figure 3.15: Mice dip in the hole board

3.4.10 Statistical Analysis

In Statistical Analysis use ANOVA test and the result obtained Dunnett method,

CHAPTER-4

RESULT AND DISCUSSION

4.1 .Analgesic activity Test

4.1.1. Result of Analgesic activity by acetic acid induced writhing method

Analgesic activity of the methanolic extract of the root part of the plant *G. densiflorum studied* in different doses (200 and 400 mg/Kg body weight) levels of *n*-hexane fraction and DCM fraction of the extract, using acetic acid induced writhing. The extract produced % inhibition of writhing at doses of 200 and 400 mg/kg body weight respectively (Table 4.1, 4.2 and Fig. 4.1). The result was found to statistically significant.

Table4.1: Analgesic activity of *Geodorum densifloram*

Animal Group	Writhing count					Mean ± SEM	% of writhing	% of inhibition
	M1	M2	M3	M4	M5			
Negative Control 1% tween 80 in saline water	19	21	23	21	19	20.6 ±1.3784	100	0
Standard (Diclofenac)	2	4	2	5	2	3**±1.3784	14.56	85.44
***n*-hexane-200**	17	15	13	18	14	15.4*±1.3784	74.75	25.25
***n*-hexane-400**	5	4	7	6	3	5**±1.3784	24.27	75.73
DCM-200mg	14	17	18	12	20	16.2***±1.3784	78.64	21.36
DCM-400mg	5	3	4	8	9	5.8**±1.3784	28.16	71.84

Values are expressed as Mean±SEM (n=5); *: $p<0.05$, **: $p<0.001$ and ***: $p<0.01$ dunnett t-test as compared to Control

Table 4.2: Multiple Comparisons

Dependent Variable: No._Writhing

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	**Lower Bound**
Dunnett t (2-sided)(a)	1	6	-5.2000(*)	1.3784	.004	-8.915	**-1.485**
	2	6	-15.6000(*)	1.3784	.000	-19.315	**-11.885**
	3	6	-4.4000(*)	1.3784	.016	-8.115	**-.685**
	4	6	-14.8000(*)	1.3784	.000	-18.515	**-11.085**
	5	6	-17.6000(*)	1.3784	.000	-21.315	**-13.885**

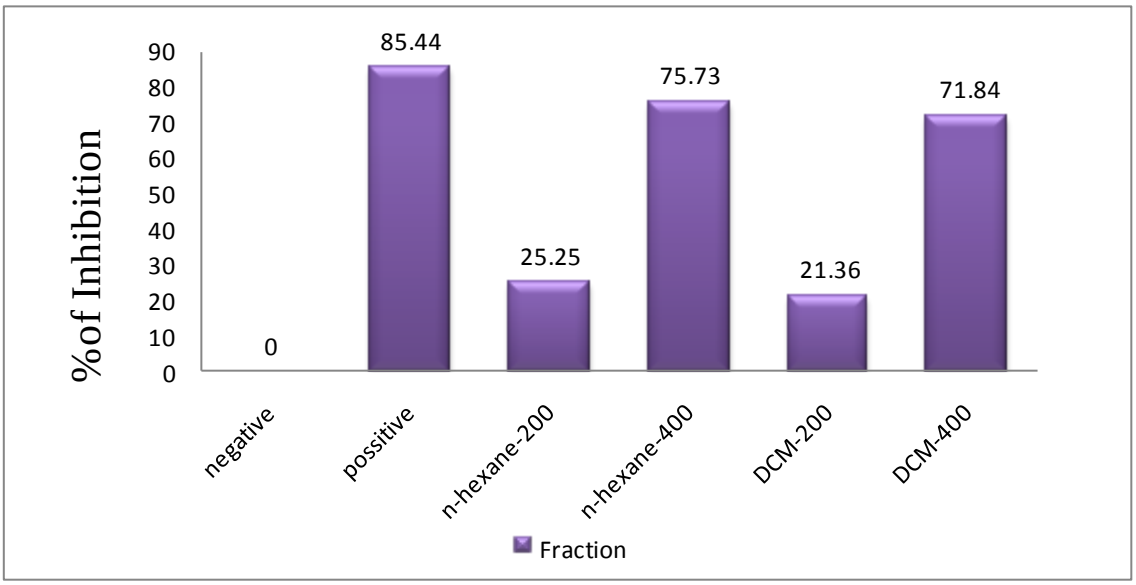


Figure 4.1: Graphical representation of % of inhibition.

In acetic acid-induced writhing test, the n-hexane fraction of *G. densiflorum at* doses 400 mg/kg body weightshowed highly significant (p<0.001), but in 200mg/kg showed normal significant (p<0.05), and the DCM fraction at does 400 mg/kg body weight showed highly significant

(p<0.001), but in 200mg/kg showed very significant (p<0.01).The inhibition of writhing response (table 4.1) induced by the acetic acid after oral administration in a dose dependant manner

4.1.2 Evaluation of analgesic property

Pain is probably the most prevalent symptom in clinical practice, and characterization of pain is of major importance in the diagnosis and choice of treatment (Thumshirn *et al*., 1999). In the treatment of diseases associated with pain, the clinical effects typically guide the selection of the analgesics and titration of the dose. However, in practice, the different symptoms of the underlying diseases confound the characterization of pain. These confounders may include complaints relating to psychological, cognitive and social aspects of the illness, as well as systemic reactions such as fever and general malaise (Drewes *et al*., 2003). Furthermore, treatment with analgesics often causes sedation and other side effects. This may bias the clinical evaluation, as the patients tend to interpret other effects of the medication– such as an effect on the anxiety and depression relating to the disease – as a relief of pain (Le Bars *et al*., 2001). Because of these confounding factors, experimental pain models are often advantageous in preclinical investigations of analgesics. With these models, the investigator can control the experimentally induced pain (including the nature, localization, intensity, frequency and duration of the stimulus), and provide quantitative measures of the psycho-physical, behavioral or the neurophysiological responses (Drewes *et al*., 2003). Experimental pain models have been used in *animal studies.* In these experiments, the neuronal nociceptive activity can be recorded or behaviour can be assessed (Sengupta & Gebhart 1994). However neuronal recordings or reactions do not reveal all aspects of pain, since pain is the net effect of complex multidimensional mechanisms that involve most parts of the central nervous system (Le Bars *et al*., 2001). Nociceptive reflexes or electrophysiological recordings from selected pathways in the animal nervous system are important in basic research and screening of analgesics. However, animal experiments typically suppress central pain mechanisms and associated complex reactions seen in man. Furthermore, the neurobiology of nociceptive systems differs between

species, and this limits the extrapolation of findings from animal studies to man even further (Le Bars *et al*., 2001).

Acetic acid-induced writhing in mice is a model of visceral pain which is highly sensitive and useful for screening peripherally acting analgesic drugs. *G. densiflorum* plant extracts caused dose-dependent antinociception against chemical induced pain in mice. *n*-hexane of the root part of the plant at the dose of 400 mg/kg body weight was found to exhibit the highest (75.73%) writhing response inhibitory effect (Table 4.1).

G. densiflorum is reported to contain sterols, alkaloids, caffeine, glycosides, flavonoids, tannin, glucosides, carotenoids (Habib *et al.* 2011)). These compounds may be responsible for the analgesic activity. Recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species form phagocytes invading the inflammation sites (Parke and Sapota, 1996). Many natural and synthetic antioxidants are in use to prevent the lipid peroxidation. There is also reports on the role of flavonoid, a powerful antioxidant (Brown and Rice-Evans, 1998; Vinson et al., 1995), in analgesic activity (Adedapo et al., 2008; Parmar and Ghosh, 1978; Mutalik et al., 2003; Venkatesh et al., 2003) primarily by targeting prostaglandins (Rajnarayana et al. 2001; Galati et al., 1994; Rao et al., 1998). Carotenoids are also reported to possess antioxidant action (Stahl and Sies, 2003; Duh et al., 1999; Veeru et al., 2009). So it can be assumed that their Cyclooxygenase (COX) inhibitory activity and antioxidant activity may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system, which is responsible for the synthesis of prostaglandins, and ultimately relieve pain-sensation.

4.1.3 Mechanism of pain induction in Acetic acid induced writhing method

Intraperitonial administration of acetic acid causes localized inflammation in mice. Following inflammation, there is biogenesis of prostaglandins (cyclooxygenase pathway) & leukotrienes (lipooxigenase pathway). The released prostaglandin, mainly prostacyclin (PGI_2) & prostaglandin E have been reported responsible for pain sensation (Le Bars *et al*. 2001).

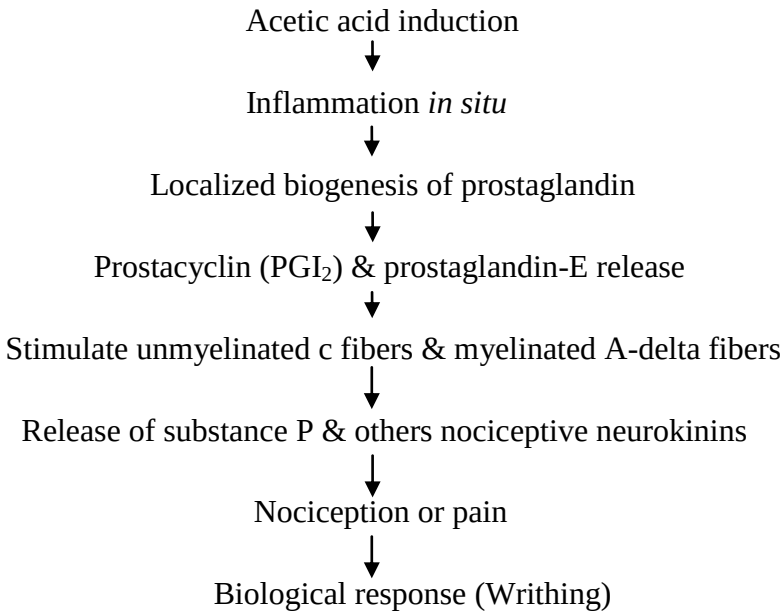


Figure 4.2: Schematic diagram of pain induction

4.2. CNS Activity Test

4.2.1. Result of CNS depressant activity on *n*-hexane part of *Geodorum densifloram* by hole cross method

CNS of the methanolic extract of the root part of the plant *G. densiflorum* studied in different doses (200 and 400 mg/Kg body weight) levels of n-hexane fraction and DCM fraction of the extract, using diazepam as a positive control. The extract produced effects at doses of 200 and 400 mg/kg body weight respectively (Table 4.3, 4.4 and Fig. 4.3). The result was found to statistically significant

Table 4.3: Data of CNS depressant activity by hole cross method

Groups	AVERAGE ± SEM				
	0 min	30 min	60 min	90 min	120 min
Control group 1%tween in saline	6.2 ±.3637	7.4±.3637	7±.3637	8±.3637	7.2±.3637
Positive control Diazepam 1mg/kg	5.6*±.3637	5.6*±.3637	6.4*±.3637	6.2*±.3637	6.4*±.3637
***n*-hexane 200mg/kg**	5**±.3637	4.8**±.3637	5**±.3637	5**±.3637	4.8**±.3637
***n*-hexane 400mg/kg**	6.4*±.3637	6.2*±.3637	6*±.3637	6.2*±.3637	5.8*±.3637
DCM-200mg	5.2±.3637	5±.3637	4.6±.3637	6.8±.3637	7±.3637
DCM-400mg	3.6**±.3637	3.6**±.3637	4**±.3637	3.2**±.3637	4**±.3637

Values are expressed as Mean±SEM (n=5); *: $p<0.05$, **: $p<0.001$ and ***: $p<0.01$ dunnett t-test as compared to Control.

Table 4.4: Multiple Comparisons (Post Hoc Tests)

Measure: Hole cross

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
Dunnett t (2-sided)(a)	2	1	-1.120(*)	.3637	.021	-2.100	-.140
	3	1	-2.240(*)	.3637	.000	-3.220	-1.260
	4	1	-1.040(*)	.3637	.035	-2.020	-.060
	5	1	-1.440(*)	.3637	.003	-2.420	-.460
	6	1	-3.480(*)	.3637	.000	-4.460	-2.500

Based on observed means.
* The mean difference is significant at the .05 level.
A Dunnett t-test treats one group as a control, and compares all other groups against it.

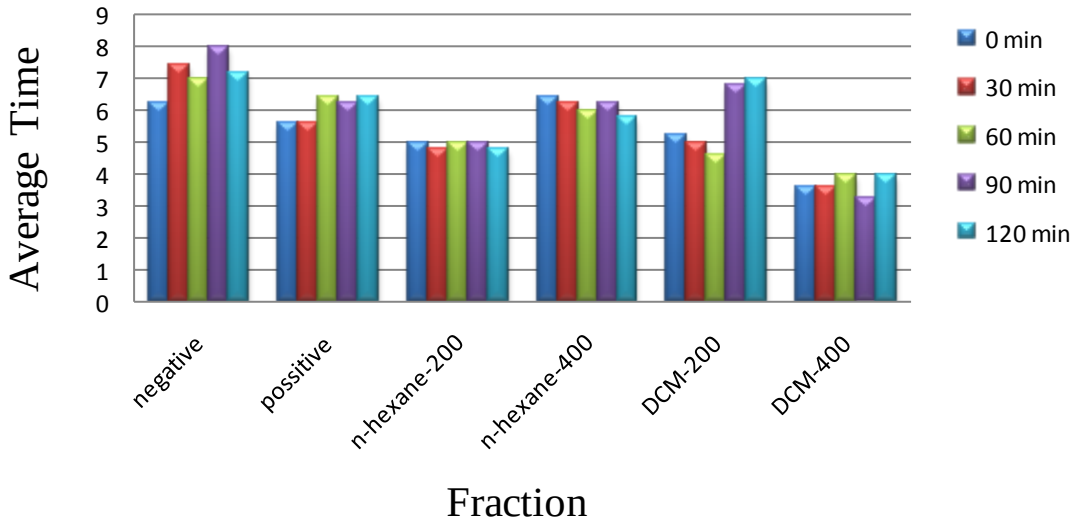


Figure4.3: Graphical representations of CNS depressant action by hole cross method

In the hole cross test, the *n*-hexane fraction of *G. densiflorum* at dose 200 mg/kg body weight showed statistically highly significant (p<0.001) (table 4.3). But the extract at 400 mg/kg body weight dose showed normal significant (p<0.05) effect.

The DCM fraction at dose 400 mg/kg body weight showed highly significant (p<0.001) effect (table 4.3). But in 200mg/kg body weight showed no significant effect.

4.2.2 Evaluation of CNS depressant property

A large number of compounds, drugs are available which depress the central nervous system (CNS) and hypotonic effects (Wafford *et al*., 2008). In smaller doses many of these drugs can produce a state of drowsiness, and when used in this manner they are referred to as sedatives. A sedative compound decreases activity, moderates excitement and calms the recipient when used in larger doses; hypnotics may produce anesthesia, poisoning and death. These progressive dose-related effects may be indicated as follows:

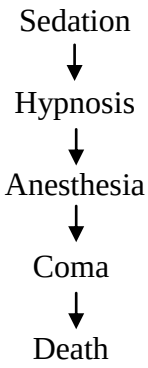


Figure 4.4: Progressive dose-related effects of CNS depressant drug

The sedatives and hypnotics are used to allay nervousness, to induce sleep, if pain is absent and control convulsions. The hypnotics suppress cerebral activity sufficiently to blunt the patient awareness of the environment thereby establishing conditions favorable for sleep. The general action of the hypnotics and sedatives is that of the depression of the CNS, which begins with the cortex and descends with increasing dosages to medullary centers. Certain compounds act at different points in the cortex and give the best therapeutic effect. The hypnotics and sedatives are usually classified into two categories: the barbiturates and non-barbiturates. Barbiturates reduce cerebral activity, which again reduces the cerebral metabolic rate probably by activating chloride channels and potentiating GABA's effects on these channels. Protection of the brain against hypoxia might theoretically occur by this mechanism, by vasoconstriction or by inhibiting calcium or glutamate (Steen, 1991).

It seemed possible that some compounds might prolong the effects of certain hypnotics by affecting vascular mechanism, for example, the absorption of the drug from the site of injection, its penetration into the 'blood-brain barrier', or its breakdown or excretion. Due to the proliferation of a granular endoplasmic reticulum (GER) in the cytoplasm of tubules of kidney, degeneration of mitochondria, and the reduction of P-450 content in the liver by many chemicals prolonged sleeping time in mice (Terasako *et al*., 1994). It has been shown that drugs which

increase brain 5-hydroxytryptamine (5-HT) usually increases sleep, whereas drugs which decrease brain 5-HT, induce a state of permanent wakefulness in mice (Lessin and Parkes, 1987). The hypothalamic action is produced by dopaminergic mechanism in the central nervous system (Yehuda and Wurtman, 1972).

The CNS depressant agent is generally associated with hypothermia and reduction in the norepinephrine content; reduction in the norepinephrine content is also associated with analgesic action. The present investigation is to study the sedative & hypnotic action.

4.2.3 Mechanism of action of Diazepam

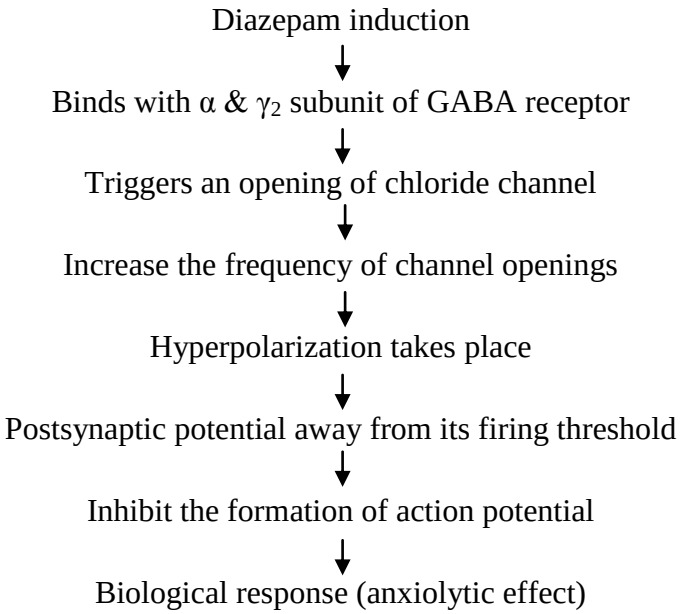


Figure 4.5: Schematic diagram of development of anxiolytic effect

4.2.4 CNS activity test by hole board method

CNS activity of the methanolic extract of the root part of the plant *G. densiflorum* studied in different doses (200 and 400 mg/Kg body weight) levels of *n*-hexane fraction of the extract, using Diazepam. The extract produced effect at doses of 200 and 400 mg/kg body weight respectively (Table 4.5, 4.6 and Fig. 4.3). The result was found to statistically significant

Table 4.5: Data of CNS depressant activity by hole board method

Animal Group	Frequency of Deeping					Mean± SEM	% of Frequency	% of Inhibition
	M1	M2	M3	M4	M5			
Negative Control 1% tween 80 in saline water	70	67	64	60	62	64.6 ±2.1229	100	0
Standard (Diazepam)	35	32	38	34	39	35.6**±2.1229	55.1	44.9
***n*-hexane-200**	44	46	51	48	49	47.6**±2.1229	73.7	26.3
***n*-hexane-400**	68	67	71	75	70	70.2*±2.1229	108.7	-8.7
DCM-200mg	18	19	18	22	21	19.6**±2.1229	30.34	69.66
DCM-400mg	55	45	50	49	57	51.2**±2.1229	79.26	20.74

Values are expressed as Mean±SEM (n=5); *: $p<0.05$, **: $p<0.001$ and ***: $p<0.01$ dunnett t-test as compared to Control.

Table 4.6 Multiple Comparisons

Dependent Variable: Head_dipping

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval

			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
Dunnett t (2-sided)(a)	1	6	-17.0000(*)	2.1229	.000	-22.722	-11.278
	2	6	5.6000	2.1229	.057	-.122	11.322
	3	6	-45.0000(*)	2.1229	.000	-50.722	-39.278
	4	6	-13.4000(*)	2.1229	.000	-19.122	-7.678
	5	6	-29.0000(*)	2.1229	.000	-34.722	-23.278

* The mean difference is significant at the .05 level.
A Dunnett t-test treats one group as a control, and compares all other groups against it.

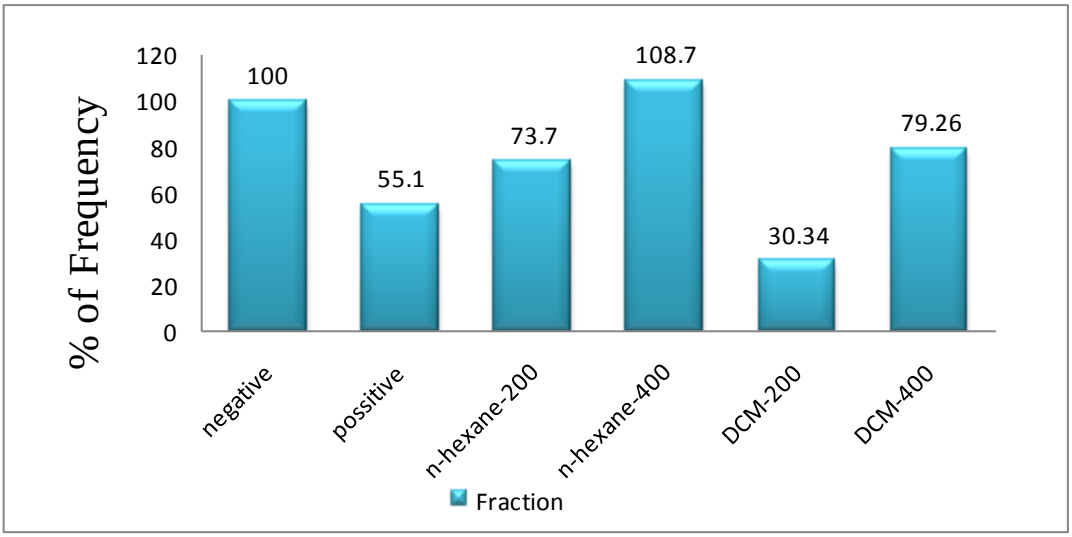


Figure4.6: Graphical representation of % of frequency

In hole board test, the *n*-hexane fraction of *G. densiflorum* at dose 400 mg/kg body weight showed an increase in latency until the first head dipping (70.2±2.1229) behavior compared with the control group which was statistically not significant ((p<0.05) (table 4.5). But the extract at dose 200 mg/kg body weight showed a decrease in latency until the first head dipping (47.6±2.1229) behavior compared with the control group which was also statistically highly

significant (p<0.001) (table 4.5), and in DCM fraction both 200mg/kg and 400mg/kg body weight were highly significant compare to control group.

4.2.5 CNS activity test by Open field method

CNS activity of the methanolic extract of the root part of the plant *G. densiflorum* studied in different doses (200 and 400 mg/Kg body weight) levels of *n*-hexane fraction of the extract, using Diazepam. The extract produced effect at doses of 200 and 400 mg/kg body weight respectively (Table 4.7, 4.8 and Fig. 4.7). The result was found to statistically significant

Table 4.7: Data of CNS depressant activity by open field

Groups	**AVERAGE ± SEM**				
	0 min	30 min	60 min	90 min	120 min
Control group 1%tween in saline	113.8 ±7.89785	72±7.89785	93.2±7.89785	85.8±7.89785	79.6±7.89785
Positive control diazepam 1mg/kg	119±7.89785	116.8±7.89785	99.4±7.89785	89.6±7.89785	93.2±7.89785
***n*-hexane 200mg/kg**	49**±7.89785	56.6**±7.89785	54.5**±7.89785	39.4**±7.89785	60**±7.89785
***n*-hexane 400mg/kg**	64.4±7.89785	59.8±7.89785	60.2±7.89785	50.4±7.89785	62.6±7.89785
DCM-200mg	26.4**±7.89785	31.6**±7.89785	28**±7.89785	30.8**±7.89785	30.6**±7.89785
DCM-400mg	103.8±7.89785	113.2±7.89785	85±7.89785	131.8±7.89785	84.6±7.89785

Values are expressed as Mean±SEM (n=5); *: p<0.05, **: p<0.001 and ***: p<0.01 dunnett t-test as compared to Control.

Table 4.8 Multiple Comparisons

Measure: line.crossing

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
Dunnett t (2-sided)(a)	2	1	14.7200	7.89785	.251	-6.5673	36.0073
	3	1	-37.0000(*)	7.89785	.000	-58.2873	-15.7127
	4	1	-29.4000(*)	7.89785	.005	-50.6873	-8.1127
	5	1	-59.4000(*)	7.89785	.000	-80.6873	-38.1127
	6	1	14.8000	7.89785	.247	-6.4873	36.0873

Based on observed means.

* The mean difference is significant at the .05 level.

a Dunnett t-tests treat one group as a control, and compare all other groups against it.

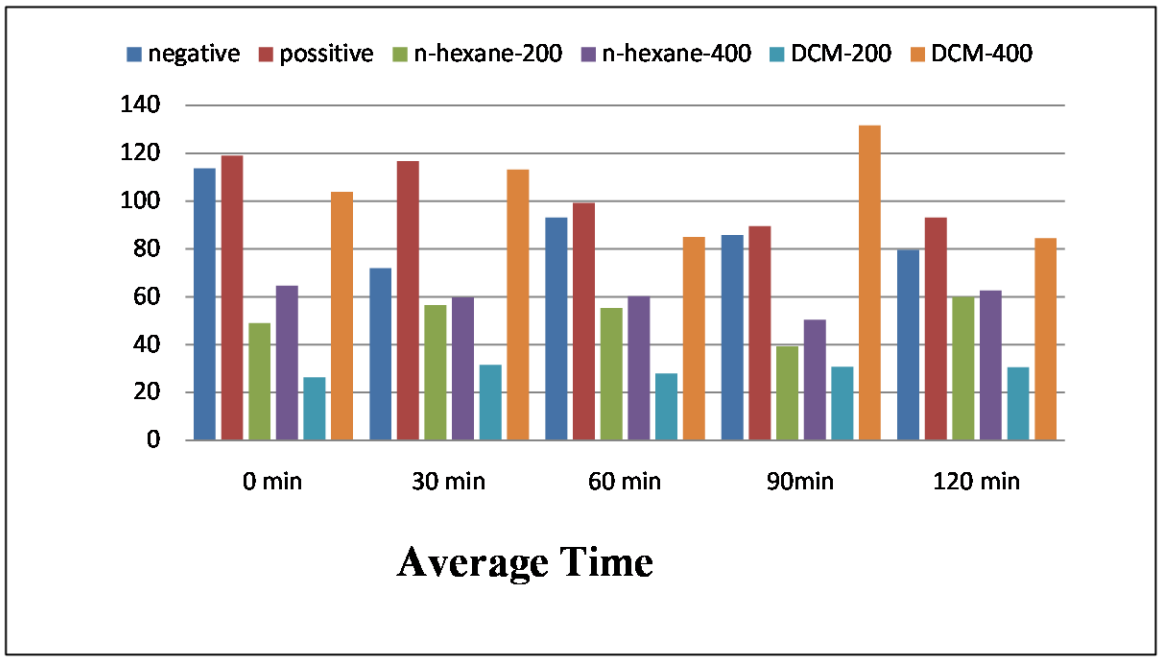


Figure 4.7: Graphical representations of CNS depressant action by open field method

In open field the *n*-hexane fraction of *G. densiflorum* at dose 200 mg/kg body weight and DCM fraction of *G. densiflorum* at dose 200 mg/kg body weight showed statistically highly significant (p<0.001) (table 4.7). But the *n*-hexane and DCM extract at 400 mg/kg body weight dose showed no significant effect.

4.2.6 CNS activity test by Elevated Plus-Maze model

CNS activity of the methanolic extract of the root part of the plant *G. densiflorum* studied in different doses (200 and 400 mg/Kg body weight) levels of *n*-hexane fractionand DCM of the extract, using Diazepam. The extract produced effect at doses of 200 and 400 mg/kg body weight respectively (Table 4.5, 4.6 and Fig. 4.3). The result was found to statistically significant

Table 4.9 Data of CNS depressant activity by elevated plus-maze model

	Mean no. of entry in (counts/5minutes)					
	Open arm duration	Open arm frequency	Close arm duration	Close arm frequency	Centre square duration	Centre square frequency
Control 1% tween in saline	2.6± 3.714	0.8± 1.095	257.8± 23.101	8.4±3.130	43±25.367	9.4±2.880
Positive control Diazepam	2.4± 5.366	0.2± 0.447	261.8± 17.584	7.6±4.037	35±13.28	6.8±3.768
***n*-hexane-200**	4.2± 9.3915	0.6± 1.342	249.8± 40.425	6.4±3.5781	46.8±31.140	5.6±3.912
***n*-hexane-400**	7.4± 12.992	0.8± 1.304	162.8± 69.149	7.6±4.098	68.8±31.838	8±4
DCM-200	20.2± 15.0735	5.8± 3.114	177.6± 56.416	7.8±1.924	60±23.473	9.8±2.280
DCM-400	3.4± 4.722	1.4± 2.073	202.6± 119.036	61.2±127.360	23.4±26.875	2.8±2.774

Open arm duration: F=2.570

P= 0.053

Open arm frequency: F=6.959

P= 0.000

Closed arm duration: F= 5.089

P= 0.003

Closed arm frequency: F= 2.829

P= 0.038

Centre square duration: F= 4.648

P= 0.004

Centre square frequency: F= 3.078

P= 0.028

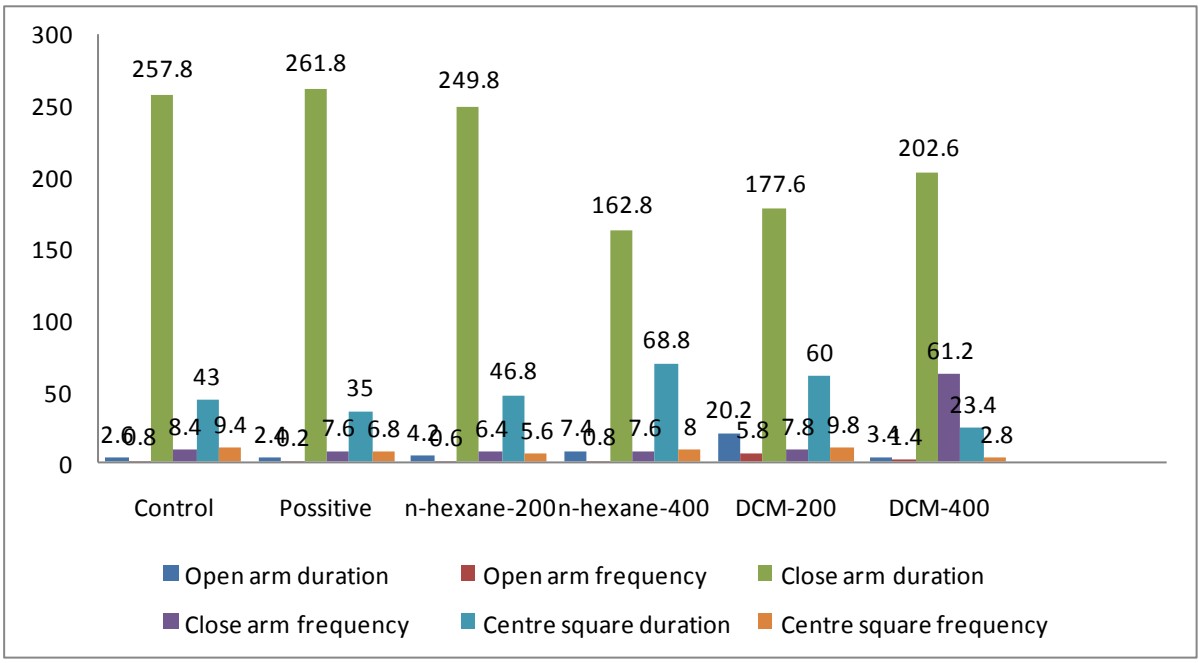


Figure4.8: Graphical representations of CNS depressant action by elevated maze test

In Elevated maze test the *n*-hexane fraction of *G. densiflorum* at dose 200 mg/kg and 400mg/kg body weight and DCM fraction of *G. densiflorum* at dose 200 mg/kg and 400mg/kg body weight showed statistically highly significant ($p<0.001$) (table 4.9) because the open arm duration is larger than the positive control, so this fraction has anxiolytic activity.

CHAPTER-5

CONCLUSION

Conclusion

Plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectancy.

Crude extract of *Geodorum densiflorum* root of the family *Geodorum* is traditionally used in various disease conditions. There are a few established research reports regarding the phytochemical and pharmacological properties of this product. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

CHAPTER-6

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