Preliminary phytochemical screening and evaluation of sedative activity of methanolic extract of *Clerodendrum viscosum* root

A project submitted
by
Kaniz Taskina Trisha
ID: 13146049
Session: Spring 2013
to
The department of pharmacy
In partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh

July, 2017

Certification Statement

This is to certify that this project titled 'Preliminary phytochemical screening and evaluation of sedative activity of methanolic extract of *Clerodendrum viscosum* root' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University, constitutes my own work under the supervision of Dr. Hasina Yasmin, Associate Professor, Department of Pharmacy, BRAC University and this project is the result of the author's original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the project paper itself.

Signed,

_____

Countersigned by the supervisor

_____

Acknowledgement

I would like to begin by expressing my gratitude to Dr. Hasina Yasmin madam, Associate Professor of Department of Pharmacy, BRAC University, for her relentless support and guidance throughout the project work. Her constant encouragement and motivation with her knowledge on photochemistry, pharmacology and medicinal chemistry has made me more passionate about the project. I would also like to thank her for her consistence patience in me during every stage of project-work in the past five months, whilst I learned and performed in the lab.

I would also like to thank Dr. Eva Rahman Kabir madam, Chairperson of Department of Pharmacy, BRAC University, for giving me this opportunity to carry out the project individually and for making Dr. Hasina Yasmin madam, my project supervisor.

Furthermore, I would like to express my gratitude to all the lab officers and lab assistants for their prolonged help in the lab-work. I would also like to extend my thanks to the National Herbarium Bangladesh, for enduring with us and helping us to identify and collect the rare medicinal plant which has been worked with in this project. Without the Grace and Mercy of The Almighty nothing would have been possible in good health.

Lastly, I would forever remain in debt to the faculty members of Department of Pharmacy at BRAC University, my friends and my family for their moral support, motivation and patience that inspired me to complete my project-work successfully.

Abstract

Clerodendrum viscosum is mostly used in traditional system of medicines particularly the leaves and roots are widely used as antiseptic, anti-inflammatory, antipyretic, against leprosy and in skin diseases. Extracts from all parts of the plant is being used intricately in Ayurveda and Unani for treating various diseases. Numerous phytochemicals were separated from many parts of the plant. Certain extracts and chemicals derived from the plant has beneficial effects such as antimicrobial, anti- inflammatory, cytotoxic, anti-hyperglycemic etc. The objective of this study was phytochemical screening and evaluation of sedative activity of methanolic extract of root of *Clerodendrum viscosum* for the first time. The preliminary phytochemical screening of methanolic root extract of *Clerodendrum viscosum* showed the presence of alkaloids, glycosides, phenols, tannins, flavonoids, coumarins and sterols. Further sedative activity was studied where the methanol extract of roots of *Clerodendrum viscosum* was determined by using diazepam as the standard drug. The time of onset of sleep was reduced by 47.67% and 53.48% at 200 and 400 mg/kg b.w. respectively in comparison to the control group. On the other hand, the total sleeping time was increased by 44.87% and 55.83% at 200 and 400 mg/kg b.w. respectively. The result suggested that the root of *Clerodendrum viscosum* is a potential source for sedative agents.

Table of contents

List of tables

List of figures

Abbreviations

ABTS- 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonate)

b.w.- Body weight

CCl_4 – Carbon tetrachloride

CH_2Cl_2 – Dichloromethane

CNS- Central nervous system

DPPH- 1, 1- diphenyl, 2-picryl hydrazyl

GABA- gamma-aminobutyric acid

gm- Gram

kg- Kilogram

H_2O_2- Hydrogen peroxide

LC- Lethal concentration

MECV- Methanolic extract of *Clerodendrum viscosum*

mg- Milligram

mL- Millilitre

µg- Microgram

NO- Nitric oxide

UV- Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Plant kingdom has a wide biochemical diversity than animals which comprises at least four fifth of secondary metabolites that originate from herbal world. This is perhaps due to the connection between soil and plants, so these have to grow various adaptation mechanisms. To date, around 40% of recent monomolecular drugs originate directly or indirectly from plants. The phytotherapy at present constitutes the most common medical practice of complementary medication, and in several countries its progress is unceasing (Balick *et al*., 1996).

Phytotherapy is the management of diseases with the help of plant. While moving around the earth for food to survive, man found through experimentation the therapeutic uses of plants and herbs. Herbal plant were utilized to mend scars and wounds on the primitive phases of human life has now advanced to set the establishment of pharmacotherapy i.e. the management of sicknesses by using drugs. Subsequently mankind has effectively utilized plants and plant products as effective therapeutic tools for fitting against diseases (Balick *et al*., 1996).

Plants have given a basis of motivation to novel medication mixes, as plant inferred solutions have made substantial commitments to human wellbeing and prosperity (Nelson,1982). The pharmaceutical properties of all the plants are attributed to its chemical constituents that have shown to retain biological properties for example antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Kose *et al.,* 2010; Pisutthanan *et al.,* 2005 and Cheng *et al.,* 2004).

The estimated number of advanced plant species (both angiosperms and gymnosperms) on this planet is 250,000 (Ayensu and De Filipps, 1978), at a lower level of 215,000 (Cronquist, 1981 and Cronquist, 1988) and at an upper level is as high as 500,000 (Tippo *et al.,* 1977 and Schultes, 1972). Of these, exclusively around 6% have been selected for biologic movement and a revealed 15% have been assessed phytochemically (Verpoorte, 2000).

A few compounds broadly adopted from plants have been accounted for to apply various organic impact, including cancer prevention agent, free radical rummaging capacities,

mitigating, hostile to cancer-causing and so forth. Bangladesh, having a large variety of plant kingdom provided the ancient culture for the practice of herbal medicines. (Munguti, 1997).

1.2 Phytotherapy

Phytotherapy laid the foundation stone of all forms of medical treatment that are practiced today. With the development of human civilization, the implementation of phytotherpy exhibits a stepwise development, which can be enumerated as –

1st stage: The crude drugs were selected, organized in the roughest means, for example powdered willow leaves in the management of pain.

2nd stage: Crude drugs were transformed into more active as well as into manageable forms, fro instance extracts or solutions, watery or alcoholic.

3rd stage: The uncontaminated active principles parted from the crude drug were employed, e.g. salicylic acid.

4th stage: Attempt to synthesize the active drug in the laboratory and indeed structural modification, e.g. aspirin, the wonder drug.

A medicinal plant can be defined as a plant which contain substance that can be utilized for remedial reason or which may act as a precursor for production of helpful drugs. In other words the plants that employ favorable pharmacological impacts on the animal body are usually titled as "Medicinal plants." It has now been proven that the plants which normally integrate and possess some secondary metabolites, similar to tannins, volatiles oils, alkaloids, glycosides and contain minerals and vitamins, have medicinal properties. (Ghani, 2005).

1.3.1 History of medicinal plants

Medicinal plants are accepted as a rich assets of constituents which can be utilized as a part of drug advancement either pharmacopoeial, non-pharmacopoeial or manufactured medications. Besides that, these plants assume a basic part in the advancement of human societies around the entire world. In addition, a few plants are measured as essential source of nourishment and because of that they are prescribed for their remedial values. Plants like

ginger, green tea, walnuts, aloe, pepper and turmeric and so on incorporate. A few plants and their subsidiaries are considered as essential basis for the active ingredients which are utilized as a part of headache medicine and toothpaste and so on (Ghani 2005).To the extent records go Babylonians (around 3000 BC) knew about countless therapeutic plants and their properties. Many of these plants are still being used in nearly a similar way and for the reason. As obvious from the Papyrus Ebers (1500 BC) the primitive Egyptians had a decent learning about the therapeutic properties of many plants. Some important plant drugs that are used at present time like henbane (Hyoscyamus spp) mandrake(Mandragora officinarum) opium (latex of papaver somniferum fruit), pomegranate(punica granatum), Castrol oil(oil of ricinus commiunis seeds), aloe(of juice Aloe spp), onions (Allium sepa) in many other were in common use in Egypt about 4500 years ago (Ghani 2005).

The most primitive use of the medicinal plants in the Indian subcontinent is found in the Rig Veda (4500-1600 BC) which includes more than 500 medicinal plants. The earliest known Chinese pharmacopeia the pen taso (1122BC) described the use the chaulmoogra oil used in the leprosy treatment, among its many other listing are hemp opium, rhubarb, and aconite. The Arabian physician Al-Razi and Ibn Sina (9^{th} to 12^{th} Ad) made a revolution in the advancement of medicine by utilizing medicinal plants. (Ghani 2005). Traditional medicine system is improved by introduction of new plants and possesses beneficial effects though there are some problems to identity the correct medicinal plants used in traditional medicine. Some traditional medicines with their sources are listed in Table 1.1.

Table 1.1 List of some crude drugs used as medicine in Bangladesh (Ghani, 2003)

Bengali name	**Scientific name**	**Plant part**	**Used**
Assamlata	*Makania cordata*	Leaves	Dysentery.
Arahar	*Cajanus cajan*	Leaves, seeds	Jaundice, mouth sore and leprosy.
Arjun	*Terminalia arfuna*	Bark	Heart disease
Amloki	*Phyllanthus emblica*	Bark flower, fruit	Hair tonic, cough, diuretic, stomach ache, dysentery,

			jaundice, dermatitis.
Basak	*Adhatoda vasica*	Root, leaves, flowers	Cough, asthma, arthritis, dysentery, and malaria.
Bohera	*Terminalia Billerica*	Fruit, bark	Constipation, diarrhea, dysentery, leprosy, rheumatisms and piles.
Bherenda	*Ricinus communis*	Roots, seeds	Constipation and rheumatisms.
Ghandabadal	*Paederiafoetida*	Leaves	Diarrhea, uriticaria, paralysis, piles and toothache
Ghritokumari	*Aloe indica*	Leaves	Constipation, anthelmintic, fistula, piles, leucorrhoea, burns and jaundice
Haritaki	*Terminalia chebula*	Fruit, Bark	Indigestions, jaundice, piles, skin disease and ulceration of gum.
Jogyadumur	*Ficus hispida*	Bark, root	Insects bites, boils, asthma, piles, cough, bronchitis, and diarrhea.
Lajjabati	*Mimosa pudica*	Whole plant	Blood purification, toothache, convulsion fistula and piles
Nayantara	*Catharanthus roseus*	Flowers	Cancer, insomnia, blood pressure and diabetes.
Sarpagandha	*Rauvolfia serpentian*	Root	Pressure and dysentery

Nishinda	*Vitex negunda*	Leaves, barks	Weakness, cough, headache,malaria,and kalazar
Amloki	*Phyllanthus emblica*	Bark flower, fruit	Hair tonic, cough, diuretic, stomach ache, dysentery, jaundice, dermatitis.
Kalojira	*Nigella sativa*	Seeds	Common cold, rheumatisms, galactagogue and carminative
Halud	*Curcuma longa*	Rhizomes	Blood purification, skin disease, eye disease, tonic, and stomach ache
Tulshi	*Ocimum sanctum*	Leaves, flower, seeds	Stomach disorder, malaria, common cold, and hypertension.
Thankuni	*Cliotoria ternatea*	Whole plant	Weakness, dermatitis, jaundice and stomach disorder
Shatamuli	*Asparagus racemosus*	Roots	Cancer, bacterial and fungal disease, tonic, appetizer, jaundice and diabetes.

1.3.2 Exploration of medicinal properties of plants

The investigation of the therapeutic properties all through of the ages was refined essentially through cautious perception and coincidental experimentation. Observation of animals' instructive separation amongst harmful and acceptable plants may likewise have helped

primitive man in picking those plants which are advantageous from nutritive and medicinal points of view and in this procedure humankind over the nations has made a tremendous legacy of knowledge and experience on medicinal plants in different cultures and civilizations. The vast majority of such indigenous information was passed on, through the ages, by at first orally and later in composed from as papyri, luxuriated mud tablets materials, original copies, lastly printed herbals, pharmacopeias and through different works. (Ghani, 1998).

1.3.3 Chemical constituents of medicinal plants

The commonly occurring chemical substances which are responsible for the medicinal (as well as toxic) properties of plants include the following (Ghani, 1998).

1. Volatile or essential oils
2. Fixed oils
3. Gum-resins and mucilage
4. Alkaloid and amines
    a. Pyridine group
    b. Tropane group
    c. Isoquiloline group
    d. Quinolone group
    e. Quinolizidine group
    f. Indole group
    g. Steroidal group
    h. Phenyl ethylamine group
    i. Alkaloid amines
5. Glycosides:
    a. Anthraquinone glycoside
    b. Cardiac glycoside
    c. Saponin glycoside
    d. Thiocyanate glycoside
    e. Other glycoside
6. Vitamin and mineral

1.4 Drug development from medicinal plants

Various techniques have been used to procure compounds to discover drugs which includes separation from plants and other normal sources, combinatorial science, and sub-atomic demonstration (Ley and Baxendale, 2002; Geysen et al., 2003; Lombardino and Lowe, 2004). In spite of the current enthusiasm for sub-atomic demonstrating, combinatorial science, and other engineered science procedures by pharmaceutical organizations and subsidizing associations, normal items, and especially restorative plants, remain a vital wellspring of new medications, new medication leads, and new synthetic substances (NCEs) (Newman et al., 2000, 2003; Butler, 2004). In both 2001 and 2002, around one fourth of the top of the line drugs worldwide were common items or gotten from regular items (Butler, 2004). A few phases include in the accompanying way improvement exercise might be summarize as takes after:

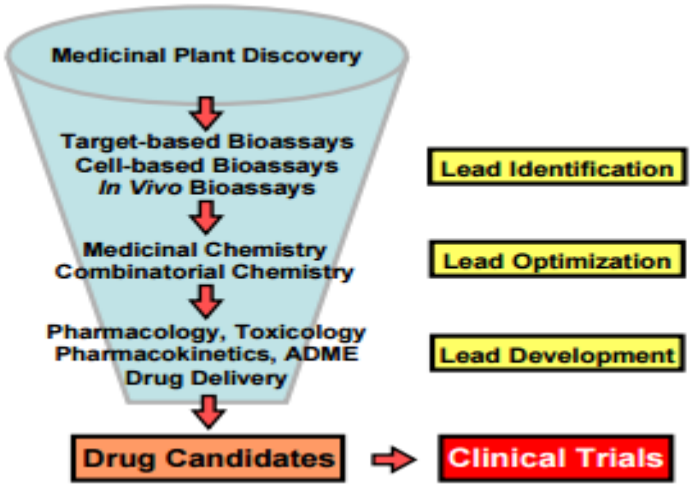


Figure 1.1: Stages involved in the drug development exercise

1.5 Current status of medicinal plants

1.5.1 National status (medicinal plants of Bangladesh)

(i) About 500 restorative plants have been accounted for to happen in Bangladesh.

(ii) Almost 80% of country people are subject to medicinal plants for their essential health insurance.

(iii) The local individuals preserve conventional learning through their experience and practices which is passed on orally with no documentation.

(iv) Over abuse of wild restorative plants has turned into a risk to its termination.

(v) In Bangladesh there is no efficient development procedure of protection techniques about restorative plants (Ayensu, 1978).

Being generally endowed by a rational topical atmosphere and appropriate prolific soil Bangladesh have a rich tropical plants. Around 5000 types of phanngrams and pteridophyties develop in its timberlands, wildernesses, squander grounds and street sides as indigenous, naturalized and developed plants. Among them more than a thousand have been asserted to have medicinal or toxic properties of which 547 have lately been specified with their therapeutic properties (Yusuf M. et at., 1994). In any case it has been watched that numerous others therapeutic plants developing in the nation have not been distinguished and there are huge numbers of them which have not been synthetically inspects and no consideration has yet been paid to pharmacognosy. Subsequently it is normal that the quantity of therapeutic plants developing or accessible in Bangladesh might be growing progressively that what has so far been identified. The list of medicinal plants that grow in different parts of Bangladesh are given in Table 1.2.

Table 1.2 List of medicinal plants that grow in Bangladesh (Rahman, 2015).

Scientific name	Bengali name	English name	Used parts	Used as patent drugs
Winthania somnifera Dunal	Ashwagandha	Winter Cherry	Root, Leaf, Fruit, seed, whole plant	Syrup Masturin, Arq Ashwaganda
Aloe vera Tour. Ex Linn.	Ghritokumari	Aloe	Leaf	Tablet Suranjan, Tablet Mudir, Syrup Belgeri
Andrographis panniculata Wall.ex Nees.	Kalomegh	Creat	Leaf, stem, Whole plant	Syrup Safi, Syrup Kuruchi
Asparagus racemosus Willd.	Satomuli	Aspargus	Tuberous root, Leaf, Flower, Fruit	Tablet Abiaj, Khisandha

Plumbago zeylanoca Linn.	Chita		Root	Syrup Kurchi
Adhatoda zeylancia Nees.	Vasak	Vasaka	Leaf, Stem, Bark, Root, Flower	Tablet Abiaj, Khisandha
Rauvolfia serpentine (Linn.) Benth.	Swarpagandha	Snakeroot	Root	Majoon Falasefa, Syrup Kurchi
Glycyrrhiza glabra Linn.	Jastimodhu	Liqourice root	Root, stem	Syrup Saduri, Tablet Kafiur

1.5.2 International status (medicinal plants in world context)

A count of the WHO from the late 1970s recorded 21000 therapeutic species. However in China alone 4941 to 26092 local species are utilized as medication in Chinese customary prescription (Ayensu, 1978) a shocking 18.9 percent. On the off chance that this extent is ascertained for other surely understood restorative greeneries and after that connected to the worldwide aggregate of 422,000 blooming plant species it can be appraise that the quantity of plant species utilized for therapeutic designs is more than 50,000.

1.6 Bioactivity guided research of medicinal plants

Bioactivity guided phytochemical approach, has three phases of investigation. First, biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones. Second, the crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, till unadulterated compounds are attained. Third, the chemical structures of unadulterated compounds are determined (Mazen *et al*., 2010).

Only the bioactive extracts or fractions would be of connotation for next phytochemical and pharmacological analysis. So in medicinal plants research, bioactivity guided phytochemical approach might be a rational approach. In a large portion of the conventional pharmaceutical practice, the therapeutic plant including the new or dried part, entire, cleaved, powdered or a propelled type of the herb is typically made by means of extraction by a dissolvable solvent,

for example, water, ethanol or a natural solvent. The solvent assumes a noteworthy part and constitutes the foundation of conventional prescription (Daniel *et al.,* 2001). Below a list of plant parts used as a medicine is given in Figure 1.1.

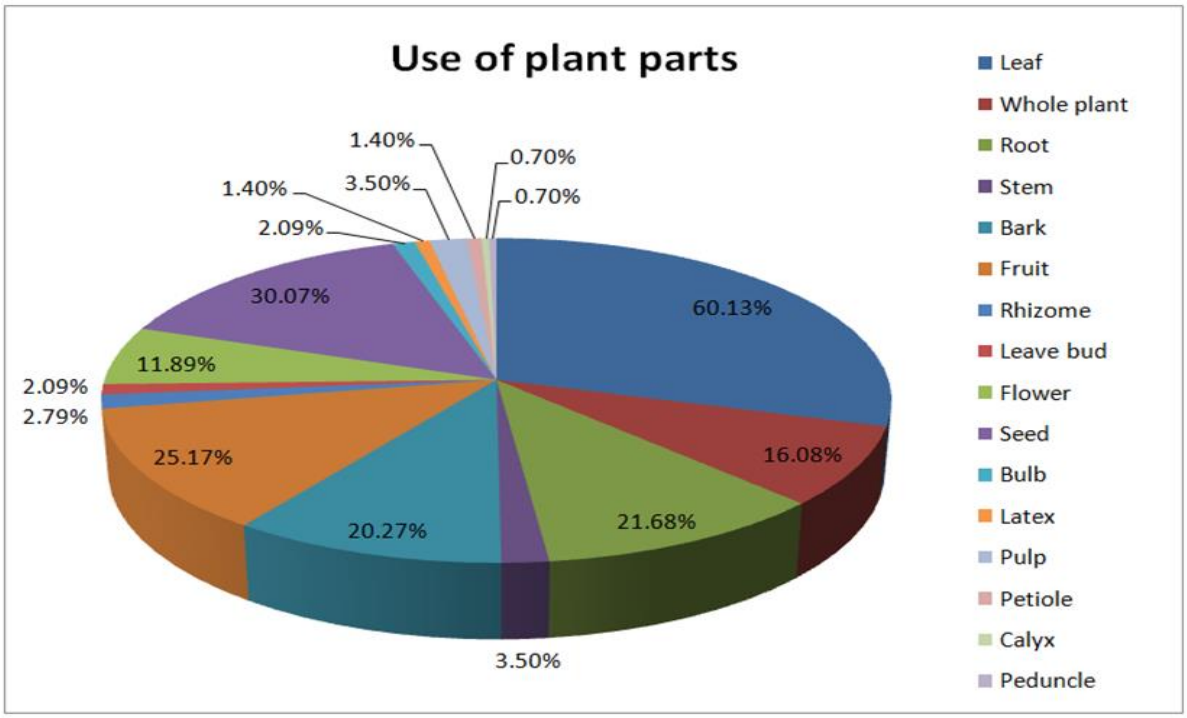


Figure 1.2 Representation of plant parts used for medicinal purpose (Rahman, 2015)

1.7 General description of the plant Clerodendrum viscosum

1.7.1 Classification

Kingdom: Plantae

Division: Angiospermae

Class: Magnoliopsida

Order: Lamiales

Family: Verbenaceae

Genus: Clerodendrum

Species: *Clerodendrum viscosum*

1.7.2 The Family Verbenaceae

The family to which *Clerodendrum viscosum* has a place is Verbenaceae which is otherwise called the teak family. It includes 35 genera and 1,200 species discovered principally in all over the world. (Heywood et al., 2007). Ponders on the concentrates of various types of the family Clerodendron have been completed by various scientists round the globe. (Shrivastava and Patel T., 2007). Roots and leaf concentrates of *Clerodendrum indicum, Clerodendrum phlomidis, Clerodendrum serratum, Clerodendrum trichotomum* and *Clerodendrum petasites* have been utilized for the treatment of stiffness, asthma and other fiery ailments. (Hazekamp et al, 2001; Kang et al, 2003). The individuals from the family are trees, bushes and herbs noted for heads, spikes or groups of little blooms, a large number of which have fragrant smell. (Cantino *et al.*, 1992). The family is firmly identified with the Lamiaceae family. These families share various characters including inverse leaves, zygomorphic corollas, and a bicarpellate ovary that is partitioned into four locules. (Wagstaff and Olmstead 1997; Cronquist, 1981).

Table 1.3: Recent taxonomic revisions of the family include these genera (Cantino *et al.*, 1992).

• *Clerodendrum brachystemon* • *C. bracteatum* . • *C. bungei* . • *C. canescens* • *C. chinense* • *C. colebrookianum.* • *C. confine* • *C. cyrtophyllum* • *C. ervatamioides* • *C. floribundum* .	• *Clerodendrum glabrum* • *C. globuliflorum* • *C. griffithianum* • *C. hainanense* • *C. henryi* • *C. indicum* • *C. infortunatum L.* • *C. intermedium C.* • *C. japonicum* • *C. kaichianum* • *C. kiangsiense*	• *Clerodendrum mandarinorum* • *C. paniculatum L.* • *C. peii* • *C. phlomidis* • *C.quadriloculare Merr.* • *C. speciosissimum* • *C. splendens G.Don* • *C. subscaposum* • *C. tibetanum* • *C. thomsoniae* . • *C. tomentosum* .

• *C. formicarum* • *C. fortunatum* • *C. fragrans* • *C. garrett.* • *C. thomsonae,* • *C. fragrans,* • *C. ugandense* • *C. chinense. Ianum* • *C. bungei Stued* • *C. myricoides*	• *C. kwangtungense* • *C. lindleyi* • *C. longilimbum* • *C. luteopunctatum* • *C. inerme,* • *C. phlomidis,* • *C. paniculatum* • *C. trichotomum* • *C. colebrookianum* • *C. trichotomum*	• *C. trichotomum .* • *C. villosum Blume* • *C. wallichii* • *C. colebrookianum,* • *C. wildii,* • *C. mandarinorum* • *C. uncinatum* • *C. infortunatum*

1.7.3 The Genus Clerodendrum

The variety Clerodendrum is broadly conveyed in the tropical and warm calm districts of the world, with a large portion of the species happening in tropical Africa and Asia. It happens in Northern Africa, in Egypt, and spreads through the rest of Africa and Madagascar. The family contains little trees, bushes and herbs and it is outstanding for its fancy employments. The primary portrayal of the class was given by Linnaeus in 1753, with the distinguishing proof of *Clerodendrum infortunatum*. Clerodendrum is a vast and various sort with around 580 distinguished species. It is the biggest sort of the tribe Teucrieae. (Steane *et al*., 1999).

1.7.4 Vernacular names:

Scientific Name: *Clerodendrum viscosum* Vent.

Synonyms: *Clerodendrum infortunatum* Gaertn.

Bengali: Bhat

English: Glory tree

Gujarati: Bharangee

Hindi: Thuner, Bhat

Tribal name: Veg, kho pa che, Baita gach.

Malayalam: Cheruthekku

Marathi: Bharangee, Bharang

Oriya: Bhania

Punjabi: Bhadangee

Tamil: Cheruteku

Urdu: Bharangi, Baharangi

Whole plant of *Clerodendrum viscosum*

Leaves of *Clerodendrum viscosum*

Flower of *Clerodendrum viscosum*

Fruit of *Clerodendrum viscosum*

Figure 1.3 *Clerodendrum viscosum*

1.7.5 Description of *Clerodendrum viscosum*

Height- The height of Clerodendrum viscosum plant is of 0.9-2.4 meter.

Stem- Clerodendrum viscosum is a woody shrub with quadrangular stem. It comprises of 4-angled stem.

Leaves- Leaves are in sessile, whorls, and glabrous. Leaves normally three at a hub, once in a while elliptic or opposite oblong, serrate.

Flowers- Flowers are purple with white pyramid molded terminal panicles.

Fruits- Fruit products are 4 lobed purple drupe, fairly succulent. Drupe, dark, about globose situated on amplified pinkish calyx.

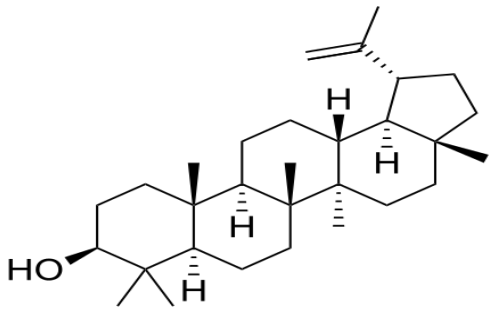
Ecology- Clerodendrum viscosum are gregarious brownish villous bush found all through tropical and subtropical districts. (Pankaj *et al*, 2007). This plant is found all through evergreen to semi-evergreen parts of forests and mixed deciduous. It is found in shallow grounds, typical weed of product fields, along the streets and railroad tracks (Warrier *et al.* 1996).

Cultivation-The bush develops on dry grounds and in an assortment of natural surroundings and soil. It will develop well in regions with low moisture. It is often developed as a hedge (Nandi and Lindem, 2015).

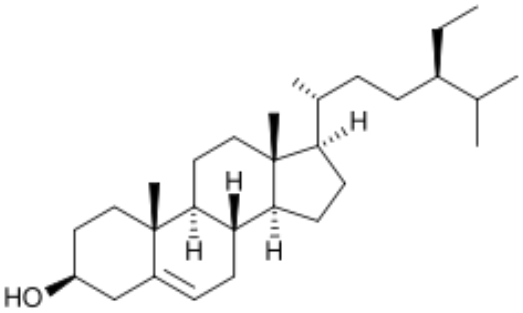
Parts used- Flower, leaf, fruit, root and stem bark of this plant are used in therapeutic purpose and medicinal treatment.

1.7.6 Chemical constituents

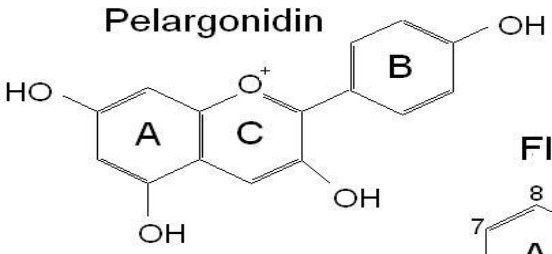
The herb comprises saponin, flavonoids, alkaloids, a new glycoside, lupeol, benzoic acid derivatives and ß-sitosterol. It also comprises cholesterol, clerodolone, clerodone. The leaves have protein, free reducing sugar, a bitter principle, clerodin a sterol, oleic, stearic and lignoceric acids, tannin, glucuronide and gallic acid. Roots comprise lupeol & ß-sitosterol, the antifungal flavonoids, cabruvin and quercetin. The seeds have fatty oil where the main fatty acids are palmitic, oleic and linoleic acids. Clerodin and hentriacontane is being separated from flowers. (Ghani, 2003; Rastogi & Mehrotra, 1990 & 93).

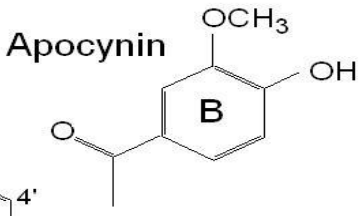


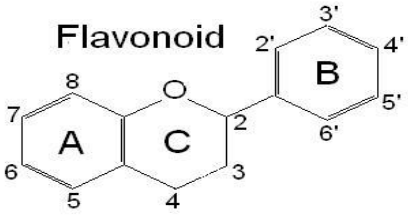
Lupeol

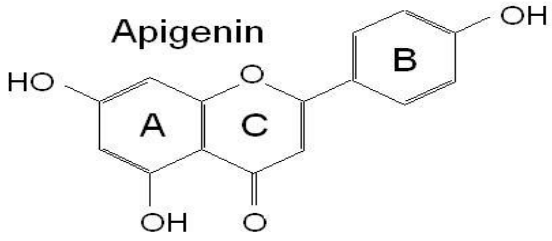


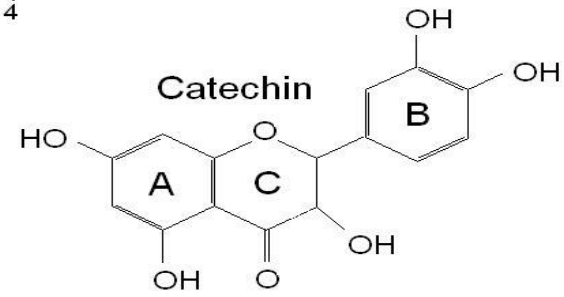
ß- sitosterol

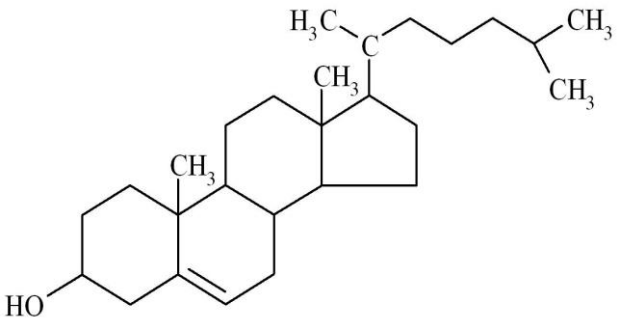












Cholesterol

Figure 1.4: Some chemical constituents obtained from *Clerodendrum viscosum*

1.8 Pharmacological activities of *Clerodendrum viscosum*

All phytocompounds of *Clerdendrum viscosum* have not been established yet. A large number of the pharmacological practices recorded the trials of alcoholic and aqueous extracts of it. Table 1.4 listed some pharmacological uses noticed by various authors.

Table 1.4: Pharmacological activities of *Clerodendrum viscosum*

Pharmacological activities	Plant Part	Extract	Reference
Antioxidant	Root	Ethanol, Methanol	Rahman *et al.* (2011), Modi *et al.* (2010), Ghosh *et al*, (2014).
Anthelmintic	Leaves, root	Ethanol, Methanol	Modi *et al.* (2010), Shamsul *et al.* (2013), Das *et al*. (2011).
Antimicrobial	Leaves, root, stems	Ethanol	Oly *et al*. (2011), Lobo *et al.* (2010), Modi *et al.* (2010), Ghosh *et al.* (2014).
Insecticidal	Leaves, stems	Ethanol	Roy *et al.* (2010), Husain & Rahman, (2006); Talukdar *et al.* (2014), Husain & Hasan (2008).
Analgesic	Roots	Ethanol	Das, *et al.* (2011), Prasanth *et al*. (2012), Sayeed *et al.* (2015).
Hypoglycaemic	Leaves	Methanol	Sayeed *et al*. (2015), Ahmed & Rahman (2014).
Anti-inflammatory	Leaves	Ethanol	Chandrashekar, R.and Rao (2013), Prasanth K.G., *et al.* (2012)
Antidiarrheal	Leaves	Ethanol	Rahman *et al.* (2011)
Cytotoxic	Root	Methanol	Rahman *et al.* (2013)
Antinociceptive	Leaves	Methanol	Khatry *et al.* (2005), Rahman et al. (2011)
Sedative	Leaves	Methanol	Ahmed *et al.* (2007)

1.8.1 Anthelmintic activity

Shamsul *et al.* (2013) examined the antihelmintic activity of aqueous and methanolic extract of *Clerodendrum viscosum* leaves against *Pheretima posthuma* of five different concentrations of the extracts. In this study, both extracts showed significant anthelmintic activity at a highest concentration of 50 mg/mL in comparison to albendazole (20 mg/mL). In another study (Das *et al*.2011), significant anthelmintic action of aqueous and ethanolic extract of *Clerodendrum viscosum* roots and leaves were established on *Ascardia galli* and *Pheretima posthuma*. Both the extracts showed dose-dependent activity. In this study, it was found that ethanolic root extract (200 mg/mL) showed better anthelminthic activity against the worms than the standard drug piperazine citrate (10 mg/mL).

1.8.2 Antimicrobial activity

In a study conducted by Ghosh *et al*. (2014), the antimicrobial action was examined for methanolic extract as well as chloroform, n-hexane, ethyl acetate fractions of *Clerodendrum viscosum* using folin-ciocalteu, agar diffusion and aluminium chloride colorimetric method against different bacterial strains. The maximum zone of inhibition for *E.coli*, *B.aureus*, *K.pneumonia*, *S.subtilis* were 28, 27, 23, 25 mm for the fraction of ethyl acetate whereas n-hexane exhibited lowest antimicrobial action. Oly *et al.* (2011) examined the antimicrobial action of crude extract of *Clerodendrum viscosum* against bacterial and fungal strains by using the technique of micro broth dilution and disc diffusion and successfully concluded that the extracts had antimicrobial action with different potency against the pathogenic microorganisms.

1.8.3 Antioxidant activity

Rahman *et al*. (2011) conducted the in vitro antioxidant property of methanolic extract of *Clerodendrum viscosum* by determining scavenging activity of nitric oxide, total antioxidant capacity and reducing power test in animal model. In this study, two different doses of 250 and 500 mg/kg body weight were used and the extract of plant showed the antioxidant activity in comparison to the standard drug ascorbic acid. In another study, Ghosh *et al*. (2014) reported that the antioxidant action of methanolic extract as well as ethyl acetate,

chloroform, n-hexane and aqueous fraction of *Clerodendrum viscosum* leaf was determined by using ABTS (2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonate), DPPH (1, 1- diphenyl, 2-picryl hydrazyl), NO (nitric oxide) and H_2O_2 (hydrogen peroxide) radical scavenging assay and compared it with ascorbic acid. In this study, all the fractions and extract except n-hexane showed significant result in DPPH assay. In NO, ABTS and H_2O_2 assay, highest scavenging activity was shown by ethyl acetate against ascorbic acid and ABTS.

1.8.4 Insecticidal activity

Roy *et al.* (2010) tested insecticidal effect of aqueous leaf extract of *Clerodendrum viscosum* against two tea pests such as *Oligonychus coffeae* and *Helopeltis theivora*. In this study, the extract of leaves decreased the pest population in comparison to the activity of Azadirachta indica (another medicinal plant) and acaricide (synthetic pesticide). In another study, biopesticidal activity of petroleum ether, chloroform and ethyl acetate leaf extracts was examined in different insects (Haque *et al*.2010), which showed that the *Rhizopertha dominica* and *Sitophiulus oryzae* had highest insecticidal action than the *Tribolium castaneus*.

1.8.5 Analgesic activity

Sayeed *et al.* (2015) determined the analgesic activity of leaf extract of *Clerodendrum viscosum* by noticeable decreases in abdominal writhings or constrictions in acetic acid-induced model in mice. In this study, three different doses of leaf extract (100, 200 and 400 mg per kg body weight) were used and the extract of leaves decreased the constriction of abdomen by 29.6%, 37.0%, and 59.3% in comparison to the standard pain relieving drug, aspirin.. Sumi *et al*. (2015) examined the analgesic activity of *Clerodendrum viscosum* by acetic acid induced writhing in animal model. In this study, two different doses of 250 and 500mg/kg body weight were used and found that the writhing inhibition was 38.59% and 59.07% at two different doses (250 and 500 mg/kg body weight) of root extracts in comparison to diclofenac sodium.

1.8.6 Hypoglycaemic activity

Sayeed *et al*. (2015) examined the hypoglycaemic activity of methanolic extract of *Clerodendrum viscosum* leaves in animal model. In this study two different doses of 200 and 400 mg per kg body weight were used and the extract of leaves decreased the level of blood glucose to 25.2 and 33.3%, respectively in comparison to the standard drug glibenclamide (10 mg per kg body weight). Ahmed & Rahman (2014) reported that the decrease of glucose level of diabetic mice by injecting the methanolic extract of *Clerodendrum viscosum* leaves at two different doses of 250 and 500 mg per kg body weight.

1.8.7 Anti-inflammatory activity

In a study conducted by Chandrashekar & Rao (2013) examined the anti-inflammatory action of the ethanolic concentrate of *Clerodendrum viscosum* leaves in Wistar Albino rats via carrageenan induced paw oedema. After administering ethanolic leaf extracts 63.75% inhibition of the oedema was observed at 3rd hour aftr administering the dose of 150 mg/kg. It is seen in another report that the ethanolic concentrate of *Clerodendrum viscosum* root anti-inflammatory activity was examined via carrageenan instigated paw oedema in Swiss albino mice and gave a good result ($p<0.001$) at a dose 200 and 400 mg/kg. (Prasanth K.G., *et al.* 2012)

1.8.8 Sedative activity

Ahmed *et al.* (2007) carried out the sedative activity on mice of the methanolic extract of *Clerodendrum viscosum* leaves conducting phenobarbital induced hypnosis test by following the method of Williamson *et al.* (1996). The test animals were divided into three groups namely control group I and the other two were experimental groups II and III respectively. The experimental groups were treated with methanolic extract of *Clerodendrum viscosum* leaves at the doses of 250 and 500 mg/kg body weight whereas the control group were treated with distilled water containing 0.1% (v/v) Tween-80 at the dose of 10 mL/kg of body weight. The extract potentiated the pentobarbital-induced sleeping time in mice. The total sleeping time was about 37 and 67 min at doses of 250 and 500 mg/kg of body weight, respectively ($p < 0.001$), whereas in the control group it was about 20 minutes.

1.8.9 Antidiarrheal activity

The antidiarrheal movement of ethanolic extract of *Clerodendrum viscosum* leaf was examined by Rahman *et al.* (2011) in mice by castor oil-induced method. Moderate anti-diarrheal activity was noticed at both 250 mg/Kg and 500 mg/Kg body weight doses of the ethanolic leaf extract of *Clerodendrum viscosum* compared to the standard antidiarrheal drug Loperamide (50 mg/kg body weight). A significant reduction ($p< 0.001$) in gastric motility was seen in mice in charcoal test. Below a mechanism of antidiarrheal activity is showed in Figure 1.5.

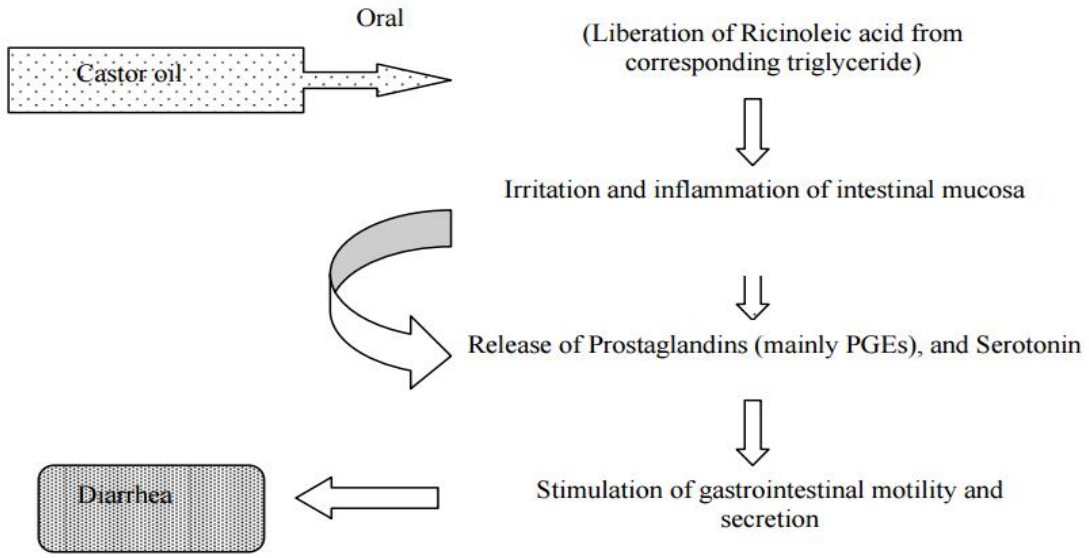


Figure 1.5: Mechanism of diarrheal action of castor oil

1.8.10 Cytotoxic activity

In a study conducted by Rahman *et al.* (2013), the in-vitro cytotoxic action of root concentrate of *Clerodendrum viscosum* was examined. The methanolic concentrate of the root of plant showed cytotoxicity using brine shrimp lethality bioassay with LC_{50} estimation of 3.696 µg/mL contrasted with vincristine sulphate (LC_{50} estimation of 0.773 µg/mL).

1.8.11 Antinociceptive activity

Ahmed *et al.* (2007) in his study mentioned the antinociceptive effect of methanolic plant concentrate of the plant in Swiss albino mice by in-vivo method. At a dose of 250 and 500

mg/kg of body weight methanol extracts of *Clerodendrum viscosum*, were administered orally 30 min prior to the intraperitoneal injection of 0.7% acetic acid which showed about 49% and 62% writhing inhibition respectively ($p < 0.001$). In another study it is seen that by inducing acetic acid on Swiss albino mice at doses of 150 and 300 mg/kg body weight, exhibited statistically significant ($p<0.001$) inhibition by 37.95 and 54.91% respectively in writhing. (Khatry *et al.* 2005).Rahman *et al.* (2011) reported that the methanolic extract of plant leaves produced 83.57% and 73.91% writhing inhibition at the doses of 500 mg/kg and 250 mg/ kg body weight respectively, in acetic acid induced mice which are comparable to Diclofenac sodium (67.65% at the dose of 25 mg/kg represents the antinociceptive activity of *Clerodendrum viscosum*.

1.9 Rationale of the study

Clerodendrum viscosum (Family: Verbenaceae) is usually found in both tropical and subtropical areas of the world mostly in India. Moreover, it also grows along the road sides and unused lands in Bangladesh which is locally known as bhat. Extracts from all parts of the plant is being used intricately in Ayurveda and Unani for treating various diseases. Numerous phytochemicals were separated from many parts of the plant. This plant is mostly used in traditional system of medicine. Particularly the leaves and roots are widely used as antiseptic, anti-inflammatory, antipyretic, against leprosy and skin diseases. Certain extracts and chemicals derived from the plant has beneficial effects such as antimicrobial, anti-inflammatory, cytotoxic, hypoglycemic etc. The project will focus on the phytochemical investigation and evaluation of sedative activity of methanolic extract of *Clerodendrum viscosum* root.

1.10 Aim of the study

The purpose of the study is to investigate the phytochemical constituents and assessment of sedative activity of methanolic extract of *Clerodendrum viscosum* root.

1.11 Objective of the study

After studying the literature review relating to the former findings of the plant the goals of

the project were set which were given as follows with respect to the methanolic of *Clerodendrum viscosum* root:

- Carrying out phytochemical screening for ensuring the presence of compounds
- To determine the bioactivity of the selected plant parts using in-vitro/in-vivo method
- To determine the sedative activity in animal model.

CHAPTER TWO

METHODOLOGY

2.1 Experimental design of *Clerodendrum viscosum*

The study was conducted on *Clerodendrum viscosum*.

Name of plant	Family	Plant part
Clerodendrum viscosum	Verbenaceae	Roots

The investigations of the plant will be discussed in two different sections.

- Phytochemical Investigation, and
- Biological Investigation.

2.2 Phytochemical study of *Clerodendrum viscosum*

2.2.1 Preparation of the plant part

The whole plant of *Clerodendrum viscosum* from was collected from Moshinda, Charpara and identified (Accession No. 41878) by taxonomist of National Herbarium, Bangladesh situated at Mirpur in Dhaka. The sample was preserved in the Phytochemical Laboratory of BRAC University of Bangladesh for further reference. For better granulating the roots were dehydrated few days and later dehydrated for 24 hours at low temperature (less than 40 °C) in oven. With the help of powerful grinder, the dehydrated roots were ground to rough fine powders.

2.2.2 Extraction of the plant material

The powder (454gm) was transferred in a conical flask and soaked in 1.5 L of methanol. The flask was secured by aluminum foil and kept for 2 days with time to time shaking and mixing. Occasional shaking was very carefully done in these two days so that the soaking is not hampered. After that, the total mixture was filtered with the help of fresh plug of cotton and filter paper. Then the refined material was transferred to the rotatory evaporator for solvent evaporation at 37˚ Celsius.

2.2.3 Solvent-Solvent partition of crude extract by Modified Kupchan Partition method (Van Wagenen et al., 1993)

Following the protocol established by Kupchan and developed by Van Wagenen *et al.*, (1993) solvent-solvent partitioning was performed. The whole partitioning process is schematically shown in Figure 2.1. First of all 5 gm of crude extract was taken in a 500 mL beaker. In another beaker 90 mL of methanol was mixed with 10 mL of water. Then slowly this mixture was added to the extract to make an aqueous methanol solution. The mixture was then partitioned with petroleum ether, carbon tetrachloride and dichloromethane. Each of the five portions were vaporized to dryness and used for further study.

Partitioning with petroleum ether

The mother solution was transferred in a separating funnel. 100 mL of the petroleum ether was added to it and the funnel was shaken for 5 minutes and left uninterrupted for 15-20 minutes. The organic portion was collected. The procedure was repeated thrice (100 mL x 3) and the fractions collected were evaporated together. The aqueous portion was taken in a beaker.

Partitioning with carbon tetrachloride

To the mother solution left after partitioning with petroleum ether, 12.5 mL of distilled water was added and mixed. The mother solution was then taken in a separating funnel and 100 mL of carbon tetrachloride (CCl_4) was added to the funnel and shaken for 5 minutes which was then left undisturbed for 15-20 minutes. The procedure was repeated for three times (100 mL x 3). The carbon tetrachloride fractions were collected and vaporized. The aqueous fraction was kept for the next step.

Partitioning with dichloromethane

To the mother solution that left after washing with petroleum ether and carbon tetrachloride, 16 mL of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with dichloromethane (CH_2Cl_2) which was

repeated for three times (100 mL X 3). The CH_2Cl_2 soluble fractions were collected together and evaporated. The aqueous methanol fraction was preserved as aqueous fraction.

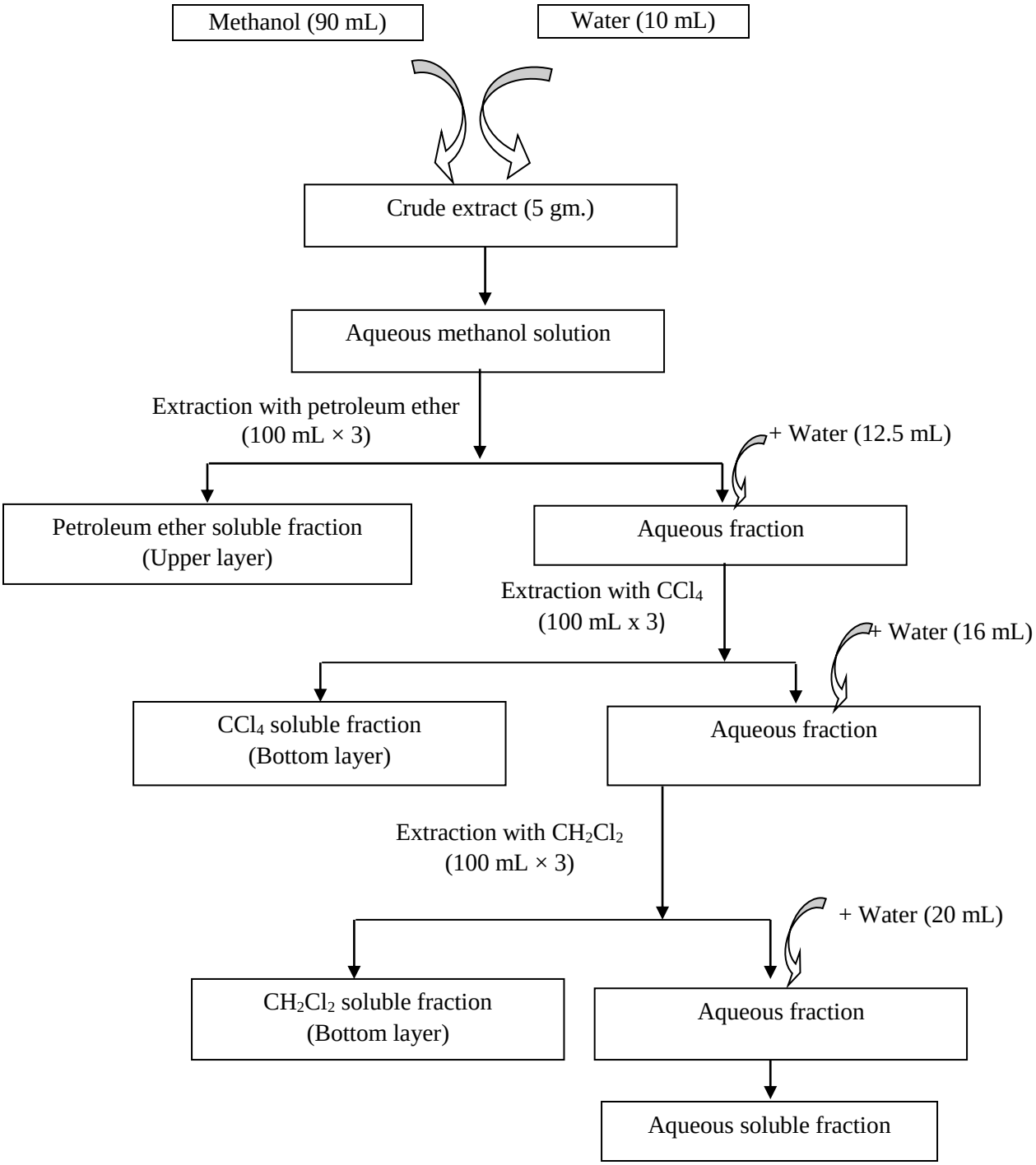


Figure 2.1: Schematic representation of the modified Kupchan Partitioning of methanolic crude extract

2.3 Phytochemical screening

Preliminary screening was performed for the secondary metabolites in different fractionates of *Clerodendrum viscosum* in order to identify its chemical constituents namely, alkaloids, flavonoids, glycosides, coumarins, tannins, phenols and sterols. The experiments were carried out according to the common phytochemical methods described by Trease and Evans, 2002.

2.3.1 Tests for alkaloids:

Mayer's test: 1 mL of each fraction was taken in a test tube and drops of Mayer's reagent (Potassium Mercuric Iodide Solution) were added. The deposition of a white precipitate indicates the existence of alkaloids.

Wagner's test: 1 mL from each fraction was treated with 1 mL of Wagner's reagent (Iodine in potassium iodide). Development of a brown or reddish brown precipitate confirms the existence of alkaloids.

Dragendorff's reagent test: 2 mL of Dragendorff's reagent was mixed with 1ml of each fraction with an addition of 2 mL of dilute HCl. An orange colored precipitate was formed which specifies the existence of alkaloids.

Hager's test: 2 mL of each fraction was treated with few drops of Hager's (Saturated picric acid solution) reagent which results in bright yellow shaded precipitate and indicates the existence of alkaloids.

Tannic acid test: 10% of tannic acid was added with 1 mL of each fraction and by its pale yellow- brown colored precipitation confirms the presence of alkaloids.

$FeCl_3$ test: 1-2 mL of all the fractions was added to few drops of neutral ferric chloride solution. Yellow precipitation confirms the existence of alkaloids.

2.3.2 Tests for glycosides:

Keller Killiani test: 1 mL of glacial acetic acid was used to dissolve 1 mL of the fraction, it was dissolved and cooled for few minutes. After that, 2-3 drops of ferric chloride, 2ml of

conc. H_2SO_4 was further added. Arrival of reddish brown shaded ring at the intersection of two layers confirms the existence of glycosides.

Conc. H_2SO_4 test: 1 mL of conc. H_2SO_4 was added with 1ml from each fraction, and permitted to standstill for 2 minute which gives a reddish color precipitate specifies the existence of glycosides.

Molish's test: To all the fractions 2-3 drops of molish reagent was added up and mixed well. To this, a few drops of conc. H_2SO_4 was added carefully. Development of reddish-purple shaded ring at the junction of two layers shows the existence of glycosides.

2.3.3 Tests for phenols:

Ellagic acid test: All the fractions were treated with few drops of 5% (w/v) glacial acetic acid accompanied by 5% (w/v) $NaNO_2$ solution. Formation of muddy brown color specifies the existence of phenols.

Phenol test: 2 mL of the fractions was independently treated with 1 mL of $FeCl_3$ solution where the establishment of a deep color approves the existence of phenols.

2.3.4 Tests for tannins:

Alkaline reagent test: A solution of sodium hydroxide was added with 2-3 ml of all the fractions. Presence of creamy to red color specifies the existence of tannins.

Ferric chloride test: Minute drops of $FeCl_3$ solution was added up to all the fractions. Development of black precipitate specifies the existence of tannins.

2.3.5 Tests for flavonoids:

Zinc-HCl reduction test: All the fractions were treated with a pinch of zinc dust along with few drops of conc. HCl. Development of intense red color shows the existence of flavonoids.

Lead-acetate test: Minute drops of basic lead acetate solution was added to 1-2 mL of all the fractions. Establishment of crimson brown precipitate shows the existence of flavonoids.

2.3.6 Test for coumarins:

1-2 mL of all the fractions were transferred in isolated tubes and was secured with a bit of paper absorbed NaOH and warmed. After that these tubes produce a yellow fluorescence in UV light shows the existence of coumarins.

2.3.7 Test for sterols:

Liebermann-Burchard test: To 1-2 mL of each of the fractions, a couple of drops of acetic anhydride was incorporated. This mixture was then treated with a couple of drops of conc. H_2SO_4 deliberately. Development of reddish dark colored ring at the intersection of two layers demonstrates the existence of steroids.

Salkowski test: 5 mL of chloroform was added to 1-2 mL of each of the fractions. The mixture was then treated with 1 mL of conc. H_2SO_4 which results in the establishment of crimson color in the lower layer indicates the existence of steroids.

2.4 Biological investigation

2.4.1 Sedative activity

There are various neuropharmacological test models for determining central nervous system (CNS) actions, for example, phenobarbitone induced sleeping time test, open field test, the hole cross test and muscle relaxant movement test. The aim of the present study is to investigate sedative activity of the methanol extract of *Clerodendrum viscosum* root in Swiss albino mice using diazepam as the standard reference drug.

Preparation of test materials

Pure diazepam and normal saline water was purchased from local market. For the preparation of diazepam at the dose of 25 mg/kg-body weight, the supplied diazepam injection (200 mg/mL) was diluted to 10 mL with saline water. To manage the extract at dosages of 200 mg/kg body weight and 400 mg/kg body weight of mice, the precise amount of extracts were measured separately and triturated in unidirectional route together with little amount of Tween-80 (a suspending agent). After legitimate blending of the extract and

suspending agent, normal saline was gradually included. The last volume of the suspension was finalized to 3.0 mL and blended well by vortex mixture.

Experimental animals

Swiss-albino mice of both sex weighing 25-35 g, matured 4-5 weeks, were collected from the Animal Resource Branch of the International Center for Diarrheal Diseases and Research, Bangladesh were utilized for the experimentation. They were kept in standard polypropylene confines and maintained at 24 ± 2ºC with relative humidity of 60-70% in a 12 hour light-dim cycle and were nourished following the guidelines of International Center for Diarrheal Diseases and Research, Bangladesh. As these creatures are exceptionally delicate to environmental variations, they are set aside before the test for no less than 3-4 days in the place where the investigation will occur. These animal experimentations were approved by the rules of the Institutional Animal Ethics Committee.

Figure 2.2 Swiss albino mice

Figure 2.3: Oral administration

Experimental Design

Twelve test animals were arbitrarily chosen and isolated into ten groups indicated as group I, group II, group III, group IV, comprising of 3 mice in each group. Each group got a specific treatment. Before any treatment, each mouse was weighed legitimately and the measurements of the test samples and control materials were balanced likewise. As it was hard to watch the biologic reaction of three mice at the moment receiving same treatment, it was important to recognize single animal of a group throughout the treatment. The animals

were categorized in the accompanying way (Figure 2.4) and set apart as M-1=Mice 1, M-2=Mice 2, and M-3=Mice 3.

Figure 2.4: Numbering of mice

Experimental Procedure

The experimental animals were divided into four groups comprising of three mice for each group. Group I was the control group, group II was the standard group whereas group II and III were experimental groups. The test groups were directed with test samples made with typical saline water and tween-80 at the measurements of 400 and 200 mg/kg b.w. , whereas the control group was regulated with ordinary saline water containing 1% Tween 80 solution. After that the standard group was treated with diazepam (25 mg/kg body weight) intraperitonially to induce sleep. The commencement of sleep and cumulative sleeping time were noted for both control group and treated groups.

CHAPTER THREE

RESULT

3.1 Preliminary phytochemical screening

Methanolic root extract of *Clerodendrum viscosum* was successively partitioned with petroleum ether, dichloromethane and carbon tetrachloride according to Modified Kupchan Partition method (Van Wagenen *et al.,* 1993). Preliminary phytochemical analysis was performed for all the fractionates showed the presence of alkaloids, glycosides, tannins, flavonoids and steroids which are listed in Table 3.1.

Table 3.1: Phytochemical analysis of different fractionates of *Clerodendrum viscosum*

Phytochemical test		Petroleum ether fraction	Dichloro methane fraction	Carbon tetrachloride fraction	Aqueous fraction
Alkaloids	Mayer's test	++	++	++	++
	Wagner's test	++	++	++	++
	Dragendorff's test	_	++	+	+
	Hager's test	_	++	+	+
	Tannic acid test	++	+	_	_
	Ferric chloride test	++	_	_	_
Glycosides	Keller killani test	++	++	++	++
	Conc. H_2SO_4 test	++	++	+	+
	Molish's test	_	++	++	++
Phenols	Ellagic acid test	_	+	+	_
	Phenol test	_	+	+	_
Tannins	Ferric chloride test	+	++	+	++

	Alkaline reagent test	_	++	++	++
Flavonoids	Zinc-HCl reduction test	_	_	+	++
	Lead acetate test	+	+	_	+
Coumarins	Test for coumarins	++	++	_	+
Steroids	Liebermann-Burchard test	++	++	++	+
	Salkowski test	++	++	++	++

3.2 Determination of sedative activity

The methanolic extract of *Clerodendrum viscosum* (MECV) root at 400 mg/kg and 200 mg/kg dose was used to determine the sedative activity (Table 3.2). The time of onset of sleep and total sleeping time of each mouse in different test groups are shown in Table 3.3. Test samples MECV at 200 and 400 mg/kg b.w. showed 44.87% and 55.83% increase respectively in total sleeping time in comparison to the control group whereas standard drug diazepam showed 87.68% in total sleeping time in comparison to the control (Table 3.4).Thus the experimental finding suggested that the methanol extract of *Clerodendrum viscosum* roots has strong sedative activity in comparison to standard diazepam.

Table 3.2: Particulars of test materials used

Test sample	Group	Identification	Dose (mg/kg b.w.)	Route of administration
Distilled Water	I	Control group	0.2 ml/30 gm of body weight	Oral
Diazepam	II	Sleep inducer	25	Intraperitoneal

MECV I	III(A)	Test group	200	Oral
MECV II	III (B)	Test group	400	Oral

Table 3.3: Screening of sedative activity by measuring the onset of sleep and total sleeping time after intraperitoneal administration of diazepam

Animal Group	**Time of onset of sleep (min.)**			**Total sleeping time (min.)**		
	M- 1	**M-2**	**M-3**	**M-1**	**M-2**	**M-3**
Control	78	44	50	78	124	90
Standard	17	13	23	174	191	183
MECV I	31	31	28	101	144	148
MECV II	20	25	35	178	107	110

M-1, M-2 and M-3 denotes first, second and third mice respectively.

Table 3.4: Sedative effect of methanol extract of roots of *Clerodendrum viscosum*

Animal group	**Time of onset of sleep (min.)**	**Reduction in time of onset of sleep (%)**	**Total sleeping time (min.)**	**Increase in total sleeping time (%)**
Control	57.33		97.33	
Standard	17.67	69.18	182.67	87.68
MECV I	30	47.67	141	44.87
MECV II	26.67	53.48	151.67	55.83

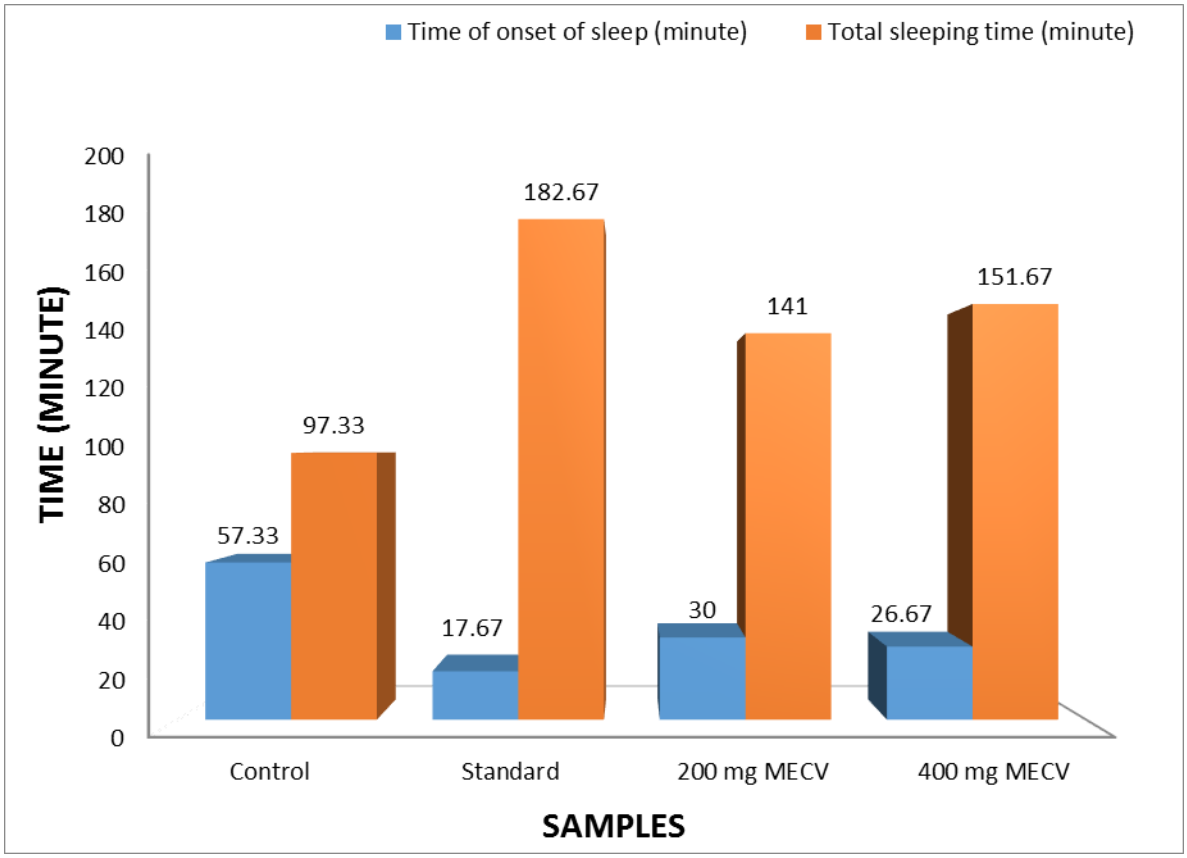


Figure 3.1: Sedative effect of MECV root.

CHAPTER FOUR

DISCUSSION

Discussion

Clerodendrum viscosum (Family: Verbenaceae) is usually found in both tropical and subtropical areas of the world mostly in Bangladesh along the road sides and unused lands. Locally it is known as bhat. It has some useful medicinal properties such as extract from leaf is used for the management of stomachache, dysentery and diarrhea. Sometimes a paste of the root is useful to treat dental caries, to reduce abdominal pain, to treat constipation, various types of skin diseases and so on. This plant is mostly used in traditional system of medicines as an antiseptic, anti-inflammatory, antipyretic, against leprosy and skin diseases (Rastogi & Mehrotra, 1990 & 93). Many researchers has reported that above mentioned activities of the extracts of the plant in animal models. However no study was found on the sedative activity of the methanolic root extract of the plant. So the study focused on the preliminary phytochemical screening and evaluation of sedative activity of methanolic extract of *Clerodendrum viscosum* root.

In this study the powder (454 gm) root of *Clerodendrum viscosum* was soaked in methanol which was further partitioned by following Kupchan and Van Wagenen *et al.,* (1993) solvent-solvent partitioning method. Later on phytochemical screening was run on the fractions of petroleum ether, dichloromethane, carbon tetrachloride and aqueous extract of root of *Clerodendrum viscosum.* The study revealed the presence of alkaloids, glycosides, phenols, tannins, flavonoids, coumarins and sterols in different fractionates. These phytochemicals are reported to have numerous biological and therapeutic properties (Katzung *et al.* 2009).

Sedative and hypnotics are central nervous system (CNS) depressants, a class of drug that reduce anxiety and deliver a sedative impact by stimulating the onset of sleep and in addition keeping up sleeping time (Katzung *et al.* 2009). There are different sorts of CNS depressants, which mostly act on the brain by inducing the neurotransmitter gamma-aminobutyric acid (GABA). GABA works by reducing brain activity. In spite of the fact that the each CNS depressants work in their own particular manner, eventually it is through their capacity to increase GABA activity that they deliver an unwinding impact that is helpful to those who suffers from tension or sleeping disorders. These days, these medications are broadly utilized as a part of treatment of various psychiatric disorders including anxiety and

sleep deprivation. Then again, constant utilization of these accessible narcotic treatments has a tendency to have severe adverse reaction extending from respiratory, stomach related, and immune system dysfunctions to degeneration of psychological function (Dhawan, 2003). In this way, advancement of new narcotic drugs with less reactions has been recommended to be a promising way to deal with diverse psychiatric issue.

The methanol extract of roots of *Clerodendrum viscosum* showed strong sedative activity in comparison to drug diazepam. The medicine diazepam is a CNS depressant (benzodiazepines) is employed to treat sleeping disorders, for example insomnia. Benzodiazepines have a coupling site on GABA receptor type ionophore complex. They reduce activity, moderate anticipation and tranquil the recipient. Substances like diazepam (the standard drug tranquilize utilized as a part of this investigation) reduce onset of and rises the time span of barbiturate prompted sleep and moderate exploratory action having possibilities as sedative (Moniruzzaman *et al.* 2015). In this trial of diazepam initiated sleep in mice, the potentiated impact of clerodendrum root extract in mice was presented. It not only elongated the sleeping time but also reduced the latency of falling asleep and increases the of sleep onset. The clerodendum root extract has provided sedation at the doses 200 and 400 mg/kg. Since the impact of diazepam on the CNS causes the initiation of the inhibition GABAergic system (Steinbach, 2001), this result recommends that a few constituents in *Clerodendrum viscosum* root extract may cause this inhibitory system.

Phytochemicals, for example flavonoids, terpenes and saponins have been found to have sedative impact (Rakhshandah, 2004). Flavonoids with anxiolytic actions have been marked in various plant species utilized in traditional medicine to depress the CNS. This impact has been credited to their affinity for the focal benzodiazepine receptors (Rakotonirina, 2001). It could be acclaimed that flavonoids of the *Clerodendrum viscosum* add to the sedative impact of this plant through central benzodiazepine receptors.

CHAPTER FIVE

REFERENCES

Ahmed, F., & Rahman, M. S. (2014). In vitro antidiabetic activity of *Clerodendrum viscosum. Pharmacreations,* 1(2).

Ahmed, F., Shahid, I., Biswas, U., Roy, B., Das, A., & Choudhuri, M. (2007). Anti-inflammatory, Antinociceptive, and Neuropharmacological Activities of *Clerodendrum viscosum. Pharmaceutical Biology*, 45(7), 587-593.

Ayensu E. S., & DeFilipps R. A. (1978). *Endangered and Threatened Plants of the United States*. Washington, DC: Smithsonian Institution Press and World Wildlife Fund, Washington, DC.

Balick J. M. & Cox P. A. (1996). *Plants, People and Culture: The Science of Ethnobotany*. Scientific American Library, New York, NY.

Butler, M. S. (2004). The role of natural product chemistry in drug discovery. *Journal of Natural Products*, 67 (12), 2141 – 2153.

Cantino, P. D., Harley, R. M. & Wagstaff, S. J. (1992). Genera of Labiatae: status and classification. *Advances in Labiatae Science*, 511-522.

Chandrashekar, R. & Rao, S. N. (2013). Chronic Anti-Inflammatory Activity of Ethanolic Extract of Leaves of *Clerodendrum viscosum* by Carrageenin Induced Paw Oedema in Wistar Albino Rats. *British Journal of Pharmaceutical Research*, 3(4), 579-586.

Cheng, S. S., Wu, C. L., Chang, H. T., Kao, Y. T. & Chang, S. T. (2004). Antitermitic and antifungal activities of essential oil of *Calocedrus formosana* leaf and its composition. *Journal of Chemical Ecology*, 30(10), 1957-1967.

Cronquist, A. (1981). *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.

Cronquist, A. (1988). *The Evolution and Classification of Flowering Plants*. (2nd ed.). The New York Botanical Garden, Bronx, New York, NY. Lawrence, Kansas.

Daniel, S., Fabricant, M., & Norman, R. (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environmental Health Perspectives*, 109(9), 234-244.

Das, J. K., Choudhury, S., Adhikary, S., Das, B., Samanta, S., Mandal, S. C., & Dey, S. P. (2011). Anthelmintic activity of *Clerodendrum viscosum*. *Oriental Pharmacy and Experimental Medicine,* 119-122.

Das, J. K., Kandar, C. C., Dey, S. P., & Mandal, S. C. (2011). Evaluation of Analgesic Activity of *Clerodendrum Viscosum* Linn. (Verbenaceae) Leaves on Experimental Animal Model. *International Journal of Pharma and Bio Sciences*.

Dhawan, K., Dhawan, S., & Chhabra, S. (2003). Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Passiflora incarnata* Linneaus: a non-habit forming anxiolytic. *Journal of Pharmacy & Pharmaceutical Sciences,* 215–222.

Geysen, D., Delespaux, V., & Geerts, S. (2003). PCR–RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of Trypanosoma species in cattle. *Veterinary Parasitology*, 110(3-4), 171-180.

Ghani, A. (1998). *Text Book of Pharmacognosy*. Rajbari Printing Press. 225-248.

Ghani, A. (2003). *Text Book of Pharmacognosy*. Rajbari Printing Press.110-135.

Ghani, A. (2005). *Text Book of Pharmacognosy*. Rajbari Printing Press.197-204.

Ghosh, A., Trivedi, P. P., Timbalia, S. A., Griffin, A. T., Rahn, J. J., Chan, S. S., & Gohil, V. M. (2014). Copper supplementation restores cytochrome c oxidase assembly defect in a mitochondrial disease model of COA6 deficiency. *Human Molecular Genetics*, 23(13), 3596-3606.

Hazekamp, A., Verpoorte, R., & Panthong, A. (2001). Isolation of a bronchodilator flavonoid from the Thai medicinal plant *Clerodendrum petasites. Journal of Ethnopharmacology,* 78, 45-49.

Heywood, V. H., Brummitt, R. K., Culham, A. & Seberg, O. (2007). Flowering plant families of the world. *Royal Botanic Gardens*, 424-456.

Husain, M. M., & Rahman M. (2006). Repellent effect of indigenous plant Bhat (*Clerodendron viscosum* L.) leaf on *Tribolium Castaneum* Herbst. *Bangladesh Journal of Science and Industrial Research*, 41(1), 67–72.

Husain, M. M., & Hasan M. R. (2008). Repellency of indigenous plant, Bhant (*Clerodendron viscosum* Vent.) leaf on *Tribolium Confusum* Duval. *Bangladesh Journal of Science and Industrial Research*, 43(2), 267–272.

Haque, M. Z., Rouf , M. A., Jalil, M. A., Islam, M. B., & Islam, M.R. (2010). Screening of phytochemical and biological potential of *Clerodendron viscosum* leaves extracts. *Bangladesh Journal of Science and Industrial Research*, 45(2), 381–386.

Kang, D. G., Lee, Y. S., Kim, H. J., Lee, Y. M., & Lee, H. S. (2003). Angiotensin converting enzyme inhibitory phenylpropanoid glycosides from *Clerodendron trichotomum. Journal of Ethnopharmacology,* 89(6), 151-154.

Katzung, B. G., Masters, S. B., & Trevor A. J. (2009). *Basic and Clinical Pharmacology, McGraw-Hill*, (11th ed.). New York, NY.

Khatry, N., Kundu, J., Bachar, S. C., Uddin, M. N., & Kundu, J. K. (2005). Studies on antinociceptive, antiinflammatory and diuretic activities of methanol extract of the aerial parts of *Clerodendron viscosum* Vent. *Dhaka University Journal of Pharmaceutical Sci*ence, 5(1), 63–66.

Kose E. O., Akta, Deniz, I. G., & Sarik, C. (2010). Chemical composition, antimicrobial and antioxidant activity of essential oil of endemic *Ferula lycia Boiss, J. Med. Plant. Res*., 4(17), 1698-1703.

Ley, S. V., & Baxendale, I. R. (2003). Organic Synthesis in a Changing World. *ChemInform*, 34(35).

Lobo, R., Chandrshakar K. S., Jaykumar, B., & Mamatha, B., (2010). *In vitro* antimicrobial activity of *Clerodendrum viscosum* (Vent). *Der Pharmacia Lettre*, 2,257–260.

Lombardino, J. G., & Lowe, J. A. (2004). A guide to drug discovery: The role of the medicinal chemist in drug discovery — then and now. *Nature Reviews Drug Discovery*, 3(10), 853-862.

Mazen, A., El-Sakka, Sahar A. M., & Hussein, P. (2010). "Phytochemistry" deals with the chemical structures of secondary metabolites, *Fourth Saudi Science Conference*, 8, 21-25.

Modi, A. J., Deore, S. L., & Khadabadi, S. (2010). In vitro anthelmintic activity of Lerodendrum infortunatum. *ResearchGate.*

Moniruzzaman, M., Rahman, A., & Ferdous, A. (2015). Evaluation of Sedative and Hypnotic Activity of Ethanolic Extract of *Scoparia dulcis* Linn. *Evidence-Based Complementary and Alternative Medicine*.

Munguti, K. (1997). Indigenous knowledge in the management of malaria and visceral leishmaniasis among the Tugen of Kenya. *Indig Knowl Dev Monitor*, 5, 10-12.

Nandi, S., & Lyndem, L. M. (2015). *Clerodendrum viscosum*: traditional uses, pharmacological activities and phytochemical constituents. *Natural Product Research*, 30(5), 497-506.

Nelson, C. (1982). *An International Journal of Plant Biochemistry*, 21(12), 2769-2998.

Newman, D. J., Cragg, G. M., & Snader K. M. (2000). The influence of natural products upon drug discovery. *Natural Product Reports*, 17 (3), 215 – 234.

Newman, D. J., Cragg, G. M., & Snader K. M. (2003). Natural products as sources of new drugs over the period 1981 – 2002. *Journal of Natural Products*, 66 (7), 1022 – 1037.

Oly, W. T., Islam, W., Hasan, P., & Parween, S. (2011). Antimicrobial activity of *Clerodendrum viscosum* Vent. (Verbenaceae). *Int J Agric Biol.* 13,222–226.

Pankaj, P., Setty, M. M., & Shirwaikar, A. (2007). Antioxidant Potential of *Clerodendrum Viscosum* Vent. Roots. *Pharmacologyonline*.

Pisutthanan, N., Liawruangrath, S., Bremner, J. B. & Liawruangrath, B. (2005). Chemical constituents and biological activities of *Chromolaena odorata*, *Chiang Mai J. Sci*., 32(2), 139-148.

Prasanth, K. G., Anandbabu, A., Johns, T., Dineshkumar, B., Krishnakumar, K., Geetha, G., & Venkatanarayanan, R. (2012). Ethanol extract of *Clerodendrum viscosum* vent

Roots: Investigation of analgesic and anti-inflammatory effects in male adult Swiss albino mice. *International Journal of Natural Products Research*.

Rahman, A. M., & Sarker, A. K. (2015). Investigation of Medicinal Plants at Katakhali Pouroshova of Rajshahi District, Bangladesh and their Conservation Management. *Applied Ecology and Environmental Sciences, 3*(6), 184-192.

Rahman, M., Hasan, M., & Sabrin, F. (2011). Evaluation of Antidiarrheal Activity of *Clerodendrum viscosum* Vent. *Journal of Innovation & Development Strategy (JIDS), 5*(2), 56-61.

Rahman, M. M., Rumzhum, N. N., & Zinna, K. (2011). Evaluation of Antioxidant and Antinociceptive Properties of Methanolic Extract of *Clerodendrum viscosum* Vent. *Stamford Journal of Pharmaceutical Sciences*, 4(1).

Rahman, M., Rahaman, A., Basunia, M. A., Fatima, N., & Hossain, S. (2013). Antihemolytic Activity of *Clerodendrum viscosum* Vent. is mediated by its Antioxidant Effect. *European Journal of Medicinal Plants,* 127-134.

Rakhshandah, H., Hosseini, M., & Dolati, K. (2004). Hypnotic Effect of *Rosa damascena* in Mice. *Iran. J. Pharm. Res*., 181 -185.

Rakotonirina V. S., Bum, E. N., Rakotonirina, A., & Bopelet, M. (2001). Sedative properties of the decoction of the rhizome of *Cyperus articulatus*. *Fitoterapia*.22-29.

Rastogi, R., & Mehrotra, B., (1990 & 93). Compendium of Indian medicinal plants. *Central Drug Research Institute, Publications and Information Directorate*.

Sayeed, R. U., Hassan, R., Anzum, N., Rahman, S., & Rahmatullah, M. (2015). Antihyperglycemic and analgesic activity studies with *Clerodendrum Viscosum* Vent. (Verbenaceae) Leaves. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(09), 216-224.

Schultes, R. E. (1972). The future of plants as sources of new biodynamic compounds. *Plants in the Development of Modern Medicine*, 103-124.

Shamsul, I. M., Rahman, M., Koushik, A. S., Jamiuddin, A., & Ariful, I. M. (2013). A Study on Cytotoxic and Anthelmintic Activities of Crude Extracts of Leaves of *Clerodendrum Viscosum. International Research Journal of Pharmacy*, 4(1).

Shrivastava, N., & Patel, T. (2007). *Clerodendrum* and Healthcare: An Overview. *Medicinal and Aromatic Plant Science and Biotechnology*, 1(1), 142-150.

Steane, D. A., Scotland, R. W., Mabberley, D. J. & Olmstead, R. G. (1999). Molecular systematics of *Clerodendrum* (Lamiaceae): its sequences and total evidence. *American Journal of Botany*, 86, 98-107.

Steinbach, J. H., (2001). Modulation of GABAA receptor channel gating by pentobarbital. *Journal of Physiology.* 715–733.

Sumi, S. A., Biswas, N. N., Islam, M. K., & Ali, M. K. (2015). Evaluation of Analgesic and Antioxidant Properties in the Ethanolic Root Extract of *Clerodendrum viscosum* Vent. *International Journal of Pharma Sciences and Research (IJPSR).*

Talukdar, M. W., Akter, M.Y., Islam M. W., & Hassan, P. (2014). Insecticidal and repellent activity of *Clerodendrum viscosum* Vent. (Verbenaceae) against Tribolium castaneum (Herbst) (Coleoptera: tenebrionoidea). *Acad J Entomol*, 7,63-69.

Tippo, O., & Stern W. L. (1977). *Humanistic Botany.* New York, NY: Norton.

Trease, G. E., & Evans W. C. (2002). *Pharmacognosy*. (15th ed.). London: Saunders Publishers.

Van Wagenen, B. C., Larsen, R., Cardellina, J. H., Ran D. D., Lidert, Z. C. & Swithenbank, C., (1993). Ulosantoin, a potent insecticide from the sponge Ulosa ruetzleri. *J. Org. Chem*., 58, 335-337.

Verpoorte, R. (2000).Pharmacognosy in the new millennium: lead finding and biotechnology. *J Pharm Pharmacol*, 52, 253–262.

Wagstaff, S. J. & Olmstead, R. G. (1997). Phylogeny of Labiatae and Verbenaceae inferred from L sequences. *Systematic Botany*, 22(1), 165-169.

Warrier, P. K., Nambbiar, V. P. K., & Ramankutty C. (1996). Indian medicinal plants I. *Hyderabad: Aryavaidyasala, Orient Longman Publication*, 160.

Williamson, E. M., Okpako, D. T. & Evans, F. J. (1996). Selection, preparation and pharmacological evaluation of plant material. *Pharmacological methods in phytotherapy research*.

Yusuf, M., Chowdhury, J. U., Wahab, M. A., & Begum, J. (1994). Medicinal plants of Bangladesh. Dhaka. *Bangladesh Centre for Scientific and Industrial Research (BCSIR).*