Investigation of antidiarrheal and hypoglycemic activities of *Clerodendrum viscosum* root extract in mice

A project submitted
by
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This work is dedicated to my parents and brother for their love and support

Certification Statement

This is to certify that this project titled 'Investigation of antidiarrheal and hypoglycemic activities of *Clerodendrum viscosum* vent. root extract in mice' 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,

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Countersigned by the supervisor

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Abstract

Clerodendrum viscosum is broadly distributed all through tropical and subtropical areas of the world. The leaves of the plant have been broadly utilized as antidandruff, ascaricide, antipyretic, vermifuge, purgative. Leaves are also utilized in chest with cough problems and troublesome expectoration. Here in we report the antidiarrheal and hypoglycemic activities of the methanolic root extract of *Clerodendrum viscosum* for the first time. In this study, the preliminary phytochemical screening of methanolic root extracts of *Clerodendrum viscosum* showed the presence of flavonoids, phenolic compounds, alkaloids, tannin, steroids, glycosides and coumarins. Loperamide HCl was used as the reference standard. Reduction of diarrheal feces in mice was observed by 45.4% and 63.4% at 200 and 400 mg/kg body weight, respectively in comparison to Loperamide (78.1%). The methanolic root extracts of the plant showed strong antidiarrheal activity in castor oil induced diarrhea in mice. In the determination of hypoglycaemic activity, glibenclamide was used as the standard drug. Reduction of blood glucose level was observed by 23.8% and 27.3% at 200 and 400 mg/kg body weight, respectively in comparison to glibenclamide (39.3%). The above results estimated strong hypoglycaemic activity. Thus, it can be said that the root of *Clerodendrum viscosum* is a potential source of drugs for antidiarrheal and antidiabetic activity.

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AA	Ascorbic Acid
ABTS	2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonate)
B.aureus	*Boletus aureus*
C. brachystemon	*Clerodendrum brachystemon*
CCl_4	Carbon tetra chloride
CH_2Cl_2	Dichloromethane
DPPH	1, 1- diphenyl, 2-picryl hydrazyl
E.coli	*Escherichia coli*
FCR	Folin-Ciocalteu Reagent
FRS	Free Radical Scavenging
GA	Gallic acid
H_2O_2	Hydrogen peroxide
Kg	Kilogram
K.pneumonia	*Klebsiella pneumonia*
LA	Lignoceric acids
LC	Lethal Concentration
mg	Milligram
mL	Milliliter
mm	Millimeter
NO	Nitric oxide
OGTT	Oral Glucose Tolerance Test

SD	Standard deviation
S.subtilis	*Splendrillia subtilis*
STD	Standard sample
TAC	Total Antioxidant Capacity
TPC	Total Phenolic Content
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Plants and human are inseparable. The utilization of plants to treat human diseases is as aged as the expansion of human civilization itself. From the starting of human civilization, medicinal plants have played an important role for the welfare of human beings. In the development of human health, the use of natural product is tremendous from the ancient time. In the early stages, people chewed plant herbs to mitigate pain and used wrapped leaves for wound healing. Ancient people in search for nourishment and to cope effectively with human sufferings started to differentiate those plants appropriate for medicinal use with particular pharmacological activity from others (Shakya, 2016).

In recent years, tremendous changes have observed in the preliminary health care sector through the improvement of medical science and technology, but still 400 cores of men are totally reliant on herbal medicine. It is reported that even in the prosperous countries 25% of the prescription drugs come from natural plant sources and 75-80% of people in the world use herbal drugs for prime health facilities because of their better compatibility with human body (Rahman and Sarker, 2015).

1.2 Pharmacological aspect of plants

Plant and man are inseparable, because natural plants not only provide with shelter, food and medicine, but also the lifesaving oxygen gas. Medicinal plants work as therapeutic tool and crude materials for the formulation of traditional and modern drugs. Phytotherapy is a one kind of science-related natural practice. People use the plant extracts or the specific part of the plants for prevention of diseases. Phytotherapy has been utilized for treatment of people and animals since a large number of years. Phytotherapy keeps on assuming an important part in the current health sector, with about 80% of total population (Rates, 2001). With the development of human civilization, phytotherapy shows a stepwise development, which can be designed as-

1st stage: Crude drugs were prepared and employed as powdered willow in the management of pain.

2nd stage: These were transformed into more potential and manageable forms, for example, watery or alcoholic, extracts or solutions.

3rd stage: The pure active forms separated from crude were employed e.g. salicylic acid.

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

1.3 Medicinal plants

Plants that have therapeutic activities and beneficial pharmacological impacts on human body can be defined as medicinal plants. As per WHO (World Health Organization), "a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis" (Sofowora *et al*., 2013). A plant can be classified as medicinal when the plant is considered as useful therapeutic agent of a medicinal preparation. Medicinal plants can synthesize and accumulate secondary metabolites such as steroids, alkaloids, flavonoids, resins, tannins, glycosides, volatile oil, quinines. They are valuable for healing and in addition for relieve (a person or animal) of the symptoms of a disease or condition. Medicinal plants are one of the important contributors to the vast majority of the medicinal formulations as crude plant materials, raw materials etc. They play an important role in pharmaceutical health care sectors to rural area's people (Motaleb *et al.*, 2011). Medicinal plants play an important role in the economy of a nation.

Table 1.1: Medicinal plant species listed by WHO which can be grown in Bangladesh commercially

Scientific name	Bangali name	Used part	Used as patent drug
Aloe vera	Ghritokumari	Leaf	Tablet Suranjan, Tablet Mudir, Syrup Belgiri.
Adhatoda zeylanica	Vasak	Leaf, Stem, Bark, Root, Flower	Syrup Saduri, Chawan Prash, Tablet Sualin, Syrup Ajaj.
Andrographis panniculata	Kalomegh	Leaf, Stem, whole plant	Syrup safi, Syrup kurchi.
Asparagus racemosus	Satomuli	Tuberous root, Leaf, Flower, Fruit	Tablet Abiaj, Khisandha.
Glycyrrhiza glabra	Jastimodhu	Root, Stem	Tablet Sualin, Mauol Hiat, Syrup Badian, Tablet Kafur.
Plumbago zeylanica	Chita	Root	Majoon Falasefa, Syrup Kurchi.
Rauvolfia serpentine	Swarpagandha	Root	Syrup Mangurin.

1.3.1 History of medicinal plants

The history of medicinal plants for treating diseases had started from remote past for the preservation and mitigation of diseases. From the past records, Babylonians (about 3000 BC) were familiar with a great number of medicinal plants and their various therapeutic properties. Some plants are still being used in practically a similar way. From the evidence of Papyrus Ebers (1500 BC), the early Egyptians had enough knowledge about the medicinal activities of many plants. Some plants that are used as drugs in present times like mandrake (Mandragora officinarum), henbane (Hyoscyamus spp), pomegranate (punica

granatum), opium (latex of papaver somniferum fruit), castrol oil (oil of ricinus commiunis seeds), aloe were commonly used in Egypt around 4500 years ago (Ghani, 2005).

In the indian subcontinent, the medicinal use of various plants is obtained in Rig veda (4500-1600 BC) which established many plants with healing power including sarpagondha. The Pen taso (1122BC) in early Chinese pharmacopeia described that the chalmoogra oil were utilized in the leprosy treatment. The utilization of Ephedra species was first recorded by it. The Greek physician Hippcrocrates (460-370BC) "materia of medica" comprises of 300 to 400 traditional plants. For preparing different medicinal recipies, the physician and Greek pharmacist Galen (131-200AD) used various medicinal plants (Ghani, 2005).

Ibn sina and Al-Razi, the Arabian physician introduced a revolution in the history of medicinal plants. In the 13th and 14th centuries, the utilization of medicinal plants was established on Doctrine of signatures or by paraselus (1490-1541). Medicinal plants utilized by the Australian aborigines developed the set of medicinal plants all over the world. The current list indicates that more than thousands of medicinal plants are growing around the world (Ghani, 2005).

1.3.2 Significance of medicinal plants as traditional medicine

Traditional medicine has been described as easily accessible and affordable source of primary health sectors for the mitigation of diseases. The roots, leaves, stems and flowers of the plant contain chemical constituents as well as desired components. The use of plants as a source of medicine is an old practice. In the whole world, medicinal plant plays an important role in indigenous medical systems. In some developing nations, a great number of people rely on traditional medicines to obtain essential health care needs. Natural products play a significant role in all over the world for treating and mitigating human diseases. In the present era, almost every plants and herbs used as either important therapeutic agent or as necessary excipient in medicinal practises to improve the activity of ingredients. The sector of traditional medicine can be developed by introduction of new medicinal plant and exhibit beneficial property (Hosseinzadeh *et al.*, 2015).

Table 1.2: Some crude drugs used as medicine in Bangladesh (Ghani, 2003)

Bangali 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	*Paederia foetida*	Leaves	Diarrhea, uriticaria, paralysis, piles and toothache
Ghritokumari	*Aloe indica*	Leaves	Constipation, antihelmintic, 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	Insomnia, diabetes, cancer, blood pressure.
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 stomachache
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.

1.3.3 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 followings (Motaleb *et al.*, 2011).

1. **Alkaloid and amines**

    a. Pyridine group

    b. Tropane group

    c. Isoquiloline group

    d. Quinoline group

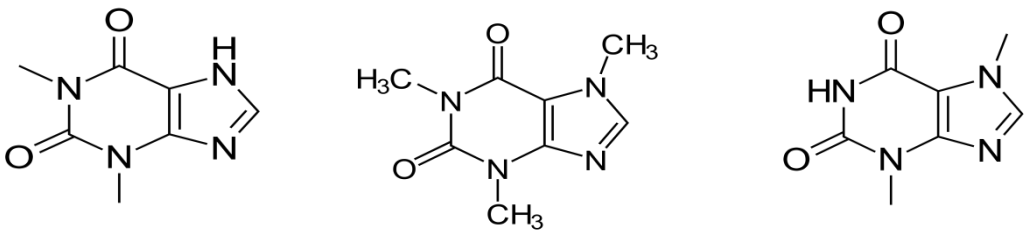
    e. Quinolizidile group

    f. Indole group

    g. Steroidal group

    h. Phenylethylamine group

    i. Alkaloid amines



Theophylline **Caffeine** **Theobromine**

Figure 1.1: Structure of some alkaloids and amines

2. **Glycosides:**

   a. Anthraquinone glycoside

   b. Cardiac glycoside

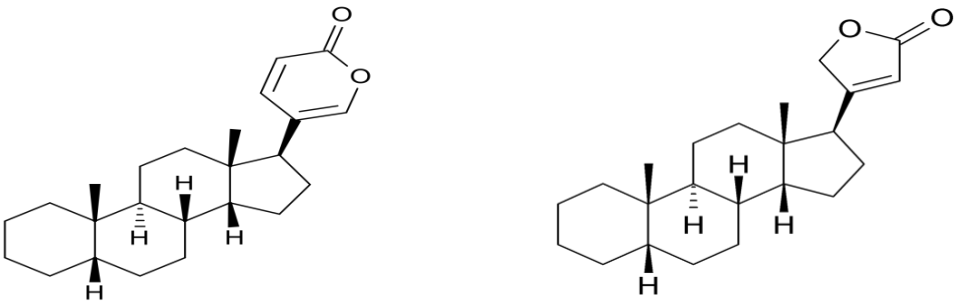
   c. Saponin glycoside

   d. Thiocyanate glycoside

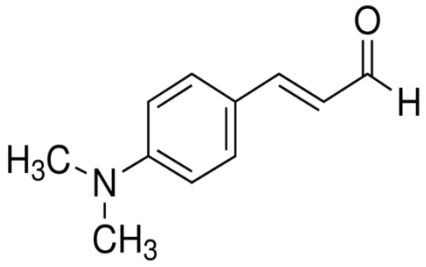
3. Volatile or essential oils

4. Fixed oils,

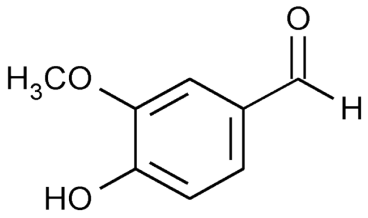
5. Gum-resins and mucilage.



Bufadienolide **Cardenolide**



Cinnamaldehyde



Vanillin

Figure 1.2: Some common glycosides and volatile oils

1.3.4 Contribution of medicinal plants to modern drugs

At present, different substances of plants are used in modern medical treatment:

- They are used as a direct source of active pharmaceutical drugs or agents – sometimes they are found as purified drugs such as morphine (extracted from the opium poppy Papaver somniferum) or as advanced extract from admixtures with other components or ingredients e.g. senna extract from *Cassia senna*.
- They are also found as blue-prints for the preparation of synthetic drugs of a same structure, for example the alkaloid of plant such as cocaine extracted from Erythroxylum coca which has given the chemical configeration for the synthesis of procaine and other anaesthetics related to it
- Help to understand pharmacological and physiological mechanisms in drug development.
- Medicinal plants are found in various dosage forms such as- pastes, powders, juices, decoctions, infusions in medicinal preparation.

Advancement of drugs from medicinal plants is sometimes time consuming and costly exercise. Pharmacological, phytochemical examination, clinical tests are important for developing drugs from natural plants. The stages include in improvement exercise –

- Selection of suitable medicinal plants and its extraction with proper solvent.
- Detection of natural activity of crude extracts and developing a bioassay framework for identification of active fraction.
- Fractionation of the crude extracts by utilizing phytochemical method and observed by biological tests identification.
- Isolation of the active compounds by chromatographic or other system and purification of compound by crystallization or repeated chemotherapy.
- Establishment of the chemical structures of the pure compound by various physicochemical techniques and determination of their biological activities by various pharmacological tests (Katiyar *et al.*, 2012)

1.4 Current status of medicinal plants

1.4.1 National status (medicinal plants of Bangladesh)

- There are about 297 Unani, 204 Ayurvedic and 77 Homeopatheic drug preparing companies in Bangladesh where the plants are utilized in semi–processed forms of medicine (Rahman & Sarker, 2015).
- The utilization of home grown drug is the most established and most common type of medicinal services in all ages in Bangladesh.
- About 70% of world population is getting their essential care of health through conventional and herbal medicines, as per WHO estimation.
- About 500 medicinal plants have been reported to occur in Bangladesh
- In Bangladesh, 75% of our populace pretty much utilize natural plants for essential healthcare.
- In Bangladesh around 550 plants species have been marked as medicinal plants having therapeutic effects of which 300 species are currently normally utilized as a part of the arrangement of traditional medicine.

Bangladesh, due to the favourable climate and location and is enriched with a large diversity of flora. About 5000 species of phanngrams and pteridophyties grow in its forests, waste lands and road sides as indigenous plants. Out of them more than a thousand have been claimed to have medicinal properties of which 546 have recently been identified with their medicinal activities and therapeutic values (Yusuf *et al.,* 1994).

Table 1.3: Medicinal plants of Bangladesh with their medicinal uses

Family	**Local name**	**Medicinal use**
Acanthaceae	Kalomegh	Bleeding piles
Anacardiaceae	Amra	Dysentery, diarrhea and vomiting
Apocynaceae	Tagarphul	Tonic to the brains, liver and spleen
Asteraceae	Gadhaphul	Rheumatism, cold, kidney troubles.
Bignoniaceae	Spathodea	Wound in healing specially burn healing
Bromeliaceae	Anaros	Antifungal, anti-inflammatory, obesity.
Caryophyllaceae	Gimashak	Useful for diabetic patient.
Combretaceae	Arjun	Heart disease.
Cucurbitaceae	Jhinga	Splenitis, haemorrhoides, ringworms,leprosy
Sapindaceae	Litchu	Tonic to the heart, brain and liver.

1.4.2 Medicinal plants of the world

Medicinal plants are comprehensively important origin of new drugs. Around 4300 plant species are said to exist in India of which 7500 plant species discover say in the recorded legends of India (Lakey and Dorji, 2016). There are more than 1300 therapeutic plants utilized as a part of Europe, of which 90 % are reaped from wild assets in the United States, around 118 of the main 150 physician endorsed medications depend on regular sources. Moreover, up to 80 % of individuals in developing nations are absolutely reliant on natural medications for their essential health services, and more than 25% of recommended prescriptions in developed nations are gotten from wild plant species (Laird, 2001).

1.5 Bioactivity guided research of medicinal plants

Bioactivity guided phytochemical examination of medicinal plants may yield more up to date chemical constituents of noteworthy therapeutic interest. Broad phytochemical examination and isolation of component(s) in the unadulterated form thus become noticeably important to evade untoward impacts and to assure safe utilization of herbal medications. With technological progression, phytochemical investigations of medicinal plants got a fast pace and the existence of numerous chemical constituents came into light. These plant-derived components can be used for synthesis of new classes of drug molecules.

One main strategy in the isolation of new leads consists of the so-called Bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds. 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, until pure compounds are obtained. Third, the chemical structures of pure compounds are determined (Mazen *et al*., 2010).

Sometimes phytochemical investigation of plants may get such chemical compounds having no marked therapeutic effects. The crude extracts of drug containing a few constituents might be observed to be ineffective in case of therapy for which it was utilized generally. For instance, *Vinca rosea*, once utilized traditionally as antidiabetic medication was found to contain hypoglycemic alkaloid standards in minute yet it was found to contain anticancer guideline vinca alkaloid in a high return. *Rauwolfia serpentina* which was traditionally utilized for variety of diseases, uncovered the presence of an antihypertensive and reserpine.

1.6 General description of *Clerodendrum viscosum*

Clerodendrum is broadly distributed all through tropical and subtropical areas of the world. *Clerodendrum* has around 580 species all through the Australia, Africa,America and Asia(Munir 1989). The genus of this plant was first illustrated by Linneus in 1753, in light

of the species of *Clerodendrum viscosum*. The name of the plant originates from two Greek words, 'Kleros' and 'dendron' (Warrier *et al.*, 1996). In Ayurvedic practise, *Clerodendrum viscosum* referred as "Bharangi" in Hindi and Bhrigubhava in Sanskrit .It is ordinarily known as Ghentu or hill glory blower in Bangladesh. It is additionally accessible in the tropical areas of Asia including Myanmar, India, Pakistan, SriLanka, Thailand.

The shrub of *Clerodendrum viscosum* is around 1–2m in height however may develop up to this length. It has branches and blunt quadrangular stems at completely developed portion of the plant. It contains alkaloids, saponion, flavonoid, lupeol, clerodendroside, lupeol, β-sitosterol and benzoic corrosive subsidiaries . It additionally includes clerodone, clerosterol and clerodolone. Plant leaves contain free reducing sugar, protein, tannin, oleic, glucuronide, stearic, and gallic acid and lignoceric acid. β-sitosterol, lupeol, antifungal flavonoids, quercetin and cabrubin have been found in plant roots (Ghani, 2003).

1.6.1 Classification

Kingdom: Plantae

Division: Angiospermae

Class: Magnoliopsida

Order: Lamiales

Family: Verbenaceae

Genus: Clerodendrum

Species: *Clerodendrum viscosum*

1.6.2 The plant family: Verbenaceae

Clerodendrum viscosum Vent is a prenatal bush of Verbenaceae family. Sometimes the members of the family known as Vervain or Verbena. The Verbenaceae are bushes, herbs or trees involving around 100 genera and 2,600 species that are further portrayed by the basic occurences of aromatic herbage and quadrangular twigs. The Verbenaceae is divided into four tribes which include Duranteae Benth. (Including Citharexyleae Briq.), Petreeae Briq.,

Verbenaceae Dumort. Lantaneae Endl. The individuals from the family are trees, bushes and herbs, flowers, spikes and many of them possess aromatic fragrance (Cantino *et al.*, 1992). The family is firmly identified with the Lamiaceae family. These families have various characters including zygomorphic corollas, opposite leaves and a bicarpellate ovary (Wagstaff and Olmstead 1997). Both families possess aromatic leaves. The traditional distinction between the two families is the ovary. The Lamiaceae have a profoundly four - lobed ovary while the ovary of Verbenaceae is unlobed.

1.6.3 Different names of *Clerodendrum viscosum*

Hindi Name- Thuner, Bhant

Malayalam- Cheruthekku

Bengali- Bhat

English- Glory tree

Sanskrit Name- Barhibarha, Sthauneya, Sukabarha, Sirnaroma, Kukkura Suka

Marathi- Bharangee, Bharang

Punjabi- Bhadangee

Telugu- Ganttubrarangee

Leaves

Flowers

Fruits

Figure 1.3: *Clerodendrum viscosum*

Table 1.4: Recent taxonomic revisions of the family includes the following genera:

• *Clerodendrum bungei* • *C. brachystemon* • *C. canescens* • *C. colebrookianum* • *C. chinense* • *C cyrtophyllum* • *C. confine* • *C. ervatamioides* • *C. formicarum* • *C. floribundum* • *C. garrettianum* • *C. fragrans* • *C. fortunatum* • *C. myricoides* • *C. intermedium* • *C. paniculatum*	• *C. globuliflorum* • *C. glabrum* • *C. griffithianum* • *C. henryi* • *C. hainanense* • *C. indicum* • *C. intermedium* • *C. infortunatum* • *C. japonicum* • *C. kiangsiense* • *C. kaichianum* • *C. kwangtungense* • *C. luteopunctatum* • *C. longilimbum* • *C. lindleyi* • *C. cyrtophyllum* • *C. ugandense*	• *C. mandarinorum* • *C. paniculatum L.* • *C. peii* • *C. phlomidis* • *C. quadriloculare.* • *C. speciosissimum* • *C. splendens* • *C. subscaposum* • *C. tibetanum* • *C. thomsoniae* • *C. tomentosum.* • *C. trichotomum* • *C. villosum* • *C. wallichii* • *C. yunnanense* • *C. chinense. Ianum* • *C. bungei Stued*

1.6.4 Description

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 oate, leathery and hardy with five petals and flower inflorescence (Nandi and Lyndem, 2015)

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

Fruits- Fruit products are 4 lobed purple durpe, 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).

Seeds- The seeds of the plant have greasy oil, in which the real unsaturated fats are oleic, linoleic and palmitic acid.

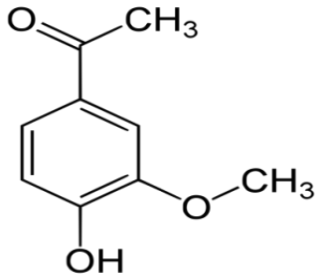
Cultivation-The bush develops on dry grounds and in an assortment of natural surroundings and soil. It will develop well in regions with low moisture. It is often developed as a hedge (Nandi and Lindem, 2015).

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

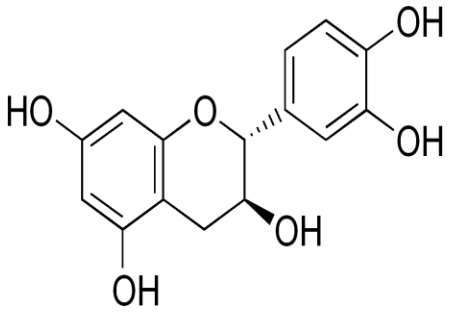
1.6.5 Chemical constituents of *Clerodendrum viscosum*

Clerodendrum viscosum contains alkaloid, flavonoids, saponin, lupeol, glycoside, benzoic corrosive subordinate, ß- sitosterol and clerodendroside. Leaves of the plant contain free reducing sugar, protein, oleic acid, a bitter principle, stearic and lignoceric acids, gallic acid and glucuronide. Roots also contain ß-sitosterol and lupeol, quercetin, cabruvin and the antifungal flavonoids. The seeds contain greasy oil, in which the real unsaturated fats

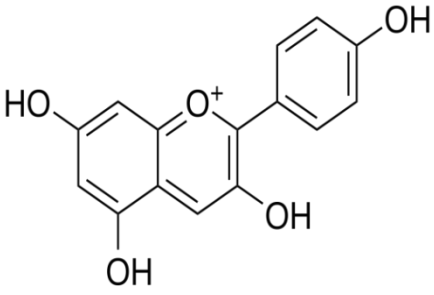
oleic, linoleic and palmitic acids. Hentriacontane and clerodin have been detached from blooms (Ghani, 2003). Different compounds of flavonoids e.g. quercetin, acacetin, apigenin, cabruvin, and scutellarin and various phenolic compounds, for example, stearate, fumerate, anthraquinones. Other components have also been originated from the root extract of plant such as clerodone, clerosterol etc.Other than steroids, some compounds e.g. scutellarin, acacetin, apigenin (Sinha *et al.*, 1981), saponin (Pal *et al.*, 2009), viscosene (Dutta-Choudhury *et al.*, 2009) have also been found (Nandi and Lindem, 2015)



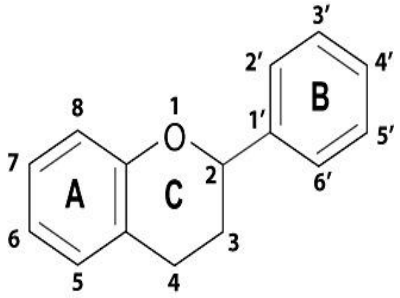
Apocynin



Catechin



Pelargonidine



Flavonoid

Figure 1.4: Some chemical constituents of *Clerodendrum viscosum*

1.6.6 Traditional uses of the plant *Clerodendrum viscosum*

The plant is used as antipyretic, tonic and anthelmintic. The root and leaf have been broadly utilized as tumors,asthma, antidandruff, ascaricide,antipyretic,vermifuge, purgative and in diagnosis of diabetes, shaking,gravel, scabies, malaria,skin infections, spasm,sore, scorpion sting, tumor and snake bite. Mixture of leaves is utilized as sharp antiperiodic and tonic in malaria. Leaves are likewise utilized in chest with cough problems and troublesome expectoration. Root squeeze alongside ginger is given to treat colic torment by the Garo in Bangladesh. In Thai solution the roots and leaves are known to be used as diuretic and utilized to treat infection in intestine and kidney disfunction (Sumi *et al.*,2015).

Phytocompounds of *Clerodendrum viscosum* have not been examined widely in pharmacology. A large number of the pharmacological practices recorded the trials of alcoholic and aqueous extracts as it were. Table 1.5 shows some pharmacological exercises noticed by various authors.

Table 1.5: Traditional uses of *Clerodendrum viscosum*

Plant part	**Traditional uses**	**Reference**
Whole plant	Asthma, bronchitis, diseases of the blood, fever, epilepsy, inflammation, burning sensation.	Modi *et al.,* (2010).
	Reduces blood sugar level, blood purifier.	Mohammed *et al.,* (2010).
	Anti-inflammatory, Anti-septic vermifuge, anti-pyretic, leprosy, expectorant.	Jirovetz *et al.,* (1999), Gouthamchandra *et al.,* (2010), Warrier *et al.,* (1996).
	Excellent laxative cholagogue, anthelmintic,anti-periodic, febrifuge, malarial fever, torpidity of liver, dysentery.	Haque *et al.,* (2010)

Leaves	As treatment of malaria, vermifuge. Skin diseases, inflammation, small pox. Anti-periodic, bitter tonic, laxative, pain killer. Traditional expectorant pills, tumours, scorpion stings.	Goswami *et al.,* (1998). Sannigrahi *et al.,* (2009). Gupta and Gupta (2012). Yusuf *et al.,* (1994).
Roots	Laxative, , analgesic, diuretic, anti-inflammatory, antibacterial, anti-tumour activities	Aley *et al.,* (2011).

1.7 Pharmacological activities of *Clerodendrum viscosum*

1.7.1 Anthelmintic activity

Shamsul *et al.,* (2013) examined the antihelmintic activity of aqueous and methanolic extract of *Clerodendrum viscosum* leaves against *Pheretima posthuma*. 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 antihelmintic 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 antihelminthic activity against the worms than the standard drug piperazine citrate (10 mg/mL).

1.7.2 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* by measuring the diameter of inhibition zones using folin-ciocalteu reagent (FCR), agar diffusion and aluminium chloride colorimetric method against different bacterial strains. In this study, 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 fraction of methanolic extract exhibited lowest antimicrobial action against *K.pneumonia, B.subtilis*. 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 methods showed that the extracts had antimicrobial action with different potency against the pathogenic microorganisms.

1.7.3 Antioxidant activity

Rahman *et al.,* (2011) conducted the in vitro antioxidant study 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 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), 1, 1- diphenyl, 2-picryl hydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) 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.

1.7.4 Insecticidal activity

Insecticidal effect of aqueous leaf extract of *Clerodendrum viscosum* against two tea pests such as *Oligonychus coffeae* and *Helopeltis theivora* were investigated by Roy *et al.,* (2010). 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 such as *Rhizopertha dominica, Sitophiulus oryzae, Tribolium castaneus* (Haque *et al.,* 2010), which showed that the *Rhizopertha dominica* and *Sitophiulus oryzae* had highest insecticidal action than the *Tribolium castaneus*.

1.7.5 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 500 mg/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.

1.7.6 Antihyperglycemic activity

Sayeed *et al.,* (2015) examined the antihyperglycemic 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 glucose level of diabetic mice were determined by injecting the methanolic extract of *Clerodendrum viscosum* leaves at two different doses of 250 and 500 mg per kg body weight. At the dose of 250 mg/kg significantly reduce at 1st hour to 3rd hour 541 to 470 mg/dl and for a dose of 500 mg/kg 130 to 36 mg/dl .In another study it is seen that in case of starved mice the methanolic concentrate of the leaves of the plant at the dose of 400mg/kg body weight reported reduction of blood glucose level. The methanolic extract and petroleum ether fractionate showed reduction in blood glucose level at 46.99% and 36.69% respectively (Panigrahi *et al.,* 2015).

Table 1.6: Common pharmacological activities of *Clerodendrum viscosum*

Pharmacological activities	Plant Part	Extract	References
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), 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 activity	Leaves, stems	Ethanol	Roy *et al.*, (2010), Waliullah *et al.*, (2014), Husain & Hasan (2008).
Analgesic	Roots	Ethanol	Pal *et al.*, (2009), Das *et al.*, (2011), Prasanth *et al.*, (2012), Sayeed *et al.*, (2015).
Antihyperglycemic	Leaves	Methanol	Sayeed *et al.*, (2015), Ahmed & Rahman (2014), Panigrahi *et al.*, (2015).
Anti-inflammatory	Leaves	Ethanol	R. and Rao (2013), Prasanth *et al.* (2012)
Antidiarrheal	Leaves	Ethanol	Rahman *et al.*, (2011)
Cytotoxic	Root	Methanol	Rahman *et al.*, (2013)
Antinociceptive	Leaves	Methanol	Khatry *et al.*, (2005)

1.7.7 Anti-inflammatory activity

To examine intense anti-inflammatory action of the ethanolic concentrate of *Clerodendrum viscosum* leaves, Chandrashekar and Rao (2013) used carrageenan induced paw oedema model in Wistar Albino rats. After administering ethanolic leaf extracts at measurements of 150 and 300 mg/kg the rate of inhibition of the oedema was 63.75 % at 3rd hour for the dose of 150 mg/kg and 46.30 % for the dose of 300 mg/kg indicated anti-inflammatory activity. It is seen in another report that the ethanolic concentrate of *Clerodendrum viscosum* roots was subjected to phytochemical examination. 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 *et al.*, 2012)

1.7.8 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 noteworthy degradation ($p< 0.001$) in gastric motility was seen in mice in charcoal test. The ethanolic extract likewise reduced the quantity of intestinal discharge by castor oil in test individuals ($p< 0.001$). Prevention of fluid secretion and gastrointestinal propulsion suggested that the leaf extract may have applied its antidiarrheal effect by antisecretory system.

1.7.9 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 with LC_{50} estimation of 0.773 µg/ml. This study recommends that the root extracts of *Clerodendrum viscosum* have some cytotoxic activity

1.8.10 Antinociceptive activity

Antinociceptive effect of methanolic plant concentrate was examined 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 Purpose of the study

From the starting of human civilization, medicinal plants have played an important role for the welfare of human beings. In the development of human health, the use of natural product is tremendous from the ancient time. In the early stages, people chewed plant herbs to mitigate pain and used wrapped leaves for wound healing. Medicinal plants are one of the important contributors to the vast majority of the medicinal formulations as crude plant materials, raw materials etc. *Clerodendrum viscosum* is a medicinal plant. The leaves of the plant have been broadly utilized as antidandruff, antipyretic,vermifuge. Many bioactive properties such as antioxidant, antimicrobial, insecticidal, analgesic, antihyperglycemic, anti-inflammatory, antidiarrheal, cytotoxic, antinociceptive, anthelmintic activity study of *Clerodendrum viscosum* was performed before. Most of the above mentioned activities are studied on leave extracts of *Clerodendrum viscosum* and no results were found for hypoglycemic and antidiarrheal activity of the root extract of the plant in previous findings. In this study, we investigated the antidiarrheal and hypoglycemic activity of *Clerodendrum viscosum*.

CHAPTER TWO

METHODOLOGY

2.1 Experimental design of *Clerodendrum viscosum*

A plant species representing to the family: *Clerodendrum viscosum* was investigated in this study.

Name of the 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 screening of *Clerodendrum viscosum*

2.2.1 Collection and preparation of the plant material

Clerodendrum viscosum was collected from Moshinda Charpara and identified (Accession No. 41878) by taxonomist of National Herbarium, Bangladesh located at Mirpur in Dhaka. The sample was preserved in the Phytochemical laboratory of BRAC University of Bangladesh. The roots were air dried for several days and then oven dried for 24 hours at considerably low temperature (not more than 40^0C) for better grinding. The dried roots were then ground to a coarse powder using high capacity grinding machine.

2.2.2 Extraction of the plant material

The powdered material of root (454gm) was taken in a conical flask and soaked in 1.5 L of methanol. The flask was sealed by aluminium foil and kept for a period of 2 days with occasional shaking and stirring. The whole mixtures were then filtered through a fresh cotton plug and finally with a filter paper and transfer the mixture to rotary evaporator for solvent evaporation at 37˚C.

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

Solvent-solvent partitioning was done by using Kupchan Partitioning method by Van Wagenen *et al.*, (1993). Firstly, 5 gm of the crude extract was taken in a 500 mL beaker. In another beaker 90 mL methanol and 10 mL warter was added and mixed properly. Then this mixture was added slowly in the crude extract (5gm) to dissolve it and made an aqueous methanolic solution. It was extracted with Petroleum ether, then with carbon tetrachloride and finally with dichloromethane. The whole partitioning process is schematically shown in Figure 2.1. All the five fractions were evaporated to dryness and were used for further analysis.

2.2.3.1 Partitioning with petroleum ether

The mother solution was taken in a separating funnel. 100 mL of the petroleum ether was added to it and the funnel was shaken for 5-10 min and then kept undisturbed for 10-20 min. The organic portion was collected. The process was repeated thrice and the fractions were evaporated together and the aqueous portion was taken in a separating funnel.

2.2.3.2 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 extracted with carbon tetrachloride and shaken for 5 min and kept undisturbed for 15-20 min. The process was repeated thrice (100 ml x 3). The carbon tetrachloride fractions were collected from the bottom layer and evaporated. The aqueous fraction was preserved for the next step.

2.2.3.3 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) (100 ml X 3). The CH_2Cl_2 fractions were collected from bottom layer and evaporated and aqueous part was preserved.

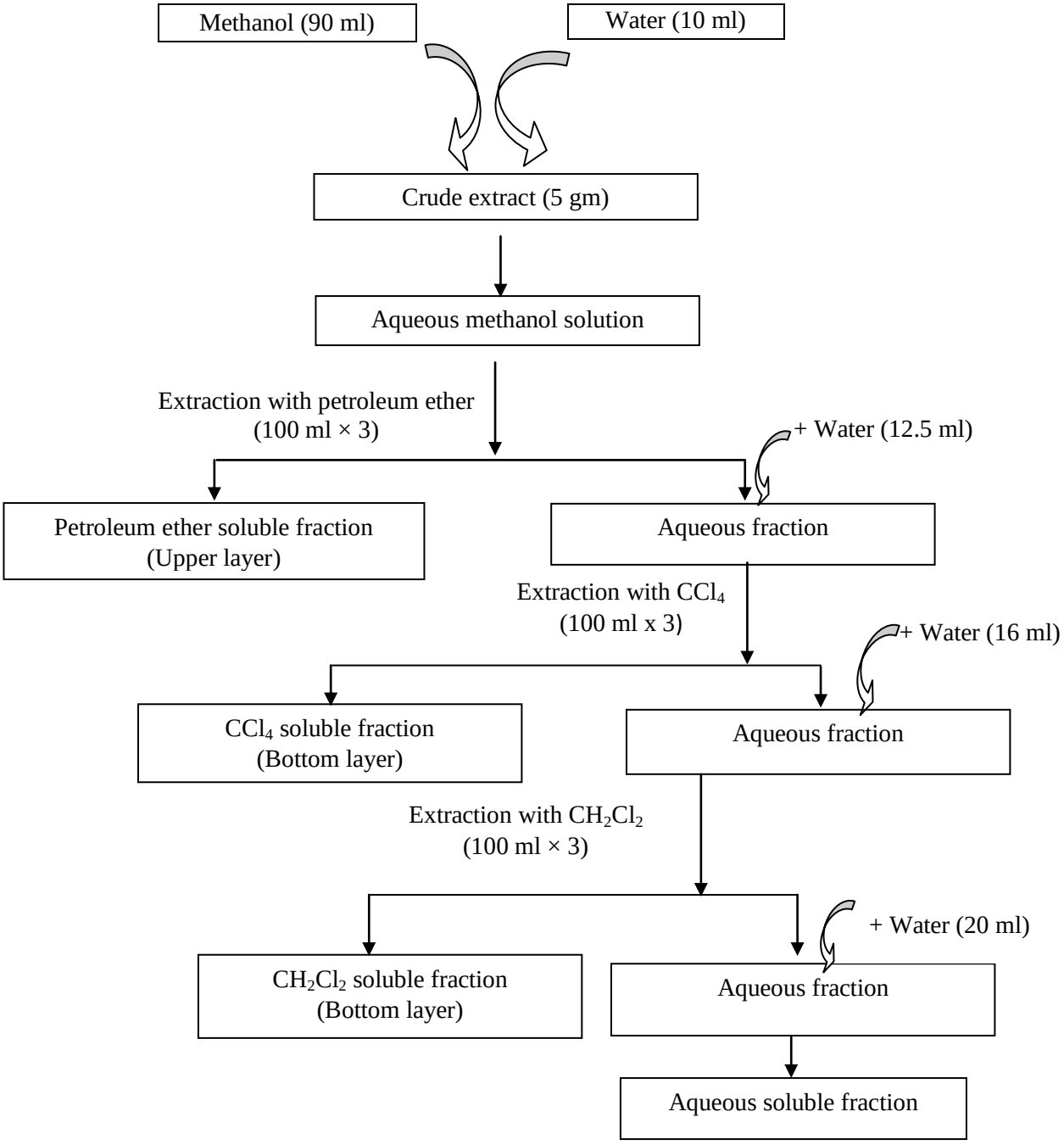


Figure 2.1: Diagram of modified Kupchan Partitioning of methanolic crude extract of *Clerodendrum viscosum*

2.3 Phytochemical screening

Phytochemical screening was performed on the crude extract of *Clerodendrum viscosum* in order to obtain its qualitative chemical composition such as alkaloids, glycosides, steroids, coumarins, tannins, flavonoids, phenols.

2.3.1. Test for alkaloids:

Mayer's test: According to Evans (1997), cream color precipitation was obtained after adding 1mL of each extract with few drops of Mayer's reagent (Potassium Mercuric Iodide Solution) which indicated the presence of alkaloid.

Wagner's test: To 1 mL of Wagner's reagent (Iodine in potassium iodide) was added to 1mL of each extract and formed reddish brown precipitate which indicated the presence of alkaloids.

Dragendorff's reagent test: 1ml of each extract was mixed with 2 mL of Dragendorff's reagent. Then the mixture was added with 2 mL of diluted hydrochloric acid and formed an orange colored precipitation which indicated that the alkaloids were present.

Hager's test: A bright yellow colored precipitation was obtained after adding 2 mL of each extract with few drops of Hager's (Saturated picric acid solution) reagent which indicated the presence of alkaloids.

Tannic acid test: The extracts were treated with 10% tannic acid and found a pale yellow-brown colored precipitation which conformed that the alkaloids were present.

$FeCl_3$ test: Few drops of ferric chloride solution were added with 2 mL of each extracts. Formation of yellow colored precipitation indicated that the alkaloids were present.

2.3.2 Test for glycosides:

Keller Killiani test: 1ml of the extracts were dissolved in equal amount of glacial acetic acid and left it for cooling and added 3 drops of ferric chloride into it. After that 2 mL of conc. sulfuric acid was added along the walls of the test tube. The presence of glycosides was confirmed by the formation of reddish brown colored ring at the junction of two layers.

Conc. H_2SO_4 test: 1mL of each extracts were treated with 1mL of conc. sulfuric acid and allowed to wait for 2 min. A reddish color precipitation was obtained which indicated the presence of glycosides.

Molish's test: 3 drops of molish reagent was mixed with the extracts and the mixture was treated with a few drops of conc. sulfuric acid. The presence of glycosides was confirmed by the formation of reddish-purple colored ring at the junction of two layers.

2.3.3 Tests for phenols:

Ellagic acid test: According to Evans (1997), muddy brown color was obtained after treating few drops of 5% (w/v) glacial acetic acid followed by 5% (w/v) sodium nitrite solution with the extracts which indicated the presence of phenols.

Phenol test: An intense color was developed after treating 2mL of the extracts with 1mL of ferric chloride solution which confirmed the presence of phenols**.**

2.3.4 Tests for tannins:

Ferric chloride test: Few drops of ferric chloride solution were treated with the extracts and the development of blackish precipitate indicated the presence of tannins.

Lead acetate test: To 1mL of extracts, few drops of 1% lead acetate solution and the formation of bulky red precipitate indicated the presence of tannins.

Alkaline Reagent test: Yellow to red color precipitation was obtained after treating 2 mL of extracts with a solution of sodium hydroxide which indicated the presence of tannins.

2.3.5 Test for flavonoids:

Zinc-HCl reduction test: To all the extracts, a pinch of zinc dust, few drops of conc. HCl was added. Formation of deep red color indicated the presence of flavonoids.

Lead- acetate test: A reddish brown color precipitation was obtained after adding 1 mL of all extracts with few drops of lead acetate solution which indicated the presence of flavonoids.

2.3.6 Test for coumarins: 2 mL of all extracts were taken in a separate test tube and covered with filter paper treated with 1 N sodium hydroxide solution and heated. Formation of a yellow fluorescence under the ultraviolet light indicated the presence of coumarins.

2.3.7 Test for sterols:

Liebermann Burchard's test: 1 mL of all the extracts was mixed with few drops of acetic anhydride solution. Then a few drops of conc. sulfuric acid were treated along the walls of test tube and the presence of steroids was confirmed by the formation of reddish brown ring at the junction.

Salkowski test: To 1mL of extract, 2mL of chloroform, 1mL of sulfuric acid were added. The appearance of reddish color indicated the presence of steroids.

2.4 Biological investigation

2.4.1 Anti-diarrheal activity

Principle

Castor oil induced method of diarrhea was used to evaluate the antidiarrheal activity of the root extract of *Clerodendrum viscosum* in mice. As per the method, each mouse was given 1mL of pure analytical quality of castor oil to induce diarrhea. The numbers of feces were noted for separate mouse. The observation of the treatment groups was compared with that of the positive control group to evaluate the antidiarrheal activity.

Experimental animal

For this experiment, Swiss-albino mice (young) with average weight of 25-35gm were needed. The mice were purchased from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). The mice were kept in suitable environmental condition in a temperature of 21±1°C with 12 h dark or light cycle and fed properly. The mice were treated with proper diet food. They were kept in a suitable environmental condition for 3-4 days due to their sensitivity to the changes of the environment. The ethics for use of experimental animals were followed carefully.

Figure 2.2: Swiss albino mice

Experimental design

Twelve mice were selected at random and divided them into four groups indicated as group I, II, III and IV consisting of three mice in individual groups. Each group was treated with particular treatment e.g. control, positive control or standard and methanolic crude extracts in two different doses (200 and 400 mg/kg body weight of *Clerodendrum viscosum* roots. Before starting the experiment, all the mice were weighted accurately and the control and test samples were accommodated properly.

Preparation of Test Materials

For the preparation of extracts at doses of 400 mg/kg body weight and 200 mg/kg body weight of mice, the extracts were weighted accurately and dissolved in 0.8 mL distilled water and orally administered to the mice. Loperamide HCl (2 mg/kg body weight) was used as standard. The standard was dissolved in 100 mL distilled water and administered to the mice orally.

Figure 2.3: Oral feeding of test sample to mice

Procedure

All the mice were divided into control, positive control and test group consisting of three mice in each group. In the experiment, group-I or the control group received distilled water at dose of 0.2 mL/kg orally. Loperamide HCl, a standard anti-motility drug (2mg/kg-body weight) was received by Group II or positive control group.

The test groups (Group III and Group IV) received methanolic root extracts of *Clerodendrun viscosum* at 200 and 400 mg/kg body weight orally. Individual mouse of each group were kept in individual case having absorptive paper under the cases. The absorptive paper was changed every hour. Castor oil was given at a dose of 1mL per mice 1 hour after the administration of root extracts, Loperamide HCl and water. The mice were examined for observing the effect of diarrhea every hour in 4 hr after administration of castor oil. Any fluid material or stools from mice are the signal of induction of diarrhea. Number of diarrheic feces that stained the adsorptive paper was computed at every successive hour and was noted for each mouse.

2.4 Hypoglycemic activity

Experimental animal

Swiss albino mice weighing about 25-35gm were taken from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B).

They were housed and controlled under the temperature of 22 ± 5°C with 35 to 60% humidity and maintained in 12-h dark/light cycle. The mice were treated with proper diet. Prior to the examination, they were accommodated to laboratory condition for 3-4 days. The research protocols were maintained under the guidelines of Institutional Animal Ethics Committee.

Experimental design

Twelve mice were divided into four groups with three mice in each. They consisted of negative control (group I), positive control (group II) and two treatment groups (group III and group IV). Each mouse was weighed appropriately before starting any treatment and the measurement of the control materials and samples were balanced accordingly. It was important to identify each mouse separately because three mice received the similar treatment at a time. The three mice in a group were denoted as M-1, M-2, and M-3.

Figure 2.4: Three mice in a group

Preparation of Test Materials

The standard Dibenol (Glibenclamide) was collected from Square Pharmaceutical Ltd., Bangladesh for this study. Glucometer (Accu-chek active) (Roche Diagnostic Corporation, Germany) glucostrips were used. Analytical graded chemicals were utilized for the study.

For the preparation of extracts at doses of 400 mg/kg body weight and 200 mg/kg body weight of mice, the extracts were weighted accurately and dissolved in dissolved in 0.8 mL distilled water and orally administered to the mice. For the preparation of Dibenol (Glibenclamide) at the dose of 5-mg/kg body weight, was dissolved in 100 mL distilled water and administered to the mice orally.

Procedure

The following procedures were maintained for the evaluation of hypoglycemic activity-

Figure 2.5: Pricking of mice's tail

Glucometer was used to collect blood samples at zero min, followed by fasting condition. Then the mice were treated orally with glucose at a dose of 1mL/kg body weight by using a long needle.

⇩

After 25 minutes, extract of two concentrations was administration into the test group, Glibenclamide solution into positive control group and water was administration into negative control group.

⇩

Samples of blood were taken from tail vein after 35, 60 and 120 min of glucose loading

⇩

Blood sugar level was determined by using glucometer.

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 above fractionates and 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	++	++	_	+
Steroides	Liebermann-Burchard test	++	++	++	+
	Salkowski test	++	++	++	++

3.2 Antidiarrheal activity

The methanolic extract of *Clerodendrum viscosum* root at 400 mg/kg and 200 mg/kg dose was used to determine the antidiarrheal activity (Table 3.2). The total number of diarrheal feces given by each mouse is shown in Table 3.3. Test samples of methanolic extract of *Clerodendrum viscosum* at 200 and 400 mg/kg body weight showed 45.4% and 63.4% reduction respectively in total number of diarrheal feces in comparison to the standard group (78.1%) (Table 3.4). Thus, the experimental finding suggested that the methanolic extract of *Clerodendrum viscosum* roots has strong antidiarrheal activity in comparison to standard Loperamide HCl (Figure 3.1).

Table 3.2: Study design in evaluation of antidiarrheal activity

Code no.	Test Samples	Group	Identification	Dose (mg/kg body weight)*
Control	Water	I	Control Group	0.1 ml/15 g of body weight
Standard	Loperamide HCl	II	Standard Group	2 mg/kg of body weight
MECV I	Methanolic extract of *Clerodendrum viscosum*	III	Test Sample	200 mg/kg of body weight
MECV II	Methanolic extract of *Clerodendrum viscosum*	IV	Test Sample	400 mg/kg of body weight

Table 3.3: Data showing the total number of diarrheal feces given by each mouse

Group	No. of mice	Number of diarrheal feces				Total	Mean
		1st hour	2nd hour	3rd hour	4th hour		
I(Control)	1 2 3	2 1 1	4 3 4	6 5 6	7 8 8	19 17 19	18.3
II(Standard)	1 2 3	0 0 0	0 1 0	1 2 1	2 3 2	3 6 3	4
III(Test group) 200mg/kg	1 2 3	0 1 0	1 2 1	3 4 3	4 6 5	8 13 9	10
IV(test group) 400mg/kg	1 2 3	0 0 0	1 2 1	2 3 2	3 3 3	6 8 6	6.7

Table 3.4: Effect of methanolic root extract on mice by the method of castor oil induced diarrhea

Treatment	**Dose (mg/kg body weight)**	**Number of diarrheal feces (Mean ± SD)**	**% reduction of diarrhea**
Control (water)	0.1 ml/15 g	18.3 ± 1.15	___
Standard	5 mg/kg	4 ± 1.73	78.1
Methanolic extract of *Clerodendrum viscosum*	200 mg/kg	10 ± 2.65	45.4
Methanolic extract of *Clerodendrum viscosum*	400 mg/kg	6.7 ± 1.15	63.4

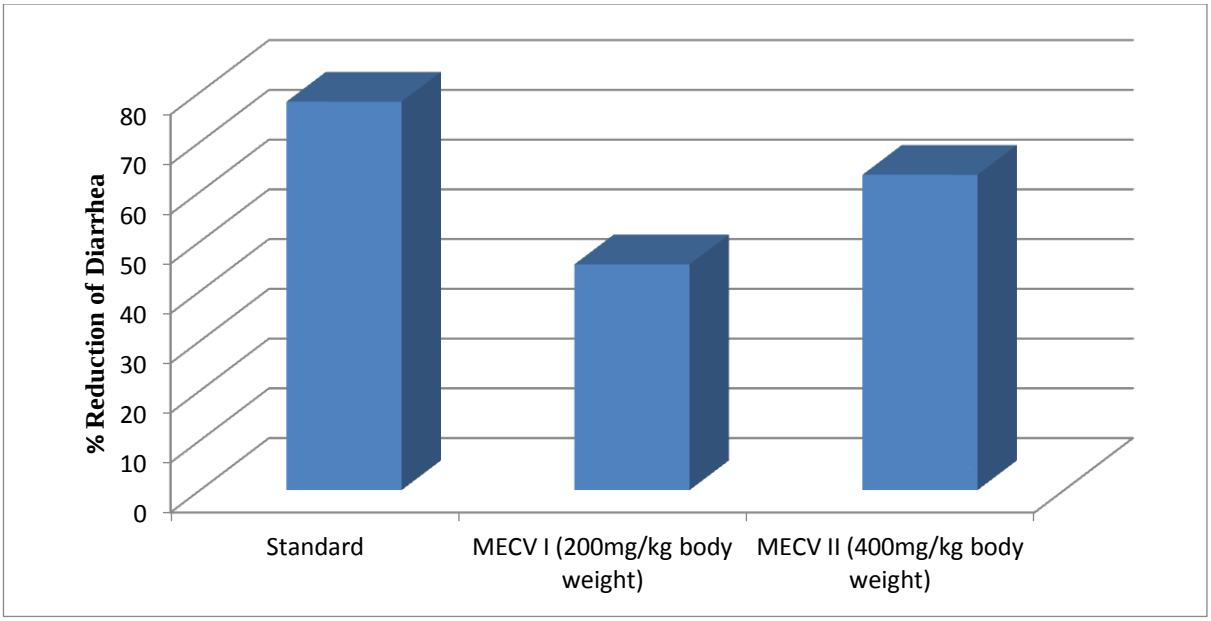


Figure 3.1: % reduction of diarrhea by different fractions of *Clerodendrum viscosum*

3.3 Hypoglycemic activity

Effects of methanolic extract of roots of *Clerodendrum viscosum* at 200 and 400 mg/kg dose on blood sugar level were monitored. Experimental design is shown in Table 3.5 and changes in blood sugar level (mmol/L) of mice at different time intervals are shown in Table 3.6.

Table 3.5: Test materials utilized for the evaluation of hypoglycemic activity of root of *Clerodendrum viscosum*

Code no.	Test Samples	Group	Identification	Dose (mg/kg)*
CT	Water	I	Control Group	0.2 mL/30 g of body weight
STD	Glibenclamide	II	Standard Group	10 mg/kg body weight
MECV I	Methanolic extract of *Clerodendrum viscosum*	III	Test Sample	200 mg/kg body weight
MECV II	Methanolic extract of *Clerodendrum viscosum*	IV	Test Sample	400 mg/kg body weight

Table 3.6: Change in blood sugar level (mmol/L) of mice at different time intervals

Group		0 Min		25 min		35 min		60 min		120 min	
		Data	Mean	Data	Mean	Data	Mean	Data	Mean	Data	Mean
Control	M-1	4.7	5.03	14.7	17.70	11.2	13.10	6.6	6.53	2.5	4.17
	M-2	4.4		19.2		10.6		4.9		3.9	
	M-3	6.0		19.2		17.5		8.1		6.1	
Standard	M-1	5.0	4.67	14.7	13.83	11.6	10.30	5.3	4.97	2.2	2.53
	M-2	4.6		15.6		14.5		4.0		2.2	
	M-3	4.4		11.2		4.8		5.6		3.2	
MECV I	M-1	4.7	3.87	12.1	16.17	11.2	12.47	6.4	5.67	3.2	3.17
	M-2	3.1		25.4		16.8		6.2		3.6	
	M-3	3.8		11.0		9.4		4.4		2.7	
MECV II	M-1	3.8	4.53	15.2	17.27	15.0	15.67	8.7	6.17	3.9	3.03
	M-2	4.7		25.4		18.7		4.5		2.5	
	M-3	5.1		11.2		13.3		5.3		2.7	

Table 3.7: Effect of methanolic root extract on mice by oral glucose tolerance test

Treatment	**Dose (mg/kg body weight)**	**Reduction of blood glucose level at 120 min (Mean ± SD)**	**% reduction of blood glucose level at 120 min**
Control (water)	0.1 ml/15 g	4.17 ± 1.8	–––
Standard	5 mg/kg	2.53 ± 0.6	39.3
MECV I	200 mg/kg	3.17 ± 0.5	23.8
MECV II	400 mg/kg	3.03 ± 0.8	27.3

Test samples of methanolic extract of *Clerodendrum viscosum* at 200 and 400 mg/kg body weight showed 23.8% and 27.3%reduction, respectivelyin blood glucose level in comparison to standard glibenclamide (39.3%). Thus, the experimental finding suggested that the methanolic extract of *Clerodendrum viscosum* root has strong hypoglycemic

activity

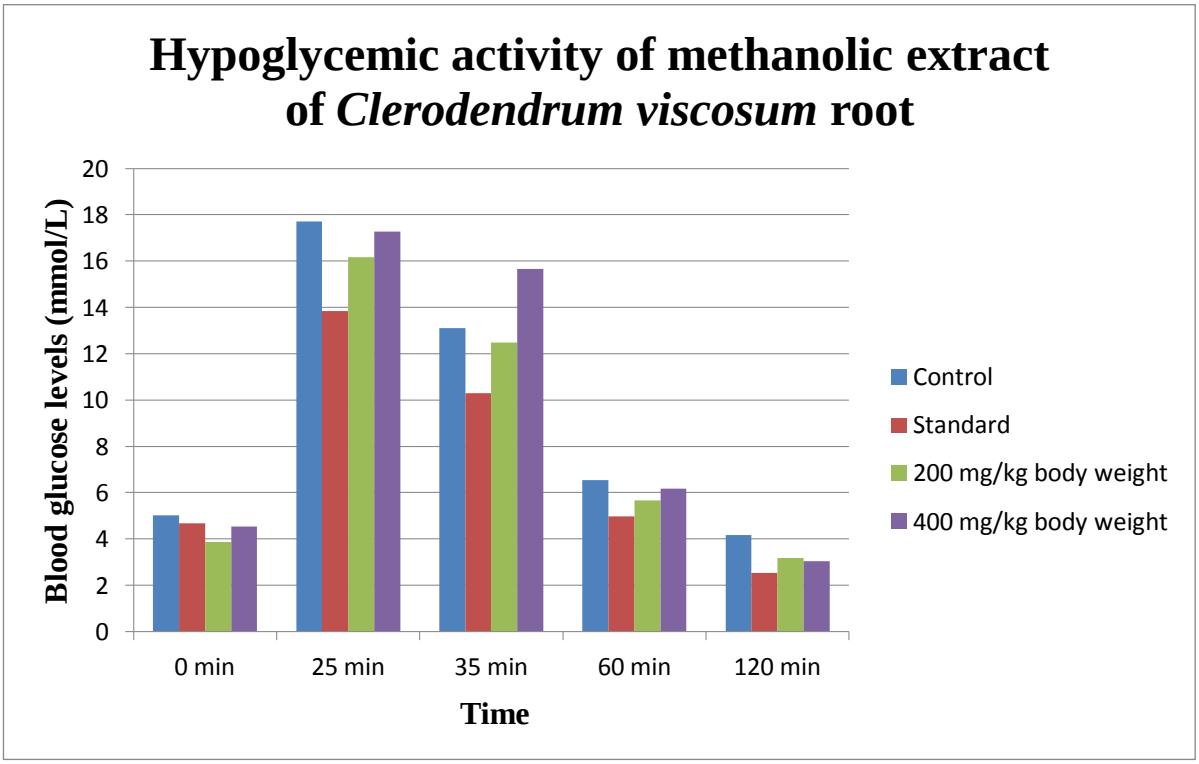


Figure 3.2: Glucose level of mice at different times

CHAPTER FOUR

DISCUSSION

Clerodendrum viscosum is a traditional herbal medicine. The plant is broadly distributed all through tropical and subtropical areas of the world. The young leave extract of the plant is used in the treatment of diarrhea, stomachache, dysentery and the root paste is used in dental caries. Leaves are also utilized in chest with cough problems and troublesome expectoration. Root of the plant is used to treat colic torment by the Garo in Bangladesh.

Abnormal passage of fluid or stool at an extended frequency is defined as loose bowels or diarrhea: animal and plant toxin, infectious agents, gastrointestinal disorders that increase gastrointestinal tract secretions can triggers the effect of diarrhea. Diarrhea is usually caused by different types of viral, bacterial and parasitic organisms. Castor oil produces diarrhea due to the presence of ricinolic acid, an active metabolite, stimulates peristaltic movement in the small digestive tract, promoting changes in the permeability of electrolytes in intestinal mucosa. It also increases the release of prostaglandin which stimulates gastrointestinal secretion and motility. It also stimulates the release of prostaglandin (Rahman *et al.,* 2011). Previous studies have reported the antidiarrheal activity of flavonoids, tannins, alkaloids, terpenes and sterols containing plant extracts.

In current study, antidiarrheal activity was examined on mice by using the method of castor oil induced diarrhea where root extracts were used to determine the amount of reduction of diarrheal feces. Methanolic extract of *Clerodendrum viscosum* roots showed the reduction of diarrheal feces in mice by 45.4% and 63.4% at 200 and 400 mg/kg doses. Loperamide HCl was used as positive control or standard in the present study. The present study exhibited that methanolic root extract of *Clerodendrum viscosum* at a dose of 200 and 400 mg/kg causes degradation of diarrheal feces compared to Loperamide HCl (78.1%). Phytochemical screening of the root extract of *Clerdendrum viscosum* in the present study showed the presence of alkaloids, glycosides, phenols, tannins, flavonoids, coumarins and steroids. It was revealed in different literature that the plant derived compound such as flavonoids and phenols were responsible for the antidiarrheal properties (Rahman *et al.,* 2011). Thus the antidiarrheal activity of the methanolic root extract of *Clerodendrum viscosum* was obtained due to the presence of the above compounds.

Diabetes mellitus is an endocrine disorder found in both men and women. It is the most widely known heterogenous metabolic disorder, which affects 143 million individuals and its occurrence is expanding consistently with changes the way of life. Many oral hypoglycemic drugs such as biguanides and sulphonylurea are used for treatment of diabetes. Some drugs exhibit side effects so that researchers have already started their research on different herbal agents which are getting popularity in the treatment of diabetes mellitus (Kayarohanam & Kavimani, 2015).

The current study evaluated the hypoglycaemic action of methanolic root extract of *Clerodendrum viscosum* and a strong reduction in the blood glucose level was found from 25 min to 120 min after the oral administration of glucose. Literature search showed that flavonoids, phenols and coumarines exhibit hypoglycemic activity. That is why, the root extract of *Clerodendrum viscosum* showed strong hypoglycemic activity. Further study of the plant may establish its antidiarrheal and antidiabetic activity.

CHAPTER FIVE

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