

Investigation of *in-vivo* antidiarrheal and *in-vitro* insecticidal activity of *Trewia nudiflora* leaf extracts

A project submitted

by

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Inspiring Excellence

Dhaka, Bangladesh

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This work is dedicated to my parents and siblings for their love and constant

Support... ..

Certification Statement

This is to certify that this project titled **“Investigation of *In-vivo*antidiarrhoeal and insecticidal activity of *Trewianudiflora* leaf extract”** submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of FarhanaAlamRipa, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the foreign language, thoughts or writings of another.

Signed,

Countersigned by the supervisor

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SayezaYeara Duyti

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Abstract:

Trewianudiflorais the plant that we choose for our thesis work. From the previous experiment it has been seen that leaf extracts of this pant contains tannins, cardiac glycosides, flavonoids etc and many more. These components shows different kind of phamacological action against various disease which is clinically proven. Keeping this in mind our current study was designed to find out Anti-diarrheal and Insecticidal activity from different solvent extracts (ETE-Ethanol, EAE-Ethyl-acetate, and CHE-Chloroform) of leaves of this plant.

Diarrhea is one of the alarming disease of Bangladesh. Diarrhea is defined by the World Health Organization(WHO) as having three or more loose or liquid stools per day, or as having more stools than is normal for that person. Sometimes this secretion is so high that it may threaten people's life especially children. Our study was aimed to investigate of anti-diarrheal effect and insecticidal effect of *Trewia nudiflora* plant leaf extracts These chemical components are actually pharmacologically active against different diseases. As this plant contains tannins and flavonoids, so it can be assumed that it can be helpful to tighten intestinal motility. To run the experiment we took ETE, EAE and CHE extract of the specified leaf. Two methods were followed to investigate antidiarrheal effect. They were: castor oil and magnesium sulphate induced diarrhea in the albino mice. In both cases we found that EAE extracts at 400mg/ml shows the best result(90.2%and 88.65%) comparing to the standard (Loperamide 5mg/kg) drugs. So we can assume that EAE extracts of this leaf shows the maximum results which is approximate to the alopathy medicine. As it is natural so it will not possesses such side-effects though we increase the dose.

Bangladesh is an agricultural country. Around 65% people's earning way is farming. Rice is the main crop that our farmers wish to grow in huge amounts. But, paddy sometimes attacked by the insects which in turn causes huge losses. Farmers are using pesticides which are not eco-friendly as well as costly also. If we can replace these pesticides with some natural remedies it will be beneficial for both farmers and environment. We followed Abbot's law to get the result and after plotting it in the graph it is clearly seen that EAE at highest dose gives the best result(83%).So, above experimental results indicate that bioactive compounds are present in those three extracts of *Trewia nudiflora* leaves including antidiarrheal activity and insecticidal activity and could be accounted for pharmacological effects.

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List of Abreviation:

WHO: World Health Organization

ETE: Ethanol extract

EAE: Ethyl-acetate extract

CHE: Chloroform extract

mg: Miligram

ml: Mililiter

Chapter 1

Introduction

1.1.General introduction:

Human are directly or indirectly dependent on plants for their own interest. From leaf to bark each and every part of plant have individual and unique uses. Some may be used as food, some may used as core ingredient in herbal medicine.

From the prehistoric stages people used plants specially medicinal plants for curing different kind of diseases. Though dramatically changes occurs due to the development of science specially in medical science and technology to meet up the requirements of over populated world. But, till today 400 cores of people of the world is totally dependent on medicinal plants for their treatment (Hasan et al, 2014). It is uncovered that even in the developed nations 25%, of the recommended drugs originate from plant sources and natural prescriptions are utilized by around 75-80% of the world's population for essential medicinal services because of their better social agreeableness, better similarity with human body and lesser reactions (Hasan et al, 2014).

The way how a medicinal plant work is still unknown. But, some scientist assumes that the whole plant or some part of plant work together to possess pharmacological effect on human as well as on other plants to save them from insects. The therapeutic properties of medicinal plants are accustomed by the presence of various kind of active substances like: alkaloids, tannins, flavonoids, vitamins and so on. These compounds physiologically affect the bodies of humans and animals or which is biologically active in relation to cause some disease also (Ghani, 1998).

1.1.1.Medicinal plants:

Literally, medicinal plants refer those plants which show pharmacological activity in animals or insects or even in human health. It can be defined in another way like, plants whose roots, leaves, bark or other constituent possesses therapeutic, tonic, purgative, or other pharmacologic activity when administered to animals are known as medicinal plants.

A number of definitions have been stated for medicinal plants. According to the definition of medicinal plants has been stated by WHO (World Health Organization)-

“A medicinal plants is any plant which is one or more of its organ, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs.”(Sofwara,1982).

In another way it can be stated that-“Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes”.

These therapeutic plants considered as a rich assets of fixings which can be utilized as a part of medication advancement and blend. Other than that these plants assume a basic part in the improvement of human societies around the entire world. Medicinal plants every now and again utilized as crude materials for extraction of dynamic fixings which utilized as a part of the incorporation of various medications (Sing, 2015).

1.1.2. The active constituents of medicinal plants:

Huge numbers of the plants could be utilized as stimulants, toxins, drugs or as pharmaceutical due to the vicinity of one of a kind or rich organic dynamic plant chemicals(i.e. Concoction aggravates that biologically affect other life form). Chemicals that make a plant significant as restorative plants seem to be-

- (1) Alkaloids (mixes has addictive or torment killing or toxic impact and in some cases help in vital cures)
- (2) Glycosides (use as heart stimulant or extreme laxative or better sexual wellbeing)
- (3) Tannins (utilized for gastro-intestinal issues like looseness of the bowels, diarrhea, ulcer and for wounds and skin infections)
- (4) Volatile/crucial oils (improve gluttony and encourage absorption or use as clean/bug spray and creepy crawly repellent properties)
- (5) Fixed oils (present in seeds and natural products could lessen gastric/sharpness)
- (6) Gum-saps and adhesive (have pain relieving property that suppress irritation and secure influenced tissues against further harm and cause gentle laxative) and
- (7) Vitamins and minerals - (Fruits and vegetables are the wellsprings of vitamins and minerals and these are utilized prevalently as a part of herbals).

There is a short list of some drugs whose active components are derived from medicinal plants:

Table: 1.1. Drugs derived from medicinal plants used to cure different diseases

Drug	Source	Disease
Vinblastine	Rosy periwinkle	Hodgkins' disease
Vincristine	Rosy periwinkle	Leukemia
Tubocurarine	Chondodendron tomentosum	Muscle relaxant
Quinine	Cinchona ledgeriana	Anti-malarial
Pilocarpine	Jaborandi	Glaucoma
Morphine	Opium poppy	Analgesic
Scopolamine	Hyoscyamus niger	Motion sickness
Taxol	Pacific yew	Ovarian cancer
Erythromycin	tropical fungi	anti-biotic

1.1.3. Characteristics of medicinal plants:

There are two different types of characteristics of medicinal plants. They are:

- Synergic medicine: The ingredients of plants all interact instantaneously, so their uses can match or damage others or neutralize their possible negative effects. Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective (Sing, 2015).
- Preventive medicine- It has been proven that the constituent of the plants also characterize by their capability to prevent the presence of some diseases. This will help to reduce the use of the chemical therapies which will be used when the disease is already present (Sing, 2015).

1.1.4. Herbal medicine:

Herbal medicine sometimes referred to as “Herbalism” which involves the use of whole plant or plant parts like leaf, bark, steams, roots, flowers or seeds etc (Sturluson,2014). At very beginning of using herbal medicine includes to consume them as raw juices or soups. Then, people started to dried and crushed them for more convenient use and used them as medicine. But when modern science started to give a closer look the use of herbal medicine become so

refined. Now a days the physicians or herbal practitioners use them for common or not so common disorder remedies.

The use of plants for healing purposes go before human history and forms the origin of much modern medicine (Vicker et al, 1999). Many traditional drugs invented from plant source, a century ago, most of the efficient drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) (Vickers et al, 1999).

1.1.5. Uses of herbal edicine:

Herbal medicine is used to treat many conditions like: allergies, asthma, eczema, premenstrual syndrome, rheumatoid arthritis, fibromyalgia, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and recently includes cancer. Some common herbs and their uses are discussed below (Umashanker et al, 2011):

Table: 1.2. Common herbs with their uses

Name	Scientific name	Parts used	Medicinal use
1. Tulsi	<i>Ocimum sanctum</i>	Leaves	Antipyretic; antitussive
2. Neem	<i>Azadirachtan indica</i>	Dried bark extract	Gastrointestinal disease, leprosy, respiratory disorders
3. Aamla	<i>Emblica officinalis</i>	Fruit extract	Antiulcer, hair growth.
4. Ginger	<i>Zingiber officinale</i>	Rhizome	Antidiabetic, anticancer
5. Green tea	<i>Camellia sinensis</i>	Leaf extract	Antidiabetic
6. Cashew	<i>Anacardium occidentale</i>	Fruit, seed bark oil	Antipyretic, irritant;

1.1.6. Brief history of herbal medicine:

No one knows for sure can say that at what time medicine herbal had been used. The first written record was observed at 2800 B.C. in China (Sturluson, 2014). But, the first pictorial evidence of herbal medicine, on the walls discovered in France in between .13000 to 25,000 BC (Wilde, 2010).

Every culture and country has their own history of developing their evolution of herbal medicine. Some of them are given below:

Mesopotemia:

The primary composed record of herbs utilized as medications was five thousands years back by the Sumerians, in old Mesopotamia (present day Iraq). Sumerian solutions for mending utilizing herbs, for example: caraway and thyme have been found by archaeologists on tablets made of earth. At about the same time, and maybe significantly prior, home grown customs were being produced in China and India.

China:

The bases of Chinese medication, which is construct generally with respect to herbalism, likewise go back around 5,000 years prior. The Chinese head Chi'en Nung set up together a book of therapeutic plants (a "home grown") called Pen Tsao. It contained more than 300 herbs including Chinese ephedra, which is still generally utilized today and is the herb from which later changed as ephedrine medications.

India:

The bases of Indian medication were put forward in the blessed works called the Vedas, which go back similarly as the second century BC. The Indian arrangement of prescription was known as the ayurveda. As right on time as 800 BC one Indian author knew 500 therapeutic plants and another knew 760-every single indigenous plant of India. Indian herbalism or ayurveda is still sharpen today, and numerous genuine, conventional definitions are accessible outside of India.

Greeks and Romans:

The Greeks and Romans imitative a lot of their natural actualities from these early civic establishments. Primordial Greece was incredibly affected by Babylonia (or Mesopotamia), Egypt, and to some degree by India and China.

1.1.7. Efficacy and safety of herbal remedies:

In suggestion to medications, safety is the possibility of not causing harm under the anticipated conditions of use, while efficacy is the capability to persuade a clinical assistance. Both safety and efficacy depend on the drug's therapeutic indication; in principle, a substance has no clinical usefulness if it is 'safe' but lacks efficacy or if it is active on appropriate therapeutic target but its use is risky (Moreira et al, 2014).

The investigative criteria for causal affiliations incorporate biological plausibility, consistency of different kind of research results, measurement reaction impacts, reproducibility of the research in distinctive connections utilizing diverse systems, the quality of the affiliation, and the cause and effects that experimented in vivo (Woolf, 2003). Using these criteria some medicinal plants have shown their efficacy on human as well as on other animate thing. For example: tea tree oil has been found to inhibit the growth of certain dermatophytes and may be useful for fungal skin conditions (Woolf, 2003).

Chemical constituents are another main cause which posses medicinal activity. Chemicals like: alkaloids (have addictive or poisonous off effect), glycoside (use to treat as heart stimulant), tannins (used for treating Gastro-intestinal problem), volatile oils (enhance appetite and improve digestion), fixed oil (present in seeds or fruits use to decrease gastric activity (Sharmin, 2004).

1.1.8. Different forms of administration of medicinal plants:

The definite organization of herbal item is affected by the strategy of extraction. A tisane will be rich in polar segments since water is a polar dissolvable. Oil then again is a non-polar dissolvable and it will assimilate non-polar mixes. Liquor lies some place in the middle. There are numerous structures in which herbs can be regulated, these include:

Tinctures-Alcoholic extracts of herbs such as Echinacea extract. Usually obtained by combining 100% pure ethanol (or a mixture of 100% ethanol with water) with the herb. A completed tincture has a ethanol percentage of at least 25% (sometimes up to 90%). The term tincture is sometimes applied to preparations using other solvents than ethanol (Dharmananda, 1997).

Herbal wine and elixirs- Herbal wine and elixirs are alcoholic extract of herbs; generally with an ethanol with the percentage of 12-38% .Herbal wine is a maceration of herbs in wine, while an elixir is a maceration of herbs in spirits (vodka, grappa, etc.) (Dharmananda, 1997).

Tisanes-Hot water extracts of herb, such as chamomile.

Decoctions- When we boiled barks and roots for long time decoctions are formed (Dharmananda, 1997).

Macerates-It is made up by cold blend of plants with high mucilage-content as sage, thyme, etc. Plants are cut into pieces and mixed to cold water. They are then keep to stand for 7 to 12 hours (depending on herb used). For most macerates 10 hours is usually preferred (Dharmananda, 1997).

Vinegars-It is made likewise as tinctures, but here a solution of acetic acid is used as the solvent. (Dharmananda, 1997).

Topicals -

- ✓ **Essential oils**- Use of crucial oil extricates, normally weakened in a transporter oil (numerous fundamental oils can smolder the skin or are just too high measurement utilized straight – weakening in olive oil or another nourishment grade oil, for example, almond oil can permit these to be utilized securely as a topical). Treatments, oils, emollients, creams and salves - Most topical applications are oil extractions of herbs. Taking a nourishment grade oil and absorbing herbs it for anyplace from weeks to months permits certain phytochemicals to be extricated into the oil. This oil can then be made into treatments, creams, salves, or just utilized as an oil for topical application. Any back rub oils, antibacterial treatments and wound recuperating mixes are made along these lines.

- ✓ **Poultices and compresses** - A poultice or compress are made by using entire herb (or the proper part of the plant) typically crushed or dried and re-hydrated with a little amount of water and then applied straight in a dressing, cloth or just as is.

Whole herb utilization—whole herb is utilized by either in dehydrated form (herbal powder), or fresh juice, (fresh leaves and other plant parts). (Dharmananda, 1997).

Syrups -Extracts of herbs made with syrup or honey. 65 parts of sugar are mixed with 35 parts of water and herb. Then they are boiled and macerated for around three weeks.

Extracts- Comprises of liquid extracts, dry extracts and nebulizes. Fluid concentrates are made up with fluids with a lower ethanol rate than tinctures. They can (and are generally) made by vacuum refining tinctures. Dry concentrates are concentrates of plant material which are vanished into a dry mass. They can then be further refined to a container or tablet. A nebulisate is a dry concentrate made by stop drying (Dharmananda, 1997).

Inhalation - As in aromatherapy breathing can be used as a mood changing action to fight a sinus disease or cough, or to cleanse the skin on a deeper level (steam rather than direct inhalation here).

1.1.9. Future of medicinal plants:

Eventual fate of Medicinal plants have a promising future on the grounds that there are about half million plants far and wide, and the greater part of them their healing exercises have not explore yet, and their healing exercises could be definitive in the treatment of present or future studies (Hasan,2012).

1.1.10. Aspects of Herbal medicine in Bangladesh:

About 25.6% people of Bangladesh live beneath the poverty line (Begum, 2015). Majority of them live in rural Bangladesh. Somehow they live from hand to mouth. With their limited income they can hardly satisfy their hunger. They are not capable of enjoying modern health facilities since it involves huge expenditure. Besides these, their food habit, pattern of dress, economy, belief system, norms and values etc. are very much traditional. Because of belief system and traditionalism, vast majorities of the rural people are still inclined towards folk-treatments, although rich and educated people are comparatively depended upon modern

medical system. As a matter of fact, rural people usually consult quack doctors and folk physicians.

Then again, Bangladesh has exceptionally rich in Bio-differing qualities. It has more than 500 restorative plants species (Yusuf et al, 1994). Medicinal plants essentially utilized as a part of the arrangements of unani and ayurvedic drug, likewise recommended by specialists of conventional prescription in distinctive parts of the nation and others are utilized as family unit cures by the basic individuals. A plant which has dynamic constituents of medicinal properties and is utilized to treat disorder/sickness in diverse frameworks of pharmaceutical or generally utilized for the treatment of infection/ailment is considered as therapeutic plant. Plants have been utilized as pharmaceuticals from the antiquated time. Medicinal plants are generally and effectively utilized on each mainland. In Asia, the act of home grown prescription is greatly settled and reported. Therefore, a large portion of the therapeutic plants that have global acknowledgment originated from this district. Plants, plant parts and plant items served as the materials for the readiness of solution and these restorative plants and plant parts constitute a vital common abundance of a nation. They assume a noteworthy part in essential medicinal services administration to country individuals.

In Bangladesh there are around 297 unani, 204 ayurvedic and 77 homeopathic drug producing commercial ventures where the therapeutic plants are broadly utilized as a part of both crude and semi-prepared types of medication in different pharmaceutical measurement details. These plants likewise serve as vital crude materials for some cutting edge restorative arrangements. The business sector estimation of medications created by these commercial enterprises from therapeutic plants is about Tk. 300 cores (The Daily Jugantor, 2003).

1.1.11. Traditional medicine:







Traditional medication is the whole of information, aptitudes and rehearses in view of the hypotheses, convictions and encounters indigenous to distinctive societies that are utilized to look after wellbeing, and also to anticipate, analyze, enhance or treat physical and emotional sicknesses. Conventional drug that has been received by different populaces (outside its indigenous society) is regularly termed option or corresponding prescription. Natural solutions incorporate herbs, home grown materials, home grown arrangements, and completed home grown items that contain parts of plants or other plant materials as dynamic fixings.

In some Asian and African nations, 80% of the populace relies on upon conventional solution for essential medicinal services. In numerous created nations, 70% to 80% of the populace has utilized some type of option or corresponding medication (e.g. needle therapy).

Table: 1.3. Some medicinal plants of Bangladesh:

Plant	Common name / Maturity period	Botanical Name or Family	Parts Used	Medicinal Uses
	Bael / Bilva After 4-5 year.	<i>Aeglemarmelous</i> Fam: Rutaccac	Fruit, Bark	Diarrhoea, Dysentry, Constipation
	Amla After 4th year	<i>Emblicoefficialis</i> Fam - Euphorbiaceac	Fruit	Vitamin - C, Cough , Diabetes, cold, Laxativ, hyper acidity.
	Long peeper / Pippali, after two to three years	<i>Peeper longum</i> Fam : Piperaccac	Fruit, Root	Appetizer, enlarged spleen ,Bronchities, Cold, antidote.
	SarpaGandha After 2 year	<i>RarwolfiaSerpentina</i> Fam: Apocynaccac	Root	Hyper tension, insomnia.
	Sandal Wood Thirty years onward	<i>Santalum Album</i> Fam: santalinaccac	Heart wood, oil	Skin disorder, Burning, sensation, Jaundice, Cough.

Table:1.3. Some medicinal plants of Bangladesh (Contd.)

Plant	Common name / Maturity period	Botanical Name or Family	Parts Used	Medicinal Uses
	Pippermint	<i>Mentha pipertia</i> Family: Lamiaceae	Leaves, flower,	Digestive, pain killer.
	Henna/Mehdi, 1/25 years	<i>Lawsonia alarmls</i> Family: Lytharaceae.	Leaf, flower, Seed	Burning, steam, anti-inflammatory.
	Gritkumari, 2nd-5th year	<i>Aloe Vera</i> Family: Liliaceae	Leaves	Laxative, Wound healing, Skin burns & care, Ulcer.
	Harida	<i>Terminali ahebula</i> Family: Combretaceae	Seed	Wound, ulcer, leprosy, inflammation, cough.
	Neem	<i>Azardirchata indica</i> Family: Mahaceae.	Rhizome	Sedative, analgesic, epilepsy, hypertensive.
	Ashok 10 years	<i>Saracaasoca</i> Family: Caesalpinanceae	Flower, dry ark	Menstrual pain, uterine, disorder, Deiabetes.

1.2.1. Rationale of the project:

Previous study on this medicinal plant included investigation to its *in-vivo* anti-ulcerogenic effect, cerebroprotective effects, and effective on crown gall tumors and so on. No previous bioactivity study has yet been conducted on the antidiarrheal activity and insecticidal activity of *Trewia nudiflora*. In addition to that, it has found in a study that this plant consists of flavonoids, tannins have demonstrated to possess antidiarrheal activity and insecticidal activity thus; this study will focus mainly on these two activities of crude extracts of *Trewia nudiflora*.

1.2.2. Aim of the project:

The aim of the study is the investigation of *in-vivo* antidiarrheal and *in-vitro* insecticidal activity of *Trewia nudiflora* leaf extracts. Three types of extracts were taken. They were: ethanolic extract (ETE), ethyl-acetate extract (EAE) and chloroform extract (CHE).

1.2.3. Objective of the study:

After studying the literature review pertaining to the previous findings *Trewia nudiflora*, the objectives of the project were made as follows with regards of using ETE, EAE, and CHE leaf extracts of *Trewia nudiflora*.

- Identification of plant leaf from National Herbarium
- Screening of insecticidal effects on rice insects
- Investigation of antidiarrheal activity on albino mice.

1.3. Plant Review:

1.3.1. *Trewia nudiflora*:

Trewia nudiflora is medium in size, dioeciously, deciduous, woods are branchless and leaves are restricted, oval 11-20 cm by 7-12 cm, chordate, sharpen, since a long time ago pointed, when youthful leaves are shaggy and later gets to be glabrous, and stalks are 2-7 cm long. Male and female blooms create on changed trees. Male trees are normally yellow 7.5mm over, contains long slack and free inflorescences, length is 10-20 cm. The female plants are singular or 2-3 consolidated inside of the basic axillary peduncles, green in shading and around 2.5 cm long. Organic products are stout, discouraged globose, grayish green in shading, 3cm by 3.5cm in volume (Balakrishnan, 2009).

The plant sort is of the spurge family (Euphorbiaceae) containing two species, viz. *Trewia nudiflora* and *Trewia polycarpa*. It is normally found from Himalayan to Hainan Island.

Table: 1.4. Brief overview of *Trewia nudiflora*

Botanical name	<i>Trewia nudiflora</i>
Common name	False white teak
Collection Locale	Tropical areas
Altitude	Upto 180mt
Seed collection period	July-December
Seed longevity	Short lived (1-6 months)
Seed purity	99%
Seed treatment	Scarification
Usual germination	30-90%
Characteristics	Timber, plywood, fuel, matchwood.
Seed counts per KG(approx)	4000-8000
Plant average height	5ft



Figure 1: *Trewia nudiflora*(Pitali) tree with leaves and fruits.

Table:1.5. Scientific Classification:

Division	Angiosperm
Class	Eudicots
Unrated	Rosids
Order	Malpighiales
Family	Euphorbiaceae
Subfamily	Acalyphoidae
Tribe	Acalypheae
Subtribe	Rottlerinae
Genus	Trewia
Species	Nudiflora

1.3.2. Economical Importance:

- The wood is even textured. It is classed as an extremely powerless and delicate timber. It is a straight-grained timber with medium fine-surface.
- It is utilized for making drums, shoddy planking and suitable for match supports, tea mid-sections and pressing cases.
- It is likewise utilized for horticultural executes, burrowed outs, yokes, slate, and picture outlines, cut pictures, and toys. Natural products are consumable, seeds yield greasy oil. Poultice of roots connected in gout and ailment (Puskar, 2013).

1.3.4. Endomethial Information:

Trewia nudiflora found in various parts of Bangladesh. Root consists of resinous matter and fat. Decoction of root is utilized as stomachic and alterative as a part of fart, gout, rheumatism⁷ and threat particularly leukemia and hepato-biliary affections and so on. A decoction of shoots and leaves of *Trewia nudiflora* is utilized as conventional prescription to diminish swelling and to treat tooting, over the top bile and sputum. The leaves are connected on wounds to recuperate them with great efficiency (KM et al, 2002).

1.3.4. Chemical components of *Trewia nudiflora*:

The whole Plant contains a pyridine alkaloid, N-methyl-5-carboxamide-2-pyridone. Plant leaves contain alkaloid, nudiflorine. Bark yields taraxerone and betasitosterol. Seeds consists of an alkaloid ricinidine. The seeds additionally contain a maytansinoid compound, trewiasine . It displayed significant cytotoxic action against different human cell lines in vitro (Shilpi et al, 2010).

1.3.5. Biological Activity:

- Ethanolic concentrate of the leaves of *Trewia nudiflora* against Indomethacin actuated gastric ulcer. Indomethacin is a cyclooxygenase inhibitor, which lessens endogeneous prostaglandin biosynthesis and disturbs the mucosal boundary and mucosal blood stream in creatures. The outcomes got demonstrated that the mean ulcer record was fundamentally decreased *Trewia nudiflora* concentrates might fortify the emission of prostaglandins or have prostaglandins like substances. Gasric ulceration delivered by anxiety is likely intervened by the vicinity of corrosive, increment in gastric motility, pole cell degranulation, diminished gastric mucosal blood stream, diminished prostaglandin amalgamation and increased discharge of glycoprotein in the bodily fluid (Rajlakshimi et al, 2012).
- The cerebroprotective impact of ethanol concentrate of leaves of *Trewia nudiflora* against the worldwide model of ischemia in rats. In the present study, the creatures were pre treated with ethanol concentrate of leaves of *Trewia nudiflora* for a time of one week (200mg/kg and 400 mg/kg) and indicated huge ($p < 0.01$) change in the conduct design, and spatial realizing, which was affirmed in trial sessions in water labyrinth test when contrasted and the negative control bunch (Girza, 2012).
- The antimicrobial bioassay against Mycobacterium tuberculosis demonstrated huge action (Guo-Hong et al, 2004).
- The ethanolic extract of this plant influenced activity against crown gall tumors on potato discs, KB cell Culture, and P388 leukemia in mice (Powell et al, 1982).

1.3.6. Different Names:

o**English Name** - False White Teak

o**Bengali name**–

- Latim,
- Lattu,
- Bhatam,
- Pitali,
- Panigambhar,
- Meragota
- Gotagamar (Sylhet)

o**Tribal name** -

- Bol-diktak
- Bolno-khap (Garo)
- Hruprukban (Mogh),
- Chagalla-dibhangor (Chakma),
- Rinmoro (Marma),
- Pitagola (CHT).

o**Nepalese name** -Gurel

o**Hindi name** - Pindar

o**Marathi name** – Wangphop

o**Urdu name** – pindara

1.4. Related Publication on *Trewia nudiflora*:

This plant has not been used so randomly yet some experiment has been done to know whether this plant has medicinal activity or not. So, here is some list of publication on *Trewia nudiflora*

- Screening of Hypoglycemic effect and Antioxidant potential of *Trewia nudiflora* leaf extracts
- Additional New Maytansinoids from *Trewia nudiflora*: 10 Epitrewiasine and Nortrewiasine (Powell *et al*, 1983).
- Potential of biosorbent developed from fruit peel of *Trewia nudiflora* for removal of hexavalent chromium from synthetic and industrial effluent: Analyzing phytotoxicity in germinating *Vigna* seeds (Bhattacharya *et al*, 2013).
- Preliminary Phytochemical Analysis and DPPH Free Radical Scavenging Activity of *Trewia nudiflora* Linn. Roots and Leaves (Balakrishnan *et al*, 2002).
- Extraction of polyphenols from *Trewia nudiflora* L. and its antioxidant activity (Abhishek *et al*, 2015).

1.5. Present Study Protocol:

The study convention of this test is intended to watch the insecticidal and antidiarrheal action of the leaf of the tree *Trewia nudiflora*. The study convention is comprised of the escorting steps:

1. Gathering of the leaves of the craved tree.
2. Dry the leaves legitimately under shade.
3. Smash the leaves in a processor into fine powder.
4. Set up the concentrate by absorbing the solvents.
5. Determination of insecticidal effects
6. Determination of antidiarrheal effect induced by castor oil
7. Determination of antidiarrheal activity induced by magnesium sulphate.

Chapter 2

Methods and Materials

2. Methods and Materials:

2.1.1. Collection of plant leaves and identification:

The tree *Trewia nudiflora* was chosen in this research to observe some pharmacological examination. The leaves of this tree were collected from Natore, Rajshahi area of Bangladesh on September, 2015. A voucher specimen is deposited in the Bangladesh National Herbarium, Mirpur, Dhaka and is tagged with the accession number- 4500 for our example. This tree is found in different spots around Northern part of the nation.

2.1.2. Extraction of the plant leaves:

To begin, the leaves were cleaned and dried for coarse granulating. The leaves are isolated from undesired piece of tree to set them up from pulverizing. The gathered material was then totally dries for pounding. After complete drying the leaves were smashed to fine powders. At that point the powder were protected in hermetically sealed holders for further utilize and kept in a cool, dim and dry spot until examination started.

About 250gm of powdered material of the dried leaves were taken in clean, flat bottomed glass containers and each is soaked in about 600ml of ethanol. The containers with its contents were sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean white cotton material. Then it was separated by filtration through Whatnari filter paper. Two beakers are taken and equal amount of filtrate is taken in it. One is considered as ethanol extract and another is used for getting other extractions.

The second part of extraction is taken in a separating funnel and 50ml of n-hexane is poured into it. Then it is shaken for 15minutes. After every half minute the cork was opened to avoid bursting due to excessive gas. Again, 20ml of n-hexane was added and the procedure repeated. Then, the upper portion was taken as specific gravity of n-hexane is 657kg/cu.m.

Then, 50ml of chloroform was added to the separating funnel and shaken for 15minutes. Then, second portion containg the chloroform extract solution is taken in a weighted beaker. To, the first portion in the separating funnel 20ml of chloroform is added and shaken for another 15 minutes and then stand for some time. Here we got the portion in the ower layer as the specific gravity of chloroform is 1464kg/cu.m.

The portion in separating funnel is being added with 50ml ethyl acetate and shaken for 15 minutes and then it is allowed to stand for few minutes. Then the second portion is allowed to be separated and taken in pre-weighted beaker. Then to the portion remaining in the separating funnel, 20ml ethyl acetate is added and also shaken for 15 minutes allowed to, and finally the lower portion was taken in the beaker, as specific gravity of ethyl acetate is 900.60 kg/cu.m. This is the ethyl acetate extract.

The extraction obtained were evaporated under ceiling fan and in water-bath (40°-50°c) until dried. It is rendered a gummy concentrate of deep green colored extract.

2.1.3. Experimented animals:

For the pharmacological investigation about 40 mice (3-4 weeks of age, 60-90 gm of either sex) were collected from the animal Resource centre of Jahangirna2gar University, Savar and was approved by International centre for Diarrheal Disease and Research, Bangladesh (ICDDR) They were kept in standard environmental condition and fed the ICDDR formulated rodent food and water. The materials used for animal housing were polyvinyl cages by BIK Industries, India, and soft wood shavin for bedding from local timber shop.



Figure 2.1: Experimented animals (Mice)



Figure 2.2: Castor oil

2.2. Pharmacological investigation:

2.2.1. Screening of antidiarrheal activity of the extracts of *Trewia nudiflora*:

The antidiarrheal activity test was done by two methods. These two methods are:

- Anti-diarrheal activity on castor oil induced diarrhea on mice.
- Anti-diarrheal activity on magnesium sulphate induced diarrhea on rodents.

2.2.2. Anti-diarrheal activity on castor oil induced diarrhea:

Principle:

Diarrhea is the most common causes form which children mortality rate is higher every year in our country. So, to identify the new source of drug which has less side-effects become one of the main goal of modern era.

In this experiment we worked with around 40 mice and particularly in each experiment it took 20 mice. Each mice was overnight fasted and divided into six groups. Then, drug and plant extract were fed and after every one hour fecal droppings were noted. we worked with two doses which were 200mg/kg and 400mg/kg.

Table:2.1. Reagents and Chemicals:

Reagent Chemicals and Equipment	Source
Loperamide	Square Pharmaceutical Company Ltd.
Tween 80 (as suspending agent)	BDH Chemicals Ltd.
Sterile disposable syringe (1ml, 100 division)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and Digital balance	Denver instruments, M-220/USA

2.2.4. Experimental design:

Twenty experimental animals were randomly selected and divided into five groups denoted as group- I, group- II, group- III (ETE 200mg/kg) group- IV (ETE 400mg/kg), group- V (EAE 200mg/kg) , group- VI (EAE 400mg/kg), group- VII (CHE 200mg/kg) and group- VIII (CHE 400 mg/kg) consisting of per mice in control and standard group, and 3 mice in rest of the groups. Each group received a particular treatment i.e. control, standard and the dose of the extracts of the plant respectively. Prior to any treatment each mice was weighted and marked properly.

Table: 2.2 Treatment procedure

Test Samples	Group	Purpose	Route of Administration
0.2 ml of water in tween 80	I	Control	Oral
Loperamide	II	Standard	Oral
ETE 200mg	III	Extract	Oral
ETE 400mg	IV	Extract	Oral
EAE 200mg	V	Extract	Oral
EAE 400mg	VI	Extract	Oral
CHE 200mg	VII	Extract	Oral
CHE 400mg	VIII	Extract	Oral

2.2.4. Preparation of test material:

- A. Preparation of Standard drug: 2mg of tablet that is Loperamide was taken into a clean and dried mortar and pestle. Then, It was powdered until fine powder was formed. The powder is then dissolved in 100ml of distilled water. From where 0.2 ml were given orally.
- B. Preparation of dose from crude extract: To administer a crude extract the dose 200mg/kg and 400 mg/kg, the doses were measured and triturated in a unidirectional way by adding 1-2 drops of suspending agents “Tween 80” to each preparation so that they become totally miscible.

Procedure:

The animals were fasted overnight and weighed and randomly divided into five groups consisting of three mice in each extract group and 1mice for control and standard group

At first, 200mg and 400mg 0.2 of extract was given to group 3-5 according to their numbering.

All the mice were administered 0.2 ml of water and loperamide orally with a tuberculin syringe with ball shaped end

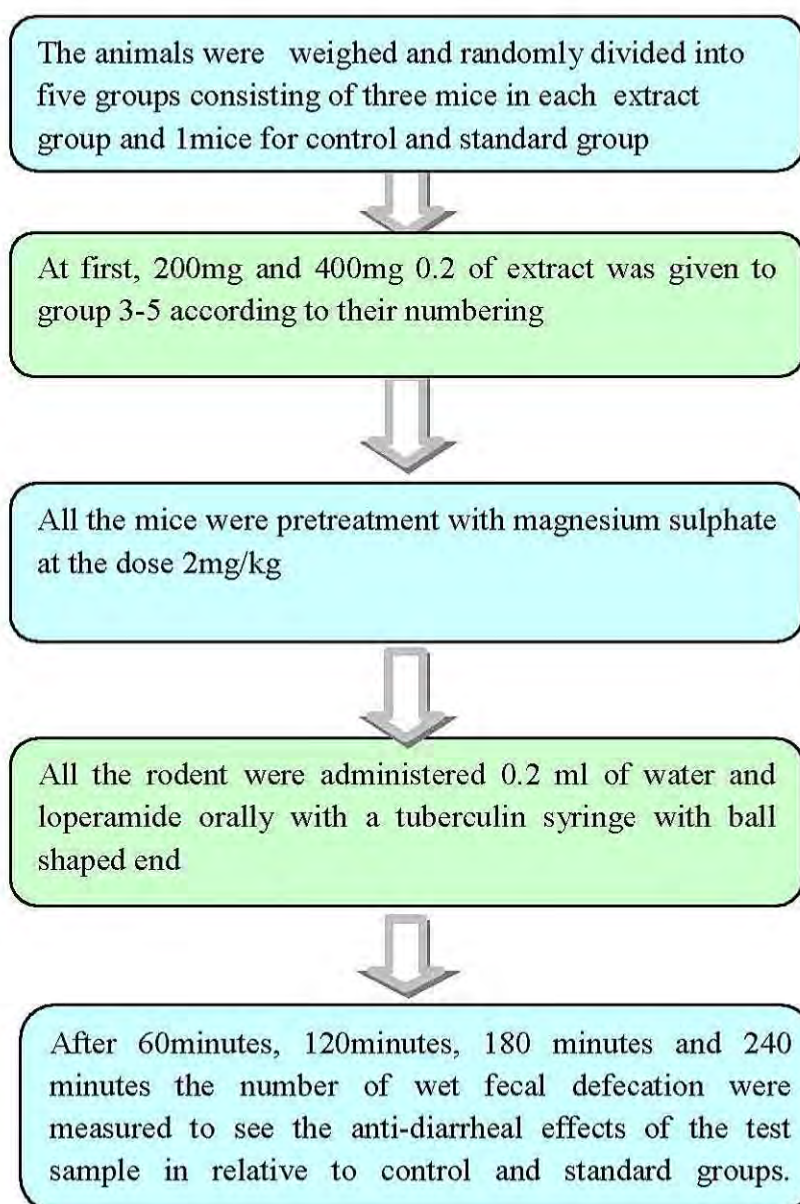
After one hour, 1ml castor oil was given to each mice where tween 80 was used a vehicle

After 60minutes, 120minutes,180 minutes and 240 minutes the number of wet fecal defecation were measured to see the anti-diarrheal effects of the test sample in relative to control and standard groups.

2.2.5. Anti-diarrheal activity on magnesium sulphate induced diarrhea:

Principle: In this experiment we worked with around 40 mice and particularly in each experiment it took 18-20 mice. Each mice was divided into five groups. Then, drug and plant extract were fed and after every one hour fecal droppings were noted. We worked with two doses which are 200mg/kg and 400mg/kg.

Procedure: The experimental procedure to determine anti-diarrheal activity induced by magnesium sulphate almost same as castor oil. The procedure are given below:



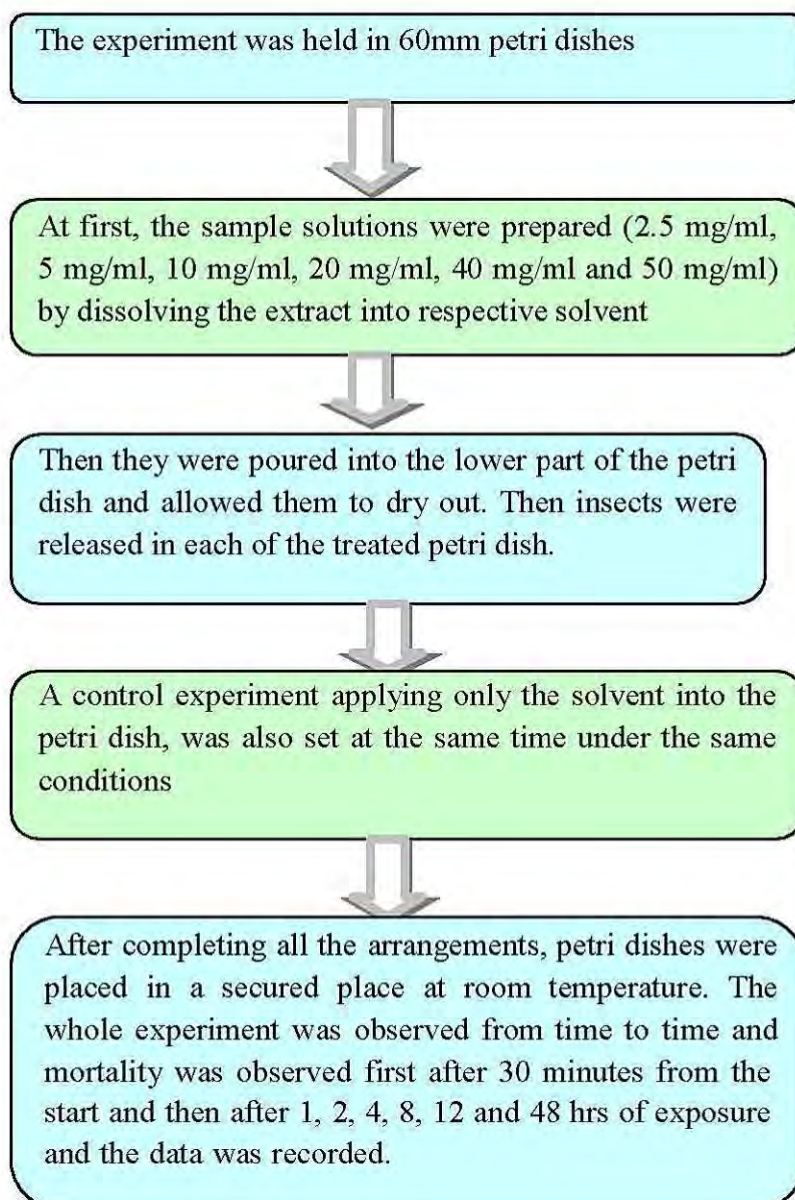
2.2.6. Screening of insecticidal activity on *Tribolium castaneum*:

Principle:

Bangladesh is an agricultural country. Till now, the main occupation of Bangladeshi people is farming mainly growing crops. But, when, the crops were in growing process sometimes the crops were being attacked by *Tribolium castaneum* which decreases the crop production. So the old farmer have to count with huge loss. They use insecticides which are much expensive and is also environment pollutant also. So, if our farmer could use herbal insecticides it may solve a lot of problems.

The present experiment was carried out using insects *Tribolium castaneum* which were collected from the stock cultures of the Crop Protection and Toxicology Laboratory, Jahangirnagar University, Savar.

Procedure:



2.3. Statistical analysis:

All the values in the test are expressed as mean \pm standard error mean (SEM). The data were statistically analyzed by ANOVA (Analysis of variance) and post-hoc Dunnett's tests with the Statistical package for Social Sciences (SPSS) program (SPSS 16.0, USA).

Chapter 3

Results

3.Results:

3.1.1. Result of antidiarrheal activity on Castor oil induced diarrhea:

By following the methods described in a journal written by Khatun et al (2014) the percentage of fecal defecation of the extracts were found.

In the castor oil–induced diarrheal experiment in mice, we administered ETE, EAE and CHE of leaves of *Trewia nudiflora* at the doses of 200mg/kg and 400mg/kg. Among them EAE at 400mg/kg gives the same result as standard drug.

Table: 3.1. Castor oil induced diarrheal model

Group	Dose	Total no of feces in 4 hrs	Percentage
G-I-Control	Distilled water	30.6± 2.374689	-----
G-II-Standard	2 mg/kg	2.4± 0.95*	92.16
G-III-ETE	(200mg/kg)	7.2±0.91*	76.47
G-IV-ETE	(400mg/kg)	5.2±1.05*	83.01
G-V-EAE	(200mg/kg)	5.4±0.74*	82.35
G-VI-EAE	(400mg/kg)	3±1.046*	90.2
G-VII-CHE	(200mg/kg)	7.4±0.74*	75.82
G- VIII -CHE	(400mg/kg)	5.6±0.74*	81.7

All the values are expressed as mean ±SEM; One-way analysis of Variance (ANOVA) followed by Dunnet's test. * $p < 0.05$ significant compared to control (Loperamide).

From this table it is clearly evident that, ethyl acetate and ethanol extract at 400mg/kg shows the most efficacy. It was a dose dependant experiment.

3.1.2. Result of antidiarrheal activity on magnesium-sulphate induced diarrhea:

In the magnesium sulphate induced diarrheal model in mice, the ethyl acetate and ethanol extract at the dose of 400mg/kg reduce the extent of diarrhea in tested animals. Those extracts were shown to reduce the total no of faeces and wet faeces at higher doses.

Table: 3.2. Magnesium sulphate induced diarrheal model

Group	Dose	Total no of feces in 4 hrs	Percentage
G-I-Control	Distilled water	28.2 ±1.61	-----
G-II-Standard	2mg/kg	3.2±1.14*	88.65
G-III-ETE	(200mg/kg)	7±0.84*	75.18
G-IV-ETE	(400mg/kg)	5.6±1.16*	80.14
G-V-EAE	(200mg/kg)	6±0.84*	78.72
G-VI-EAE	(400mg/kg)	5±0.84*	82.27
G-VII-CHE	(200mg/kg)	8.4±0.95*	70.21
G- VIII -CHE	(400mg/kg)	6.8±1.14*	75.89

All the values are expressed as mean ±SEM; One-way analysis of Variance (ANOVA) followed by Dunnet's test. * $p < 0.05$ significant compared to control (Loperamide).

So from the above table, it is evident that both the extract of ethyl acetate and ethanol at the dose of 400mg/kg shows great efficacy like standard drug loperamide.

3.1.5. Result of insecticidal activity:

Using Abbott's method, the percentages of mortality rate of the extracts were found. The mortality rate of rice insects was calculated using the following equation:

$$Pr = (Po - Pc \ / \ 100 - Pc) \times 100$$

Where, Pr = Corrected mortality%;

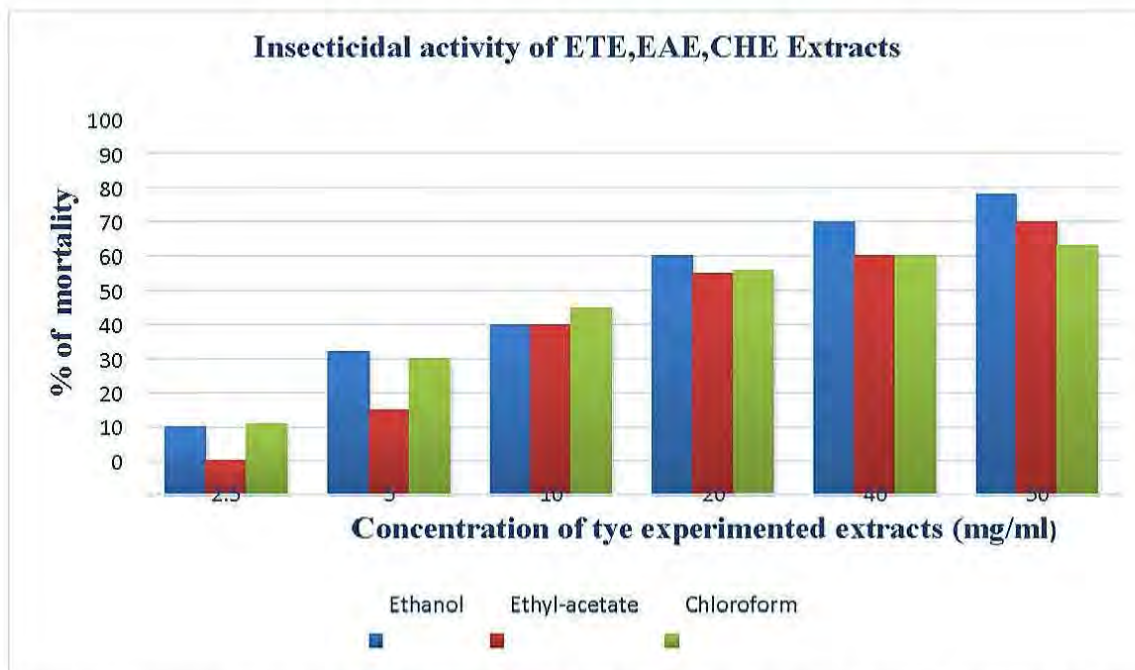
Po = Observed mortality%;

Pc = Control mortality%, sometimes called natural mortality%

Here three types of extracts were taken to observe the mortality rate of rice insects. They were observed very carefully for about 48 hours.

The percentage inhibitions obtained were plotted in a graph given below. The graph is drawn against the used concentrations of those three extracts.

Figure: 3.1. Percentage of mortality of rice insects using leaves extracts



From the above graph, it is clearly evident that the percentage of mortality rate of rice insects increases with the increasing doses. At the doses of 50mg/ml ethanol extract of *Trewia nudiflora* plant leaves shows around 88% mortality rate. From the above graph it can be said that, ethyl-acetate extract of *Trewia nudiflora* plant leaves also shows insecticidal effects. At the dose of 50mg/ml the mortality increases to around 83%. It can be also asserted that chloroform extract of experimented plants also shows insecticidal activity but not so strong as ethanol and ethyl acetate extract. It increases the mortality rate around 73% at the dose of 50mg/ml.

Chapter 4

Discussion

4. Discussion:

From the ancient time the use of herbal medicine in the treatment of diarrheal disease is a common practice in many countries across the world including Bangladesh. Therefore, the need to substantiate or otherwise the folkloric claim of *Trewia nudiflora* as an antidiarrheal and insecticidal agent using several experimental model cannot be overemphasized. The plant's different parts have been experimented to know about its potentiality against diabetes, antioxidant activity and many more but antidiarrheal and insecticidal activities have never been studied (Toyin et al, 2010).

Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saps, steroids and tannins. Earlier study showed that anti-dysentric and antidiarrheal properties of medicinal plants were due to these chemical components (Labu et al, 2015).

Looseness of the bowels can be portrayed as the anomalous successive defecation of feces of low consistency which might be because of an aggravation in the vehicle of water and electrolytes in the digestion tracts. Rather than the variety of etiologies (i) increased electrolytes secretion (secretorydiarrhea), (ii) expanded luminal osmolarity (osmotic loose bowels), (iii) unhinged intestinal motility bringing on diminished travel time, and (iv) decreased electrolytes assimilation might be in charge of pathophysiology(Agbor et al, 2004). Numerous reports affirmed that castor oil actuated diarrhoeal activity (Harapakki et al, 2005). Recently it has been proven that nitric acid which is present in castor oil is responsible for the diarrheal effect(Rahman et al, 2015). These diarrheal activity includes diminishment of ordinary liquid assimilation by hindrance of intestinal Na⁺, K⁺ ATPase activity (Izzo, 1996), enactment of adenylatecyclase or mucosal cAMP intervened dynamic secretion (Genillia,1978) incitement of prostaglandin formation (Capasso et al, 1994), platelet activating factor (Capasso et al,1986) and nitric oxide has been accounted for to commitment of the diarrheal impact by castor oil (Pinto et al, 1992). The movement of castor oil which delivers looseness of the bowels because of its most dynamic part recinoleic corrosive by a hyper secretory reaction was proved (Ammon et al, 1974).

In this experiment it is showed that the overall antidiarrheal study reveals the dose dependent activity. In our study, *Trewia nudiflora* leaves shows how it significantly reduced amount of feces in castor oil-induced in albino mice. Here, three extracts were taken. They were: ethanol extract, ethyl-acetate extract and chloroform extract. The doses were 200 mg/kg and 400mg/kg. So, they reduced the faecal defecation compared to the standard drug loperamide

(92.16%). For ethanol extract the percentage was 76.47% and 83.01% for 200 and 400mg doses. For ethyl-acetate extract the percentage was 82.35% and 90%, for chloroform extract the percentage were 75.82% and 81.7% for 200mg/kg and 400mg/kg doses respectively. So, it is clearly seen that ethyl-acetate extract of *Trewia nudiflora* plant leaves gives the approximate result to the standard drug loperamide.

Past report on the phytochemical screening of *Trewia nudiflora* leaves has demonstrated the alkaloids, tannin, glycosides and steroids are available. It was accounted for that flavonoids and tannins were in charge of the antidiarrheal movement properties. However, previous concentrates likewise have demonstrated that flavonoids have capacity to repress intestinal motility and water and electrolyte secretion (Dcarlo et al,1993). Moreover, *in vivo* and *in vitro* tests have additionally demonstrated that flavonoids can restrain prostaglandin E₂ incited intestinal emission and spasmogens instigated compression furthermore hinder arrival of prostaglandins and autocoids . Along with, flavonoids as the inhibitors of prostaglandins biosynthesis are considered to postpone castor oil-instigated loose bowels (Brijesh,2009).Tannins additionally can indicate antidiarrheal property. So, the antidiarrheal action drive in Pharmacological Sciences to the ethanolic, ethyl-acetic acid derivation and chloroform concentrate of the leaves of *Trewia nudiflora* could subsequently be because of the vicinity of flavonoids and tannins.

On the other hand, magnesium sulfate has been reported to create diarrhea by rising the amount of intestinal substance through counteractive action of reabsorption of water. It has also been reported that it encourages the release of cholecystokinin from the duodenal mucosa, which elevates the discharge and motility of small digestive tract and in this manner keeps the reabsorption of sodium chloride and water. The ethanolic extract was found to improve the diarrheal conditions in this experimented model. The extracts may boost the absorption of water and electrolyte from the gastro-intestinal tract, since it delayed the GI transit in mice as compared to the control. The delay in the GI transit provoked by the extract might have contributed, at least to some extent, to their antidiarrheal activity by allowing a greater time for absorption (Zavala et al, 1998).

For insecticidal activity *Trewia nudiflora* leaves have shown almost approximate mortality rate of *Tribolium castaneum* (Rice insects) for three extracts: ethanol, ethyl-acetate and chloroform at a dose of 50mg/ml in 12 hours. Since these extracts have strong insecticidal

activity, dose dependent activity was done, where six graded doses (viz. 50, 40, 20, 10, 5 and 2.5 mg/ml) were used.

In case of ethanol extracts, shows 88%, 80%, 70%, 50%, 42% and 20% mortality rate at the dose of 50mg/ml, 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml, respectively. It shows the highest mortality rate among three extracts. Secondly, Ethyl acetate extract shows 80%, 70%, 65%, 50%, 25% and 0% mortality rate at the dose of 50mg/ml, 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml respectively. In case of chloroform extract shows 73%, 70%, 66%, 55%, 40% and 21% mortality rate at the dose of 50mg/ml, 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5 mg/ml.

So, it is clearly evident from the above experiment that ethanol extracts of *Trewia nudiflora* leaves give almost 88-90% activity against rice insects. To show insecticidal activity sugars, saponins, phytosterol, phenol, flavonoids and tannins have to be present in medicinal plants. So, in conclusion we can say that the experimented plant leaves of crude extracts shows the poisonous impact in the considered bugs.

Chapter 5

Conclusion

5. Conclusion:

From the ancient time people used plants as medicines which have serve as natural source of treatment and therapies. Today scientists are using these renewable sources to produce a new generation of therapeutic solution. Many plants have pharmacological properties against various types of diseases but they are well known to common people. Medicinal plants se an indispensable source of medicinal preparation both preventive and curative. Careful and planned investigations of the plant are needed in order to develop new drug that would meet the criteria of modern treatment.

The *Trewia nudiflora* is a plant of natural gift that possesses many therapeutic activities. The project work was aimed to investigate the possible in vivo and in vitro pharmacological investigations of the extracts of ethanol, ethyl-acetate and chloroform in albino mice various pharmacological done were-

- Antidiarrheal activity on castor oil and magnesium sulphate induced diarrhea
- Screening of insecticidal activity on *Tribolium castaneum*

Antidiarrheal activity induced by castor oil was evaluated by observing the effects of above mentioned extracts by following journal. After 240 minutes from administration of ethanol, ethyl acetate and chloroform extract (200mg/ml and 400mg/ml) the number of fecal defecation by mice were reduced with time. The *Trewia nudiflora* has shown good antidiarrheal activity compared to the standard drug loperamide.

Again antidiarrheal activity induced by magnesium sulphate was done in the same was as castor oil and after 240 minutes the number of faecal defecation were counted which shows *Trewia nudiflora* have antidiarrheal activity at high doses.

So, it can clearly be said that, *Trewia nudiflora* have antidiarrheal activity which can reduce fecal defecation. As it is previously proven that, this plant contain glycosides, tannins, steroids flavinoids etc. among them flavinoids and tannins have the antidiarrheal activity. With increasing doses they show more antidiarrheal activity when compared with standard drug. Loperamide is considered as standard drug in this experiment.

In case of insecticidal activity, *Trewia nudiflora* shows good response to increase mortality rate with high doses. Here, we worked with three extracts which are: ethanol, ethyl-actate and

chloroform extracts. This plant contains, tannins, flavonoids, saponins which shows insecticidal activity against rice insects.

From this experiment it can be said that, *Trewia nudiflora* leaves extracts have several biological activities. But, as we have conducted our studies with crude extracts we still do not know which phytochemicals of particular extracts shows potency. So, in future our concern is to conduct further comprehensive investigation as well as illustration of active components and initiates preformulation studies for development of the potential dosage form from this crude extracts.

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