Project on

Evaluation of the aqueous extract of leaves of *Averrhoa carambola* for pharmacological activity

A dissertation Submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University

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APPROVAL

This project, **Evaluation of the aqueous extract of leaves of *Averrhoa carambola* for pharmacological activity**, submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Master of Pharmacy and approved as to its style and contents.

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Md. Abdullah-Al Faisal Arif

Author

DEDICATION

Dedicated to

My Parents and

Supervisor

Abstract

The aim of the study was to screen different chemical groups in aqueous extract of leaves of *Averrhoa carambola* and evaluate the pharmacological activities. The plant was reported earlier to contain anti-inflammatory and hypoglycemic properties. The present study was conducted in vivo to evaluate the analgesic and anti-diarrheal activity of the aqueous extracts of leaves. Phytochemical screening of the sample indicated the presence of carbohydrates, tannins, and alkaloids. When tested for its analgesic effects on acetic acid-induced writhing in mice, the extract produced significant analgesia at doses of 250 and 500 mg/kg body weight and percentage (%) of inhibition was 32.78% and 36.89% respectively compared to the standard diclofenac at a dose of 10 mg/kg body weight. And when tested for its anti-diarrheal effects on castor oil induced defecation in mice, the extract produce antidiarrheal effects at dose 250 mg/kg body weight and percentage (%) of activity was 62.5% respectively compared to the standard imotil at a dose of 5 mg/kg body weight.

The overall result obtained from the study suggests the analgesic and anti-diarrheal properties of leaves of the plant. The effect of both analgesic and anti-diarrheal of the aqueous extract was significant.

Keywords: *Averrhoa carambola,* aqueous extract, leaves extract, analgesic, acetic acid, writhing, anti-diarrheal, defecation, castor oil

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CHAPTER 01

INTRODUCTION

1.1 Ethno botany and herbal medicines

Ethno (as in 'ethnic') refers to people, culture, and a culture's collective body of beliefs, aesthetic, language, knowledge, and practice.Botany is the study of plants—from the tiniest fern or blade of grass to the tallest or oldest tree. Botany includes all the wild plants and the domesticated species. Domesticates are species that we humans have selected over time from the wild plant species, then tamed and trained to optimally produce for us: food, fibers, medicine, materials, and more. The domesticated species are both the subject and object of agriculture. [1]

1.1.1 Ethno pharmacology

Ethnopharmacology is the study of medicinal plant use in specific cultural groups or study of differences in response to drugs by different cultures.this is the naturally occurring drug compounds and practiced by various ethnic groups, especially by instinctive people.Ethno medicine and ethno pharmacology, both of these are significantly interrelated with ethno botany. [2]

1.2 Phytomedicine in global health care

Herbal medicine or phytomandisine refers to the use of plants and plants for the healing and healing of people's diseases. Registered history has been used by humans since many years ago. Although modern medicine has taken leadership from herald medicine in the treatment of treatment among people, the use of herbal products worldwide is increasing because they have a strong side effect or some side effects compared to modern medicine.[3] The healthcare potential of herbal medicine has been established by various phytochemical and pharmacological studies. Herbal medicines are generally run as a whole bone extract, as a herbal tea or fresh juice. [4] All the supplementary evidence behind the use of fatmachines is to use standard teas of plant ingredients to ensure the recycling of clinical settings. With growing interest for alternative approaches to the treatment of the disease treatment, cardiovascular medicinal products also play an important role for the new therapeutic agents. For these problems, research should be focused on: 1. Characterization of biological functioning and biofunctional activity of phytomedicines 2. Phytotesthesis Chemical properties of chemical properties, study the effects of specific processing and

extraction methods and parameters. 3. Development of quality of chemo-based and organic-based phiothomedic systems [5]

1.3 Natural product research and drug discovery

Nature is a source of treatment agents for thousands of years, and an impressive number of modern medicines is disconnected from natural sources, based on their use in many traditional medicines. In the past century, a growing role has been played by sub-scientists in the production of antibiotics and other medicines for the treatment of some serious diseases. Biodiversity of the Earth Through continuous investigation, novelization for screening will play an important role in the development of molecular diversity, most of it is not unrealistic. [6]

Natural products and related structures are the essential source of new pharmaceuticals, because of the large number of functionally related secondary metabolites of microbial and plant species. Furthermore, the development of strong analytical tools based on genomic, protofics, metabolism, bioinformatics and other technologies of the 21st Century has expanded the identification and characteristics of these natural products. [7] Different screening methods are being created to make use of natural products available for natural exploration, and data mining and virtual screening techniques are also being applied to natural product data. The more effective and efficient applications of natural products are expected to improve drug discovery [8] Natural products have always been an important factor in the discovery of drugs, but researchers are able to detect, disconnect, extract and synthesize their active compounds in new ways through modern technology and technology. They allowed the drug again, and again they again discovered the drug Most of the progress is coming. Combining the feasibility of traditional medicine with the modification of modern chemical technologies, the use of natural products can help develop the process of drug development more environmentally sound, economic, and effective drug discovery. [9]

1.3.1 Opportunities in drug discovery from medicinal plants

Drug discovery from plants involves a multidisciplinary method of mixing Botanical, ethnobotanical, phytochemical and biological techniques. Plants provide our new

chemicals (lead molecules) for the development of drugs with various pharmacological targets, including cancer, HIV / AIDS, malaria, Alzheimer's disease and pain. Some natural products of plant production use drugs in clinical, which include Pacititakele, Campetean-Enerogue analog, Arcte, Galanthamine, Tietopromium and several Phase 2 and Phase III clinical trials. Although plant-based drug discovery programs provide an important source of new drug sources, many challenges include the invention and the authentication of plant materials, the implementation of high-throating screening bisexuals and the scale-up of biological lead compounds. [10] The current research of drug discovery from the medicine plant involves a multifaceted method of plant-based plants, phytochemical, biological, and molecular techniques. Medicinal plants offer new and important sources against pharmacological targets, including drug discovery, cancer, HIV / AIDS, Alzheimer's, malaria, and pain. Although an important source of new narcotics is found in the field of medicinal plants, many challenges are faced with many challenges, including the selection of plant materials, appropriate high-throating screening bicycles and scale-up of active compounds and active compounds. [11]

1.3.2 Challenges in drug discovery from medicinal plants

Drug development discovered from medicinal plants is also inadequate for limited optimization, lead development and clinical trials, which are usually small amounts of natural products facing unique challenges. Drug discovery from the medicine plant is traditionally so time-consuming, fast and good method for collecting plants, bioassay screening, compound isolation, and compound development must be employed. Innovative techniques are needed to improve the plant collection process. Reduction of plant collecting can be a kind of strategy. Suitable, clinical-related, high-tech airspace design, determination and perception is a difficult process for all drug discovery programs. While the design of high-throop screening asses may be challenging, but if tested based on screening, compounds and libraries for biological activity may be examined. Screening of extrat libraries may be problematic, but new techniques, including extract precaution, can overcome some of these problems. The challenges of screening of Biasas are an important factor in the future of drug discovery from pharmacological plants. [12]

1.4 Bangladeshi medicinal plants

Bangladesh has very rich in Bio-diversity. It has more than 500 medicinal plants species (Yusuf *et al*., 1994). An alarmingly populous, but size-wise a very small country is rather unique in having diversified genetic resources in a wide range of habitats. The total numbers of plant with medicinal properties in the subcontinent are present stands at about 2000. About 450 to 500 of such medicinal plants name so far been enlisted as growing or available in Bangladesh. According to World Health Organization (WHO, 1993), 74% modern medicine derived from about 119 plants are used in ways directly correlated with their traditional uses as medicine. (Nyarko HD *et al.* 2012). In Bangladesh, most rural people depend on thousands of different plants for traditional medicine among the 5000 phanerogam and pteridophyte species growing in forests, wastelands and roadsides. (Ghani, 2003).[13] A number of plants used for traditional medicine have been found to contain various efficacious compounds against different diseases. Extracts from different Bangladeshi medicinal plants were studied in vitro to see the effect on the proliferation of breast cancer cell lines [14]. There are traditional uses of medicinal plants for the treatment of urinary tract infection and sexually transmitted disease [14]. A number of functional foods have been identified having pharmacological properties [15]. Some traditionally used plants have also been found to be anti-hypotensive and antidiabetic effects. There are also some plants used in respiratory problems. [16] [17]

Besides these findings, there are lot of medicinal plants available those are not discovered or studied yet which need to be brought under attention. Screening of these medicinal plants will be potential for modern medicine and scientists are considering them as alternative sources for the discovery and development of novel drugs.[18]

1.5 Introduction to *Averrhoa carambola*

Averrhoa carambola is the common plants in Bangladesh, the fruits of which are edible. The leaves and fruits of *A. carambola* are used by folk medicinal practitioners for treatment of diabetes. Since scientific studies are absent on the analgesic andantidiarrheal effects of theleaves of the plants, it was the objective of the present study to evaluate the analgesic andantidiarrheal potential of extract of leaves of the plants.

Techniques for acetaminopath are considered as the cause of the strongening and emission of acne due to the ripe fruit of Averrhoacarambola L (usually known as "Kamaranga"). Dried fruits are also used in fever; It possesses chilly and antiscorbutic properties. An effort has been made to show the results of this herb through pharmaconicostic studies. Initial phytochemical analysis also performed powdered fruit. The triangular and large unknown cavities of the presence of transparently could be used as prominent features and physical markers such as the reverse section of the fruit. The maximum increase in the length and diameter of the timing of the maturity stage was recorded and their outer color changed from green to golden yellow. Water dissolves and dissolves the fruit gradually decreases the alcoholic extractive value of the solution. In the primary phytochemical analysis, the presence of saponin, tannins, alkoeud, and flavonovide is mentioned. [19]

1.5.1 Scientificclassification[20]

- Scientific Name: Averrhoacarambola
- Kingdom: Plantae – Plants
- Subkingdom: Tracheobionta -Vascular plants
- Superdivision: Spermatophyta
- Division: Magnoliophyta – Flowering plants
- Class: Magnoliopsida – Dicotyledons
- Subclass: Rosidae
- Order: Geraniales
- Family: Oxalidaceae – Wood-Sorrel family
- Genus: AverrhoaAdans. – averrhoa
- Species: Averrhoacarambola L. – carambola

1.5.2 Chemical constituents of *Averrhoacarambola*

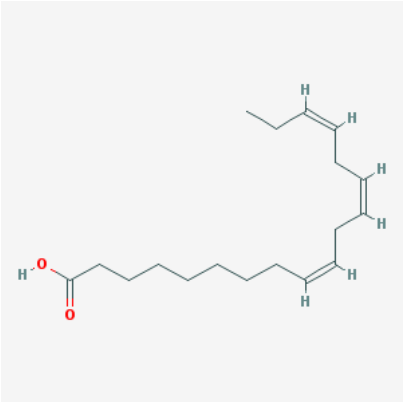

Fig 1.1: linolenic acid

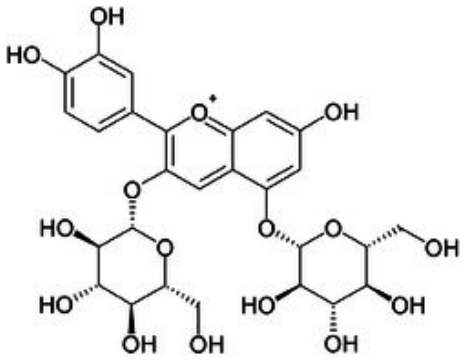

Fig 1.2: cyaniding-3-5-o-β-d-diglucoside

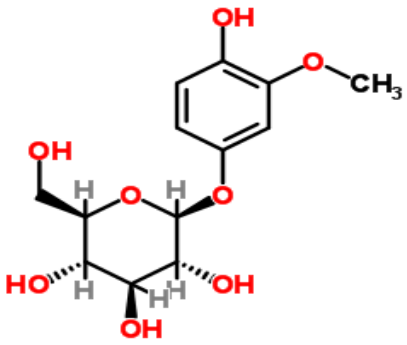

Fig 1.3: methoxy-hydroquinone-4-*β*-*D*-glucopyranoside

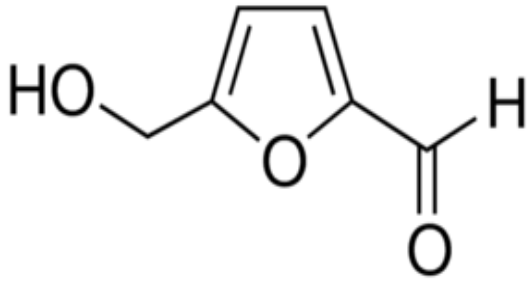

Fig 1.4: 5-hydroxymethyl-2-furfur-al

1.5.3 Pharmacology of *Averrhoa carambola*

A crabbulla is used in the disorder such as arthritis, chronic headache, swelling and pyendarmas, cold, cough, epicaxis, sperm, fever, food poisoning, gastroenteritis, malaria, malariae splenomegaly, oliguria, postpartum edema, throat throat, subcalorism and traumatic injury. The carbon dioxide pharmacological examination showed anti-inflammation, antimycrobial, antifungal, antitumor and anti-ulcer function. In addition, there are plant hypocholesterolemic, hypoglycemic, hypotonous, nephrotoxic, and neurotoxic, negative introthic and chronotropic effects.

Phytochokemic tests showed the presence of asponinas, tannins, alkoeids and flavonovides. [21]

1.5.4 Photograph of *Averrhoacarambola*

Fig 1.5:*Averrhoacarambola*

Fig 1.6: Leaves of*Averrhoacarambola*

Fig 1.7: Flowers of*Averrhoa carambola*

Fig 1.8:*Averrhoa carambola* fruits

1.6 Analgesic

Analgesic, any drug that relieves pain selectively without blocking the conduction of nerve impulses, markedly altering sensory perception, or affecting consciousness. This selectivity is an important distinction between an analgesic and an anesthetic. Analgesics may be classified into two types: anti-inflammatory drugs, which alleviate pain by reducing local inflammatory responses; and the opioids, which act on the brain. The opioid analgesics were once called narcotic drugs because they

can induce sleep. The opioid analgesics can be used for either short-term or long-term relief of severe pain. In contrast, the anti-inflammatory compounds are used for short-term pain relief and for modest pain, such as that of headache, muscle strain, bruising, or arthritis. [22]

1.6.1 Anti-inflammatory analgesics:

Most anti-inflammatory analgesics are derived from three compounds discovered in the 19th century—salicylic acid, pyrazolone, and phenacetin (or acetophenetidin). Although chemically unrelated, the drugs in these families have the ability to relieve mild to moderate pain through actions that reduce inflammation at its source. Acetylsalicylic acid, or aspirin, which is derived from salicylic acid, is the most widely used mild analgesic. It is considered the prototype for anti-inflammatory analgesics, the two other major types of which include acetaminophen (a derivative of phenacetin) and the aspirin-like drugs, or nonsteroidal anti-inflammatory drugs (NSAIDs), which include compounds such as ibuprofen, naproxen, and fenoprofen. Pyrazolone derivatives, with some exceptions, are no longer widely used in many countries, because of their tendency to cause an acute infection known as agranulocytosis. Preferences in COX selectivity and the possibility of additional molecular actions of NSAIDs may explain differences in the therapeutic effects between aspirin, acetaminophen, and NSAIDs. For example, while aspirin is effective in reducing fever, as well as relieving inflammation, acetaminophen and NSAIDs are more potent antipyretic (fever-reducing) analgesics. Acetaminophen, on the other hand, possesses inferior anti-inflammatory activity compared with aspirin and NSAIDs and thus is relatively ineffective in treating inflammatory conditions such as rheumatoid arthritis. Despite this, acetaminophen is a popular mild analgesic and antipyretic and is a suitable alternative to aspirin for patients who develop severe symptoms of stomach irritation, because it is not as harmful to the gastrointestinal tract. [22]

1.6.2 Opioid analgesic:

Opioid drugs are useful in the treatment of general postoperative pain, severe pain, and other specific conditions. The use of opioids to relieve the pain associated with kidney stones or gallstones presumably depends on their ability to affect opioid

receptors in these tissues and to inhibit contractility. By a similar mechanism, opioids are also able to relieve the abdominal distress and fluid loss of diarrhea. Low doses of opioids are also used for relief of the respiratory distress that accompanies acute cardiac insufficiency complicated by the buildup of fluid in the lungs.

Among the opioid antagonist drugs, naloxone and its longer-lasting orally active version, naltrexone, are used primarily to reverse morphine overdoses and to reverse the chemical stupor of a wider variety of causes, including alcohol intoxication and anesthesia. In opioid overdoses, these drugs provide recovery within minutes of injection. They can, however, also precipitate severe withdrawal reactions in a person addicted to opiates. [22]

1.6.3 Mechanisms of action of analgesic:

Aspirin and NSAIDs appear to share a similar molecular mechanism of action—namely, inhibition of the synthesis of prostaglandins (natural products of inflamed white blood cells) that induce the responses in local tissue that include pain and inflammation. In fact, aspirin and all aspirin-like analgesics, including indomethacin and sulindac, which are derived from a heterocyclic organic compound known as indole, inhibitprostaglandin synthesis and release. All these agents can be further divided into nonselective COX inhibitors and selective COX inhibitors. COX, or cyclooxygenase, is an enzyme responsible for the synthesis of prostaglandins and related compounds. It has two forms, COX-1, which is found in most normal tissues, and COX-2, which is induced in the presence of inflammation. Because COX-2 is not normally expressed in the stomach, the use of COX-2 inhibitors (e.g., rofecoxib, celecoxib) seems to result in less gastric ulceration than occurs with other anti-inflammatory analgesics, particularly aspirin. However, COX-2 inhibitors do not reduce the ability of platelets to form clots, a benefit associated with aspirin and other nonselective COX inhibitors.

As might be expected from their common mechanisms of action, many of the anti-inflammatory analgesic drugs share similar side effects. Hypersensitivity responses to aspirin-like drugs are thought to be due to an accumulation of prostaglandins after the pathways that break down prostaglandins are blocked. These responses can be fatal when very strong anti-inflammatory compounds are given. Inhibition of prostaglandin synthesis may result in other serious side effects, such as peptic ulcers and a reduced

ability of platelets in the blood to aggregate and form clots. The latter effect, however, has given aspirin an added use as a prophylactic antithrombotic drug to reduce chances of cardiac or cerebral vascular thrombosis—the formation of a clot in a blood vessel in the heart or brain. Some aspirin-like analgesics also have specific toxic effects: liver damage occasionally occurs after administration of acetaminophen, and renal toxicity is sometimes seen with use of NSAIDs. [22]

1.7 Diarrhea

Diarrhea is a condition that involves the frequent passing of loose or watery stools - it is the opposite of constipation and can have many causes, which may be infectious or non-infectious. Diarrhea comes from the Greek word diarrhea. Dia means "through" and rheo means "flow". The term "flowing through" was coined by Hippocrates. It is very common and usually not serious. Many people will have diarrhea once or twice each year. It typically lasts two to three days and can be treated with over-the-counter (OTC) medicines. Some people often have diarrhea as part of irritable bowel syndrome or other chronic diseases of the large intestine. It is recognized as the most frequent cause of childhood death in many topical countries. It is recognized as the most frequent cause of childhood death in many topical countries. [23]

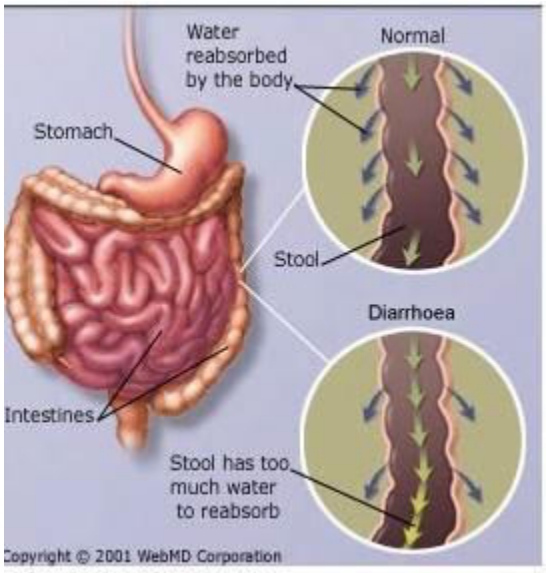

Fig 1.9: Diarrhea

1.7.1 Classification of Diarrheal Diseases:

The Millennium Development Goals call for a reduction of child mortality by two thirds between1990 and 2015.Nearly nine million children less than five years of age die each year. Approximately one in six deaths occurs among children younger than five years. Diarrheal diseases are a major cause of hospitalizations and child deaths globally particularly in the developing countries. [24]

1. Acute diarrhea, 2. Persistent diarrhea and 3. Chronic diarrhea

1.7.1.1 Acute Diarrhea:

Acute diarrhea is defined as the passage of fossilized urinary tract compared to traditional, it is not lasting longer than a fixed period, more than 2 weeks, often discomfort from the corresponding gas, wheels and rashes. Diarrhea intensity can be described as light (there is no change in the function of the patient), moderate (activity needs change but the person is able to work), or severe (patient is disabled, often restricted to beds or cells). Acute diarrhea in the United States Every one of the absences of one person per year, it is one of the most common medical disorders seen by primary physicians. [25]

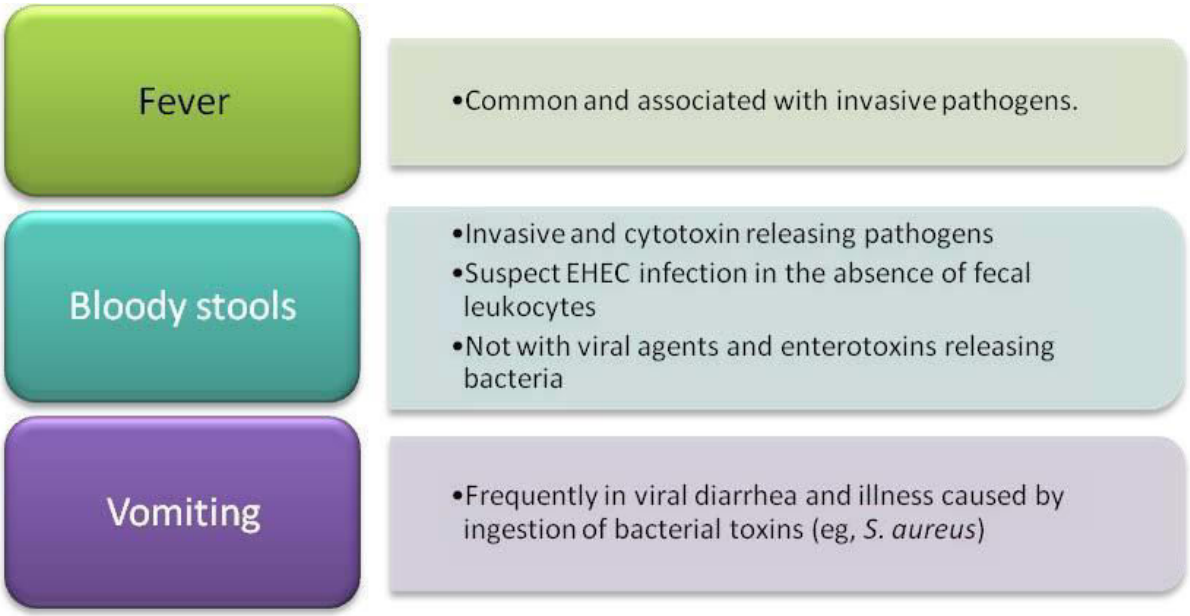

Fig1.10: The main symptoms to the causes of acute diarrhea. EHEC, enter hemorrhagic Escherichia coli.

» **Causes of Acute Diarrhea:**

The volume of the diarrhea may help to localize the disease process within the GI tract. Frequent, small-volume bowel movements typically are associated with diseases of the left colon or rectum, and watery large-volume diarrhea suggests disorders of the small bowel or proximal colon. Urgency and cramping typically indicates inflammation in the rectum. Nocturnal diarrhea may be seen in infectious colitis or severe inflammatory bowel disease, and is not a feature of irritable bowel syndrome. [25]

1.7.1.2 Persistent Diarrhea:

Persistent diarrhea is operationally defined as an episode of acute diarrhea that lasts for 14 days or longer. This is predominately a problem of children below 3 years of age and occurs in 3-21% of diarrheal episodes. It gives rise to major problems of nutritional management, growth faltering and morality and considerably increases the cost of national programmes for control of diarrheal diseases. A specific microbial etiology has not been found although an association with aggregative adherent E.coil and repeated infections may be important. Malnutrition, particularly of micronutrients and prior illness, especially measles, may be risk factors. The strategy of management of acute episode does not contribute to persistence and it is difficult to predict persistence in the individual patient. Antibiotic are of limited use. Specific nutritional deficiencies should be corrected. Fluid and dietary management is important. Algorithms have been developed and are currently being tested in several centers. [26]

1.7.1.3 Chronic Diarrhea:

Diarrhea persisting for periods longer than a month is found in a wide variety of conditions. A clinical approach to these could divide them into malabsorption syndromes, chronic diarrhea without malabsorption and chronic diarrhea of the immunocompromised.A common presentation of human immunodeficiency virus infected individuals is with chronic diarrhea, usually associated with a variety of opportunistic infections. In addition morphological studies have been suggested the possibility of HIV enteritis. Patients with irritable bowel syndrome may present with a history of chronic diarrhea which usually reflects frequent small tools without increase in faecal water and no malabsorption. The commonest types of chronic diarrhea in tropical countries are the malabsorption syndrome. The duration of chronic diarrhea is longer than a month. [27]

The absorption of nutrients is a complex process involving digestion of food, absorption of the simple molecules produced by digestion by the enterocytes and the transport of the nutrients from the gut.

1.7.2 Sign and Symptoms:

There are many different symptoms of diarrhea. These symptoms may occur in any combination, depending on the cause of the condition. [28]

- Nausea
- cramping
- bloating
- Dehydration
- Fever
- Bloody stools
- Abdominal pain

1.7.3 Medications:

Although antibiotics are beneficial for certain types of acute diarrhea, it is usually not used for specific conditions. Acrychia coli O157: There are concerns that can increase the risk of antibiotic hemolytic uremic syndrome among people with H7. In poor countries, treatment with antibiotics can be beneficial. However, some antibiotics can also cause diarrhea, and antibiotics that develop some antibiotics, especially Shigella, are the most common adverse effects of antibiotic treatment with antibiotics. [29]

1.7.3.1 Bulking Agent:

Psyllium (Metamucil, Fybogel, Generics) - Cylinders are often recommended twice a day for chicks, but it is also used in diarrhea, because it has an effect on water in the intestine, which can help in an abundance of water levels. Some doctors recommend it for the richness of IBS's amicable intake. It has the power to tighten some toxins that can cause acute diarrhea. Silium products should be avoided with silicil. [30]

1.7.3.2 Over the Counter (OTC) Drugs:

- **Bismuth (Pepto-Bismol, generics):** This preparation is sometimes recommended for traveler‟s diarrhea and chronic microscopic colitis. It decreased the number of bowel movements.
- **Loperamide (Imodium):** The safest of the opioid drugs, loperamide is available OTC in 1 and 2mg doses. Depending on age, the recommended dose is 2 mg after each loose bowel movement to a maximum of 16 mg/day. It has an opioid‟s ability to slow gut transit and improve absorption of water from the intestines. Some evidence suggests it also improves anal sphincter tone. Although it has the lowest addiction potential of all opioids, it may cause sedation, nausea, and cramps. It is the best emergency treatment for mild attacks of diarrhea, and when taken preventively it may even help avoid urgent exits during meetings or other events. [31-32]

1.7.3.3 Prescription Drugs:

- **Cholestyramine (Questran):** Cholestyramine is a powdered resin with a plastic taste that binds bile salts and has a water-holding effect. When other treatments fail, it may relieve some cases of diarrhea. Rarely, chronic diarrhea occurs after removal of the gall bladder or the lower small intestine (ileum), and cholestyramine has a beneficial effect. Usually prescribed for patients with high cholesterolblood levels, it is available in 4mg packets and is taken with water. Occasionally, a very small dose will improve diarrhea, but for most cases, loperamide is preferable. [33-35]
- Diphenxillitis (lymph): It is unpleasant due to some drug addiction feasibility, difexylate is available only by prescription. It combines its entropy so that excessive use can cause dry mouth and other unwanted side effects. It is effective if another drug fails. [36]

1.7.3.4 Zinc supplementation:

In many parts of the world, adequate sanitation and adequate sanitation means poverty is the main cause of death among children and children in uninterrupted and middle-income countries. More than one million children under the age of five die every year due to fluid related to diarrhea related deaths. It is estimated that 13% of all year-old diarrhea is lost due to illness, disability, or primary death (so-called "disability-addictive life"). [37] Good guidelines about the clinical management of diarrheal

diseases in the world's most vulnerable children are so important. There are two simple and effective treatments for managing acute panic:

• Low density oral reusable salt use (ORS)

• Regular use of zinc supplements, more than 20 milligrams per day for six-month-old children or 10 mg of dose per day in 10 to 14 days of age for six months.

Oral rehydration is a well-known and relatively easy treatment method. Zinc supplement has been found to reduce the length and intensity of diarrheal episodes and reduce the chances of subsequent infections for 2-3 months. Gene supplementation is generally accepted (regardless of the types of genital sulfate, jing acetate or zinc gluconate used in both children and caring and common zinc salts). Supplementary zinc children with brokers is beneficial because it is an essential micronutrient essential for protein synthesis, cell growth and difference, immune function and water and electrolyte intake transportation. Gene is also important for both the diarrhea and the normal growth and development of children both. [38]

CHAPTER 02

LITERATURE REVIEW

2.1 "Analgesic activity of Amorphophalluscampanulatus tuber" by J.A. Shilpi*, P.K. Ray, M.M. Sarder, S.J. Uddin

The methanol extract of Amorphophalluscampanulatus tuber, given orally at the doses of 250 and 500 mg/kg, showed significant analgesic activity in mice. D 2005 Elsevier B.V. All rights reserved.

2.2 "Antidiarrheal Activity of the Aqueous Extract of *Punicagranatum*. (Pomegranate) Peels" by E.Y. Qnais,A.S. Elokda,Y.Y. Abu Ghalyun &F.A. Abdulla

The antidiarrheal effects of the aqueous extract of *Punicagranatum*. L. (Punicaceae) peels were evaluated in rats. Studies were carried out on the isolated rat ileum, gastrointestinal motility *in vivo*., and on castor oil–induced diarrhea in rats. The results revealed that the extract exhibited a concentration-dependent inhibition of the spontaneous movement of the isolated rat ileum and attenuated acetylcholine-induced contractions. The extract (100, 200, 300, and 400 mg/kg) also caused a dose-dependent decrease of gastrointestinal transit and markedly protected rats against castor oil–induced diarrhea enteropooling. The intraperitoneal injection LD_{50} of the extract was found to be 1321 ± 15 mg/kg in mice. A preliminary phytochemical screening of the aqueous extract of *Punicagranatum*. peels gave positive tests for tannins, flavonoids, and alkaloids. The results obtained showed that the aqueous extract of *Punicagranatum*. peels may contain some biologically active principles that may be active against diarrhea, and this may be the basis for its traditional use for gastrointestinal disorders.

2.3 "pharmacognostic evaluation and physicochemical analysis of averrhoacarambola l. Fruit" by Thomas S., Patil D.A.*, Patil A.G. and Naresh Chandra

In Ayurveda, the ripe fruit of Averrhoacarambola L. (commonly known as "Kamarakh") is considered as digestible, tonic strengthening, for bleeding piles and causing biliousness. The dried fruit is also used in fever; it is cooling and possesses antiscorbutic properties. It is considered as one of the best Indian cooling medicines. An attempt has been made to highlight this medicinal fruit through the pharmacognostic studies. The preliminary phytochemical analysis has also been performed on the powdered fruit. The presence of trichomes and large oval

lysigenously formed cavities as seen in the transverse section of fruit were the distinguishing features and can be used as anatomical markers.

2.4 "**AverrhoaCarambola: An Updated Review**" by P. Dasgupta, P. Chakraborty, N. N. Bala

Increasing knowledge of metabolic process and the positive effects of plants on human physiology have enlarged the range of application of medicinal plants. From the centuries, herbal medicines have been used to treat various diseases and now they had become an item of global importance, with both medicinal and economic implications. Selecting the right scientific and systematic approach to biological evaluation of plant products, based on their use in traditional medicine is the key to ideal development of new drugs from plants. One such plant is Averrhoacarambola (Oxalidaceae), traditionally known as 'kamrakh' and commonly known as star fruit because of its peculiar shape It has widely been used in Ayurveda, preparations of its fruit and leaves are used to pacify impaired kapha, pitta, skin diseases, pruritis, worm infestations, diarrhea, vomiting, hemorrhoids, intermittent fever, over-perspiration and general debility. It is also used in traditional medicines in countries like India, China, Phillipines, Brazil for various ailments. Although review articles on this plant are already published, but the present attempt is to review and compile all the updated information on botany, phytochemical and pharmacological properties, drug interaction, contraindication and toxicity studies of Averrhoacarambola. These results are very encouraging and indicate that this plant should be studied more extensively to confirm the reproducibility of these results and also to reveal other potential therapeutic effects, along with some "leads" with possible isolation of active biomoieties and their mechanism of action.

CHAPTER 03

PURPOSE OF THE STUDY

Bangladesh is enriched with thousands of different plants among which many have medicinal properties. Rural people are still dependent in some ways on these plants for medication and these plants are sources of various folk medicines. People prefer these folk medicines over conventional treatment because of low economy and they also think that natural sources are safer than conventional medicines.

Research and development of drugs from these medicinal plants have a potential for the drug sector of the country and the mankind of the whole world as well. In developing countries, 80% people rely on natural medicine (Farnsworth *et al.* 1985).

These medicinal plants can be put under research and different drugs can be discovered and developed from them by phytochemical screening of these plants.

In addition, Bangladeshi pharma sector is developing day by day. They export pharmaceuticals in many parts of the world. But they have to import various pharmaceutical raw materials as well. Discovery and development of lead compound can make a quick progress in pharmaceutical sector and it will be economic too.

All these factors make it necessary for the screening of the plant extracts and their fractions in order to get new lead compounds and develop new drugs.

Averrhoacarambola is commonly used plant as traditional medicine and its uses as an anti-inflammatory& analgesic opens a door for the research with this plant. Besides, the plant is enriched with carbohydrates, tannins, alkaloids, etc. which can makes it significant for further study.

Besides the traditional use of this plant, the present work is designed to scientifically evaluate the plants-

- Phyto-chemical screening
- Analgesic activity
- Anti-diarrheal activity

CHAPTER 04

MATERIALS AND METHODS

5

4.1 Phytochemical screening

Phytochemical screening is the extraction, screening and identification of medicinally active compounds found in plant parts e.g., leaves, stems, barks, roots etc. or whole plant. Flavonoids, alkaloids, tannins, antioxidants, glycosides etc. are different major compounds having medicinal properties in a plant.

In modern phytochemistry, these compounds are screened and isolated using different techniques and their medicinal and pharmacological properties are studied although before the beginning of medicinal sciences, plants containing different active medicinal compounds were used for the treatment of disorders.

In phytochemical screening, extraction of organic compounds is performed and active compounds are identified, isolated and purified to get the best medicinal effect and develop new drugs.

4.1.1 Apparatus and reagents

A. Materials required

Equipment required for extractions		
Serial	**Material**	**Source**
1	Beaker (1000 ml)	Borosil, Germany
2	Funnel	GlasscoLabpratoriy Equipment, UK
3	Filter Paper	Whatnam Filter Paper
4	Beaker (500 ml)	Borosil, Germany
5	Beaker (50 ml)	GlasscoLabpratoriy Equipment, UK
6	Glass Rod	-

7	Stand with Clamp	-
8	Cotton	-
9	Measuring Cylinder	GlasscoLabpratoriy Equipment, UK

Table 4.1: Equipment required for extractions

B. Apparatus required

Apparatus required for extraction		
Serial no.	**Equipment**	**Source**
01	Electronic Balance	Shimadzu Corporation Limited
02	Grinder	Panasonic, Japan
03	Oven	MMM, Germany
04	Rotary Evaporator	LabTech, United States

Table 4.2: Apparatus required for extraction

C. Chemicals

Chemical reagents used for different chemical group tests		
Serial no.	**Name of the chemicals**	**Source**
1	α-Napthol	Merck, Germany
2	Sulfuric acid	Active Fine Chemicals
3	Hydrocloric acid	Merck, Germany

4	Sodium Hydroxide (pellet)	Merck Specialities Private Limited, India
5	Ferric Chloride	Merck, Germany
6	Lead Acetate	JHD Chemical, China
7	Potassium Iodide	Active Fine Chemicals
8	Mercuric Iodide	Active Fine Chemicals
9	Bismuth Nitrate	Merck, Germany
10	Cupric Sulfate	Merck, Germany
11	Sodium Citrate	Merck, Germany
12	Anhydrous sodium carbonate	Active Fine Chemicals
13	Distilled Water	Prepared in the Laboratory

Table 4.3: Chemical reagents used for different chemical group tests

4.1.2 Experimental plant

Persicariacapitata included in Polygonaceae was investigated in this study.

Experimental plant		
Plant name	**Family**	**Plant part used**
Averrhoacarambola	Oxalidaceae	Leaves

Table 4.4: Experimental plant

4.1.3 Plant collection

The experimental plant was collected from Feni in May 2018 and the genus as well as the family was identified from National Herbarium, Bangladesh (accession number is 45747).

4.1.4 Preparation of crude extract

- **Drying and grinding**

The undesirable materials, plants or plant part were removed from the experimental plant after collection. The leaves and stems were separated and the leaves were air-dried under shed for 21 days. The dried leaves were then ground into coarse powder with the help of a grinder. The powder was kept into a dry container in a dark, cool place until further analysis.

- **Extraction**

200 grams of dry powder was taken in a 1000 ml beaker and soaked with about 500 ml of water. Then the beaker was sealed using aluminium foil paper and kept in phytochemical laboratory room, Department of Pharmacy, Daffodil International University, accompanied by occasional stirring. After 2 days, the moisture was filtered through cotton followed by filter paper. The filtrate was then taken in a rotary evaporator to concentrate the extracts. The semi-solid residue was then taken in a 50 ml beaker. The weight of the extract was 10 g. The extract was stored in freeze for further investigation.

4.1.5 Chemical group test

Presence of different chemical groups were tested as the preliminary phytochemical study. The chemical groups were tested as per "Trease, G.E. and Evans, W.C., 1983". 10% (w/v) solution of aqueous extract in water was taken in each test tube unless otherwise mentioned in individual test.

4.1.6 Reagents used for different chemical group test

Reagents used for the chemical group tests are as follows (Trease, G.E. and Evans, W.C., 1983):

- **Mayer's reagent:**

  1.36 g of mercuric iodide was dissolved in 60 ml of distilled water and mixed with a solution containing 5 g of potassium iodide in 20 ml of distilled water.

» **Dragendroff's reagent**:

1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of water and this solution was mixed with a solution containing 16 g of potassium iodide in 40 ml of water.

» **Benedict's reagent:**

1.73 g of cupric sulphate, 1.73 g of sodium citrate and 10 g of anhydrous sodium carbonate in water and the volume was made up to 100 ml using water.

» **Molisch's reagent:**

2.5 g of pure α-naphtha was dissolved in 25 ml of ethanol.

4.1.7 Testing procedures for identifying chemical groups

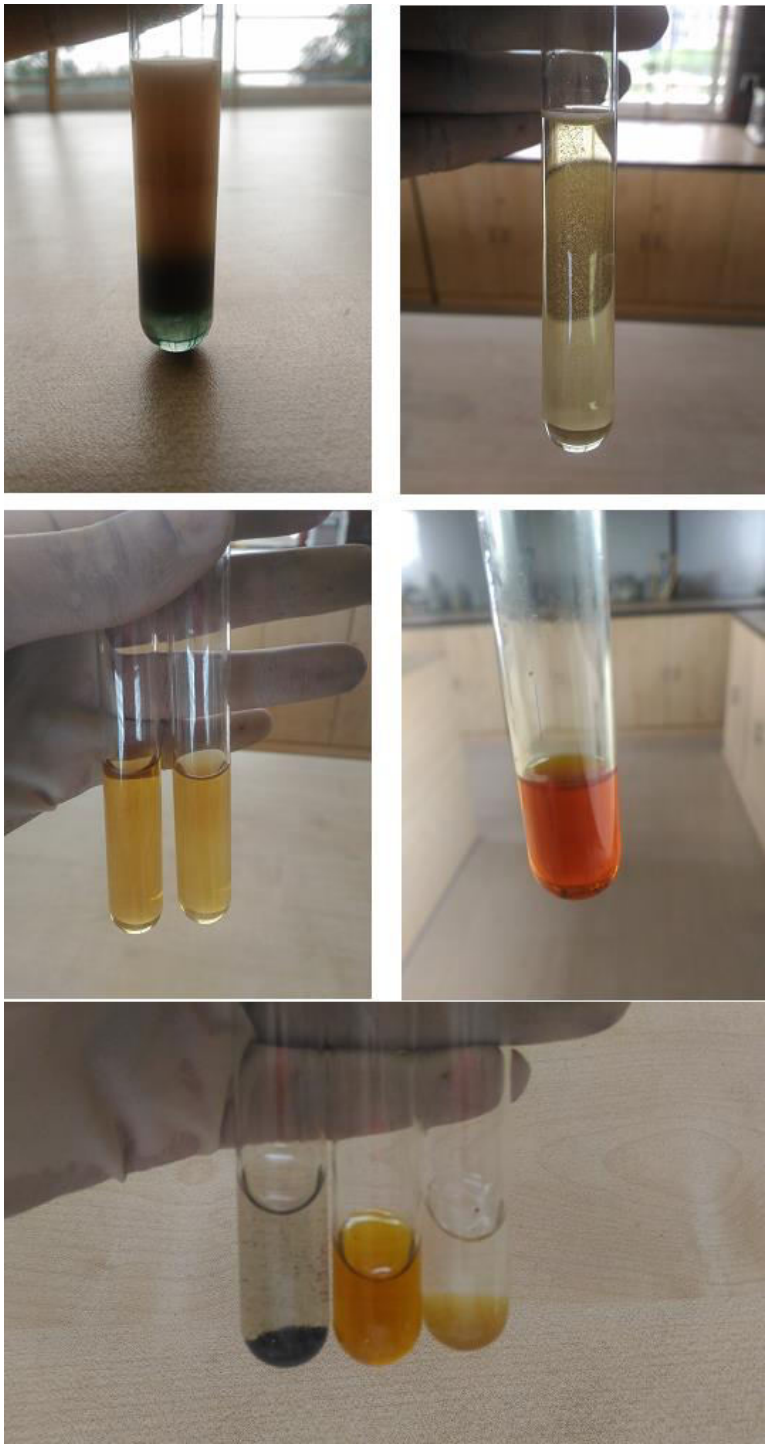

Fig 4.1: Different chemical group test of the crude extract

Test for carbohydrates

» **Molisch's test:**

2 drops of freshly prepared 10% alcoholic solution of α-napthol was added to 2 ml solution of crude extract taken in a test tube and then sulphuric acid was added to the flown side of the inclined tube in such a way that the acid forms a layer beneath the aqueous solution. A red or reddish violet ring would form at the junction of the two layers which confirms the presence of carbohydrate. A dark purple solution would form upon shaking or standing. The test tube was allowed to stand for 2 minutes and then diluted using 5 ml of distilled water. A dull violet precipitation confirms the presence of carbohydrate.

Tests for tannins

» **Ferric chloride test**

In a test tube, 1 ml of 5% Ferric solution was added with 5 ml of aqueous solution of the crude extract. Presence of Greenish black precipitation confirmed the presence of tannins.

» **Potassium dichromate test**

5 ml of aqueous solution of the crude extract was taken in a test tube and 1 ml of 10% potassium dichromate solution was added to it. Formation of yellow precipitation confirmed the presence of tannins.

» **Lead acetate test**

1% solution of lead acetate was added to 5 ml of aqueous solution of the crude extract and formation of yellow or red precipitation confirmed the presence of tannins.

Test for steroids

» **Salkowski test**

2 ml of chloroform of solution of crude extract was taken in test tube and 1 ml of sulphuric acid was added to it. Emergence of red colour would confirm the presence of steroids.

Test for alkaloids

» **Mayer's test**

0.2 ml of concentrated hydrochloric acid was added to 2 ml of aqueous solution of the crude extract. 1 ml of Mayer's reagent was added to it and formation of yellow colour confirmed the presence of alkaloids.

» **Dragendroff's test**

» 0.2 ml of concentrated hydrochloric acid was added to 2 ml of aqueous solution of the crude extract. 1 ml of Dragendroff's reagent was added to it and formation of yellow colour confirmed the presence of alkaloids.

Test for glycosides

» Small amount of crude alcoholic extract was added with 1 ml of distilled water and few drops of aqueous sodium hydroxide was added to it.Fomation of yellow colour indicated the presence of Glycosides.

Different chemical group tests performed and the results				
Test	**Sample**	**Test solution**	**Observation**	**Inference**
Carbohydrate	**2 ml of extract solution and 2 drops of 10% alcoholic α-napthol with 0.5 ml of sulphuric acid**		**Red ring formation at the junction of two layers**	**Presence of Carbohydrate**
Tannin	**5 ml of aqueous solution of extract with 1 ml of 5% ferric solution**		**Greenish black precipitation**	**Presence of Tannin**

	5 ml of aqueous solution of extract with 1 ml of 10% potassium dichromate solution	**Yellow precipitation**	**Absence**
	5 ml of aqueous solution of extract with few drops of 1% lead acetate solution	**Red precipitation**	**Absence**
Steroids	**2 ml of chloroform solution of extract with 1 ml of sulphuric acid**	**No red colour formation**	**Absence of Steroids**
Alkaloids	**0.2 ml of concentrated HCl and 2 ml of aqueous solution of extract with 1 ml of Mayer's reagent**	**No yellow precipitation formed**	**Presence of Alkaloids**
	0.2 ml of concentrated HCl and 2 ml of aqueous solution of extract with 1 ml of Dragendroff's reagent	**No brown precipitation formed**	**Presence of Alkaloids**
Glycoside	**Small amount of alcoholic extract with 1 ml of distilled water and few drops of aqueous sodium hydroxide**	**Formation of yellow precipitation**	**Absence of Glycoside**

Table 4.5: Different chemical group tests performed and the results

4.2 Analgesic test

Analgesics are agents that reduce pain. The analgesia is characterized by the writhing of the mice and it is induced by injecting 1% acetic acid. The analgesic activity is evaluated by visual observation of reduction in frequency and number of writhing after administration of the test sample of crude extract. The total count of writhing is compared with the standard group. Analgesic agents decrease the writhing count.(B. A. Whittle, 1964)

4.2.1 Materials and methods

Apparatus and reagents	
Name	**Origin**
Acetic acid	Merck, Germany
Diclofenac	Opsonin Pharmaceutical Ltd.
Distilled water	Prepared in laboratory
Needle	
Syringe	
Electronic balance	Shimadzu Corporation Limited
Hand gloves	
Face masks	
Boxes for mice	

Table 4.6: Apparatus and reagents

4.2.1.1 Animal collection

Swiss albino mice (male) were collected from Jahangir Nagar University, Dhaka, Bangladesh. The mice weighed between 25-30 grams. The mice were acclimatized for 7 days prior to experiment. The study was conducted following approval by the Institutional Animal Ethical Committee of Daffodil International University, Dhaka, Bangladesh.

4.2.1.2 Environment control

The animals were kept in a stainless steel cage under controlled temperature (24 ± 2°C), in a 12 hours light-dark cycle. ICDDR,B formulated rodent food and pure water was given to the mice since these animals very sensitive to environmental changes.

4.2.2 Design of the experiment

i. Preparation of Control: Laboratory prepared distilled water was used as control.

ii. Preparation of Standard: Ampoule of Diclofenac solution containing 75 mg of Diclofenac per 3 ml of solution was collected.

iii. Preparation of Sample: For mice weighing 25 g, to prepare the doses of 250 and 500 mg/kg body weight, 250 mg of crude extract was dissolved in distilled water using magnetic stirrer. The mixture was filtered to obtain a clear solution.

Fig 4.2: Swiss albino mice

4.2.3 Methodology

i. Randomly chosen test animals were divided into four groups having three animals in each.

ii. The groups were separated as-

Group-I: Control Group

Group-II: Standard Group

Group-III: Test Group-1

Group-IV: Test Group-2

iii. All the mice of different groups were marked with different identification codes and separated into different cages.

iv. 30 minutes prior to the injection of acetic acid-

- » Group-I received 10 ml/kg body weight equivalent of distilled water.
- » Group-II received 10 mg/kg body weight equivalent of diclofenac.
- » Group-III and Group-IV received the test sample solution at 250 and 500 mg/kg body weight respectively.

v. After administration of acetic acid followed by 5 minutes of waiting, the mice were observed for 10 minutes and writhing were counted.

Experimental profile to observe the analgesic effect of leaves of *Averrhoa carambola* on acetic acid induced mice				
Code	**Group**	**Identification**	**Sample**	**Dose (mg/kg)**
CTRL	I	Control	Distilled Water	10 ml/kg
STD	II	Standard	Diclofenac	10
EXT-1	III	Test Sample	Extract	250
EXT-2	IV	Test Sample	Extract	500

Table 4.7.1: Experimental profile to observe the analgesic effect of leaves of *Averrhoa carambola* on acetic acid induced mice

Experimental profile to observe the analgesic effect of leaves of *Averrhoacarambola* on acetic acid induced mice					
Group	**Weight of Mice**	**Distilled Water at 10 ml/kg (ml)**	**Standard at 10mg/kg (mg)**	**Extract at 250mg/kg (mg)**	**Extract at 500mg/kg (mg)**
Control	28	0.28	-	-	-
	28	0.28	-	-	-
	29	0.29	-	-	-
Standard	29	-	0.29	-	-
	30	-	0.30	-	-
	29	-	0.29	-	-
Group 1	29	-	-	7.25	-
	30	-	-	7.5	-
	30	-	-	7.5	-
Group 2	27	-	-	-	13.5
	28	-	-	-	14
	27	-	-	-	13.5

Table 4.7.2: Experimental profile to observe the analgesic effect of leaves of *Averrhoacarambola* on acetic acid induced mice

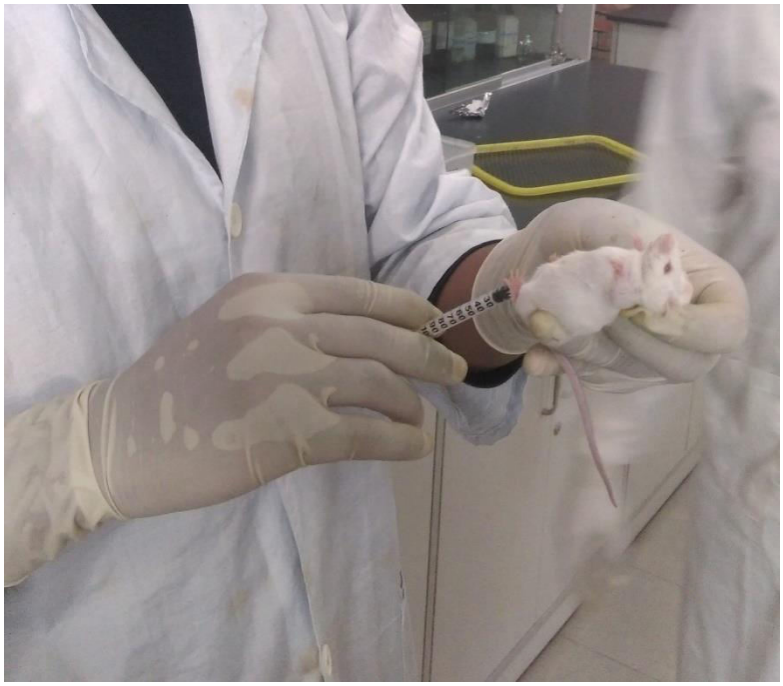

Fig 4.3: Injecting acetic acid

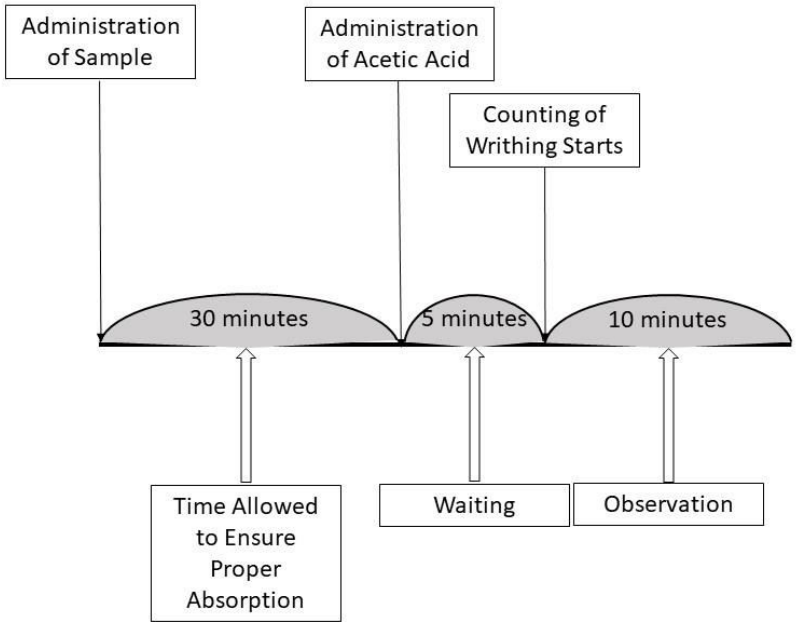

Fig 4.4: Schematic representation of study design of analgesic activity observation in test animal with acetic acid induced writhing

4.3 Anti-diarrheal test

The diarrhea is defined by the presence of steel or any fluid content, which is the staining of the absorbing paper kept under the cage. The time taken before the first ban is 'secret time'. Comparison of the total counting styles and testing groups compared to the positive control group. Anti-diarrheal agent reduces the dormant duration and decreases total stall count.

4.3.1 Materials and methods

Apparatus and reagents	
Name	**Origin**
Castor oil	

Imotil	Square Pharmaceutical Ltd.
Distilled water	Prepared in laboratory
Mice feeder	
Saline	
Electronic balance	Shimadzu Corporation Limited
Hand gloves	
Face masks	
Boxes for mice	

Table 4.8: Apparatus and reagents

4.3.1.1 Animal collection

Swiss albino mice (male) were collected from Jahangir Nagar University, Dhaka, Bangladesh. The mice weighed between 25-30 grams. The mice were acclimatized for 7 days prior to experiment. The study was conducted following approval by the Institutional Animal Ethical Committee of Daffodil International University, Dhaka, Bangladesh.

4.3.1.2 Environment control

The animals were kept in a stainless steel cage under controlled temperature (24 ± 2°C), in a 12 hours light-dark cycle. ICDDR, B formulated rodent food and pure water was given to the mice since these animals very sensitive to environmental changes.

4.3.2 Design of the experiment

I. **Preparation of control:** Normal saline was bought from local market and dissolved in 100ml water. Then 1 ml tween 80 was dissolved in saline solution

II. **Preparation of Standard:** To prepare standard at the doses of 5mg/kg per body weight, loperamide was dissolved in saline solution and the final volume of was made 10 ml.

III. **Preparation of Sample:** To prepare suspension of the test samples at the doses of 400 & 200 mg/kg per body weight,for 26gm mice 52 & 26 mg of samples were measured respectively. The extract was first dissolved in distilled water. The final volume of the suspension was made 10 ml.

4.3.3 Methodology

- Tested animals randomly and divided into three groups, each experiment group has three mouse,
    i. Group -1 or 1% of TB 80 containing only 1 ml of saline water
    ii. Group-2 or A Oral suspension as a lopamide at a dose of body weight of 5 milligram / kg, taking standard-antimetolytic drug, standard 2 millilia.
    iii. Group-3 or testing groups are treated with hydro-extract of 2 ml permanently, which is affected by oral dosage of 250 mg / kg-body weight.

- The dose of mice was feeded with sample from the oral management of the spinal area one hour earlier. Every individual animal in each clan was kept in a separate cage, which contains papaya paper and is tested for diarrhea in five hours.
- The number of stools or any fluid that is stuck on the adsorbent paper is calculated within each incremental hour of 4 hours and is noted for each mouse.
- Each mouse's latent period is also calculated. At the beginning of each hour, new papers were kept for old people.

Experimental profile to observe the anti-diarrheal effect of leaves of *Averrhoacarambola* on castor oil induced mice			
Animal Group	***Treatment***	***Dose (/kg-body wt.)***	***Route Of admin.***
I (Control) n=3	Saline Water containing 1% tween 80	10ml	Oral
II (Standard) n=3	Loperamide	5 mg	Oral
III & IV *Test sample* n=3	Aqueous Extract of whole plant	200mg	Oral
	n=no. of mice		

Table 4.9: Experimental profile to observe the anti-diarrheal effect of leaves of *Averrhoacarambola* on castor oil induced mice

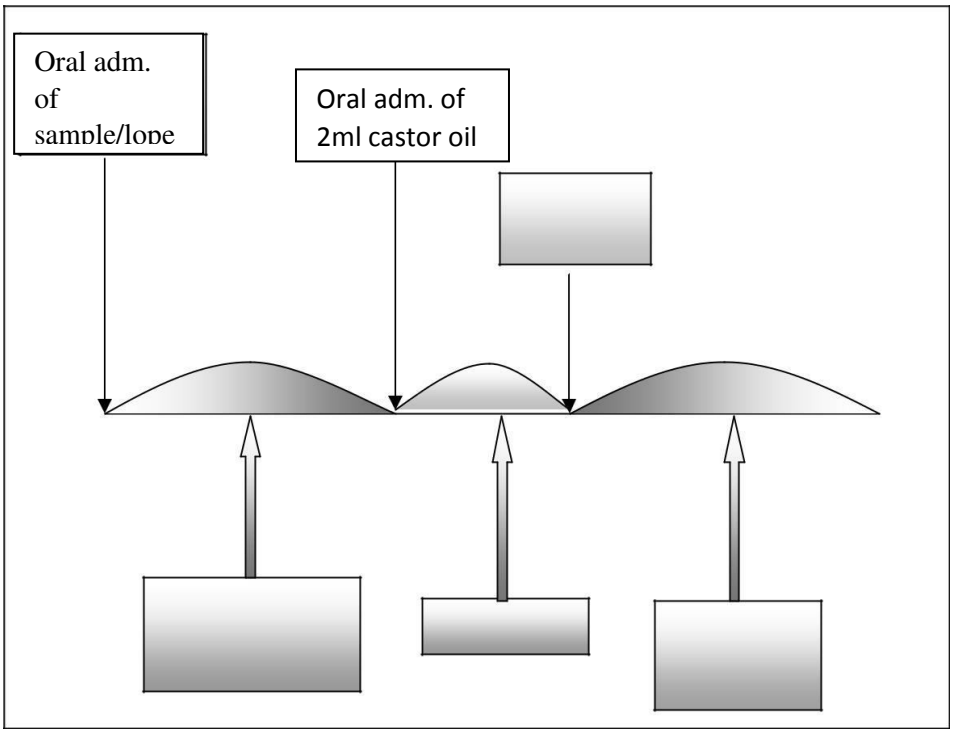

Fig 4.5: Schematic representation of study design of anti-diarrheal activity observation in test animal with castor oil induced defecation

Test material used				
Code no.	**Test Sample**	**Group**	**Identification**	**Dose(mg/kg)**
CTL	**1% Tween-80 in normal saline**	**1**	**Control Group**	**10 ml/kg of body wt.**
STD	**Lopiramide**	**2**	**STD group**	**5**
AEL	**Aqueous Extract of Leaves**	**3**	**Test Sample**	**250**

Table 4.10: Test material used

CHAPTER 05

RESULT AND DISCUSSION

5.1 Result and discussion of chemical group test

Major classes of therapeutically important compounds like carbohydrate, tannins, and alkaloids were found in the plant leaves after performing different chemical test for identification of the compounds. The phytochemicals present in the plant leaves are outlined in the following table:

Different chemical group test results	
Test for carbohydrate	
Molisch's test	Positive
Test for tannin	
Ferric Chloride test	Positive
Potassium Dichromate test	Negative
Lead Acetate test	Negative
Test for alkaloid	
Mayer's test	Positive
Dragendroff's test	Positive
Test for glycosides	Negative
Test For steroids	Negative

Table 5.1: Different chemical group test results

5.2 Result and discussion of analgesic test

The extract was also subjected to Swiss albino mice to study the analgesic activities against acetic acid induced writhing and analgesic actions were found with oral doses of 250 and 500 mg/kg which is shown in the following table:

Result obtained from evaluation of analgesic action				
Group	**Treatment**	**Dose**	**Writhes counted**	**% of Inhibition**
Control	Normal saline	-	24.3	-
Standard	Diclofenac	10	15.0	38.27
Test group 1	Leaves extract	250	16.33	32.75
Test group 2	Leaves extract	500	15.33	36.89

Table 5.2: Result obtained from evaluation of analgesic action

5.3 Result and discussion of Anti-diarrheal test

In case of castor oil-induced diarrheal test, the AEL showed a potent anti-diarrheal effect in the mice. The leaves extract (AEL) showed anti-diarrheal effect, in the mice upon administration of extract at doses 250 mg/kg compared to the standard group at the dose of 5 mg/kg. The above discussion indicates that *Averrhoa carambola* have Anti-diarrheal activities which prove the traditional use of this plant.

Result obtained from evaluation of anti-diarrheal action									
Code No.	**Mice no**	**1st hour**	**2nd hour**	**3rd hour**	**4th hour**	**Average**	**Total Average With SEM**	**Std. deviation**	**% Reduction diarrhea with SD**
CTL	1	1	1	1	2	1.25	4±0.083	0.144	-
	2	1	0	3	2	1.5			
	3	1	2	0	2	1.25			
STD	0	0	0	0	0	0	0	0	100
	0	0	0	0	0	0			
	0	0	0	0	0	0			
AEL	1	0	0	2	0	0.5	1.5±0	0.25	62.5
	2	0	0	1	0	0.25			
	3	0	1	0	2	0.75			

Table 5.3: Result obtained from evaluation of anti-diarrheal action

CHAPTER 06

CONCLUSION

Conclusion

The phytochemical screening confirmed the presence of several organic compounds. Pharmacological studies for the corresponding effects of the compounds may lead to the discovery of medicinally active lead compound.

Acetic acid-induced writhing test confirmed significant analgesic activity compared to standard diclofenac by reducing the writhing count. From this test, the peripheral analgesic action of the plant had been confirmed.

Castor oil induced defecation confirmed significant anti-diarrheal activity compared to standard imotil by reducing defecation. From this test, the anti-diarrheal action of the plant had been confirmed.

The results of the current studies indicate that further isolation and purification of the crude extract may lead to the discovery of lead compounds with potential activity.

CHAPTER 07

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