Phytochemical investigation and in vitro evaluation of antioxidant property and in vivo evaluation of cytotoxic, anthelmintic and antidiarrheal activity of Urena sinuata

DISSERTATION

(This report presented in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy)

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APPROVAL

This is notified that a Project report, Phytochemical investigation and in vitro evaluation of antioxidant property and in vivo evaluation of cytotoxic, anthelmintic and antidiarrheal activity of Urena sinuata is submitted by "151-29-791 to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents."

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DECLARATION

I hereby declare that, this project report is done by me under the supervision of Farhana Israt Jahan, Assistant professor, Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. "I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree."

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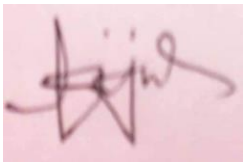

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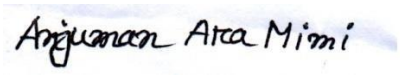

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Praise to Almighty Allah who has given me strength, patience and ability to make this project possible and for his providence, grace and mercy upon my life.

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Anjuman Ara Mimi

Dedicated to

My beloved parents & respected supervisor

Abstract

Urena sinuata is commonly known as Burr mallow and Bon-Okra (Bengali), one of the vital medicinal herb of Bangladesh. Its root is used as emollient, leave is used as anti-inflammatory and flowers is used in bronchitis. The current study deals with the phytochemical investigation and in vitro evaluation of antioxidant property and in vivo evaluation of cytotoxic, anthelmintic and antidiarrheal activity of methanolic extracts of *Urena sinuata* based on conventional use of plant. Thus the aim is to authenticate its traditional uses and representing the great potential of this species for further expansion within the pharmaceutical industry. Antioxidant activity was evaluated according to DPPH free radical scavenging method in which the positive control ascorbic acid showed IC50 value of 12.44µg/ml. On the other hand, the crude methanolic extract of whole plant showed promising DPPH free radical scavenging activity with IC50 value 30.73 µg/ml.. Methanol extracts of whole plant was most toxic &LC50 of 145.91µg/ml. The extract have significant anti-diarrheal activity with 77.27% and 68.18%% reduction of diarrheal feces in both 75mg/ml and 150mg/kg body wt. compared to standard loperamide which reduce 72.73%. Methanolic extract of the leaves of *Urena sinuata* shows anthelmintic activity against helminthes. Death time of standard Albendazole is 14min (25mg/ml). On the other hand, with increasing the concentration of the plant's extract decrease the death time (at 250mg/ml death time is 48.33min). In a nut shell, this study suggest that the extracts of *Urena sinuata* retains anti-oxidant, anti-diarrheal, cytotoxic and anthelmintic properties, which upkeep its use in traditional medicine. In future the plant should be further investigated for its pharmacologically active compound.

Key words: Antioxidant, cytotoxic, antidiarrheal, anthelmintic, phytochemical

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Phytochemical investigation and in vitro evaluation of antioxidant property and in vivo evaluation of cytotoxic, anthelmintic and antidiarrheal activity of *Urena sinuata*

CHAPTER 1

INTRODUCTION

1. Introduction

1.1 Introduction to Phytomedicine

"Herbal-based modern medical practice that uses various plant materials in modalities careful both preventive and therapeutic. Phytomedicine or the use of herbal medicine with beneficial properties has played a important role throughout history. Although its usage greatly reduced during the dawn of the scientific era, there is a stimulation of interest in its potential by late 20th century, especially in the expansion of new drugs".[1]

1.2 Phytomedicine in global health care

Plants have been the origin of many traditional medicine systems all over the world for thousands of years and continue to deliver manhood with new remedies. Plant based medicines initially distributed in the form of crude drugs such as essences, teas, poultices, powders, and other herbal formulations, now serve as the basis of novel drug detection. Phytomedicine, popularly known as herbal medicine, denotes to the use of plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. According to World Health Organization (WHO), from 119 plant-derived medicines, about 74% are used in modern medicine in ways that compare directly with their traditional uses. WHO also estimates that 4 billion people, 80% of the world's population, presently use herbal medicine for primary health care. Herbal medicine is a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional oriental, Native American and Indian medicine. Even among prescription drugs, at least 25% contain at least one compound derived from higher plants. The percentage might be developed if we comprise over-the counter (OTC) drugs (Barrett et al., 1999).

"In developing countries including Bangladesh, about 75% of the populations depend on different forms of traditional medicine for their crucial health care (Matu and Staden, 2003). The high cost of imported conventional drugs and/ or inaccessibility to western health care facility, imply that traditional mode of health care is the main form of health care that is affordable and available to our rural people".

1.3 Prospect of natural products and phytomedicine

"Several methods have been used to gain compounds for drug discovery, including isolation from plants and other natural sources, synthetic chemistry, combinatorial chemistry and molecular modeling. Despite the recent interest in molecular modeling, combinatorial chemistry

and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, natural products and particularly medicinal plants, continue an important source of new drugs, new drug leads and new chemical objects (NCEs). According to Newman et al. (2003), 61% of the 877 small-molecule NCEs introduced as drugs worldwide during 1981–2002 was inspired by natural products. These include: natural products (6%), natural products derivatives (27%), synthetic compounds with natural products-derived pharmacophore (5%) and synthetic compounds designed from natural products (natural products mimic, 23%) (Butler, 2004; Geysen et al., 2003;Lombardino and Lowe, 2004). New drugs derived from natural sources have been thrown on the market during the last twosome of years.

These new drugs have received approval for the treatment of cancer, neurological diseases, infectious diseases, cardiovascular and metabolic diseases, immunological, inflammatory and related diseases, and genetic disorders, which involve many of the common mortal diseases".

1.4 Role of phytomedicine in human society:

"People on all landmasses have used hundreds to thousands of native plants for treatment of illnesses since primitive times [2]. Aboriginal healers often privilege to have learned by perceiving that sick animals change their food favorites to bite at bitter herbs they would normally reject [3]. Coastal brutes take 90% of their regime from the fruits of Aframomum melegueta, a relative of antiviral, antibacterial, antifungal and anthelminthic properties, a reasonable case can be made for self-medication by animals in the wild[4]. A plant that is bland to a particular animal may not be safe for humans to ingest [5]. A reasonable conjecture is that these discoveries were the ginger plant that is a potent antimicrobial and apparently keeps shigellosis and similar infections at bay" .[6]

1.5 Herbalism

"Herbalism makes orientation to as phytotherapy or botanical medicine, is one of the initial systems of medicine known. It is the practice of making or prescribing plant based herbal therapies for medical conditions. Today's medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs. Although phyto-therapy may spread over modern standards of usefulness testing to herbs and drugs derived from natural sources, few high-quality clinical trial and standards for purity, efficacy, potency and dosage existence. Herbalism is the use of organic crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be total, continuous or powdered.[7] [8]

Phytotherapy varies from plant-derived medicines trendy standard pharmacology because it does not isolate and regiment the compounds from a given plant supposed to be biologically active.[9]

A medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils some rare compounds like hydroxycoumarins, napthoquinones, acylphloroglucinols and sterones are also dispersed among the plant kingdom. The active constituents are usually secondary metabolites, derived from biosynthetic pathways present within the plant tissue. In 1985 it was verified that 74% of the 119 plant derivative drugs were exposed as a result of chemical studies to isolate the vigorous substances accountable for their traditional use. [10]. *Urena sinuata* is used as a traditional medicine from the ancient time. This plant considered to be a chemical factory of alkalods, glycosides, resins, gums,flavonoid,tannin etc."

Figure 1.1: Herbal plants

1.6 History of Herbalism

"Traditional medicines has a very long history.A number of traditional medicine leading to the allopathic medicine at the end of the twentieth century such as The Siddha and Ayurvedic medicine systems from various South Asian Countries [11], Chiness herbal medicinal , native American medicine[12] .According to the WHO about 80% need of the medicine is satisfied

from the herbal medicine sources. In fact, 70% of German physicians recommend plant-based medicines for some part of their main healthcare."[13]

"400 B.C.– In herbal medicine the Greeks joined first by Greece scientist Hippocrates of Kos, also known as Hippocrates. Hippocrates blowout out the ideas that diet, exercise, and overall happiness formed the foundation of wellness.

1800 A.D.–. The National Association of Medical Herbalists was designed, and later renamed the National Institute of Medical Herbalists (NIMH.)

2000 A.D.– EU took achievement on regulation and testing of herbal medicines alike to those used for pharmaceuticals.

Since 4000 years , the herbal medicines have remained documented. These medicines have lasted real world testing and thousands of years of human testing. Some medicines have been banded due to their toxicity and adverse effect, while others have been modified or combined with additional herbs to neutralize side effects'.[16][17][18]

1.7 Medicinal plant

"The medicinal plants means that the plant contain active compound, which have biological activity in our body. Different parts may hold this active compound such as leaves, barks, roots, seeds, fruits, stem etc. are used to medicinal purpose. The medicinal plants also created some compound for their own defence. [14] A plant whose parts encompassing active chemical constituents or substance can be used for medical purpose is called medicinal plant. Alternatively, this plant can act as pioneer for the synthesis of other drugs. Medicinal plants have been in performance a great role in curative process for many years. From the origin of medicinal plant, they were connected with religion. Nobody knows that accurately when medicinal plants were used for the first time. Most medicinal plants were discovered as a result of search of new type of food. First written text on the use of medicinal plant is about 5,000 years old and was written by Sumerians, in ancient Mesopotamia".[15]

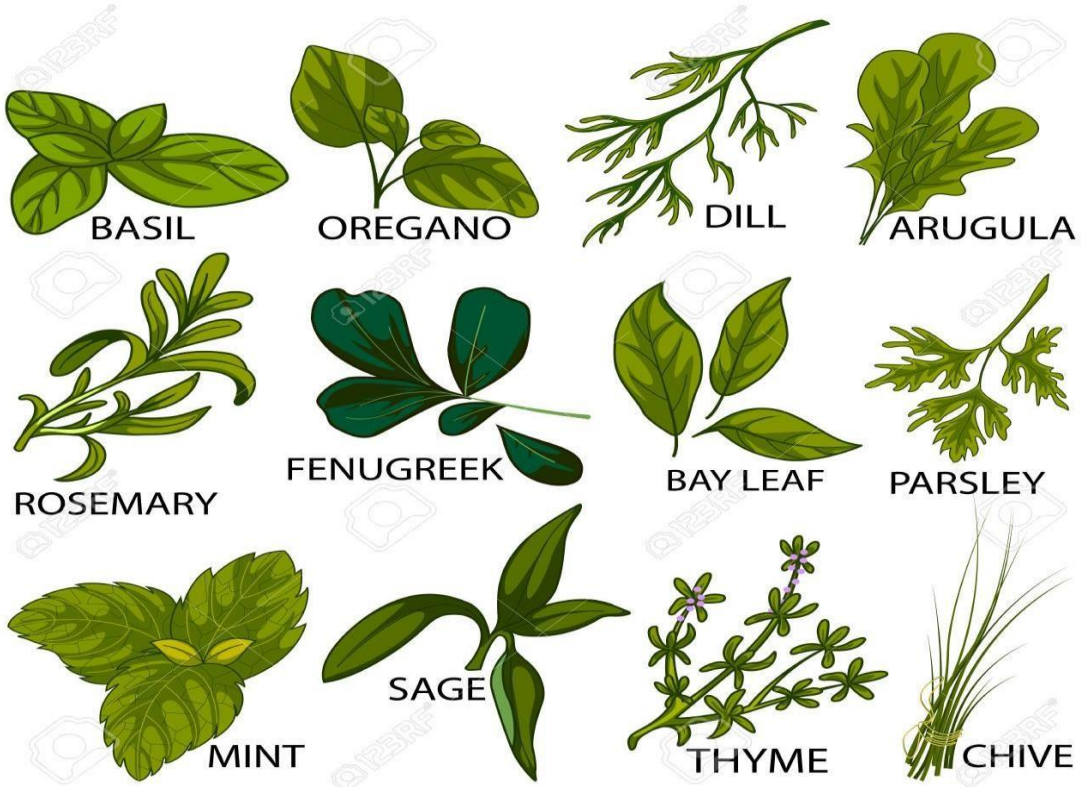


Figure1.2: Some medicinal plant

1.8 Natural product research and drug discovery

"Nature seems to be a therapeutic abundance to treat indulgence of diseases ranging from common cold to diverse type of illness since the dawn of evolution. Irresistible evidence has accumulated showing that natural products from plants, microorganisms and marine organisms encompass major portion of the total range of the available therapeutic drugs. Products of natural origins are often called natural products.Natural products include: an entire organism (e.g., a plant, an animal, or a microorganism) that has not undergone any kind of processing or treatment other than a simple process of preservation (e.g. drying), part of an organism (e.g.leaves or flowers of a plant, an isolated animal organ), an extract of an organism or part of an organism, and exudates, and pure compounds (e.g. alkaloids, glycosides, sugars, flavonoids, coumarins, lignans, steroids, terpenoids, etc.) isolated from plants, animals, or microorganisms (Samuelsson, 1999)".

" Natural products have played a strategic role in drug discovery research, as many medicines are either natural products or derivatives there of. Indeed, it is assessed that about 40% of all medicines is one or the other natural products or their semi-synthetic derivatives. This may not be astonishing as herbal medicine is a tradition of healthcare since ancient times and natural extracts screening has been one of the roots of drug discovery research, where erythromycin and rifampicin (bacterial infections), statins (hyperlipidemia), quinines and artimesinin (malaria), paclitaxel, vinblastine and vincristine (cancer), are a few well-known natural products-based medicines.

For bacterial infections, over 80% of all medicines in medical use is either natural products or their derivatives, while for anticancer agents over 60% of all drugs is either natural products or derivatives ; examples of numerous potential lead molecules are vincristine, vinblastine, taxol, camptothecin, etc which have been isolated from plants for effective use in cancer treatment (Newman and Cragg, 2007; Butler, 2004; Newman et al., 2000).In earlier times, all drugs and medicinal agents were derived from natural constituents, and most of these medications were obtained from higher plants."

1.9 Approaches to natural product research and drug discovery

"Drug discovery from plants involves a multidisciplinary approach merging botanical, ethnobotanical, phytochemical and biological techniques. The search for bioactive chemicals from the natural part of the plant kingdom can be shown essentially with three methods (Cotton, 1996); the random method involves the collection of all plants found in a given area of study, phylogenetic pointing means the collection of all members of those plant families which are known to be rich in bioactive compounds, and the ethno botanical approach is based on the traditional knowledge of medicinal plant use. It has been suggested that the ethno-directed sampling is most likely to succeed in distinguishing drugs for use in the conduct of gastrointestinal, inflammatory and dermatological protests.. These can be broadly divided into two categories:"

1.9.1 Older approach

- "Intensive on chemistry of compounds from natural sources, but not on activity.
- Conventional forward isolation and identification of compounds from natural sources followed by testing of biological action in animal model.
- Selection of organisms mainly based on ethnopharmacological information, folkloric characters, or traditional uses.''

1.9.2 Modern approach

- "Bioassay-engaged (mainly in vitro) isolation and identification of active lead compounds from natural sources.
- Production of natural products libraries.
- Production of active compounds by cell or tissue culture, genetic manipulation, natural combinatorial chemistry and so on.
- More focused on bioactivity.
- Selection of organisms based on ethnopharmacological information, folkloric reputations, or traditional uses, and also those indiscriminately selected".

1.10 Opportunities in drug discovery from medicinal plants

"When ethnobotanical or ethnopharmacological approach is utilised, additional specific necessities that relate to prior informed consent, recognition of Indigenous Intellectual Property and Indigenous Intellectual Property Rights as well as short- and long-term benefit distribution need to be taken into account (Patwardhan, 2005).

In order to screen thousands of plant species at one go for as many bioassays as possible, we must have a collection of a large number of extracts. Worldwide, there is a need to build natural products extract libraries. The extract libraries offer various advantages, such as reduction in cost and time for recurrence collection of plants and availability of properly encoded and preserved extracts in large numbers for biological screening in terms of high-throughput screenings and obtaining hits within a short period.."

1.11 Medicinal plants in Bangladesh

"Bangladesh has an huge amount of medicinal plants. Inappropriately, there are not sufficient systematic efforts to enlarge and develop this appreciated potential, except for some intermittent institutional trials. The researcher's original information was one of the most difficult to use, and the obtainability and use of medicinal plants for therapeutic purposes in this country. Medicinal plants mostly used in the preparation of Unani and Ayurvedic medicines are also prescribed by the doctors of traditional medicine in different parts of the country and others are used as domestic remedies by the general public. Traditionally, these plants have been collected in forests and wild areas, but there have been no systematic efforts to improve the cultivation practices of these plants.

297 Unani, 204 Ayurvedic and 77 Homeopatheic, some mutual medicinal plant in Bangladesh that used as therapeutic purpose. Medicinal plant species is listed by WHO &it can be grown in Bangladesh commercially."[19]

1. Table :Medicinal plant species list

Scientific name	Bengali name	Used parts	Used as patent drug
Winthania somnifera Dunal	Ashwagandha	Root, Leaf, Fruit, Seed	Syrup:Masturin,Arq Ashwaganda.
Aloe vera Tour. ex Linn.	Ghritokumari	Leaf	Tablet:Suranjan,Syrup :Belgiri
Andrographis panniculataWall.ex Nees.	Kalomegh	Leaf, Stem,whole plant	Syrup :Safi, Kurchi
Asparagus racemosus Willd.	Satomuli	Tuberous root, Leaf, Flower, Fruit	Tablet:Abiaj, Khisandha, Ka-4, Sufoof Gigian
Glycyrrhiza glabra Linn.	Jastimodhu	Root, Stem	Tablet:Sualin,Mauol,Hiat, Syrup: Badian,

1.12 Plant review

1.12.1 Introduction of plant

Urena sinuata is a genus of plants which cultivate in various tropical and subtropical areas all over the world. Some view the plant as a weed, but others make use of its fiber for numerous purposes. The leaves and flowers are also a scarcity food in Africa. Its seeds are blowout by animals.[20]

The common name of *Urena sinuata* is Burr Mallow.It is an vertical subshrub to 1-2 m tall. Leaves are palmately deeply lobed, the sinuses (curves) are rounded. Leaves are downy grayish teenage with star-shaped hairs, 4-8 cm long. Flowers are pinkish-violet, about 1 cm across, clustered. Flowers in close fascicles on the axis of terminal or axillary racemes, which are 10-40 cm long.Calyx 4-5 mm long, corolla 6-8 mm long, purple [21][22]

Fruit is velvety and covered with hooked bristles. The genus name Urena comes from its Malayalam local name Uren. This plant is thoroughly related to *Urena lobata*, which has leaves with lobes pointed, and not rounded.[21][22]

Figure 1.3: Urena sinuata

1.12.2 Taxonomic classification

Kingdom: Plantae

Division: Tracheophyta

Order: Malvales

Family: Malvaceae

Class: Mangoliopsida

Subfamily: Malvoideae

Tribe: Hibisceae

Genus: Urena

Species: Urena sinuate[23][24]

Common name: Burr mallow, Caesarweed, Congo jute, Hibiscus burr, Pink burr, Pink Chinese burr, Urena burr

Synonyms: Urena aculeata, Urena morifolia, Urena muricata, Urena paradoxa, Urena swartzii [25]

1.12.3 Geographical distribution

Generally distributed over the hotter parts of India and tropics of both hemispheres.

1.12.4 Morphological features

The plant is shrub of 1-2 m high, leaves 2.5-7.S cm long, more or less stellately hairy on both surfaces, cordate or shorten at the base, irregularly lobed to below the middle; lobes 3-5 or more, expanded upwards, with curved sinuses, indent or toothed, pale beneath, with in most cases, a gland near the base of the midrib and sometimes on one or both of the adjoining nerves, petioles 2-4 cms long, flowering time is from Oct-Nov, pedicels short, axillary, flowers clustered, involucral bracts 1.5cms and connate at the base, linear- oblong, acute, as long as, or slightly longer than the calyx and alternate with its lobes, calyx minutely pubescent, lobes lanceolate. Corolla rose-coloured, connate below, united to the staminal tubes. [26]

1.12.5 Medical uses of Urena sinuata

The roots are considered emollient and refrigerant. They are castoff in external uses for lumbago. The leaves are prescribed in inflammation of the intestines and bladder. In Brazil, the plant is attributed with emollient and refrigerant. An infusion of the flowers is used in bronchitis Urena sinuate is used for unspecified male problem [26][27]

1.12.6 Chemistry of Urena sinuata

Urena sinuataL. consists of three 6-membered rings for the aglycone fragment and one 6 membered ring from the glycoside unit, which exhibits a chair conformation. The packing arrangement is governed by twelve (12) conventional hydrogen bonds, three (3) of them are conventional intra-molecular hydrogen bonds and nine (9) conventional intermolecular hydrogen bonds. In addition, four (4) intra-molecular non-conventional interactions are clearly displayed.[28]

The major components are the flavonoids: quercetagetin-6,7-O-dimethylether-3'-β-D-gluco-pyranoside (I), quercetagetin-6,7-O-dimethylether-4'-β-D-glucopyranoside (II), and quercetagetin-6,7-O-dimethylether-3-β-D-glucopyranoside (III). These products we characterized through their physical constants, UV, MS, and one- and two-dimensional NMR studies.[29]

CHAPTER 2

PURPOSE OF THE STUDY

2. PURPOSE OF THE STUDY

The purpose of this study is that investigation of the anthelmintic, cytotoxicity,anti-oxidant and antidiarrheal activity of the *Urena sinuata*. In Bangladesh this plant is available and suitable for growing that is why I selected this plant because if this plant shows any activity that will be helpful for our country people. In Bangladesh about 25% population live in poverty line they are not capable to visiting hospitals and taking modern medicine.

It is absolutely needed to find out the safe, less or no side/adverse effect of the herbal drugs, because of the natural products of higher plants may definitely give a new source of cytotoxic, antidiarrheal, anthelmentic, anti-oxidant activity.

Cytotoxicity is the quality of being toxic to cells. Chemotherapy is a type of cancer treatment that uses one or more anti-cancer drugs to kill cancer cells [30], but it has some potential side effects. Cancer treatment is also very expensive. For this reason, Urena sinuata plant is tested for its cytotxic effects so that the study can help the researcher to discover a safer alternative for the treatment of cancer at low cost.

Chemical composition of this plant contains flavonoid, tannins. They are some type of anti-oxidant. Antioxidants that are reducing agents can also act as pro-oxidants. Antioxidants are nature's way of protecting the body and cells from damaging free radicals. Free radicals are unstable molecules that are generated by sun exposure, stress and as part of the natural aging process. Free radicals damage cells, DNA and collagen. Free radicals may play arole in heart disease, cancer and other diseases.[31]

The wormicidal activity of alcohol extract against earthworms suggests that it is effective against parasitic infections of humans. It would be interesting to identify the active principle responsible for the anthelmintic activity and to study its further pharmacological actions.[32]

At the beginning of nation, secondary metabolites of plants have been used for mankind as remedies. These plants were including the secondary metabolites like alkaloids, glycosides, flavonoids, tannins, steroids and terpenoids. For this reason, If I identify the two pharmacological effect for the people that they can also use of this plant as a drug against diarrheal ,cancer cell,helminthic,aging.

Diarrheal disease is often a significant source of mortality and morbidity, particularly among children in emerging countries affecting a major healthcare problem. As per recommendation of WHO, management of diarrhoea with traditional medicine is the main effort of our current studies. The current study is planned to evaluate the anti-diarrheal activity of crude methanol extract *of Urena sinuata* [42]

CHAPTER 3

LITERATURE REVIEW

1. **Title: "Flavonoids from Urena sinuataL".**

Authors: "Adakarleny Sosa & Carmelo Rosquete* Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias".

"In this work it is exposed the obtained results of the phytochemical study of the fresh leaves of Urena sinuataL. (dog wart). The major components are the flavonoids: quercetagetin-6,7-O-dimethylether-3'-β-D-gluco-pyranoside (I), quercetagetin-6,7-O-dimethylether-4'-β-D-glucopyranoside (II), and quercetagetin-6,7-O-dimethylether-3-β-D-glucopyranoside (III). These products were characterized through their physical constants, UV, MS, and one- and two-dimensional NMR studies. By other hand, the obtained results of the Artemia salinacytotoxicity bioassay carried out to the isolated products are exposed".[28]

2. **Title: "Crystal Structure Analysis of 6,7-di-O-Methyl-Quercetagetin-3-O-DGlucopyranoside dihydrate Isolated from Urena sinuata L"**

Authors: "Adakarleny Sosa1, Carmelo Rosquete1, Julia Bruno, Luis Rojas, Laurent Pouységu, Stéphane Quideau,Jean-Michel Leger, Stéphane Massip, Marie-Aleth Lacaille-Dubois, Anne-Claire Mitaine-Offer"

"In the present work, the structural analysis of 6,7-di-O-methyl-quercetagetin-3-O-β-D-glucopyranoside dihydrate(I), which was isolated from Urena sinuata L. (dog wart) collected in Táchira-Venezuela, was achieved by single crystal X-ray diffraction. Compound I crystallizes in the monoclinic system, space group C2 (No. 5) with unit cell parameters a = 29.289(3) Å; b = 6.6352(7) Å; c = 14.6533(13) Å; β = 113.636(6)°; V = 2608.8(5) Å 3; Z = 4. The structure refinement converged to R = 0.0421, wR2 = 0.1195, S = 1.02. This is the first X-ray report of thiscompound obtained from U. sinuata L".[28]

3. **Title: "Sedative, Anxiolytic, Antinociceptive,Anti-inflammatory and Antipyretic Effects of a Chloroform Extract from the Leaves ofUrena sinuata in Rodents"**

Authors: "Talha Bin Emran,Tajbiha-E-Mowla, Shahriar Ahmed4, Sumyya Zahan, Ahmed Rakib, Mohammed Shamim Hasan, Mohammad Nurul Amin, Tasmih Rob Mow and Mir Muhammad Nasir Uddin"

Context: "Urena sinuateL. (Malvaceae) is a well-known medicinal plant and it is used in traditional medicine systems. Objectives: The current study unravels the neuropharmacology, antinociceptive, anti-inflammatory and antipyretic effects of the chloroformextract of Urena sinuataleaves (CEUS) in rodents and alsoOriginal Research Article determine the possible mechanism of antinociception which is involved inits acute toxicity and phytochemical studies.Materials and Methods: Neuropharmacological activities of CEUS were performed by hole cross, open field test, elevated plus-maze test and Thiopental Na inducedsleeping time test. For the analgesicactivity of CEUS,different methods likehot plate test, acetic acid induced test, formalin-induced test, tail immersion test and glutamate-inducednociception were used. Additionally,a possiblemechanism of nociception was identified by cyclic guanosine monophosphate (cGMP) and ATP-sensitive K+ channel pathway analysis. Carrageenan-induced rat paw edemaand cotton pellet-induced granuloma test were conducted to detect anti-inflammatory activity and brewer's yeast induced pyrexia test for antipyretic activity. Before 60 min of subjection to the respective test, the extracts (200 and 400 mg/kg) were given orally.Results: The obtained results showed that CEUS produced significantly($p < 0.05$) neuropharmacological, anti-inflammatory and antipyretic activity with low or no toxicity. Moreover, in all the thermal and chemical-induced nociception models, antinociceptive response was exhibited. Furthermore, it involved cyclic guanosine monophosphate (cGMP) as well as ATP-sensitive K+ channel pathway mediated antinociceptive effect. Conclusions:These data show that CEUS has significant neuropharmacological, anti-inflammatory and antipyretic effects that appear to have a relation with the inhibition of the glutamatergic system. Thus, the leaves of Urena sinuatacould be used in the treatment of several types of inflammation in intestines and bladder".[33]

4.Title: "Evaluation of the anti-diarrheal activity of methanol extract and its fractions of Urena sinuata L. (Borss) leaves"

Authors: "Mir Muhammad Nasir Uddin, Sumyya Zahan, Md. Ashiqul Islam, Shahriar Ahmed, Tajbiha-E-Mowla1,Mohammad Sofiqur Rahman, Ramiz Ahmed Sultan, Talha Bin Emran,"

Objectives: "Diarrhoeal disease is often a leading source of mortality and morbidity, especially among children in developing countries causing a major healthcare problem. As per suggestion of WHO, treatment of diarrhea with traditional medicine is the main focus of our present studies. The present study is designed to evaluate the anti-diarrhoeal activity of crude methanol extract of Urena sinuata L. (U. sinuata) and its fractions.

Methods: Crude methanol extracts are obtained by in vacue methods and its fractionating is done by Kupchan partitioning method. The anti-diarrhoeal activity is screened by castor oil-induced diarrhoea, castor oil-induced enteropooling and gastrointestinal motility test.

Results: The diarrheal severity was reduced significantly ($P < 0.01$) by ethyl acetate fraction by 37.01% whereas 44.78% inhibition was found for standard drug loperamide at 5 mg/kg. The two fractions namely hydro methanol, ethyl acetate and crude methanol extract also significantly ($P < 0.01$) reduced the intestinal volume in case of castor oil induced enteropooling.

Conclusion: It is concluded that, leaves of U. sinuata contains bioactive natural substances with anti-diarrhoeal properties. These attributes may give a justification for your use of U. sinuata in diarrhoea management by traditional healers."[34]

5.Title: " Insecticidal and repellent activities of the chloroform extracts of Urena sinuata L. against Tribolium castaneum (Herbst) adults"

Authors: "M Abdullah, A Kumar Pk, D K M A Saleh, R Islam, A R Khan and N Islam"

Abstract: “The insecticidal and repellent activity tests of the chloroform extracts of fruit, leaf, root and stem of Urena sinuata L. against the red flour beetle Tribolium castaneum L. adults have been conducted. The leaf, root and stem extracts showed high toxicity by giving mortality of the beetles within 30 min. of exposure. The LD50 values for the 12, 24, 36 and 48h of exposures were 0.354, 0.262, 0.209 and 0.196mg cm2 for the fruit extract; 0.587, 0.413, 0.355 and 0.299mg cm2 for the leaf extract; 0.440, 0.389, 0.340 and 0.268mg cm2 for the root extract and 0.968, 0.661, 0.491 and 0.362mg cm2 for the stem extract. The insecticidal activity can be arranged in the order of fruit> root> leaf> stem extracts. The root and stem showed repellent activity against T. castaneum adults at P<0.01 and P<0.05 levels of significance, while the fruit and the leaf extractsdid not show repellency at all’’.[35]

6.Title: “Sedative, anxiolytic and analgesic effects of Urena sinuata L. leaf extract in animal models”

Authors: “Emran, T. B. and Rahman,”

Abstract:

“The sedative and analgesic potential of Urena sinuata L. was investigated for the first time in this study. The crude methanol extract of Urena sinuata L. leaves was evaluated for its central nervous system (CNS) depressant effect using rodent behavioral models. Methanol extract of Urena sinuata at a dose of 400 mg/kg body weight, displayed a suppressive effect on motor activity, exploratory behavior (in hole cross and open field tests) and prolongation of thiopental induced sleeping time in mice. In the elevated plus-maze (EPM) test, the same dose of methanol extract significantly ($p < 0.05$) increased the time spent by the treated mice in EPM open arms. Analgesic potential of the extract was also evaluated for centrally acting analgesic activity using formalin induced licking response model and for peripheral analgesic action using acetic acid-induced writhing test and tail immersion tests. In formalin induced licking response model, a significant ($p < 0.05$) inhibition of pain compared to reference drug diclofenac sodium was observed. In acetic acid-induced writhing test and tail immersion test, the extract at 200 mg/kg body weight produced a significant reduction of writhing response and pain respectively. These results evidenced the potential sedative and analgesic effects of Urena sinuata leaves”.[36]

7.Title:"PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT ACTIVITY OF CHLOROFORM EXTRACT OF URENA SINUATA (L.)''

"Authors: Dibyajyoti Saha, Swati Paul "

Abstract:

"The present study was designed to investigate antioxidant properties of chloroform extract of Urena sinuate along with phytochemical study for the presence of phytochemical constituents. Phytochemical analyses were found to be positive for carbohydrates and gum, reducing sugar, alkaloid, steroid, glycoside and flavonoids.Antioxidant potential was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assays. In DPPH scavenging method, scavenging of DPPH was observed in different concentrations (20, 40, 60, 80, 100, 200, 400, 800μg/ml). Plant extract found to demonstrate significant scavenging activity which was found to increase with concentration of the extract with IC50 value of 10.64μg/ml while IC50 value of the reference ascorbic acid was 1.61μg/ml.''[37]

CHAPTER FOUR

METHODS AND MATERIALS

4. Materials & Methods

4.1 Materials required for extraction:

The phytochemical evaluation of a plant can be divided into following steps:

a) Collection of the plant sample.

b) Drying of the plant leafs at room temperature.

c) Grinding of the plant leafs and preparation of powdered plant material.

d) Preparation of the plant material.

e) Extraction of the plant material by rotatory evaporator.

4.2 Collection of the plant leaves

Urena sinuata was collected from Asulia, Dhaka.

4.3 Drying and grinding

"The plant leafs were separated from the unwanted materials and dried at room temperature for two weeks. The plant leafs were ground into a granular powder with the help of suitable grinder".

4.4 Methanol extraction by the plant powder

"About 800gm of powder materials was taken in a clean and dried glass beaker and soaked in 1000 ml of methanol. The container with its contents was sealed and kept for a period of 15 days".

4.5 Evaporation of the solvent

"A rotary evaporator is a device used in chemical laboratories for the competent and gentle removal of solvents from samples by evaporation. Evaporation of the solvents through rotatory evaporator at 60 degree temperature".

Figure:Methanolic extract.

Fig: Rotatory evaporator

4.6 Materials Required

4.1.1 Table: Materials required

Serial no.	Materials	Source
1.	Amber Glass Jar (2litres bottle)	Used Reagent Bottle
2.	Beakers (0.5 litre)	Borosil, Germany
3.	Funnel	Glassco Laboratory Equipments,UK
4.	Filter Paper	Whatnam Filter Paper
5.	Beaker (100ml)	Borosil, Germany
6.	Beaker (50ml)	Glassco Laboratory Equipments,UK.
7.	Glass rod	--
8.	Dropper	--
9.	Stand with clamp	Glassco Laboratory Equipments, UK
11.	Measuring cylinder	--
13.	Oven	MMM, Germany
14.	Laminar air flow	--
15.	Autoclave	--
16.	Spatula	--
17.	Test tube	--
18.	Uv- visible spectrophotometry	--

4.7 Solvents Required

4.1.2 Table: Solvents Required

Serial no.	Solvents	Source
1.	Methanol	Merck Germany
2.	Ethanol	Merck Germany

4.8 Experiment Plant

Urena sinuate included in Malvaceae was investigated in this study.

Plant Name	Family	Plant part used
Urena sinuate	Malvaceae	Whole plant

4.9 Chemicals required

4.1.3 Table: Chemicals required

Serial no.	Chemicals name	Source
1.	α-napthol	Merk, Germany
2.	Ethanol	Merk, Germany
3.	Sulphuric Acid	Active Fine Chemicals
4.	Copper sulphate	Merk, Germany
5.	Sodium Potassium tartarate	JHD Chemical, China
6.	Sodium hydroxide (pellet	Merck Specialities Private Limited, India
7.	Sodium Citrate	Merk, Germany
8.	Sodium Carbonate Anhydrous	Active Fine Chemicals
9.	Mercuric Iodide	Active Fine Chemicals
10.	Potassium iodide	Merck Specialities Private Limited, India
11.	Bismuth Nitrate	Merk, Germany
12.	Tartaric Acid	JHD Chemical, China
13.	Ferric Chloride	Merk, Germany
14.	Lead Acetate	JHD Chemical, China
15.	Hydrochloric Acid	Merk, Germany
16.	Distilled Water	---

4.10 Chemical group test

"Testing of different chemical groups contain in extract represent the preliminary phytochemical studies.The following reagents were used for the different chemical group test "

4.10.1 Mayer's reagent:

"1.36 gm. mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm. of potassium iodide in 20 ml of water".

4.10.2 Dragendroff's Reagent:

"1.7 gm. basic bismuth nitrate and 20 gm. tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm. potassium iodide and 40 ml water".

4.10.3 Fehling's solution A:

"34.64 gm. copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml".

4.10.4 Fehling's solution B:

"176 gm. of sodium potassium tartarate and 77 gm. of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use'.

4.10.5 Benedicts Reagent:

"1.73 gm. cupric sulphate, 1.73 gm. sodium citrate and 10 gm. anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water"

4.10.6 **Molish Reagent**: 2.5 gm. of pure α-naphtha was dissolved in 25 ml of ethanol.

4.1.4 Table: **Different chemical group tests performed and the results are mentioned**

Sample	Test solution	Observation
# **Test for Alkaloids:**	0.1 ml of Mayer's	Yellowish buff colored precipitate

2 ml solution of the extract and 0.2ml of dilute hydrochloric acid	Reagent.	indicate the presence of alkaloid
# 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid.	0.1 ml of Dragendroff's reagent.	Orange brown precipitate indicate the presence of alkaloid
Test For Glycosides: A small amount of an alcoholic extract was taken in 1ml of water.	A few drops of aqueous NaOH was added.	A yellow color indicate the presence of glycoside
# A small amount of an Alcoholic extract was taken in water and alcohol.	Boiled with Fehling's solution.	Brick-red precipitate Indicate the presence of glycoside
Test for Steroids: # 10 mg extract dissolved in 1 ml chloroform.	1 ml sulfuric acid.	Chloroform layer acquired reddish brown color indicate the presence of steroid
Tests for Gums : # 5 ml solution of extract.	Molish reagent and sulfuric acid.	If Red –violet ring produced at the junction of two liquid,it indicate the presence of gum.
Tests for Flavonoids: # 1 ml solution of ethanolic extract.	Few drops of conc. HCl was added to the extract.	If Immediate red color is formed, it indicate the presence of flavonoid
Tests for Saponins: # 1 ml solution of the was diluted distilled water to 20 ml.	Shaken in a graduated cylinder for 15 minutes.	If centimeter layer of foam is formed, it indicate the presence of saponin.
Tests for Tannins: # 5 ml solution of extract	1 ml of 5% of Ferric chloride solution.	Greenish black precipitate indicate the presence of tannin

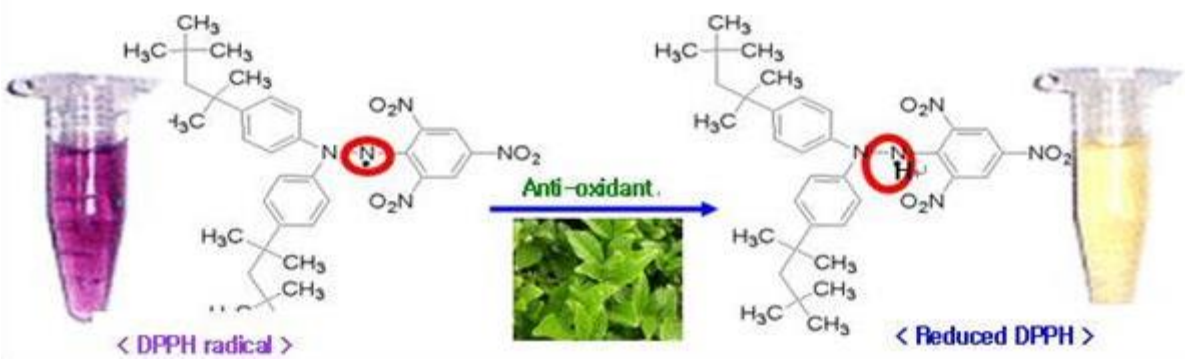
ANTI-OXIDANT ACTIVITY

5. ANTIOXIDANT EVALUATION BY DPPH METHOD

5.1 Principle

"The current study was planned at evaluating the In vitro free radical scavenging activity of *Urena sinuata* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the method of Brand-Williams. 2.0 ml of a methanol solution of the extract at dissimilar concentration were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of ascorbic acid by UV spectrophotometer".

"The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptive of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. DPPH radical scavenging activity is described as IC50 which is the concentration of samples to produce 50% reduction of the DPPH".



5.2 Materials and methods:

DPPH was used to evaluate the free radical scavenging activity of various compounds and medicinal plants.

5.2.1 Materials

1,1-diphenyl-2-picrylhydrazyl	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Distilled water	Test tube
Ethanol	Light-proof box
Amber reagent bottle	Pipette (5 ml)
Foil paper	Micropipette (10-1000 µl)

5.2.2 Methods

- "At first ten test tubes were taken and washed properly.

- 10mg plant extract was taken in test tube and dissolved with 10ml methanol with continuously stirring. Here the concentration was 1000 µg/ml. This solution was use as sample mother solution.

- 10mg ascorbic acid was taken in 10ml volumetric flask and dissolved with methanol with continuously stirring and placed in a dark placed. Here the concentration was 1000 µg/ml. This solution was used as mother solution for standard.

- 2ml methanol was taken in each ten test tube and 2 ml Plant extract was taken from mother solutions in first test tube then serial dilution was done by using following concentration:- 500,250,125,62.5,31.25,15.625,7.813,3.906,1.953a,and 0.9775µg/ml.

- Same work done for ascorbic acid each ten test tube by using following concentration:500,250,125,62.5,31.25,15.625,7.813,3.906,1.953,and0.9775µg/ml., which was used as a standard.
- 0.004gm DPPH was taken in 100ml volumetric flask and dissolved with ethanol with continuously stirring, and placed in a dark placed and placed in a dark placed for 15 minutes. Here the concentration was 20 µg/ml.
- After 15 minutes 2 ml DPPH were added to all 20 test tubes of sample and ascorbic acid and kept in dark place for 30 minutes.

- A Control solution was Prepared by using 3ml DPPH and 2ml methanol, which is used as a blank.

- After 30 minutes, absorbance was taken for both plant extract and ascorbic acid at 517nm. Methanol was used to set the spectrophotometer and also to set zero".

- Free radical scavenging activity was conveyed as inhibition percentage and was calculated using the following formula:

$$\%\ of\ Inhibition = \frac{\mathrm{Ab} - \mathrm{Aa}}{Ab} * 100$$

Where, Ab is the absorbance of the control (without test samples) Aa is the absorbance of test samples.

5.3 At a glance- free radical scavenging activity:

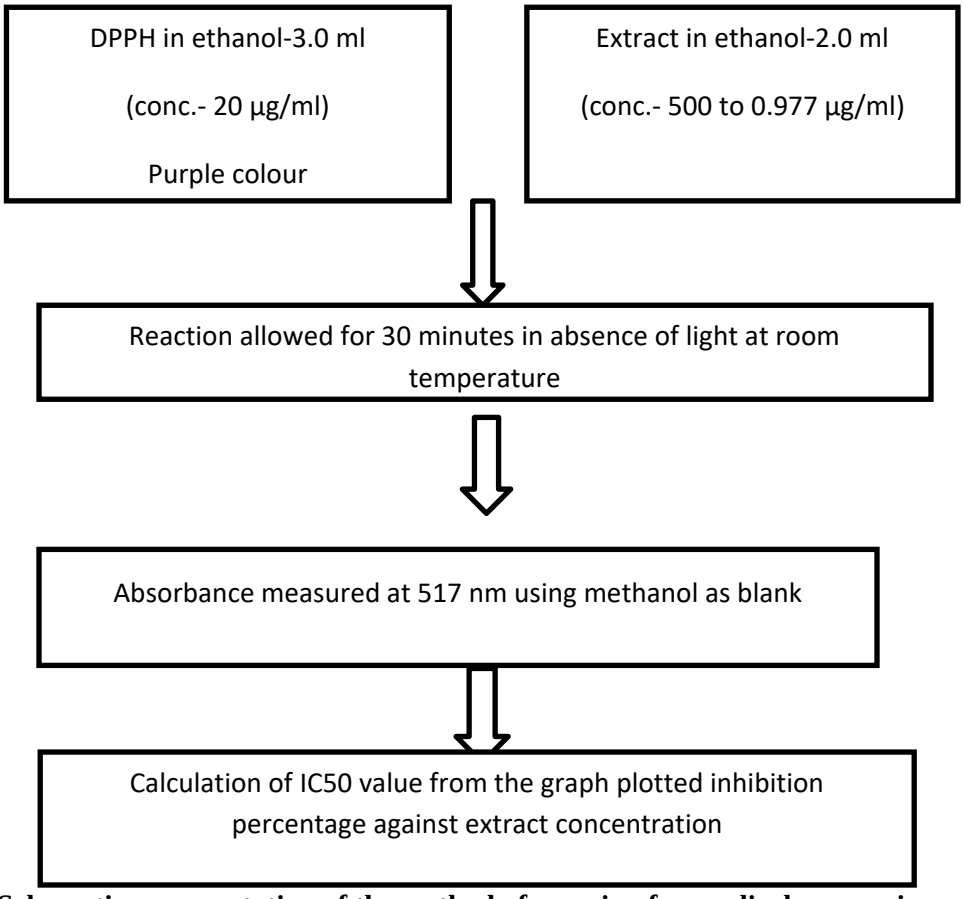


Figure: Schematic representation of the method of assaying free radical scavenging activity.

ANTHELMENTIC ACTIVITY

6. Test for anthelmintic activity by *Urena sinuata* extract

6.1 Principle:

Helminthic infections are among the most communal infections in man, affecting a large proportion of the world´s population. In developing countries they attitude a large threat to public health, and contribute to the occurrence of malnutrition, anemia, eosinophilia, and pneumonia. Parasitic diseases causing severe morbidity contain lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomias. These infections can disturb most populations in endemic areas with major economic and social consequences (Bundy, 1994).

Anthelmintic or anti-helminthic are drugs that dismiss parasitic worms (helminths) from the body, by either striking or killing them. They may also be called vermifuges (stunning) or vermicides (killing). Helminths infections are among the most widespread infections in humans, difficult a huge population of the world.

The objective of the present study is to evaluate anthelmintic activity of ethanolic extract of Gynura Procumbens as well as to rationale the use in helminthiasis in folk medicine. **[34][35]**

6.2 Experimental animal

“Earthworms were collected from freshly slaughtered cattle at local abattoirs and identified by experts. After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.54 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na2HPO4 and 0.27 g KH2PO4 in 1 liter of distilled water at 37±1°C”.

6.3 Study design

“Anthelmintic activity of the extract was investigated on live parasites.The parasites were divided into different groups consisting of three parasites in each group. Extract at the concentrations of 100, 150 and 200 mg/mL and reference standard Albendazole (collected from Square pharmaceuticals Ltd., Bangladesh) at the concentrations of 25 mg/mL in PBS were prepared and transferred to Petri dishes. Control group was treated with water. Three parasites were placed in each Petri dish and detected. The time of paralysis was noted when no movement was observed unless shaken vigorously. The death time was documented after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity is conveyed as the time required for paralysis and death of parasites as compared to control”.[38]

6.4 Preparation of sample

"To prepare the suspension of Methanolic extract of *Urena sinuata* .Theconcentrations of 100,150 and 200mg/ml; .1, .15 and .2g of extract were taken and triturated with Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration with PBS. For the preparation of standard albendazole at concentrations of 15 mg/ml; 150 mg of albendazole powder were taken and triturated with of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration with PBS".[38][39]

Fig: Sample of extract (DIU Pharmacy lab.)

Fig: Standard of albendazole (DIU pharmacy lab.)

6.5 Methodology

1. 10 ml of control, standard and extract of each concentration were taken in different petri dishes.
2. 3 parasites of both types were taken in each different petri dishes.
3. Time was taken for paralysis for each parasite was recorded.
4. Time was taken for death for each parasite & was recorded.

Fig: parasite with extract(DIU Pharmacy lab)

Antidiarrheal activity

7. ANTIDIARRHEAL ACTIVITY OF *URENA SINUATA* BY CASTOR OIL INDUCED DIARRHEA IN MICE:

7.1 Principle:

"Diarrhea is a disorder of loosing of fluid from our body and the most common reasons of diarrhea contain Bacteria from contaminated food or water, Viruses such as the flu, norovirus**,** or rotavirus. Rotavirus is the most common reason of severe diarrhea in children. Parasites, which are small organisms found in polluted food or water. Medicines such as antibiotics, cancer drugs, and antacids that contain magnesium. [40]

Food intolerances and sensitivities, which are problems digesting certain ingredients or foods. An example is lactose intolerance, Diseases that affect the stomach, small intestine, or colon, such as Crohn's disease , Problems with how the colon functions, such as irritable bowel syndrome.

Diarrhea is treated by exchanging lost fluids and electrolytes to prevent dehydration. Depending on the cause of the problem & needing medicines to stop the diarrhea or treat an infection.

According to the WHO and UNICEF reports, there are about 2.5 billion cases of diarrheal disease worldwide every year, and 1.9 million children below 5 years of age die from diarrhea each year, of whom most are from developing countries. Of all child deaths from diarrhea, 78% occur in African and Southeast Asian regions" [41].

7.2 Mechanism of diarrheal action of castor oil:

"Upon oral administration, castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglyceride. A small amount of ricinoleic acid is absorbed from the gastrointestinal tract and metabolized like any other fatty acid but most rests in the intestine where it produces its anti-absorptive or secretory effect. The ricinoleic acid thus unconventional readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The ricinoleate salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. The precise with serotonin (5-HT) have been termed as "diarrhoeangenic hormones mechanism "of how ricinoleate salts induce diarrhoea is yet to be unknown. But most agreed view is that it stimulates the intestinal epithelial cell's adenyl cyclase, release prostaglandins and specially prostaglandins of the E series along".

7.3 Materials and Method

Name	**Origin**
Tween 80	
Loperamide	Square Pharmaceuticals Ltd., Bangladesh
Syringes	
Needle	
0.9% sodium chloride solution	Orion Infusions Ltd., Bangladesh
Castor oil	WELL's Heath Care, Spain
Boxes for mice	
Electronic balance	
Hand gloves	
Castor oils	
Face Musk's	

7.4 Animal Collection:

"Swiss albino mice, which weighed between 25-30g, were used in the present study. The animals were obtained from Pharmacy department Jahangirnagar University. The animals were acclimatized for Six days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of Daffodil International University, Dhaka, Bangladesh".

7.5 Environment control:

"These are kept in standard polypropylene cages and kept under controlled room temperature (24 ±2°C), relative humidity (RH) 60-70% in a 12 hours light-dark cycle and feed ICDDR, B formulated rodent food and water as these animal are very sensitive to environment changes".

7.6 Preparation of control:

"Normal saline was accepted from local market and dissolved in 100ml water. Then 1 ml tween 0 was dissolved in saline solution and the final volume of was made 10 ml".

7.7 Preparation of Standard:

"To formulate typical at the doses of 10mg/kg per body weight was melted in saline solution and the final volume of was made 10 ml".

7.8 Preparation of Sample:

"To prepare the test samples at the doses of 75& 150 mg/kg per body weight. were measured respectively. The extract was first dissolved in 1 ml methanol then the distilled water was gradually added. The final volume of the solution was made 10 ml".

7.9 Methodology:

- "The test animals were randomly chosen and divided into three groups having three mice in each. Of the experimental groups, Group-I or the control received only 2ml saline water containing 1% Tween-80.
- Group-II or the positive control received 2ml standard antimotility drug, loperamide at a dose of 10mg/kg-body weight as oral suspension.
- Group-III, IV or The test groups were treated with 2ml solution of Methanol extract of whole plant at the oral dose of 75 & 150mg/kg-body weight.
- The mice were fed with the samples 1 hour prior to the oral administration of castor oil at a dose of 2ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in four hours study after the castor oil administration.
- Number of stools or any fluid material that blemished the adsorbent paper were counted at each sequential hour during the 4-hour period and were noted for each mouse.
- The latent period of each mouse were also counted. At the start of each hour new papers were taken for the old ones".

Figure-4: Mice feeding and injection.

Fig: After identification extract were given and started identification the number of diarrheal droppings.

7.9.1 Table : Experimental profile to observe the effect of Urena sinuate on castor oil induced diarrhea in mice.

Animal Group	Treatment	Dose (/kg-body wt.)	Route of admin.
I (Control) n=3	Saline Water containing 1% tween 80	10ml	Oral
II (Positive control) n=3	Loperamide	5 mg	Oral
III & IV Test sample n=3	Methanolic Extract of whole plant	75 & 150mg	Oral

n=number of mice

CYTOTOXIC EFFECT

8. Cytotoxic Effect

8.1 INTRODUCTION

"Cytotoxicity is accomplished designed for evaluating the level of toxicity. A number of novel antitumor and pesticidal natural products have remained using this bioassay. Brine Shrimp lethality bioassay tests to discovery out the presence of chemical that has a cytotoxic effects on cell line. Antioxidant activity tests to find out Reducing power & CUPRAC Assay also evaluated cytotoxic properties by brine shrimp lethality bioassay as it appears to be a suitable, rapid and cheap method for the determination of the cytotoxic belongings of the medicinal plant extracts".

8.2 Materials

- Needed Anemia salina Leach. (Brine eggs),
- Needed Sea salt (NaCI)
- Minor tank
- Floor lamp to attract Shrimps
- Pipettes (5, 10ml) and Micropipette (5-50nl), (10-100ul)

8.3 Preparation of seawater

At first taken 38 gm. sea salt (without iodine) was weighed, liquefied in one liter of distilled water and sieved off to get clear solution".

8.4 Hatching of Brine Shrimp

"Artemia salina leach (brine shrimp eggs) collected from pet shops (Katabon) was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatched shrimps are attracted to the light (phototaxis) and so nauplii free from egg shell was collected from the illuminated part of the tank. The nauplii was taken from the fish tank by a pipette and diluted in fresh clear sea water to increase visibility and 10 nauplii were taken carefully by micropipette".

8.5 Preparation of test solutions with samples of experimental plants

1. "At first,4 mg of plant extract was taken and dissolved in 200µ l of pure dimethyl sulfoxide (DMSO). Thus the concentration of the stock solution was 20000µg/ml.

2. 100 µl stock solution and 100 µl DMSO was taken in another test tube and followed by serial dilution was done for 10 times (100 µl from 1st test tube to 2nd test tube with 100 µl DMSO and so on.)

3. Then 10 nauplii were added to each test tube.

4. Finally 4.9 ml of sea water was filled to make final volume 5ml and final concentrations were 400, 200, 100, 50, 25, 12.5, 6.25,3.125 µg/ml and kept in normal temperature for 24 hours".

Table: 8.5 Preparation of test solutions with samples of experimental plants

Concentrations(µg/ml)	Extract Solution (µl)	Sea water containing 10 nauplii (ml)	Final volume(ml)
400	100	4.9	5
200	100	4.9	5
100	100	4.9	5
50	100	4.9	5
25	100	4.9	5
12.5	100	4.9	5
6.25	100	4.9	5
2.13	100	4.9	5
1.55	100	4.9	5
0.78	100	4.9	5

8.6 Preparation of control group

"Control groups were recycled in cytotoxicity investigation to permit the test method and confirm that the results acquired were only due to the activity of the test agent and the properties of the other probable factors remained nullified".

Two types of control groups were used

i)Positive control

ii)Negative control

8.7 Preparation of the positive control group

" Positive control in a cytotoxicity study is a commonly accepted cytotoxic agent and the result of the test agent is related with the result obtained for the positive control. In the present study vincristine sulfate was used. As vincristine is a very cytotoxic alkaloid it was evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06 µg/ml)"

8.8 Preparation of the negative control group

"50 µl of DMSO was added to each of three premarked test tubes containing 4.95 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as in effective as the nauplii died due to some cause other than the cytotoxicity of the compounds."

8.9 Counting of nauplii

"After 24 hours, the test tube were examined using a magnifying glass against a black background and the number of lasted nauplii in each tube was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration using following equation".

$$\% \, Mortality = \frac{\mathrm{N.} - \mathrm{N}}{N.} * 100$$

Here, N. =Number of nauplii taken
N =Number of nauplii Live

CHAPTER 5

RESULT AND DISCUSSION

9.1 Preliminary phytochemical evaluation:

After finishing wide range of chemical test for the identification of major classes of therapeutically compounds such as alkaloid,glycosids,carbohydrate,gums,flavonoid were found in the plant. The following table will give us a broad idea about phytochemicals present in these plants.

9.1 Table: Preliminary phytochemical evaluation:

Test	**Result**
Test for Carbohydrate Fehling test Molish test	+ +
Test for Flavonoid	+
Test for Saponin	-
Test for Tannin	-
Test for alkaloid	
With Dragendroff's reagent	+
With Mayer's reagent	+
Test for Glycosides	+
Test for Steroid	-
Test for Gum	+

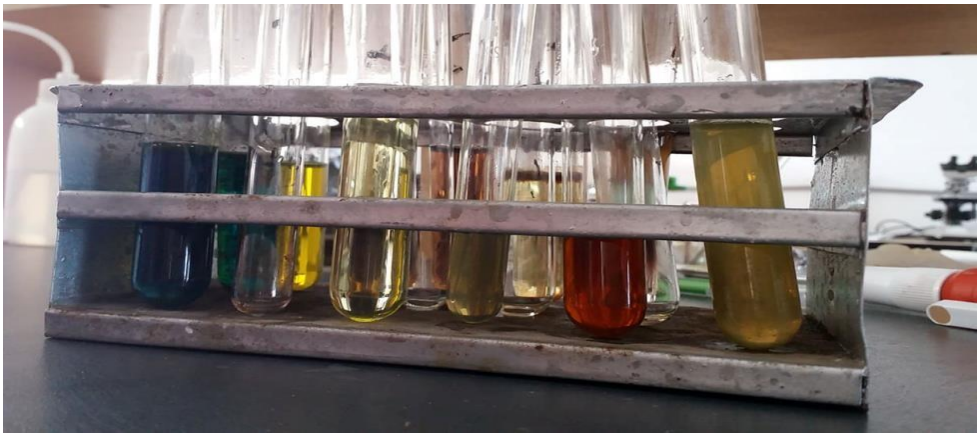


Figure:Chemical group test

9.2 ANTI-OXIDANT ACTIVITY

9.2.1 DPPH Free Radical Scavenging Activity

"The test of DPPH is recognized on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Virtually, the reaction brings about the reduction of DPPH radicals to the consistent hydrazine, it is validated by a color change from violet to yellow, which is detected spectrophotometrically. It is superficial from the table that the % scavenging of DPPH radical was found to rise with increasing concentration of the samples. The positive control ascorbic acid of which IC50 value is 12.44 µg/ml. On the other hand, the methanol extract exposed encouraging DPPH free radical scavenging activity with IC50 value".

9.2.2 IC50 value of ascorbic acid:

SI NO.	Absorbance of control	Concentrations (µg/ml)	Mean Absorbance of Ascorbic acid	% of Inhibitions	IC50 (µg/ml)
1.	0.506	500	0. 032	93. 67	12.44
2.		250	0.057	88.73	
3.		125	0.068	86.56	
4.		62.5	0.088	82.60	
5.		31.96	0.133	73.71	
6.		15.63	0.172	66. 0	

7.		7.18	0.189	62.64	
8.		3.90	0.242	52.17	
9.		1.90	0.263	48.02	
10.		0.97	0.288	43.08	

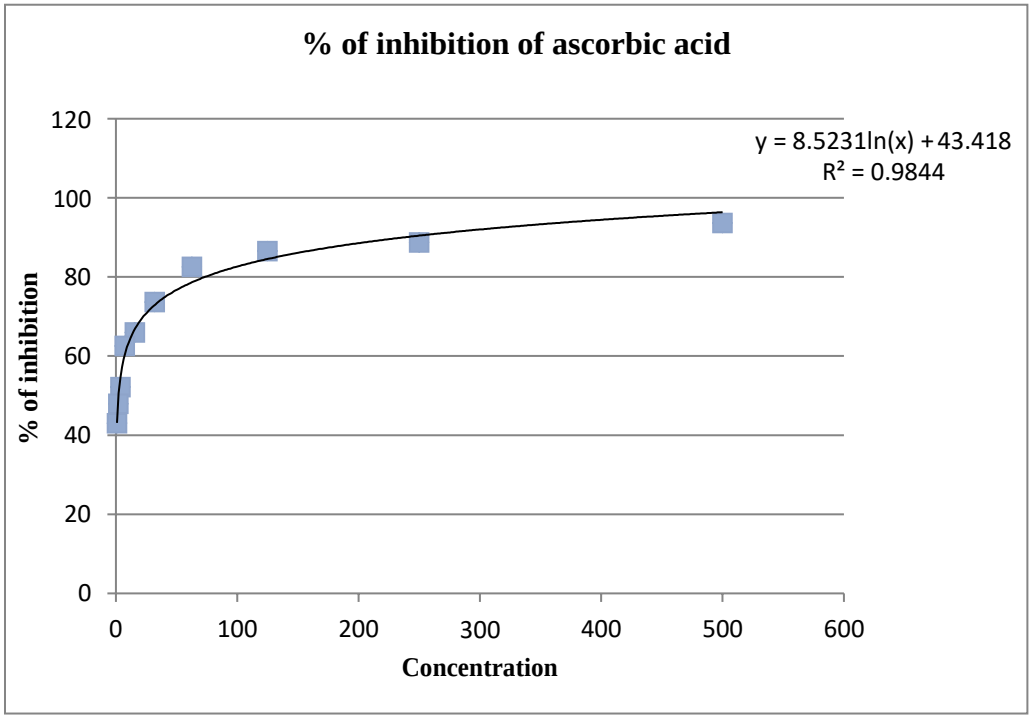


Figure: % of inhibition of ascorbic acid

9.2.3 IC50 value of methanolic extract of whole plant

SI NO.	Absorbance of control	Concentrations (µg/ml)	Mean Absorbance of whole plant	% of Inhibitions	IC50 (µg/ml)
1.	0.506	500	0. 057	88.74	**30.73**
2.		250	0. 085	83.20	
3.		125	0. 121	76.08	
4.		62.5	0. 144	71. 54	
5.		31.96	0.165	67.39	
6.		15.63	0.185	63.43	

7.		7.18	0.232	54.15	
8.		3.90	0.245	51.58	
9.		1.90	0.274	45.84	
10.		0.97	0.343	37.74	

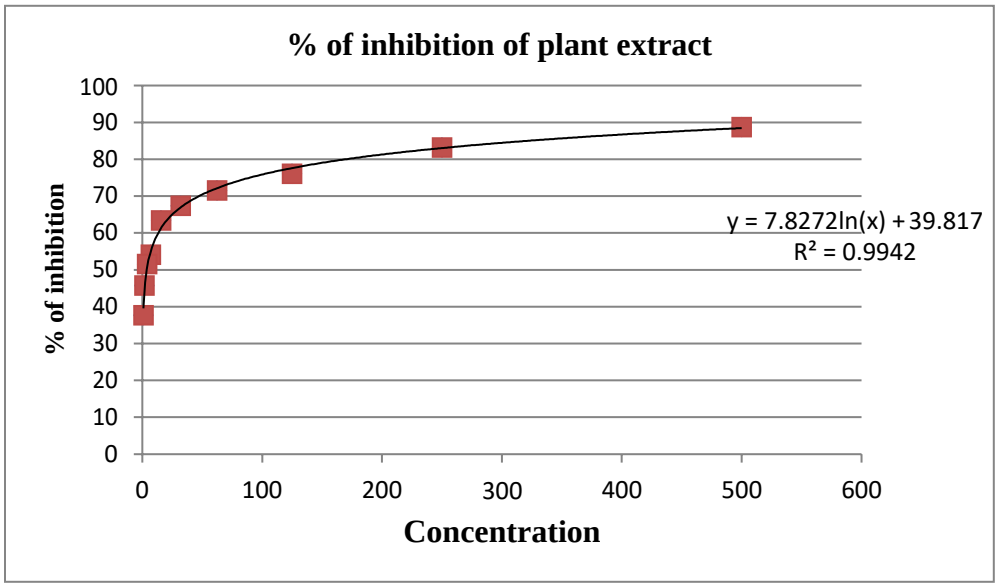


Figure:% of inhibition of plant extract

9.2.4 Discussion:

"DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging process is used to visualize the total antioxidant potential of crude plant extract without the use of any test. DPPH radical has the capacity to provide hydrogen and electron in order to remove reactive oxygen species and oxidative stress and to inhibit lipid peroxidation of the body.

After UV-Spectrophotometer analysis, it was originate that Crude methanolic extract of whole plant have a high percentage of Free Radical Scavenging activity which show the presence chemicals having antioxidant properties.

The Crude methanolic extract of whole plant showed strong DPPH free radical scavenging activity with an IC50 value of 12.44 µg/ml and compared to the standard ascorbic acid with an IC50 value of 30.73 µg/ml.The significant results with the previous result of the presence of flavonoids shows the high presence of antioxidant compound of the plant extract. These antioxidant performance not only vital role in the body by anti-inflammatory, anti-microbial, free radical scavenging potentiality, these plants upon exposure to environment have huge role in eliminating volatile organic compounds which are hypothetically harmful as they can be inhaled through the human body unintentionally and their possibly to induce oxidative stress by producing reactive oxygen and nitrogen species resulting in lipid peroxidation and removing these compound can help in minimization of the adverse health effect over the human at a large scale".

9.3 CYTOTOXIC ACTVITY

"All the extracts were also endangered to Brine Shrimp lethality bioassay designed for possible cytotoxic action. In this study, methanol extract of whole plant was found to be the most toxic to Brine Shrimp nauplii.Methanol extracts of whole plant LC50 of 6 µg/ml The high toxicity of methanolic extract of whole plant probably attributed to the alkaloid that is confirmed in phytochemical screening".

9.3.1 Table: Cytotoxicity of whole plan

Concentration	LogC	No.of nauplii taken	Dead	Live	%of mortality	LC50(µg/ml)
400	2.602059991	10	8	2	80	145.91
200	2.301029996	10	7	3	70	
100	2	10	6	4	60	
50	1.698970004	10	5	5	50	
25	1.397940009	10	4	6	40	
12.5	1.096910013	10	3	7	30	
6.25	0.795880017	10	2	8	20	
3.13	0.495544338	10	2	8	20	
1.56	0.193124598	10	1	9	10	
0.78	-0.107905397	10	1	9	10	

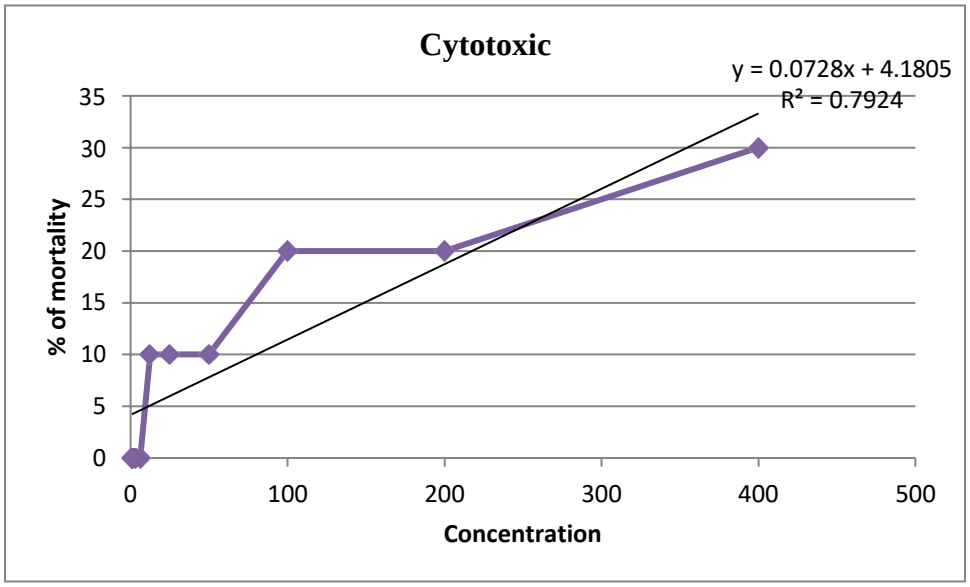


Figure: Cytotoxicity of whole plan

9.3.2 Table: Cytotoxicity of negative control

Concentration	LogC	No.of nauplii taken	Dead	Live	%of mortality	LC50(µg/ml)
400	2.602059991	10	3	7	30	629.38
200	2.301029996	10	2	8	20	
100	2	10	2	8	20	
50	1.698970004	10	1	9	10	
25	1.397940009	10	1	9	10	
12.5	1.096910013	10	1	9	10	
6.25	0.795880017	10	0	10	0	
3.13	0.495544338	10	0	10	0	

1.56	0.193124598	10	0	10	0	
0.78	-0.107905397	10	0	10	0	

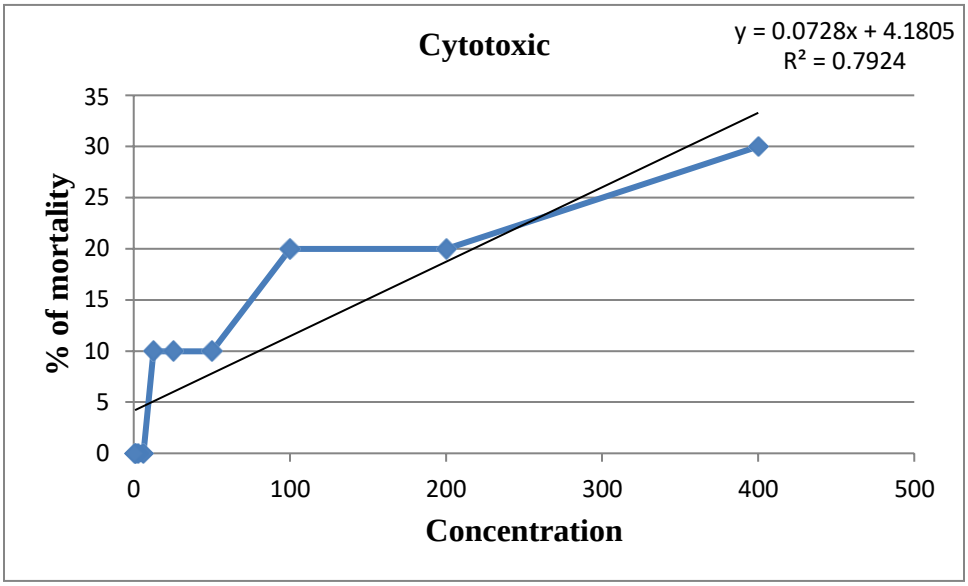


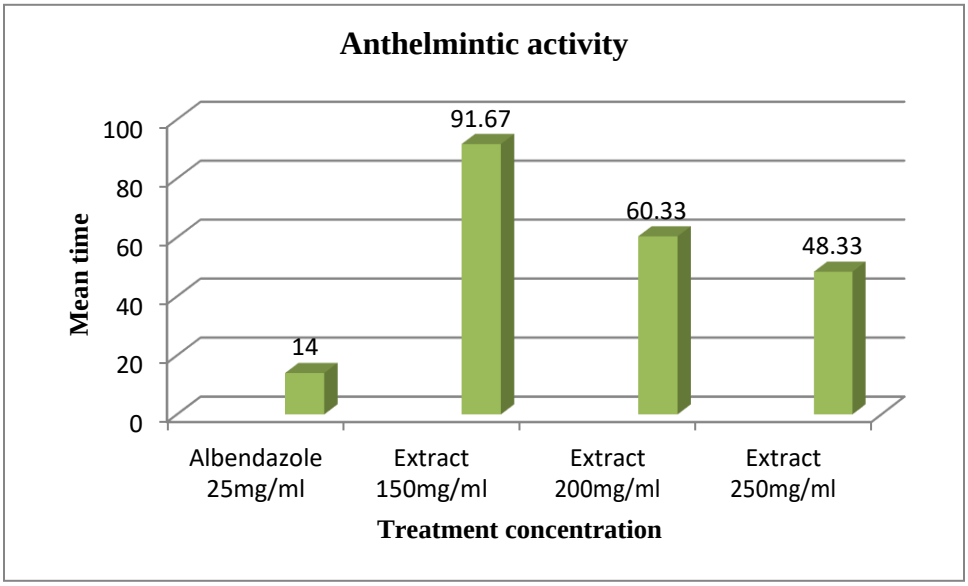
Figure: Cytotoxicity of negative control group

9.4 ANTHELMINTIC ACTIVITY

Treatments Concentration(mg/ml)	Worm No.	Time taken for death in min.	Mean time taken for death in min.
Control in water	**C1**	--	--------
	C2	--	
	C3	--	
Standard Albendazole 25 mg/ml	**S1**	**13**	**14**
	S2	**14**	
	S3	**15**	
Plant's extract 150mg/ml	**E1**	**90**	**91.67**
	E2	**90**	
	E3	**95**	
Plant's extract	**E1**	**60**	**60.33**

200mg/ml	**E2**	**60**	
	E3	**61**	
			48.33
Plant's extract 250mg/ml	**E1**	**45**	
	E2	**50**	
	E3	**50**	

Figure: Anthelmintic activity of Urena sinuate the basis of death time on helminthic.



9.4.1 Discussion

"Methanolic extract of *Urena sinuata* whole plant was verified for anthelmintic activity. Standard Albendazole drugs were used for comparative study. The table showed that Methanolic extract of *Urena sinuata* demonstrates anthelmintic activity against helminthes. Death time of standard Albendazole is 14min (15mg/ml) and on the other hand, if we rise the concentration of the plant's extract that reduces the death time at 250mg/ml concentration death time is 48.33min".

9.5 ANTIDIARRHEAL ACTIVITY

Treatment	First diarrhea in minute(latent time)	Total number of feces	% inhibition of defecation
Control	2	22	----------
Loperamide(10mg/kg)	1hour	6	72.73%
Urena sinuate (75mg/kg)	2hours	7	68.18%
Urena sinuate(150mg/kg)	3hours	5	77.27%

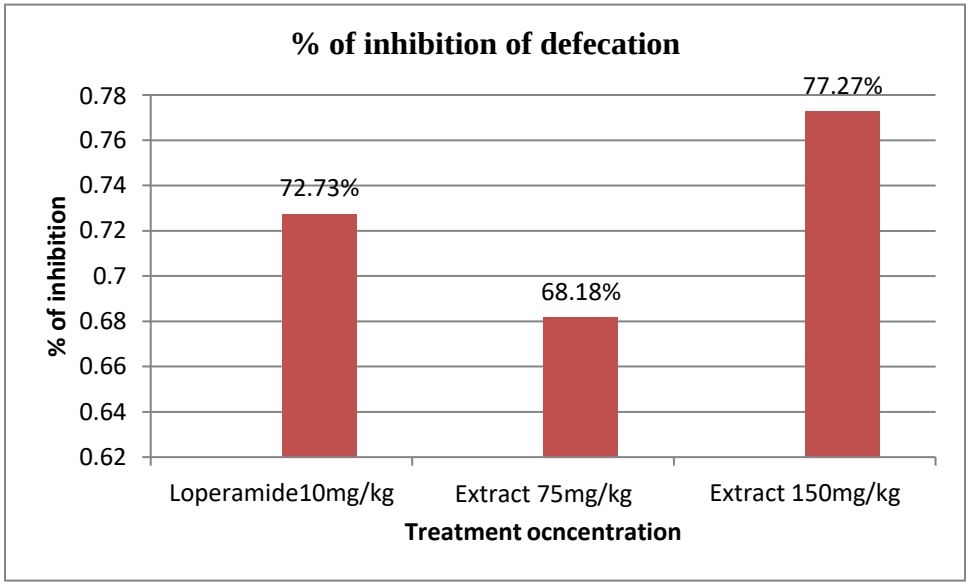


Figure: % of inhibition of defecation

"The methanol leaf extract of *Urena sinuata* was used for castor-oil diarrhea test and the following data were noted . The extract have significant anti-diarrheal activity with (77.27%) and (68.18%) decrease of diarrheal both 75mg/ml and 150mg/kg body wt. compared to standard loperamide (72.73%)".

CHAPTER 6

Conclusion

Now a days appealing of phytomadicine is enhanced day by day. Nature is giving us medicinal gift which act as natural source of modern drugs. *Urena sinuata* which contains so many pharmacological activities. The current study was prearranged to examine the anthelmintic, cytotoxic, antidiarrheal and anti-oxidant activity of the *Urena sinuata*. In those study, methanolic extract of plant presented cytotoxic and anti-diarrheal activity and good anti-oxidant,anthelmintic activity compare to standard. So, we can use this plant for cancer and diarrheal patient by further clinical experiment.

“The Crude methanolic extract of whole plant showed strong DPPH free radical scavenging activity. The remarkable results with the previous result of the presence of flavonoids shows the high presence of antioxidant compound of the plant extract. These antioxidant play not only vital role in the body by anti-inflammatory, anti-microbial, free radical scavenging potentiality, these plants upon exposure to environment have huge role in eliminating volatile organic compounds and their potentially to induce oxidative stress by producing reactive oxygen and nitrogen species. It can help in minimization of the adverse health effect over the human at a large scale”.

Pharmacological evaluation of the whole plant extracts of *Urena sinuata* indicates that it is an important source of bioactive compounds that may be a source novel medicine. This report may serve as a footstep to use these plants as a new source of medicine. But our work was only initial effort. It will require additional exhaustive advanced investigation.

CHAPTER 7

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