Chemical Composition and Physiological Effects of *Sterculia colorata* Components

A Project Submitted By

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ID: 14146042

Session: Spring 2014

To

The Department of Pharmacy in Partial Fulfillment of the Requirements for the Degree of Bachelor of Pharmacy

Dhaka, Bangladesh

September, 2018

Dedicated to my family, for always giving me unconditional love and support.

Certification Statement

This is to corroborate that, this project work titled 'Chemical composition and Physiological effects of *Sterculia colorata* components' proffered for the partial attainment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University, comprises my own work under the guidance andsupervision of Dr. Mohd. Raeed Jamiruddin, Assistant Professor, Department of Pharmacy, BRAC University and this project work is the result of the author's original research and has not priorly been submitted for a degree or diploma in any university. To the best of my insight and conviction, the project contains no material already distributed or composed by someone else aside from where due reference is made in the project paper itself.

Signed,

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Countersigned by the supervisor,

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Acknowledgement

I would like to express my gratitude towards Dr. Mohd. Raeed Jamiruddin, Assistant Professor of Pharmacy Department, BRAC University for giving me guidance and consistent support since the initiating day of this project work. As a person, he has inspired me with his knowledge on phytochemistry, which made me more eager about the project work when it began. Furthermore, I might want to offer my thanks towards him for his unflinching patience at all phases of the work.

I would also like to convey my gratitude towards Dr. Eva Rahman Kabir madam, Chairperson of Department of Pharmacy, BRAC University for giving me the opportunity and complete support to perform the project work at an individual level.

Moreover, I want to thank all the lab officers and lab partners for their continuous direction and assistance during lab work.

All these would not have been made possible without the grace and mercy of The Almighty, Allah, who has enabled me to finish this project work.

Last of all, I want to thank the faculty members of Department of Pharmacy at BRAC University, my classmates and my family for their ethical support, motivation and persistence that altogether empowered me to accomplish my work effectively.

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Abstract

Sterculia colorata, known as 'Odal' in Bangladesh, can be found in Southern Yunnan, Myanmar, Thailand, Vietnam, Sri Lanka, South West India eastwards to Burma and Bangladesh. It is commonly found in jungles in hill tracts as it can endure long period of dryness. This species of Malvaceae family is well known to the people living in hill tracts as the traditional medicine for the treatment of cholera and jaundice. The major influence behind this study comes from the vast use of S. colorata as traditional medicines. However, another prime reason behind the selection of this plant for study is that, it has not yet undergone any type of studies based on phytochemical and medicinal properties. In this study, to check for antioxidant activity in the leaf and bark extracts, DPPH free radical scavenging test, Total phenolic content and Total flavonoid content estimation have been performed in-vitro. Furthermore, phytochemical screening has also been conducted to identify some particular phytochemicals present in the leaf and bark extract. However, the effective use of S. colorata against cholera among tribal people has acted as a powerful stimuli to conduct the Antidiarrheal activity test for this study. The antidiarrheal activity test has been performed in-vivo; with rats in the laboratory. From this study, strong free radical scavenging activity of the bark extract, high flavonoid content in the leaf extract and highly significant antidiarrheal activity of the leaf extract in lower dose have been observed, which can be an effective substitute in the absence of other anti-diarrheal drugs.

Chapter 1:

Introduction

1.1 General Introduction

With the inspiration of encouraging a healthy life and to upgrade the standard of life, usage of medicinal herbs has increased a lot (Ashafa, Afolayan, 2009). Since the primitive era, assorted herbs comprising of medicinal qualities, were administered to cure diseases in human. Because of the natural resistance of organisms, oxidative stress takes place and in the body, assorted large molecules like nucleic acids, lipids, proteins etc. may be affected by decay of oxidation (Chiranjibi, Sudhakar, Dhal, Rashmita, 2006). As a result of this oxidative decay, assorted chronic human maladies; for example, tissue damage, congestive diseases, assorted neurodegenerative disorders, mutagenesis and the aging system take place (Namukobe, Kasenene, Kiremire, Byamukama, Mugisha, Krief, Dumontet, Kabasa, 2011).

Plants with medicinal properties are ubiquitous. For hundreds of years, civilizations of the world have acquired the skills of using plants to prevent diseases and maintain sound health (Abramovitz, 1997). These traditional medicines with ready access and origin wise significance form the basis of an available and amendable health-care practice and are a primary source of living for tribal and rural people (Balick, Mendelsohn, 1992). Medicine has reached the dimensions far ahead, beyond the imagination of our forefathers. The marvel of present medication has brought about versatile investigation subjects, for instance, stem cell study is no more a prospect but an adventurous reality (Associated Press, 2006).

Moreover, medicinal plants that grow naturally have attracted scientific and commercial significance. In the US, minimum 118 prescription medicines are from natural sources (Botanic Gardens Conservation International, 1996). However, we still have much more knowledge to earn about the treasures in wild places. Less than 1% of all tropical plant species had been tested for useful pharmaceutical applications (Roberson, 2008).

Since medicinal plants are attracting increased scientific and commercial significance towards them, there is increasing pressure on the plants which are the mother sources of maximum medicinal plants (Tuxhill, 1999). Excessive harvestation has put several species to the verge of extinction. Traditional medicines has sometimes become unavailable to the indigenous people who have relied on them for centuries due to business related exploitation (Torrance, 2000).

Thus, the research and protection of medicinal plants have become increasingly important. The fast loss of species and habitat worldwide makes this situation more alarming (Plant Conservation Alliance Medicinal Plant Working Group). Approximately 15,000 medicinal plants may have been in the risk of extinction around the world. According to specialists, the earth is losing minimum one potential essential drug every two years (National Center for the Preservation of Medicinal Herbs).

According to Botanic Gardens Conservation International, in the first ever checklist of the world's plants created in 2010, around 350,000 accepted varieties of plants were listed with over 240,000 still to be confirmed (U.S. Forest Service). All of these plants provide different chemicals which can be used in different cases. Isolation of about 12000 chemicals was performed and only 10% of them were estimated (International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants).

1.2 Historical Review of Medicinal Plants:

Recuperating with therapeutic plants begins from indistinguishable period from humanity itself. The connection amongst human and their scan for drugs in nature dates from the most distant past, of which there is adequate proof from different sources: composed archives, protected landmarks, and even unique plant medications (Stojanoski, 1999). Familiarity with therapeutic plants utilization is a result of the numerous long periods of battles against sicknesses because of which humankind figured out how to seek after medications in barks, seeds, natural product bodies, and different parts of the plants. Contemporary science has recognized their dynamic interest, which has skilled current pharmacotherapy with a scope of medications of plant beginning, known by antiquated civic establishments and utilized consistently (Kelly, 2009). The information and in addition the advancement of thoughts identified with the use of restorative plants and the development of mindfulness has advanced the capacity of drug specialists and doctors to react to the rising difficulties with the spreading of expert administrations in help of human life (Petrovska, 2012).

The old European, Indian and the Chinese therapeutic frameworks, in spite of the fact that had significant contrasts, through and through trusted that lopsidedness in the human body is the reason for all ailment. In this manner each one of those frameworks mainly work to reestablish harmony after which great wellbeing will take after. Before 500 B.C,

plants were the main drugs utilized. Besides, it was trusted that plants had both custom enchantment powers and restorative characteristics. After 500 B.C., disease was step by step observed as a characteristic human condition and began to shed its heavenly characteristics (health 24, 2015).

The most established composed verification of utilization of restorative plants for planning of medications has been found on a Sumerian earth piece from Nagpur, around 5000 years of age. It comprised of 12 formulas for sedate arrangement alluding to more than 250 different plants, some of them alkaloid, for example, poppy, henbane, and mandrake. "Pen T'Sao", the Chinese book on roots and grasses composed by Emperor Shen Nung around 2500 BC, depicts 365 medications (dried parts of restorative plants), a large number of which are utilized even these days, for example, the accompanying: Rhei rhisoma, camphor, Theae folium, Podophyllum, the colossal yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra. The Indian heavenly books Vedas recommend treatment with plants, which are inexhaustible in India. Indeed, even today, various flavor plants utilized begin from India: nutmeg, pepper, clove, and so on. The Ebers Papyrus of 1550 BC, speaks to an accumulation of 800 bans alluding to 700 plant species and medications utilized for treatment, for example, pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, basic centaury, and so on. In antiquated history, the most critical author on plant drugs was Dioscorides, "the dad of pharmacognosy" who, as a military doctor and pharmacognosist, contemplated restorative plants wherever he went with the Roman Army. In 77 AD, he composed the book "De Materia Medica." This established work interpreted commonly, offers a lot of information on the restorative plants constituting the fundamental materia medica until the late medieval times and the Renaissance. Among the aggregate 944 medications portrayed, 657 are of plant cause, with discourses about the outward appearance, region, method of gathering, making of the restorative arrangements, and their remedial impacts. (Petrovska, 2012).

1.3 Significance of medicinal plants:

The significance of therapeutic plants turns out to be more apparent at the present occasions in creating nations. It is evaluated that, in Pakistan, 80% of its populace rely upon plants to fix themselves, 40% in China (Tucakov, 1990). In created nations, for

example, United States, 60% individuals utilize therapeutic plants constantly to battle certain infections. In Japan, therapeutic plants are more mainstream than present day meds (Botanical Online).

Black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are helpful to mend wounds, bruises and bubbles. Many herbs function as blood purifiers to modify or change a long-standing condition by taking out the metabolic poisons. They are otherwise called 'blood chemicals'. Some particular herbs enhance the invulnerability of the individual, in this way diminishing conditions, for example, fever (Kirtikar, Basu, 1918).

To lessen fever and the generation of abundance body warm, certain antipyretic plants, for example, Chirayta, dark pepper, shoe wood and safflower are suggested by conventional Indian drug professionals. Sandalwood and cinnamon are powerful astringents separated from being fragrant. Sandalwood is particularly valuable in capturing the release of blood, bodily fluid and so forth (Moralles, Padilla, Falconi, 2016).

Some herbs are utilized to invalidate the corrosive delivered by stomach. Herbs, for example, marshmallow root and leaf fill in as acid neutralizers. The satisfactory sound gastric corrosive required for appropriate processing is held by such herbs. Ginger and cloves are utilized in certain hack syrups for their expectorant property, which advances the diminishing and launch of bodily fluid from the lungs, trachea and bronchi. Eucalyptus, cardamom, wild cherry and cloves are additionally expectorants (OFDC).

Specific restorative herbs have disinfectant property. They restrain the development of pathogenic microorganisms in charge of transferable sicknesses. Herbal drug experts endorse calmative herbs, which give a mitigating impact to the body. They are regularly utilized as tranquilizers (Hassan, 2012).

Specific fragrant plants, for example, aloe, brilliant seal, barberry and chirayata go about as mellow tonics. The sharpness of such plants decreases poisons in blood. They are useful in battling contamination too. Some herbs are utilized as stimulants that expansion the movement of a framework or an organ, for instance herbs like cayenne, red chillies, myrrh, camphor and guggul (Herb Society of America).

1.4 Medicinal Plants of Bangladesh:

According to estimation, there are around 722 medicinal plants in our country. They are diverse on the basis of size, shape, habitat, adaptation power, flowering, production season, somatical element and in the effects on human life. These medicinal plants can be detected and distinguished easily for their diversity. In the subcontinent, the total number of medicinal plants stands at about 2000 at present. From them, 400-500 plants enlisted are growing or available in Bangladesh (Ghani, 1998). Folk medicines based on indigenous knowledge composes a very diverse characteristic in Bangladesh (Sadi, 2012).

Scientific Name	**Local name**	**Medicinal Uses**
Zingiber officinale	Ada	Fever, Bronchitis
Calotropis procera	Akanda	Piles
Mangifera indica	Am	Toothache
Phyllanthus emblica	Amloki	Burning sensation, vomiting, indigestion, cough, jaundice
Oxalis corniculata	Amrul	Anemia, cough
Ananus comosus	Anarosh	Abortion. Diuretic, fever, helminthiasis, worm.
Achyranthes aspera	Apang	Abortion, diuretic, eczema
Cajanus cajan	Arhar	Diabetes, jaundice
Terminalia arjuna	Arjun	Burning sensation, blood pressure, heart disease, worm
Micania cordata	Assamlata	Cut, injury
Annona squamosa	Ata	Abscess, dysentry
Ocimum americanum	Babuitulshi	Bronchitis
Aegle marmelos	Bel	Abscess, fever, dysentry, indigestion
Adhatoda zeylanica	Bassak	Cough, fever
Terminalia bellirica	Bohera	Burning sensation, rheumatism
Clerodendrum viscosum	Bhant	Vomiting, Worm, dyspepsia
Abroma augusta	Ulatkambal	Gonorrhea, Amenorrhea, dysentry, burning urination
Andrographis paniculata	Kalomegh	Febrifuge, stomachic, liver problems, diabetes
Argemone maxicana	Shialkanta	Antifungal, Anthelmintic,

		antileprotic
Emblica officinalis	Amloki	Immunomodulatory, antiulcer, anticancer, promoting spermatogenesis, anti-aging
Geodorum densiflorum	Shonkho	Regulate menstrual cycle
Terminalia chebula	Horitoki	Asthma, bile duct disorder, heart disease, high blood pressure, high cholesterol, sexually transmitted diseases
Nigella sativa	kalojira	Asthma, bronchitis, rheumatism, boils, to increase milk production

1.5 Plant Description:

Kingdom: Plantae

Order: Malvales

Family: Malvaceae

Genus: Sterculia

Species: Sterculia colorata (Flowers of India)

Malvaceae is a family comprising of floral plants which contains 244 genus with 4225 known species. Major members of commercial significance include okra, cotton, cacao and durian. There are also some genus consisting of known ornamentals, such as Alcea (hollyhock), Malva (mallow) and Lavatera (tree mallow). The biggest genus according to number of species include Hibiscus (300 species), Sterculia (250 species), Dombeya (250 species), Pavonia (200 species) and Sida (200 species) (Bayer, 1999).

Sterculia is a variety of blooming plants in the Malvaceae family, Malvaceae: subfamily Sterculioideae. Individuals from the variety are casually known as tropical chestnuts. The logical name starts from Sterculius of Roman folklore, who was the lord of fertilizer; this is in reference to the unsavory smell of the blossoms of this family (e.g. Sterculia foetida). Sterculia might be monoecious or dioecious, and blooms unisexual or indiscriminate (Sterculia: The Plant List). Sterculia species are nourishment plants for the hatchlings of some Lepidoptera species including

the Bucculatrix xenaula, which bolsters solely on this class. Gum karaya is extricated from Sterculia species, utilized as a thickener and emulsifier in nourishments, as a purgative, and as a denture cement. In India, this begins from: Gujarat, Maharashtra, Madras, Madhya Pradesh and Chhota Nagpur (Germplasm Resources Information Network).

Sterculia colorata (Scarlet Sterculia), otherwise called Bonfire tree, Colored Sterculia and Indian almond; in Assamese Odal and in Marathi known as kaushi, is a medium-sized tree with spreading branches. Before the beginning of blooming, the tree sheds takes off. After leaf-shedding, buds grow and form into blooms (Christenhusz, Byng, 2016). The tree blossoms from March to April. Blossoms come in short thick panicles which happen at the closures of the branches. They are orange-red in shading and hang downwards. The blooming stalks together with blossoms are secured with fine fleece hairs giving the entire blossom a delicate, smooth look. Amid blossoming stage, the Scarlet Sterculia is very noticeable and presents a splendid sight in view of its orange-red blooms against a leafless state (Harper, 2012).

The blooms are huge, 30 mm long. The blossom tube is 13 mm long, tubular at the base and lobed at the tip. Its edge is encompassed by white delicate hair. The corolla appears as though it is joined inside with the tubular sepals at the base. From the focal point of the calyx tube, a staminal section projects bearing at its summit 30 anthers. Red Sterculia is regularly found in the backwoods of Western Ghats and the Deccan (Thorne, 1992).

1.6 Purpose of this study:

Therefore, developing the herbal medicine with potential antioxidant property from plants like *Sterculia colorata* is the rationale behind choosing such experimental work.

This study focuses into the phytochemical and biological investigations of leaf and bark extracts of *Sterculia colorata* which includes:

 Extraction of dried powder of leaf and bark of *Sterculia colorata* with methanol by cold extraction method.

 Estimation of entire phenolic content of methanolic mixture of *Sterculia colorata* by Folin-Ciocalteu reagent.

 In vitro assessment of crude methanolic extract of *Sterculia colorata* for antioxidant activity by DPPH radical scavenging assay.

 Phytochemical screening of the leaf and bark extracts of *Sterculia colorata*. .

 In-vivo assessment of antidiarrheal activity of *Sterculia colorata.*

1.7 Importance of potential antioxidant originating from natural sources:

In our daily life, natural antioxidants can be found from assorted vegetable and food sources. Such antioxidants are hostile to a reaction taking place in the body named as oxidation. This reaction forms an atom in the body, which is very unsteady and injurious and known as free radicals. This harmful free radical is stimulated by oxygen and if they are left uncontrolled, there is a possibility that they can cause danger to the body. By donating an electron to the unsteady molecule, antioxidants stabilize those molecules. The antioxidants taking part to neutralize free radicals are renewed by taking an electron from another antioxidant. Later on, by departing their electrons, synthesized antioxidants lose their ability to be used in such way. Antioxidants have a characteristic to become a threatening byproduct after used up and have the tendency to lead more stress on the oxidative load. Presenile dementia, malignancy, Parkinson's etc are associated with the lack of antioxidants (Y. Feng & X. Wang, 2012).

Dietary supplements are the potential sources of antioxidants; for example, vitamin c and vitamin e. Vitamin C is an immensely strong antioxidant as it restrains fluid phase ROS before lipid peroxidation. Vitamin E is a very effective antioxidant which is also a very efficient chain-breaker of the interior part of the cell membrane. As a reliable origin of

vitamin C, herbs and fruits are utilized; grains and high quality vegetable oils are a source of vitamin E. (Wang & Quinn, 1999).

1.8 Importance of Antidiarrheal study:

Looseness of the bowels is a risky issue which influences 3-5 billion individuals for every year around the globe, particularly kids beneath 5 years. Indian writing from ayurvedic beginnings and others assert the strength of a few plants in the treatment of looseness of the bowels. The WHO together with the United Nations Children's Fund has started Diarrhea Disease Control Program to control the runs in creating nations because of high mortality and dreariness rate, particularly in kids. Oral rehydration arrangement, zinc arrangement, probiotics and particular anti-infection agents have lessened death rate by huge proportion in diarrheal illness. In any case, incessant looseness of the bowels is as yet a dangerous issue in a few districts of the world. Shockingly, the program does not reach to the poor world, and the illness is as yet a noteworthy test before essential wellbeing specialists and analysts. Along these lines, the distinctive conventional standards of prescriptions, for example, Chinese solution, Japanese medication, needle therapy treatment, and ayurvedic drug can assume an awesome part in antidiarrheal crusade. A few therapeutic plants from the distinctive locales of the world have been utilized to fix looseness of the bowels (Mishra, Seth, Mauriya, 2016).

Chapter 2:
Methodology

2.1 Chemical Task:

The chemical process performed on the leaves of *Sterculia colorata* includes the following steps:

- Collection and identification of plant sample properly.
- Processing of plant samples.
- Extraction of the leaves and bark of the plant with methanol.
- Evaporation of solvent that yields crude methanolic extract.
- Estimation of total phenolic content.
- Antioxidant assay of the extract.

2.1.1 Collection and Identification of plant sample:

Sterculia colorata leaves and bark were gathered from a forest of Tangail district during December, 2017. The accession number for the plant is 45443.

2.1.2 Processing of plant sample:

After gathering the leaves and barks of the plant, those were scoured with water from tap and cleansed. Then, they were dried out by sun light for a week. Later on, these dried leaves and barks were converted into powdered form with the help of grinding mill. The rough powder was then stored appropriately in different containers which were closed well so that air cannot enter, and were preserved in a cool and dry place.

2.1.3 Extraction of the leaves and bark of the plant with methanol:

The plant sample which was in the form of powder, was extracted by the cold extraction method. 700gm of test samples (leaf and bark) of the herb which was in the form of powder, were poured into two different jars and were soaked completely with double amount of methanol. For the interval of seven days the test materials were preserved in the jars and were being stirred and shaken in unidirectional way occasionally. The texture of the mixtures was colloidal. Colors of the leaf mixture and bark mixture were greenish brown and dark brown. Later on, filtering of the mixtures was done with the

help of white cloth and cotton. After this filtration, opaque liquids were obtained. To perform the final purification, the filtrates were then filtered with filter paper. This resulted in clear liquids. Right after the filtration process, the filtered leaf and bark mixtures were poured in a round bottom flask and set in a rotary evaporator beneath reduced stress at raised temperature, for 1 hour to reduce the solvent. After 1 hour, the remaining residue was poured in a methanol rinsed petridish. The residue contained very little amount of methanol. The bark extract residue was deep dark brown in color, sticky and viscous in nature. The leaf extract residue was slightly viscous, yellow colored with brown spots. The extracts are known as crude methanolic extract (CME). Both the extracts were kept in laminar airflow for drying and stored in refrigerator.

2.1.4 Antioxidant Assay

2.1.4.1 Estimation of total phenolic content:

The estimation of the elements of all the phenolic compounds of the various extracts in the plant can be done by the utilization of Folin–Ciocalteu Reagent (FCR). Still the entire chemical elements of the FCR is not known but the presence of Heteropolyphosphotung states- molybdates is assumed. In a species containing phenolic contents, a sequence of versatile reduction reactions of one or two electron take place. A complex reaction takes place in the middle of Mo (VI) and reductants, where Molybdenum is easily reduced.

2.1.4.1.1 Reagents used:

- Folin-ciocalteu reagent
- Sodium carbonate
- Methanol
- Gallic acid

Other materials:

- Distilled water
- Beaker

 Test tube

 Pipette

 Pipette

 Micropipette

2.1.4.1.2 Procedure:

- At first, a test tube which can hold 2.5 ml of a reagent which is named Folin - ciocalteu

(Mixed well with water to make it diluted 10 times) was taken.

- Folin – ciocalteu reagent was diluted 10 times with distilled water.
- Then 2.5 ml Folin – ciocalteu reagent solution was withdrawn in a test tube.
- 7.5% (w/v) Sodium Carbonate solution was prepared.
- Then 2ml Sodium Carbonate solution was withdrawn in a test tube.
- 2mg bark extract and 2mg leaf extract were taken in different test tubes.
- Then 1ml water was added in each test tubes and solutions were prepared.
- 0.5ml solution were withdrawn from bark and leaf extract solutions.
- Next, 2ml sodium carbonate solution, 2.5ml Folin- ciocalteu solution, 0.5ml bark/leaf extract solutions were mixed in a test tube.
- This whole process was repeated 3 times.
- Thus 3 test tubes of bark extract and 3 test tubes for leaf extract: total 6 test tubes were kept in dark for 30 minutes.
- Previously, as soon as Folin reagent solution was added to bark and leaf extract solutions, color changed to dark-bluish.
- After 30 minutes, UV absorbance of six solutions was measured at 760 nm, using distilled water as blank.

Constituents of Folin- ciocalteu reagent:

1. Water- 57.5%
2. Lithium Sulfate- 15.0%
3. Sodium Tungstate Dihydrate- 10.0%
4. Hydrochloric Acid>=25% -10.0%

5. Phosphoric Acid 85 % solution in water- 5.0%
6. Molybdic Acid Sodium Dihydrate- 2.5%

2.1.4.1.3 Preparing Standard Curve:

Here, Gallic acid was utilized as a standard. The solution of Gallic acid was made up with Folin-Ciocalteu reagent (diluted 10 times) and also 2.0 ml of a chemical named Na2CO3 (7.5 % w/v) solution was mixed with Gallic acid solution of 0.5 ml. After that, the combination was incubated at normal temperature for the time interval of 30 minutes. Consequently, the absorbance was determined at 760 nm. A linear relationship was obtained subsequently by graph; the absorbance in contrast to the concentration, which was utilized as a curve, which is considered as a standard with the purpose of finding out the entire phenolic constitutes of the sample which was tested.

2.1.4.2 DPPH (1, 1-diphenyl-2-picrylhydrazyl) Assay:

On the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the technique of Brand Williams Et al., 1995 determined the antioxidant capacity or the free radical scavenging activities. In this method, methanol solutions of different concentrations were taken at the amount of 2.0 ml and were mixed up with DPPH methanol solution of 3.0 ml (20µg/ml). Methanol mixture of DPPH having the appearance of purple was bleached by the plant extract as contrasted to that of BHT or in broad tert-butyl-1-hydroxytoluene and Acetyl Salicylic Acid and UV spectrophotometer was utilized for the assaying of potential antioxidant.

2.1.4.2.1 Required Materials:

- 1, 1- diphenyl-2-picrylhydrazyl tert-butyl-1-hydroxytoluene (BHT)
- Ascorbic acid
- Distilled water
- Methanol
- Chloroform
- Carbon tetra chloride
- n-hexane
- UV-spectrophotometer
- Beaker

- Amber reagent bottle
- Test tube
- Light-proof box
- Pipette
- Micropipette

2.1.4.2.2 Control Group Preparation

ASA and BHT were utilized as a positive control. For obtaining a mixture as mother solution that has a density of 1000 µg/ml of ASA and BHT. The quantity of those chemicals was calculated by consequently dissolving in methanol. Using the mixture as mother solution, with the purpose of obtaining assorted density, ranging from 500.0 to 0.977 µg /ml, a sequence of dilution was generated.

2.1.4.2.3 Test Sample Preparation

At first, 2mg leaf and bark extracts were both dissolved in 4ml methanol differently to obtain the density of 500 µg /ml. This is the mother solution. Next, 2ml was withdrawn from mother solution and diluted with 2ml methanol; 250 µg /ml density was obtained. Similarly, serial dilution was performed multiple times so that the final diluted solution had the density 0.977 µg /ml. They were kept marked.

2.1.4.2.4 DPPH Solution Preparation

DPPH solution was prepared by dissolving 5mg DPPH powder in 250 ml Methanol. The density of the solution was 20 µg/ml. In an amber bottle, the solution was prepared and kept in a box which was light proof.

2.1.4.2.5 Free radical scavenging activity assay procedure

In this method, methanol plant extract solutions of different concentrations were taken at the amount of

2.0 ml and were mixed with DPPH methanol solution of 3.0 ml (20µg/ml) in 10 different test tubes. Purple colored DPPH methanol mixture bleached by the extract of plant as contrasted to that of BHT and ASA by utilizing UV spectrophotometer the assaying of potential antioxidant was done.

Inhibition of free radical scavenging in percent (I%) was determined as follows:

(I%) = (1 – Asample/Ablank) X 100

Where Ablank is represented as the control reactions absorbance, which consist of all reagents except from the meterial to be tested. From the graph where percentage of inhibition was plotted against the density of the extract, calculation of the density of extract that gives 50% inhibition (IC50) was done.

2.1.4.3 Flavonoid content estimation:

2.1.4.3.1 Control group preparation

- 10% Aluminium Chloride solution needed to be prepared to the amount of 100 ml. At first, 10g of Aluminium Chloride was weighed and taken in a 100ml volumetric flask. Then it was dissolved in distilled water taken up to the mark of 100ml.
- 100 ml of 1M Potassium Acetate solution was also needed. 9.815g of potassium acetate was weighed and taken in a 100 ml volumetric flask. Then it was dissolved in water up to the mark of 100 ml.

2.1.4.3.2 Sample solution preparation

120 mg of leaf and bark extracts were taken in different test tubes. Then they were dissolved in 10ml methanol separately. So 12mg/ml or 12000 µg/ml sample stock solutions were prepared. Both stock solutions were serially diluted in same way.

- At first, 1ml stock solution was withdrawn in a test tube and 9ml methanol was added to make the volume up to 10ml. So, V2= 10ml.

  S1= 12000/10= 1200 µg/ml

  S1V1=S2V2

  1200*V1= 800*10

  V1= 6.67ml
- Secondly, 6.67ml solution was withdrawn from the first diluted sample solution and it was diluted with 3.3ml methanol to obtain the volume of 10ml.

  So, V1= 400*10/800= 5ml.

- Next, 5ml solution was withdrawn from the second diluted stock solution and it was diluted with 5ml methanol to obtain the volume of 10ml.
  So, V1= 200*10/400= 5ml
- Lastly, 5ml solution was withdrawn from the third diluted sample solution and was diluted with 5ml methanol to obtain the volume of 10ml.

2.1.4.3.3 Standard Preparation

Quercetin solutions were prepared in same way as the sample solutions.

2.1.4.3.4 Blank Preparation

200 µl of Aluminium Chloride and 200 µl of Potassium Acetate was mixed with 5.6ml of distilled water and 4ml of methanol.

2.1.4.3.5 Assay Solution Preparation

4 test tubes were taken. Consecutively, 1ml sample and 1ml standard solutions were withdrawn from each diluted sample and standard solutions serially in 4 test tubes. Then 3ml methanol, 5.6ml of distilled water, 200 µl of Aluminium Chloride and 200 µl of Potassium Acetate were added in 4 test tubes and mixed. Lastly, UV absorbance was recorded of these 4 solutions.

2.1.5 Phytochemical Screening tests

By observing the characteristic color change, the main chemical groups were identified according to the standard procedure mentioned (Ghani, 2003).

2.1.5.1 Carbohydrate Screening

a. **Molisch Test:** In 2 test tubes, 2ml solutions of leaf and bark extracts were taken separately. After that, freshly prepared 10% alcoholic solution of alpha napthol was added 2 drops to both the test tubes. This step was followed by addition of sulphuric acid to the mixture to the flown side of the inclined tube so that the acid forms a layer beneath the aqueous solution. If carbohydrate present, a red or reddish violet ring would form at the junction of the two layers.
b. **Fehling's test:** Equal volumes of Fehling's solution A and B were taken to make a 1ml mixture. Then this mixture was added to 2 ml solutions of bark and leaf

extracts separately and boiled for a few minutes. If carbohydrate present, then red or brick-red precipitate would form immediately.

2.1.5.2 Tannin Screening

a. **Ferric Chloride Test:** 5ml aqueous solutions of leaf extract and bark extract were taken in 2 separate test tubes. After that, 1ml of 5% ferric chloride solution was added to both the extracts. If tannin was present, greenish black precipitate would form.
b. **Potassium Dichromate Test:** 5ml aqueous solutions of leaf extract and bark extract were taken in 2 separate test tubes. Next, 1ml of 10% Potassium Dichromate solution was added to both the solutions. If tannins present, presence of yellow precipitate was seen.
c. **Lead Acetate Test:** 5ml aqueous solutions of leaf extract and bark extract were taken in 2 separate test tubes. This was followed by addition of few drops of 1% Lead Acetate solution to both the extract solutions. If tannins present, yellow or red precipitate would form.

2.1.5.3 Flavonoid Screening

0.5ml alcoholic solutions of leaf and bark extracts were prepared separately in 2 different test tubes. After that, small piece of zinc ribbon or zinc dust with 5-10 drops of concentrated hydrochloric acid was added to both the test tubes. The solutions were boiled in water bath for a few minutes. Presence of flavonoids would be confirmed by the development of red to crimson colors.

2.1.5.4 Saponins Screening

Frothing Test: 0.5ml alcoholic solutions of leaf and bark extracts were prepared separately in 2 different test tubes. Next, both the solutions were diluted to 10ml using distilled water followed by shaking in a graduated cylinder for 3-5 minutes. If saponins present, persistence frothing would form.

2.1.5.5 Protein Screening

1 ml of aqueous solutions of leaf and bark extracts were taken separately. After that, 5-6 drops of 10% sodium hydroxide and 1-2 drops of 3% copper sulphate solution were added to both solutions. If proteins present, red or violet color would develop.

2.1.5.6 Alkaloid Screening

a. Mayer's Test: 2 ml fluid arrangements of unrefined concentrates were taken in 2 test tubes. At that point 0.2ml of concentrated hydrochloric corrosive was added to the two arrangements. From that point forward, 1ml of Mayer's reagent was added to both. In the event that alkaloids present, yellow crystalline hasten would shape.

b. **Dragendroff's Test**: 2 ml fluid arrangements of unrefined concentrates were taken in 2 test tubes. At that point 0.2ml of concentrated hydrochloric corrosive was added to the two arrangements. From that point onward, 1ml of Dragendroff's reagent was added to both. On the off chance that alkaloids present, orange dark colored encourage would shape.

c. **Hager's Test**: 2 ml watery arrangements of unrefined concentrates were taken in 2 test tubes. At that point 0.2ml of concentrated hydrochloric corrosive was added to the two arrangements. Next, 1ml of Hager's reagent was included. On the off chance that alkaloids present, yellow crystalline encourage would shape.

2.1.6 Antidiarrheal activity test

Castor oil and Magnesium Sulphate both were used separately to induce diarrhea in the process of evaluating the antidiarrheal activity of the leaf and bark extract of *Sterculia colorata* in rats. According to the method, each rat was given 1mL of pure analytical quality of castor oil/magnesium sulphate to induce diarrhea. The numbers of feces were recorded for separate rat. The outcome of the treatment groups was compared with that of the positive control group to evaluate the antidiarrheal activity.

For this experiment, rats with average weight of 25-35gm were obtained. The rats were purchased from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDRB). The rats were preserved in suitable environmental condition in a temperature of 21±1°C with 12 h dark or light cycle and properly fed. Proper diet food was provided to the rats. They were kept in this suitable environmental condition for 3-4 days due to their sensitivity. The ethics for use of experimental animals were followed with appropriate precautions.

2.1.6.1 Design of Experiment

Twenty-four rats were selected at random and were divided into four groups indicated as group 1, 2, 3 and 4 consisting of six rats in each groups. Each group was treated with particular one treatment e.g. control, positive control or standard and methanolic crude extracts in two different doses (200 and 400 mg/kg body weight of *Sterculia colorata* leaf and bark extract). Prior to the experiment, all the rats were weighted accurately and the control and test samples were accommodated properly.

2.1.6.2 Test Material Preparation

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

2.1.6.3 Process

All the rats were divided into control, positive control and test sample group comprising of six rats in each group. Group 1 or the control group was administered distilled water at dose of 0.2 mL/kg orally. Loperamide HCl, a standard anti-diarrheal drug was received by Group 2 or positive control group by 2mg/kg-body weight. The sample test groups (Group 3 and Group 4) were given methanolic leaf and bark extracts of *Sterculia colorata* at 200 and 400 mg/kg body weight orally. Individual rat of each group were kept in individual case putting absorptive paper under the cases. The absorptive paper was changed every hour. Castor oil and Magnesium Sulfate were given at a dose of 1mL per mice 30 minutes after the administration of leaf and bark extracts, Loperamide HCl and water. The rats were observed carefully for recording the effect of diarrhea every hour in 4 hr after administration of castor oil. Any fluid material or stools from rats were marked as signals of induction of diarrhea. Number of feces that stained the adsorptive paper was recorded at every consecutive hour and was noted for each rat.

Chapter 3:

Result

3.1 Antioxidant Assay of *Sterculia colorata*:

3.1.1 DPPH Free Radical Scavenging Assay of *Sterculia colorata* Leaf extract

The DPPH free radical scavenging activity of a standard antioxidant Ascorbic Acid (ASA) is shown in table (3.1.1.1). At different concentrations, the UV absorbances of ASA and DPPH mixture solutions were recorded in order to calculate the % of Inhibition. Furthermore, the IC50 (concentration required to inhibit 50% free radical scavenging activity) of ASA was calculated to estimate the inhibition activity more accurately.

Table 3.1.1.1 DPPH free radical scavenging assay- Percentage of Inhibition of Standard Ascorbic Acid (ASA)

Conc. (µg/ml)	Absorbance of Standard (ASA)	% of inhibition	IC_{50} µg/mL
500	0.031	94.97568882	75.688
250	0.043	93.03079417	
125	0.065	89.46515397	
62.5	0.184	70.17828201	
31.25	0.292	52.67423015	
15.625	0.385	37.6012966	
7.813	0.418	32.2528363	
3.906	0.464	24.79740681	
1.953	0.481	22.0421394	
0.977	0.497	19.4489465	
Blank	0.617		

Figure (3.1.1.1) shows the plotting of % of Inhibition of ASA against Concentration. From the graph, it is observed that, up to the concentration of 120 µg/ml, % of inhibition of ASA has gradually increased. But when concentration of ASA went above 120 µg/ml, the rise in % of inhibition stopped due to lens saturation. Furthermore, it also shows that, the ASA initiates the free radical scavenging activity from a very low point and the inhibition gradually rises which is due to the potent antioxidant property of standard ASA that can work for a sufficient long time.

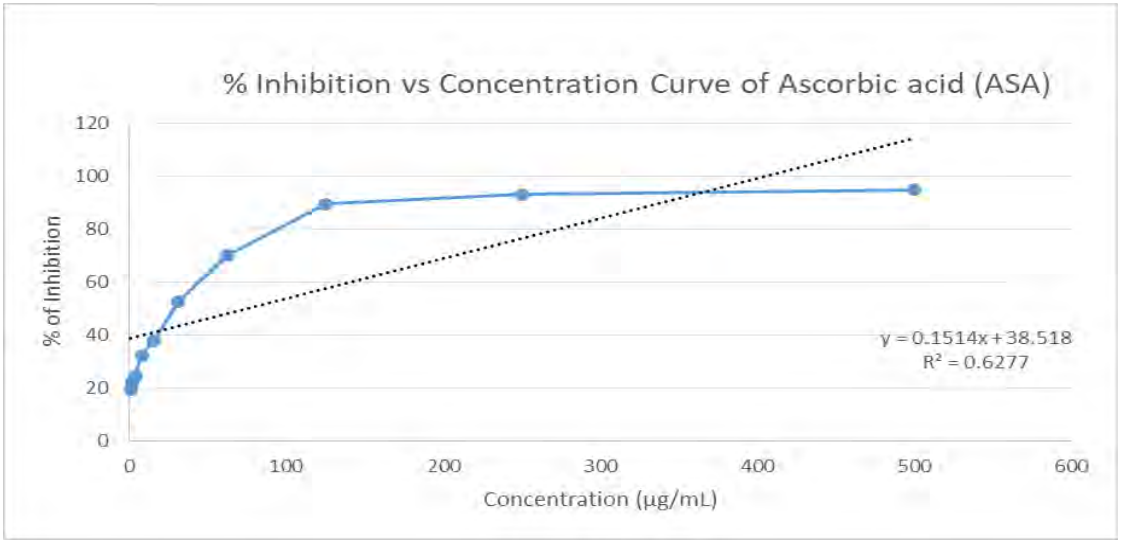

Fig 3.1.1.1 DPPH Assay- Percentage of inhibition vs. Concentration (µg/mL) for Standard Ascorbic Acid (ASA).

Table (3.1.1.2) shows the DPPH free radical scavenging activity of the crude methanolic leaf extract of S. colorata. At different concentrations, the UV absorbances of crude methanolic leaf extract and DPPH mixture solutions were recorded in order to calculate the % of Inhibition of DPPH free radical scavenging caused by the S. colorata leaf extract. Furthermore, the IC50 (concentration required to inhibit 50% free radical scavenging activity) of S. colorata leaf extract was calculated to estimate the free radical scavenging activity precisely. If we compare the DPPH free radical scavenging activity of S. colorata leaf extract with that of the standard antioxidant ASA, it can be seen that IC50 of S. colorata leaf extract is 49.9% of the IC50 of ASA.

Table 3.1.1.2 DPPH free radical scavenging assay- Percentage of Inhibition of Crude Methanolic Extract of Leaf

Conc. (µg/ml)	Absorbance of leaf	% of inhibition	IC50 µg/mL
500	0.063	89.7893	
250	0.053	91.41005	
125	0.061	90.11345	
62.5	0.105	82.98217	
31.25	0.139	77.47164	
15.625	0.185	70.01621	37.78
7.813	0.21	65.96434	
3.906	0.211	65.80227	
1.953	0.217	64.8298	
0.977	0.235	61.9125	
Blank	0.617		

Figure (3.1.1.2) shows the plotting of % of inhibition against different concentrations for methanolic leaf extract of S. colorata. It exhibits that, initially S. colorata leaf extract shows much higher % of inhibition compared to the standard ASA. But the gradual increase of inhibition activity is very less with time compared to ASA. It predicts that, S. colorata leaf extract shows free radical scavenging activity for a very shorter time compared to ASA, however the initial effect is very stronger than that of ASA.

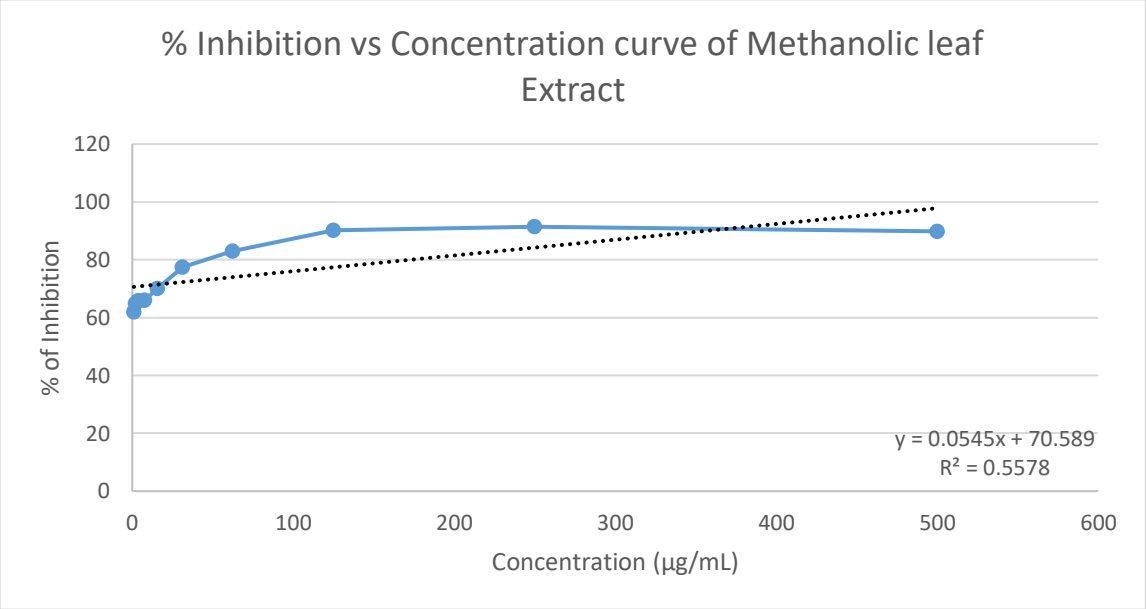

Fig 3.1.1.2 DPPH Assay- Percentage of inhibition vs. Concentration (µg/mL) for Crude Methanolic Extract of Leaf

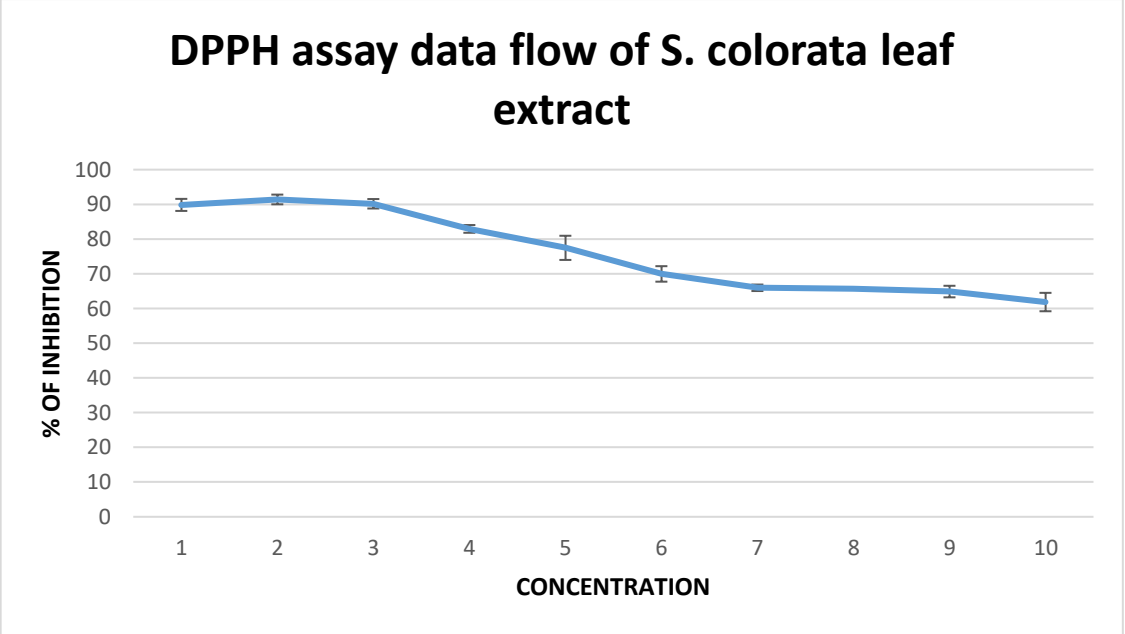

Fig 3.1.1.3 DPPH free radical scavenging assay- Data flow of S. colorata leaf extract

3.1.2 DPPH Free Radical Scavenging Assay of *Sterculia colorata* Bark extract

Table (3.1.2.1) shows the DPPH free radical scavenging activity of a standard antioxidant Ascorbic Acid (ASA). At different concentrations, the UV absorbances of ASA and DPPH mixture solutions were recorded in order to calculate the % of Inhibition. Furthermore, the IC50 (concentration required to inhibit 50% free radical scavenging activity) of ASA was calculated to estimate the inhibition activity more accurately.

Table 3.1.2.1 DPPH free radical scavenging assay- Percentage of Inhibition of Standard Ascorbic Acid (ASA)

Conc. (µg/ml)	Absorbance of Standard (ASA)	% of inhibition	IC50 µg/mL
500	0.031	94.97568882	75.688
250	0.043	93.03079417	
125	0.065	89.46515397	
62.5	0.184	70.17828201	
31.25	0.292	52.67423015	
15.625	0.385	37.6012966	
7.813	0.418	32.2528363	
3.906	0.464	24.79740681	
1.953	0.481	22.0421394	
0.977	0.497	19.4489465	
Blank	0.617		

Figure (3.1.2.1) shows the plotting of % of Inhibition of ASA against Concentration. From the graph, it is observed that, up to the concentration of 120 µg/ml, % of inhibition of ASA has gradually increased. But when concentration of ASA went above 120 µg/ml, the rise in % of inhibition stopped due to lens saturation. Furthermore, it also shows that, the ASA initiates the free radical scavenging activity from a very low point and the inhibition gradually rises which is due to the potent antioxidant property of standard ASA that can work for a sufficient long time.

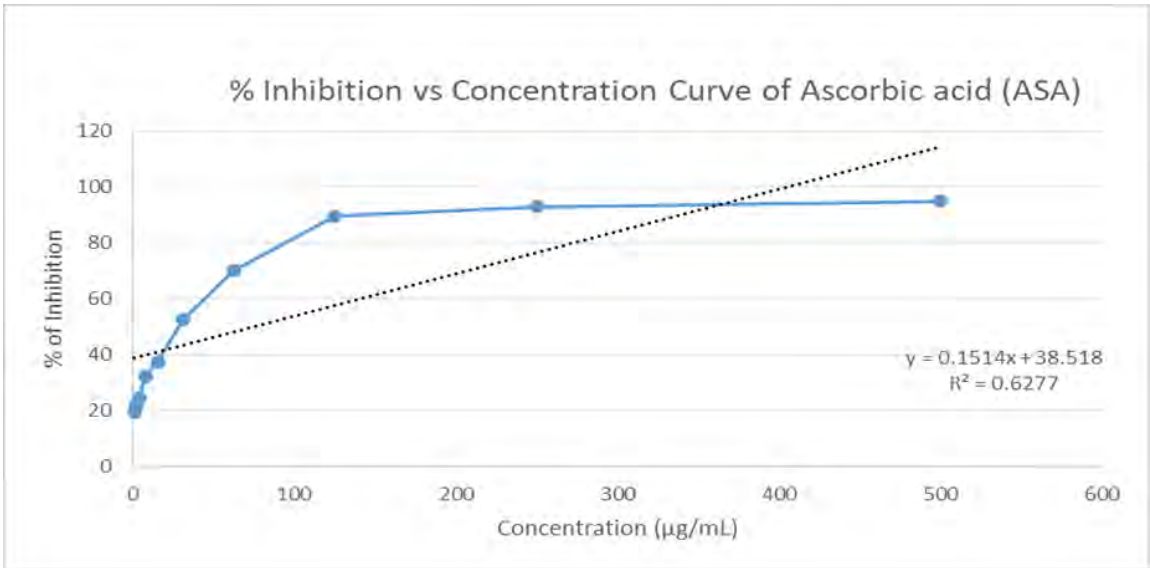

Fig 3.1.2.1 DPPH Assay- Percentage of inhibition vs. Concentration (µg/mL) for Standard Ascorbic Acid (ASA).

Table (3.1.2.2) shows the DPPH free radical scavenging activity of the crude methanolic bark extract of S. colorata. At different concentrations, the UV absorbances of crude methanolic bark extract and DPPH mixture solutions were recorded in order to calculate the % of Inhibition of DPPH free radical scavenging caused by the S. colorata bark extract. Furthermore, the IC50 (concentration required to inhibit 50% free radical scavenging activity) of S. colorata bark extract was calculated to estimate the free radical scavenging activity precisely. If we compare the DPPH free radical scavenging activity of S. colorata bark extract with that of the standard antioxidant ASA, it can be seen that IC50 of S. colorata bark extract is 80.44% of the IC50 of ASA.

Table 3.1.2.2 DPPH free radical scavenging assay- Percentage of Inhibition of Crude Methanolic Extract of Bark

Conc. (µg/ml)	Absorbance of Stem	% of inhibition	IC50 µg/mL
500	0.089	85.57536	
250	0.058	90.59968	
125	0.042	93.19287	
62.5	0.037	94.00324	
31.25	0.035	94.32739	
15.625	0.044	92.86872	1201.24
7.813	0.143	76.82334	
3.906	0.163	73.58185	
1.953	0.191	69.0438	
0.977	0.268	56.564	
Blank	0.617		

Figure (3.1.2.2) shows the plotting of % of inhibition against different concentrations for methanolic bark extract of S. colorata. It exhibits that, initially S. colorata bark extract shows much higher % of inhibition compared to the standard ASA. But the gradual increase of inhibition activity is very less with time compared to ASA. It predicts that, S. colorata bark extract shows free radical scavenging activity for a very shorter time compared to ASA, however the initial effect is very stronger than that of ASA.

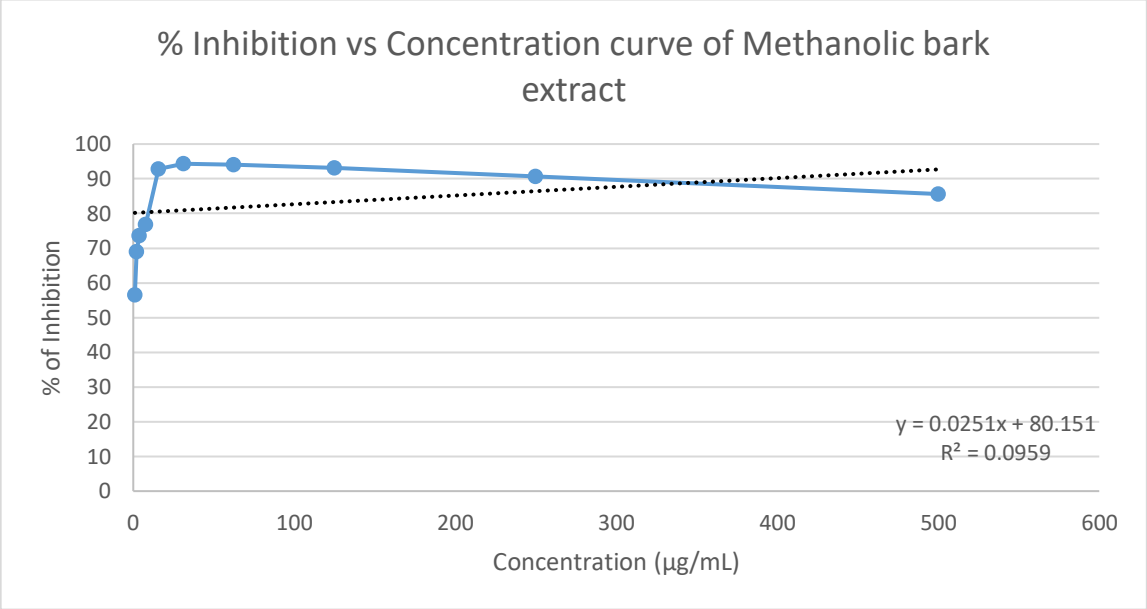

Fig 3.1.2.2 DPPH Assay- Percentage of inhibition vs. Concentration (µg/mL) for Crude Methanolic Extract of bark

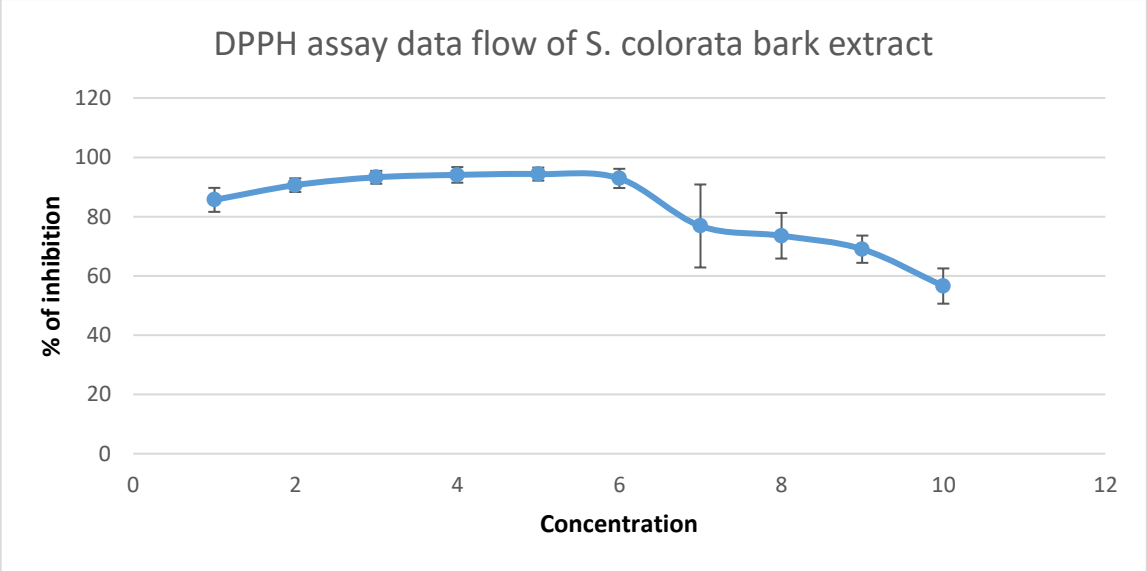

Fig 3.1.2.3 DPPH free radical scavenging assay- Data flow of S. colorata bark extract

3.1.3 Total Phenolic Content Determination of S. colorata leaf extract

Figure (3.1.3.1) is the standard curve of Gallic Acid. Gallic acid was used as a standard for total phenolic content (TPC) estimation of S. colorata leaf extract. UV absorbance was recorded for different concentrations of Gallic acid solutions. So, the obtained Absorbance values are plotted against concentrations in this standard curve and a straight line generates. The regression equation generated from the straight line is used for estimation of TPC in S. colorata leaf extract.

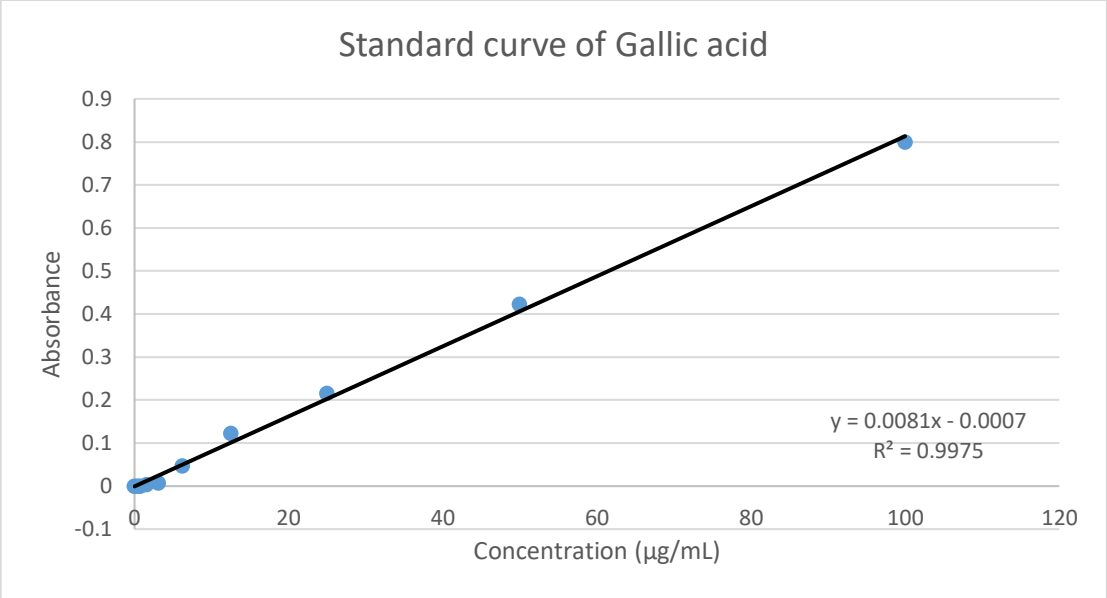

Fig 3.1.3.1 Standard curve of Gallic acid for determining Total Phenolic Content in S. colorata leaf extract

Table (3.1.3.1) shows the recorded UV absorbance values of S.colorata leaf extract and Folin-ciocalteu mixture at three different concentrations at the wavelength of 760 nm. From the regression equation, TPC is calculated for 3 different absorbances as in 3 different concentrations. It is evident that, TPC values are decreasing with decreasing concentration of S. colorata leaf extract. That means the antioxidant activity decreases with the decrease in S. colorata leaf extract concentration. Thus, the maximum TPC of S. colorata leaf extract is 105.89 (GAE) mg of Gallic acid per gram of dried extract.

Table 3.1.3.1 Total Phenolic Content (TPC) of *Sterculia colorata* Leaf extract determined from the regression line y=0.0081x-0.0007

Concentration of S. colorata leaf extract solution (µg/ml)	**Absorbance values of S. colorata leaf extract recorded at 760 nm**	**TPC (GAE) mg of Gallic acid per gram of dried extract**
200	0.857	105.89
400	0.642	79.35
800	0.581	71.81

3.1.4 Total Phenolic Content Determination of S. colorata bark extract

Figure (3.1.4.1) is the standard curve of Gallic Acid. Gallic acid was used as a standard for total phenolic content (TPC) estimation of S. colorata bark extract. UV absorbance was recorded for different concentrations of Gallic acid solutions. So, the obtained Absorbance values are plotted against concentrations in this standard curve and a straight line generates. The regression equation generated from the straight line is used for estimation of TPC in S. colorata bark extract.

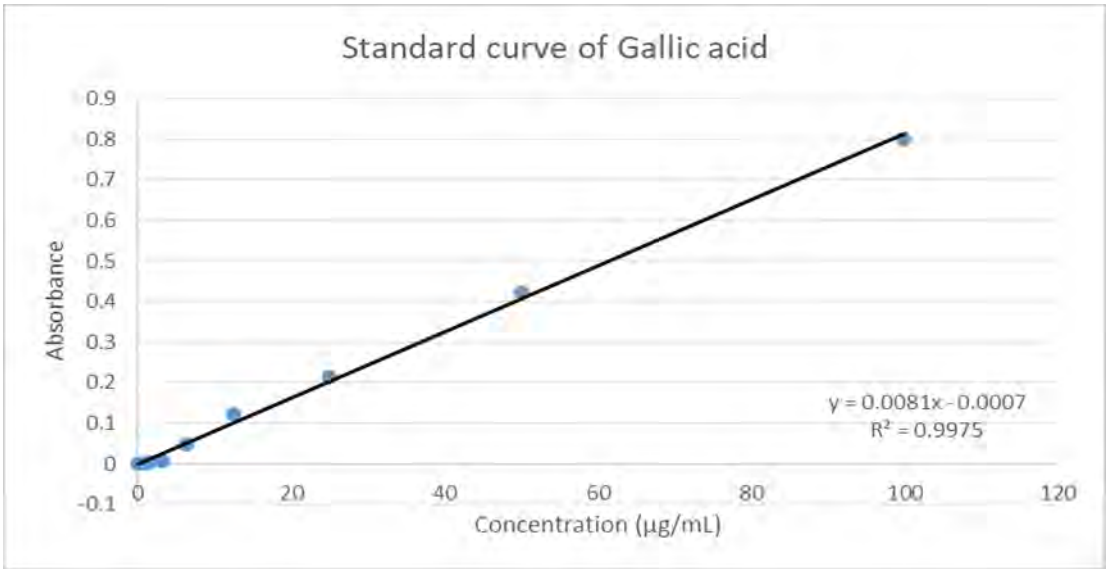

Fig 3.1.4.1 Standard curve of Gallic acid for determining Total Phenolic Content in Sterculia colorata Bark extract

Table (3.1.4.1) shows the recorded UV absorbance values of S.colorata bark extract and Folin-ciocalteu mixture at three different concentrations at the wavelength of 760 nm. From the regression equation, TPC is calculated for 3 different absorbances as in 3 different concentrations. It is evident that, TPC values are increasing with decreasing concentration of S. colorata bark extract. That means the antioxidant activity increases with the decrease in S. colorata bark extract concentration. Thus, the maximum TPC of S. colorata bark extract is 151.32 (GAE) mg of Gallic acid per gram of dried extract.

Table 3.1.4.1 Total Phenolic Content (TPC) of Sterculia colorata Leaf extract determined from the derived equation y=0.0081x-0.0007

Concentration of S. colorata bark extract solution (µg/ml)	**Absorbance values of S. colorata bark extract recorded at 760 nm**	**TPC (GAE) mg of Gallic acid per gram of dried extract**
200	1.062	131.20
400	1.081	133.54
800	1.225	151.32

3.1.5 Total Flavonoid Content Test of *Sterculia colorata* Leaf extract

Quercetin is used as a standard for total flavonoid content estimation. Different concentrations of Quercetin solutions were prepared to record the UV absorbance values. Then a Standard curve (3.1.5.1) was prepared by plotting the absorbance values against the concentrations to obtain the regression line and regression equation. The regression equation is used to estimate the TFC of S. colorata leaf extract.

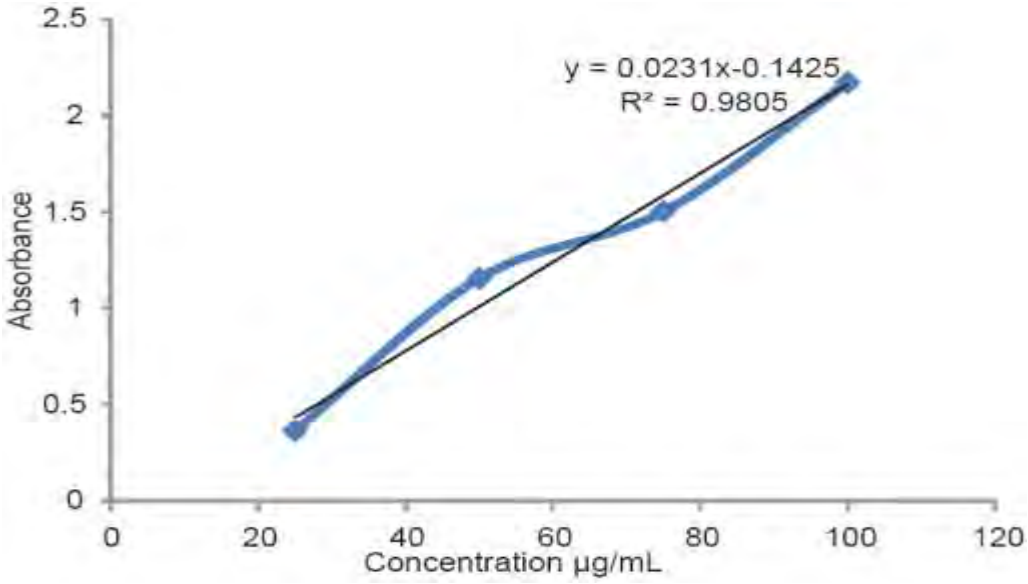

Fig 3.1.5.1 Standard Curve of Quercetin at 415 nm for determining TFC in S. colorata leaf extract

Table (3.1.5.1) shows the recorded UV absorbance values of S.colorata leaf extract and Quercetin mixture at three different concentrations at the wavelength of 415 nm. From the regression equation, TFC is calculated for 3 different absorbances as in 3 different concentrations. It is evident that, TFC values are decreasing with decreasing concentration of S. colorata leaf extract. That means the antioxidant activity decreases with the decrease in S. colorata leaf extract concentration. Thus, the maximum TFC of S. colorata leaf extract is 100.97 (QE) mg of Quercetin per gram of dried extract.

Table 3.1.5.1 Total flavonoid content (TFC) in S. colorata leaf extract determined from the derived equation y=0.0231x-0.1425

Concentration of S. colorata leaf extract solution (µg/mL)	**Absorbance values recorded at 415 nm**	**TFC(QE) mg of Quercetin per gram of dried extract**
200	2.190	100.97
400	1.515	71.75
800	1.086	53.18

3.1.6 Total Flavonoid Content Test of S. colorata Bark extract

Quercetin is used as a standard for total flavonoid content estimation. Different concentrations of Quercetin solutions were prepared to record the UV absorbance values. Then a Standard curve (3.1.6.1) was prepared by plotting the absorbance values against the concentrations to obtain the regression line and regression equation. The regression equation is used to estimate the TFC of S. colorata bark extract.

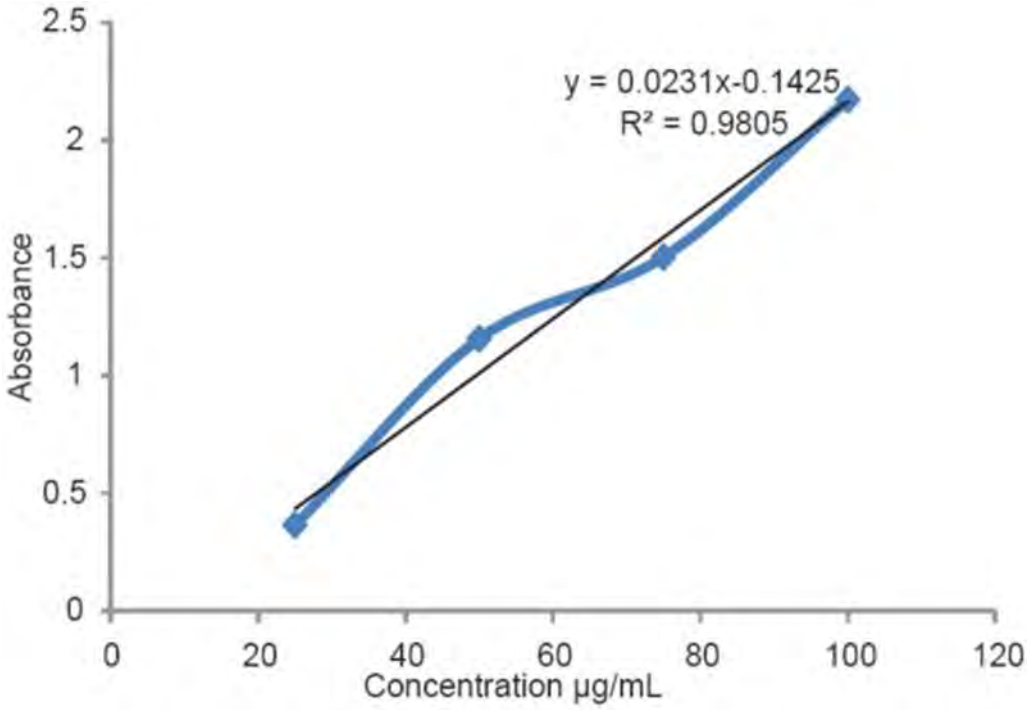

Fig 3.1.6.1 Standard Curve of Quercetin at 415 nm for determining TFC in S. colorata bark extract

Table (3.1.6.1) shows the recorded UV absorbance values of S.colorata bark extract and Quercetin mixture at three different concentrations at the wavelength of 415 nm. From the regression equation, TFC is calculated for 3 different absorbances as in 3 different concentrations. It is evident that, TFC values are decreasing with decreasing concentration of S. colorata bark extract. That means the antioxidant activity decreases with the decrease in S. colorata bark extract concentration. Thus, the maximum TFC of S. colorata bark extract is 16.65 (QE) mg of Quercetin per gram of dried extract.

Table 3.1.6.1 Total flavonoid content (TFC) in S. colorata bark extract determined from the derived equation y=0.0231x-0.1425

Concentration of S. colorata bark extract solution (µg/mL)	**Absorbance values recorded at 415 nm**	**TFC(QE) mg of Quercetin per gram of dried extract**
200	0.242	16.65
400	0.207	15.13
800	0.105	10.71

3.2 Phytochemical Screening of *Sterculia colorata* Leaf and Bark (methanolic) extracts:

Table (3.2.1) shows the result of phytochemical screening of S. colorata leaf and bark extracts can be observed. Firstly it's evident that, carbohydrate is not present in either the leaf or bark extract of S. colorata. Proteins and Alkaloids are also not present in any of the extracts. However, Tannin, Flavonoid and Saponin are present in both the leaf and bark extracts of S. colorata.

Table 3.2.1 Phytochemicals presence record of S. colorata leaf and bark extracts

Phytochemical	Screening Tests	Leaf Extract	Bark Extract
1. Carbohydrate	**1(a)** Molisch Test	–	–
	1(b) Fehling's Test	–	–
2. Tannin	**2(a)** Ferric Chloride Test	–	+
	2(b) Potassium Dichromate Test	–	–
	2(c) Lead Acetate Test	+	–
3. Flavonoid		+	+
4. Saponin		+	+
5. Protein		–	–
6. Alkaloids	**6(a)** Mayer's Test	–	–
	6(b) Hager's Test	–	–
	6(c) Dragendorff's Test	–	–

3.3 Antidiarrheal activity assay of *Sterculia colorata* Leaf extract:

Table (3.3.1) shows the percentage of inhibition values of Loperamide HCl and the S. colorata leaf extract (200 mg/kg and 400 mg/kg) observed in the diarrhea induced rats. Diarrhea was induced by castor oil and Magnesium Sulfate separately. The standard Loperamide HCl produced significant inhibition in both type of inducers. But the S. colorata leaf extract at 200 mg/kg dose produced much greater inhibition in case of castor oil induced diarrhea compared to that of magnesium sulfate. However, the leaf extract at 400 mg/kg dose produced almost similar forceful inhibition in both types of inducers. The p values were also calculated for leaf extract at both doses (200 and 400 mg/kg). From the p values, it can be noted that, S. colorata leaf extract inhibits diarrhea most significantly when castor oil induces diarrhea. Moreover, the most significant inhibition occurs in case of S. colorata leaf extract at 200 mg/kg dose compared to 400 mg/kg dose.

Table 3.3.1 Percentage of Inhibition observed in case of diarrhea induced by castor oil and magnesium sulfate

	% of inhibition of Castor oil	**% of inhibition of MgSO4**
Standard (Loperamide HCl)	54.47	49.61
Sample 200 mg/kg	41.46 (p value: 0.008223)	4.65 (p value: 2.98392E-06)
Sample 400 mg/kg	39.84 (p value: 0.010922)	37.98(p value:0.028772805)

Figure (3.3.1) shows the degree and significance of percentage of inhibition caused by S. colorata leaf extract at two different doses (200mg/kg and 400mg/kg) by comparing with the % of inhibition of the standard anti-motility drug Loperamide HCl. This comparison grounds on diarrhea induced by castor oil and by magnesium sulfate. Based on the p value calculated for leaf extracts at both doses against the standard, the degree of % inhibition is assigned a significance: which is shown by the number of stars. Firstly, the most significant % inhibition showed by two stars, is observed from the leaf extract at 200mg/kg dose; which took place in case of castor oil induction. Furthermore, the leaf extract at 400mg/kg dose gave lesser significant % inhibition represented by a single star; in case of castor oil induction. However, in case of diarrhea induced with Magnesium Sulfate, the leaf extract at 400mg/kg dose showed significant % inhibition instead of 200mg/kg dose. However, if the % inhibition of leaf extract administered at 200 mg/kg is compared with that of the standard, then the decrease in % inhibition of leaf extract is 23% (castor oil) and 91% (magnesium sulfate) respectively. For 400 mg/kg dose, this decrease is 26% (castor oil) and 23% (magnesium sulfate) respectively.

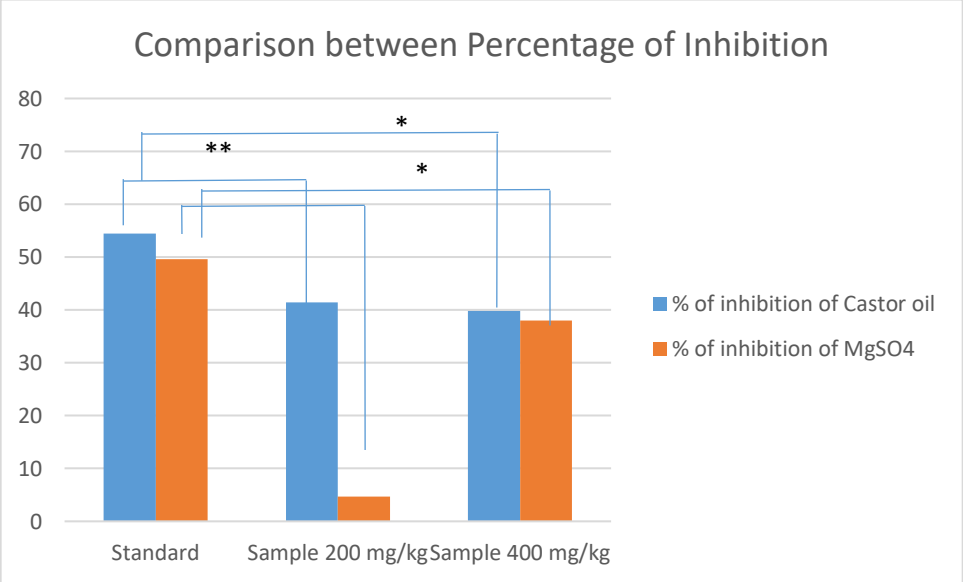

Fig 3.3.1 Comparison between Percentage of Inhibition observed in case of diarrhea induced by castor oil and magnesium sulfate on the basis of p value

Chapter 4:
Discussion

This study was conducted on the medicinal plant *Sterculia colorata.* Moreover, crude Methanolic extracts of both the leaf and bark of S. colorata were used for this study. However, the properties examined and assayed were Antioxidant activity and Antidiarrheal activity (for leaf extract only). Phytochemical Screening of the leaf and bark extracts was also performed to identify some specific biochemical constituents.

For the purpose of assaying the antioxidant activity of S. colorata leaf and bark extracts, DPPH free radical scavenging test, Total phenolic content determination and Total flavonoid content determination tests were performed.

The DPPH free radical scavenging assay of S. colorata leaf extract showed that, it possesses 49.9% free radical scavenging activity compared to standard but the inhibition is initially very rapid for a shorter duration unlike ASA. Thus, similar pattern of DPPH free radical scavenging activity can be observed in the *Sterculia foetida* leaf extract (Manivannan, Kothai, Arul, Rajaram, 2011), which represents rapid acting free radical scavenging property.

The Total Phenolic Content (TPC) estimation test of S. colorata leaf extract showed that, the maximum TPC is 105.89 (GAE) mg of Gallic acid per gm of dried extract and the TPC decreases with decreasing extract concentration. This estimated TPC is very similar to S. tragacantha leaf extract (Oblang, Misso, Atome, Ondo, Engonga, Emvo, 2017), which specifies a moderately effective antioxidant property.

The Total Flavonoid Content (TFC) determination test of S. colorata leaf extract showed that, the maximum TFC is 100.97 (QE) mg of Quercetin per gm of dried extract, which decreases with decreasing extract concentration. Significantly similar type of TFC content can be observed in S. quadrifida leaf extract (Akter, Barnes, Brophy, Harrington, 2016), which reflects quite high flavonoid content.

The DPPH free radical scavenging assay of S. colorata bark extract showed that, it possesses 80.44% free radical scavenging activity compared to standard but the inhibition is initially very rapid for a shorter duration unlike ASA. So, bark extract has much stronger DPPH free radical scavenging ability compared to the leaf extract, but the duration pattern is quite similar. Thus, similar pattern of DPPH free radical scavenging activity can be observed in the S. foetida bark extract (Khatoon, Mohapatra, Satapathy, 2016), reflecting a very potent free radical scavenging property.

The Total Phenolic Content (TPC) estimation test of S. colorata bark extract showed that, the maximum TPC is 151.32 (GAE) mg of Gallic acid per gm of dried extract and the TPC increases with decreasing extract concentration. This estimated TPC is very similar to S. quadrifida bark extract (Lulan, Fatmawati, Santoso, Ersam, 2018), proving very high amount of TPC leading to potent antioxidant activity compared to the leaf extract.

The Total Flavonoid Content (TFC) estimation test of S. colorata bark extract showed that, the maximum TFC is 16.65 (QE) mg of Quercetin per gm of dried extract, which decreases slightly with decreasing bark extract concentration. This TFC is lowest among other plants of Sterculia genus as most Sterculia plants have high flavonoid content. However, this low TFC is closely similar to the plant *Firmiana simplex* of the same family as that of S. colorata (Rahman, Sherei, Kassem, 2017). Thus, the S. colorata leaf extract has much higher TFC compared to the bark extract.

The phytochemical screening assay of S. colorata leaf and bark extracts showed that, tannins, flavonoids and saponins are present in both the leaf and bark extracts of S. colorata. However, carbohydrates, alkaloids and proteins are absent in both the extracts. This result pattern of phytochemical screening assay is quite similar to the plant S. setigera (Kubmarawa, Ajoku, Enwerem, Okorie, 2007).

From the Antidiarrheal activity test conducted on S. colorata leaf extract, it can be observed that, 200mg/kg dose of S. colorata leaf extract inhibits diarrhea induced by castor oil most significantly. However, the 400mg/kg dose proved more significant than that of 200 mg/kg dose in case of Magnesium Sulfate induced diarrhea. This observed pattern of antidiarrheal activity is quite similar to the plant S. setigera (Gera, Ume, Anyiin, Iheukwumere, 2015, Idyu, Deshi, VC, Ogundeko, 2015) which reflects effective antidiarrheal activity of S. colorata leaf extract.

Chapter 5:
Conclusion

There are about thousands and millions of medicinal plants existing all over this world. Every day, many extensive and versatile researches are being performed with many of these medicinal plants. Moreover, these researches ground on various fields including Phytochemistry, Pharmaceutics, Pharmacology, Clinical Surveys, Biological Screening, Environmental effects and so on. The selected plant for this particular study is *Sterculia colorata.* Many phytochemical and pharmacological studies have taken place on the other species of the Sterculia genus. But to this date, S. colorata has been only subjected to maximum environmental and botanical studies. This plant has not yet been subjected to phytochemical and biological studies. So, S. colorata was selected specifically for this study in the hope of discovering some useful properties in the fields of medicine and phytochemistry. This study covers antioxidant, phytochemical screening and antidiarrheal assay of S. colorata very briefly. The assays could be performed in much more detailed, authentic and extensive way. But due to constraints in time period, financial limitations and limited resources, that could not be made possible. From plant collection, extraction to performing the assays, utmost care and concern was attained to maintain the good quality of the results. Still there can be some small unavoidable errors in the results due to any unnoticed mistakes or machine errors. Instead of all the constraints, the study can play a great role in putting a new spotlight on the phytochemical and medicinal properties of *Sterculia colorata.*

Chapter 6:

References

Atakpama W, Batawa K, Dourma M, Pereki H, Wala K, Dunobe K, Akpagana K, Gbeassor M, 2012: Ethno biological Knowledge of Sterculia setigera Del. In the Sudanian Zone of Togo. ISRN Botany. Vol 2012, pp 1-5. Accessed 7th September, 2018.

Idyu I. I., Deshi E. F., Idyu V. C., Ogundeko T. O, 31st May, 2015, The Antidiarrheal Effect of Ethanolic Bark Extract of Sterculia Setigera in mice, Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos, Nigeria, Asian Journal of Science and Technology, ISSN: 0976-3376, Vol. 6, Issue 05, pp. 1397-1400. Accessed at 7th September, 2018.

Gera Y, Ume E. U., Tor-Anyiin T. A., Iheukwumere C. C, 2015, Ethno botanical survey of anti-diarrheal medicinal plants among Tiv people of Nigeria, Dept. of Biological Sciences, University of Agriculture, Makurdi, Nigeria, Dept. of Chemistry, University of Agriculture, Makurdi, Nigeria, Scholars Research Library, Archives of Applied Science Research, ISSN 0975-508X, pp 16-21, CODEN (USA) AASRC9, Accessed at 7th September.

Lulan T, Fatmawati S, Santoso M, Ersam T, Yehezkiel T, 2018, Antioxidant Capacity of Some Selected Medicinal Plants in East Nusa Tenggara, Indonesia: The Potential of Sterculia quadrifida R, Br.. Free Radicals and Antioxidants, Vol: 8 (2), pp: 96-101, Accessed 7th September, 2018.

Akter K, Barnes E, Brophy J, Harrington D, Community Elders Y, Vemulpad S, Jamie J, 2016, Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia, Evidence-based complementary and alternative medicine : eCAM, ISSN 1741-427X, Vol: 2016, pp: 4683059, Accessed at 7th September, 2018.

Obiang S.C., Misso N.M.R., Atome N.G., Ondo J, Engonga O.L., Emvo N.E., 2017, Chemical composition, antioxidant and antimicrobial activities of stem barks of Englerina gabonensis; Engler; Sterculia tragacantha Lind from Gabon, International Journal of Phytomedicine, Vol: 9 (3) pp: 501, ISSN 0975-0185, Accessed 7th September, 2018.

Manivannan E, Kothai R, Arul B, Rajaram S, July-September 2011, In-vitro antioxidant properties of Sterculia foetida Linn, Research Journal of Pharmaceutical, Biological and Chemical Sciences, Department of Pharmacology, Vinayaka Mission's Kirupanandha

Variyar Medical College, Seeragapadi Salem-636 308, Tamilnadu, India, Vinayaka Mission's College of Pharmacy, Salem, ISSN: 0975-8585, Vol 2 (3), pp 43-52, Accessed 7th September, 2018.

Kubmarawa D, Ajoku G.A., Enwerem N.M., Okorie D.A., 18TH July 2007, Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria, African Journal of Biotechnology, Department of Chemistry, Federal University of Technology, P. M. B. 2076, Yola, Adamawa State, Nigeria, Department of Pharmaceutical Microbiology, National Institute for Pharmaceutical Research and Development Abuja, Nigeria. Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. 4Department of Chemistry, University of Ibadan, Ibadan, Nigeria, ISSN 1684–5315, Vol. 6 (14), pp. 1690-1696, Accessed 7th September, 2018.

Rahman A.Y.R.A.A., Sherei M.M.E., Kassem M.E.S.A., 2017, A Pharmacognostical Study of Certain Sterculia Species (Family: Sterculiaceae), National Research Centre, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, pp 20, Accessed at 6th September, 2018.

Roberson E, Mccormack J, 2008, Medicinal Plants at Risk: Nature's Pharmacy, Our Treasure Chest: Why We Must Conserve Our Natural Heritage, Center for Biological Diversity, Tucson, pp 19, Accessed at July, 2018.

Sadi A, 24TH December 2012, প্রাণকাকলি: বাংলাদেশের ঔষধি গাছের একটি বিস্তারিত পাঠ, Medicinal Plants of Bangladesh, URL: anupsadi.blogspot.com/2012/12/medicinal-plants-of-bangladesh_24.html, Accessed at July, 2018.

Khatoon A, Mohapatra A, Satapathy A.B., 1st July 2016, Studies on In-vitro Evaluation of Antibacterial and Antioxidant Activities of *Sterculia foetida* L. Bark, International Journal of Pharmaceutical Science and Research, ISSN (Online): 0975-8232, ISSN (Print): 2320-5148, pp 2090-95, Accessed at September, 2018.

Ashafa, A.O.T., Afolayan, A.J., 2009, Screening the root extracts from Biden pilosa L.var. Radiata (Asteraceae) for antimicrobial potentials, Journal of Medicinal Plants Research 3, 568–572, Accessed at July, 2018.

Chiranjibi, P., Sudhakar, R., Dhal, N.K., Rashmita, D., 2006. Some phytotherapeutic claims by tribals of Rayagada district, Orissa, India. Ethno botanical Leaflets 10,189–197, Accessed at July, 2018.

Namukobe J, Kasenene J.M., Kiremire B.T., Byamukama R, Mugisha M.K., Krief S, Dumontet V, Kabasa J.D., 2011, Traditional plants used for medicinal purposes by local communities around the Northern sector of Kibale National Park, Uganda, Journal of Ethnopharmacology, ScienceDirect, Vol: 136, pp: 236-245, Accessed at August, 2018.

Associated Press, 2006, Slippery Elm Trees Fall Victim to Poachers, Accessed at August 9, 2006.

Abramovitz, J.N., 1997, Valuing Nature's Services, In: State Of The World 1997: A Worldwatch Institute Report On Progress Toward a Sustainable Society. New York: W.W. Norton and Co.

Balick, M.J., R. Mendelsohn, 1992, Assessing the Economic Value of Traditional Medicines from Tropical Rain Forests, Conservation Biology, Vol 6(1), pp: 128-130.

Botanic Gardens Conservation International, 1996, CITES and Medicinal Plants Study: A Summary of Findings, Web resource: www. bgci.org/wellbeing/CITES_and_Med_Plants_ Summary/.

Torrance A.W., 2000, Bioprospecting and the Convention on Biological Diversity, Harvard Law School document archive, Web resource: leda.law.harvard.edu/leda/data/258/Torrance,_ Andrew_00.pdf

Tuxhill J, 1999, Nature's Conucopia: Our State in Plant Diversity, Worldwatch Paper #148. Washington, DC: Worldwatch Institute.

U.S. Forest Service, 2001, Non-Timber Forest Product Fact Sheets, Web resource: www.sfp. forprod.vt.edu/factsheets/factsheets.htm

Plant Conservation Alliance Medicinal Plant Working Group (MPWG): www.nps.gov/ plants/medicinal/plants.htm.

Tucakov J, 1990, Healing with plants, Beograd: Rad, pp 576–8

National Center for the Preservation of Medicinal Herbs (NCPMH): home.frognet. net/~rural8/frames2.html

International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP): www.forum-essenzia.org/PDFs/ ISSC-MAP.pdf

Stojanoski N, 1999, Development of health culture in Veles and its region from the past to the end of the 20th century, Veles: Society of science and art, pp: 13–34.

Kelly K, 2009, History of medicine, New York: Facts on file, pp 29–50

Petrovska B, Jan-June 2012, Historical review of medicinal plants' usage, Pharmacognosy Review, NCBI, Vol 6(11), pp 1-5.

Importance of Medicinal Plants, Botanical Online. Accessed at August, 2018.

Morales F, Padilla S, Falconi F, 23rd November 2016, Medicinal Plants Used in Traditional Herbal Medicine in the Province of Chimborazo, Ecuador, African Journal of Traditional, Complementary and Alternative Medicines, Vol 14(1), pp 10-15, Accessed at July, 2018.

Kirtikar K.R., Basu B.D., 1918, Indian Medicinal Plants, pp.72, Accessed at July 2018.

OFDC, List of Important Medicinal Plants and their Uses, Accessed at August, 2018.

Hassan B.A.R., 10th December 2012, Medicinal Plants (Importance and Uses), Pharmaceutica Analytica Acta, ISSN: 2153-2435, Accessed at August, 2018.

Christenhusz M. J. M., Byng, J. W., 2016, The number of known plants species in the world and its annual increase, Phytotaxa, Magnolia Press, 261 (3): 201–217.

Thorne R, 1992, Classification and geography of the flowering plants, Bot Rev. 58 (3): 225–348.

Bayer C, 1999, Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: a combined analysis of plastid atpB and rbcL DNA sequences, Botanical Journal of the Linnean Society. 129 (4): 267–303

"Sterculia". The Plant List, accessed 30 June 2018.