Evaluation of Antioxidant Activity of *Trigonella foenum-graecum* Using Several In Vitro Assays

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

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APPROVAL

This Project, Evaluation for Antioxidant activity of *Trigonella foenum-graecum* using several in vitro assay systems submitted by Jannatul Ferdous Mukta 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.

BOARD OF EXAMINERS

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Internal Examiner-2

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DECLARATION

I hereby declare that, this project report is done by me under the supervision of **Dr. Md. Nurul Islam, Assistant Professor,** Department of Pharmacy, Daffodil International University ,impartial 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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DEDICATION

Dedicated to my parents

(Md.Motaleb Talukder & Jharna Begum)

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ABBREVIATION

Abbreviate	Meaning
WHO	World Health Organization
ROS	Reactive Oxygen Species
SOD	Super Oxide Dismutase
GPx	Glutathione peroxidase
CAT	Chloramphenicol Acetyl Transferase
ADP	Adenosine Di-nucleotide Phosphate
AIDS	Acquired Immune Deficiency Syndrome
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
GSH	Glutathione sulfhydryl group
ALS	Amyotrophic Lateral Sclerosis
UV	Ultra Violet
ARDS	Adult Respiratory Distress Syndrome
LDL	Low Density Lipoproteins
DNA	Deoxyribo Nucleic Acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
FCR	Folin– Ciocalteu Reagent
GAE	Gallic Acid Equivalents
QE	Quercetin Equivalents

ABSTRACT

The present study has been designed to examine the antioxidant activity of the methanol extracts of *Trigonella foenum-graecum.* Antioxidant activity was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH)radical scavenging and ferric reducing power assays. In addition, % yield, total phenolic and total flavonoid contents were also determined. Total phenolic contents were expressed as gallic acid equivalent (GAE) while total flavonoids contents were expressed as quercetin equivalent (QE) of the methanol extracts of *Trigonella foenum-graecum*. Total phenolic contents of the methanol extract of *Trigonella foenum-graecum* were found 226.66 ± 5.23 µg GAE/mg of the dried extract. On the other hand, the total flavonoid contents in the methanol extract was 237.6667 ± 6.21 µg QE/mg of the dried extract. The methanol extract of *Trigonella foenum-graecum* displayed very strong antioxidant activity by scavenging DPPH free radical with an IC_{50} value of 281.875 ± 7.32 µg/ml compared to the positive control ascorbic acid of which IC_{50} value is129.7143 ± 3.95 µg/ml. On the other hand, *Trigonella foenum-graceum* methanolic extract also showed strong ferric reducing power compared with the ascorbic Acid. Moreover, the total antioxidant capacity of the methabnol extract of *Trigonella foenum-graceum* was found to be 188.57 ± 6.47 µg AAE/mg of the dried extract. From the above results, it can be concluded that the methanol extract of *Trigonella foenum-graceum* has very strong antioxidant potential which might be associated with the high level of phenolic and flavonoids type compounds present in the extract. Therefore, *Trigonella foenum-graceum* could be used as a source of naturally occurring potent antioxidants that might ameliorate oxidative stress and related disorders. However, this plant needs further investigation in order to find out the responsible chemical compounds associated with the antioxidant activity of *Trigonella foenum-graceum.*

Chapter 1
Introduction

1.1. Phytomedicine in global health care

The practice of Traditional medicine is deeply rooted in the cultural heritage of Bangladesh and constitutes an integral part of the culture of the people of this country. Different forms of Traditional medicines have been used in this country as an essential means of treatment of diseases and management of various health problems from time immemorial. The practice of traditional medicine in this country has flourished tremendously in the recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country, particularly in the rural and semi-urban areas, still prefer to use traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighborhood. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs.

Trigonella foenum-graecum plays a major role in the medicinal properties. The plant parts of *Trigonella foenum-graecum* such as seeds, leaves are used for numerous medicinal purposes like ulcers, jaundice, diabetes and antipyretic. The leafpossesses hypoglycemic, antihyperglycemic, antioxidant properties and is also used to treat infective hepatitis. This review provides the botany, morphological character.

1.2. Approaches to research & drug discovery

A phytopharmaceutical preparation or herbal medicine is any manufactured medicine obtained exclusively from plants, either in the crude state or as pharmaceutical formulation. Although the industrial revolution and the development of organic chemistry resulted in a preference for synthetic products, World Health Organization (WHO) reports that between 70% and 95% of citizens in a majority of developing countries still rely on traditional medicine as their primary source of medication.

The role of herbal medicine started to decline after the 1960s as vast quantities of resources and money were used to promote synthetic medication. Besides this, advances in the human genome, increase knowledge of the structure and function of proteins and the notion that synthetic drugs are safer with fewer side effects (which does not necessarily be true) also contributed to the rise in the popularity of synthetic drugs. However, these advancements have several major constraints. The large number of possible new drug targets has already outgrown the number of existing compounds that could potentially serve as drug candidates and the field of chemistry has limitation when it comes to synthesizing new drug structures.

In the last decade, herbal medicine has seen some form of revival, advancing at a greater pace in community acceptance of their therapeutics effects. This field is bringing forward new lead drug discoveries as well as safe and efficacious plant-based medicines. In turn, this leads to growing

number of sales of commercialized medicinal herbs and most importantly, growing number of pharmaceutical companies that involve in the research and development of plants as a source for modern medicine. What chemists have been desperately seeking, Mother Nature has already plenty of stock. This review tries to expound on the importance of herbal medicine in modern drug development by highlighting salient topics from the history of herbal medicine and examining its roles in modern drug development. In addition, this review discusses the challenges and future of herbal medicine in modern medical practice

1.3. Methods of Phytomedicine Research

Modern natural drugs chemistry actually began with the work of Serturner, who first isolated morphine from opium poppy, Papaver somniferum. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to any known compounds. Of the estimated 250 000 to 500 000 plant species, only a fraction (about 5000 species) have been studied for medical use. Among the most important roles of herbal medicine in modern drug development is the identification of plants with useful therapeutics compounds. This is where the modern field of phytosciences comes in. This field attempts to verify health benefits of plants commonly used in traditional medicine and their mechanisms of action. As an exact science, the researchers within this field aim to explore the side effects of plant-based compounds (phytocompounds), identify the bioactive elements, estimate the appropriate dosages, as well as describe the best methods of extraction and conservation of the compounds. Up until now, a few methods have been employed to identify the potential plants for drug development. The basic technique for the study of plants used in traditional medicine is summarized in the Figure 1.

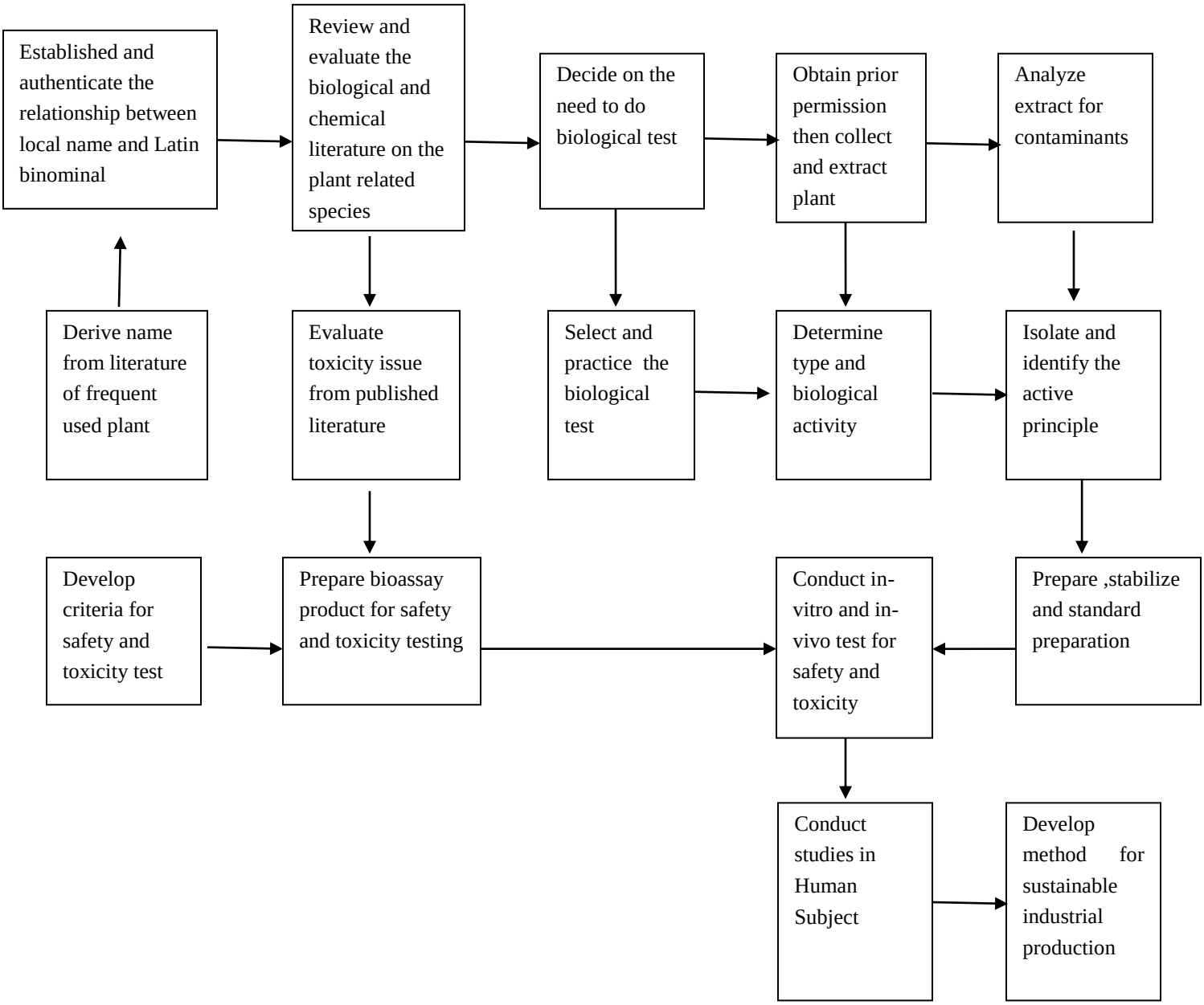


Figure 1.1: Flow chart of the basic technique for the study of plants used in traditional medicine (adapted from G.A Cordell & Colvard).

Plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems. A brief summary of the experimental conditions for the various methods of extraction is show in **Table 1.1**

Table 1.1

A brief summary of the experimental conditions for various methods of extraction for plants material

Extraction	Soxhlet	Sonification	Maceration
Common Solvents used	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water
Temperature (°C)	Depending on solvent used	Can be heated	Room temperature
Pressure applied	Not applicable	Not applicable	Not applicable
Time required	3–18 hr	1 hr	3–4 days
Volume of solvent required (ml)	150–200	50–100	Depending on the sample size

One of the methods of selecting potential plants for drug development is to decide on a well-defined pharmacological activity and perform a randomized search for this activity among plant extracts, resulting in active species to be considered for further study. At one point of time, the concept of high-throughput screening (HTS) became the paradigm of lead discovery of novel bioactive phytocompounds. HTS allows a researcher to efficiently conduct hundreds of experiments simultaneously via a combination of modern robotics and other specialized laboratory hardware. It applies a forceful method to collect a large amount of experimental observations about the reaction of phytocompounds towards the exposure of various chemical compounds in a relatively short time. However, results of HTS in producing synthetic anti-cancer drug have been less than promising. The National Cancer Institute (NCI) in the USA has tested more than 50 000 plant samples for anti-HIV activity and 33,000 samples for anti-tumour activity. It should be reminded that these plants were not screened for other pharmacological activities. Through HTS, several plant products have been identified for the production of new anti-cancer drugs, which include the vinca alkaloids, the taxanes and camptothecins derived from Cantharantus roseus, Taxus brevifolia and Camptotheca acuminata respectively. Flavopiridol, derived from the plant Amoora rohituka and Dysoxylum binectariferum is currently the most exciting discovery as it represents the first anti-cancer agent targeting cell cycle progression. Even though the discoveries mentioned above are commendable, one cannot help but

wonder about the practicality of the HTS approach in identifying beneficial natural phytocompounds. In many cases, the deficiency of the supplies of varied plant extracts and their immense chemical diversities were not appropriate to feed the HTS. This setback caused the emergence of the science of combinatorial chemistry that produces high-throughput chemical synthesis of phytocompound analogs in the late 1980s and early 1990s. This new technology initiated a decreasing interest in natural compound screening, which in turn, lead towards the complete abandonment of such programs by many companies. Combinatorial chemistry emerged as the preferred option for HTS resources, but unfortunately, this technique has not produced many high-quality drug candidates.

.The most common method of plant-based drug identification and selection remains with careful observation of the use of herbal medicine in the folk medicine of various cultures, which includes analyzing traditional ethnobotanical documentation and inventories. This is sometimes referred as the "reverse pharmacology path" because clinical experiences, observation and available data from human use are utilized as the starting-point, instead of being the end-point, in conventional drug research.

1.4. Rational and objective of the work

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. The ancient civilization of Bangladesh, India, China, Greece, Arab and other countries of the world developed their systems of medicine independent of each other but all of them were predominantly plant based. About 400to 500 medicinal plants grow in Bangladesh, where ~80 percent of the rural population depends on traditional remedies for ailments such as jaundice cough, cold, fever, headache and dysentery etc. Trigonella foenum-graecum is most important of them. I have experience about the medicinal use of TheTrigonella foenum-graecum leaves. In my village area a lot of people used The Trigonella foenum-graecumleaves and seeds are used in several purposes like , diabetes, wound healing, ulcers, , skin disease, asthma.

A pharmacognostic study is the preliminary step in the standardization of crude drugs. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained from plant.

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential, the first step towards ensuring quality of starting material authentification. Thus in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. Pharmacognostic studies have been done on many important drugs and the resulting observation has been incorporated in various pharmacopeias. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs. In recent years growing demand for herbal products has been increased and plant materials

traded within and across the countries activity. Previously the phytochemicals with unknown pharmacological activities have been extensively investigated as source of medicinal agents. Trigonella foenum-graecumis a perennial member of the family of leguminosae, commonly known as Methi shak.

The main objective of this study are as follows:

1.To know about the habitat,diversity of Trigonella foenum-graecum.

2.To know about the potentiality of the antioxidant properties

3.To know about the uses of Trigonella foenum graecum in several diseases.

1.5. Oxidative stress:

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. $O2^-$ (superoxide radical), OH (hydroxyl radical) and H2O2 (hydrogen peroxide. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

In humans, oxidative stress is thought to be involved in the development of cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction ,fragile X syndrome, Sickle Cell Disease, lichen planus ,vitiligo , autism ,infection, and chronic fatigue syndrome.However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens.Short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis.

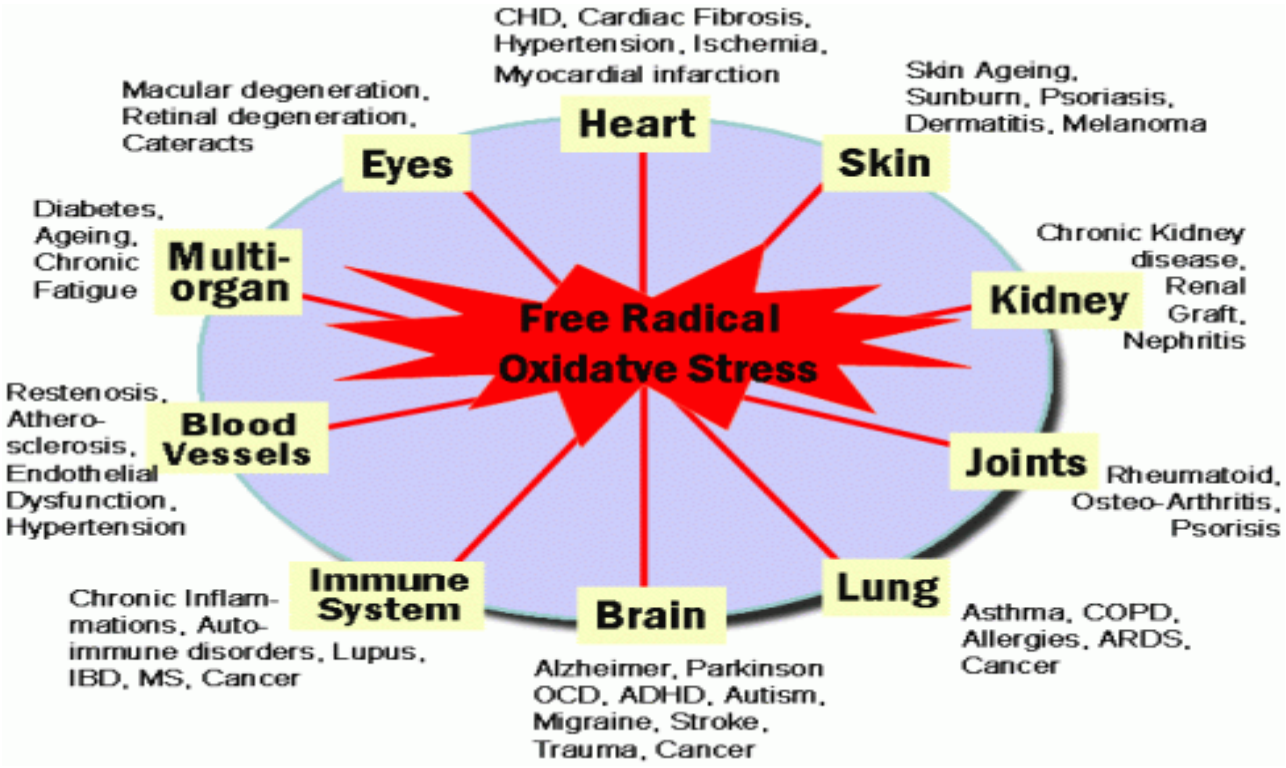


Figure 1.2:Oxidetive stress effect on animal

1.5.1 .Antioxidant

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically this means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free-radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. The antioxidant molecule may be very large (allowing it to "dilute" the unpaired electron), it may be readily neutralized by another antioxidant and/or it may have another mechanism for terminating its free radical condition.

A free radical attack on a membrane usually damages a cell to the point that it must be removed by the immune system. If free radical formation and attack are not controlled within the muscle during exercise a large quantity of muscle could easily be damaged. Damaged muscle could in turn inhibit performance by the induction of fatigue. The roles of individual antioxidants have in inhibiting this damage.

The major benefits of Antioxidant through the examination of different health benefits including:

1. Counteraction of the damaging oxidative action of low-density lipoproteins (LDLs), the so-called bad cholesterol, thereby protecting the arteries from worsening effects of atherosclerosis.

2. Protection of the endothelial cells of the arteries themselves from free radical damage,

permitting them to be compliant and reactive rather than rigid and dysfunctional.

3. Decrease in platelet aggregation (clumping), protecting the vascular system from clot formation that has potentially damaging effects such as heart attacks and strokes

4. Counteraction of the oxidation-promoting effects of stress hormones such as the catecholamines (epinephrine and nor epinephrine), often secreted in high amounts during chronic stress.

5. Counteraction of free radical damage to many cells of the body that could potentially trigger undesired proliferation in the form of cancer.

6. Protection from some of the damaging effects of aberrant metabolism that can contribute to the triggering of type II diabetes.

7. Protection of important connective tissues of the body to help counteract many age-related forms of deterioration.

8. Protection and enhancement of immune responses important in protective responses against viral infections and surveillance and protection from the formation or spread of many types of cancer.

9. Counteraction of damaging effects of inflammatory responses in diverse systems of the body, including joints (arthritis), and brain (Alzheimer's disease).

10. Protection against degenerative processes in the brain that can lead to specific neuronal damage associated with Parkinson's disease and Alzheimer's disease.

1.5.2 .Antioxidant Enzymes

Even though the production of antioxidant enzymes in the body is a complex process that is not yet totally understood, there are several processes that we are aware of and which seem to constitute a large part of the finished system.
The antioxidant enzyme defense system consists of hundreds of different substances and mechanisms. This is why only an adequate combination of whole foods, such as sprouted food concentrates, will contain all of the known and unknown nutritional factors that the body requires to enhance its antioxidant enzyme supply.
Enzymes are:

- **Superoxide-Dismutase & Catalase**
- **Glutathione Peroxidase**
- **Methionine Reductace**

1.5.3. Clinical applications of antioxidant enzymes

1. **Chronic Inflammation:** Chronic inflammatory diseases such as rheumatoid arthritis are self-perpetuated by the free radicals released by neutrophils. Both corticosteroids and non-steroids anti inflammatory drugs interfere with formation of free radicals and interrupt the disease process.
2. **Acute Inflammation:** At the inflammatory site, activated macrophages produce free radicals. Respiratory burst and increased activity of NADPH oxidase are seen in macrophages and neutrophils.
3. **Respiratory Diseases:** Breathing of 100 % oxygen for more than 24 hr produces destruction of endothelium and lung edema. This is due to the release of free radicals by activated neutrophils (Vasudevan et al., 2006). In premature newborn infants, prolonged exposure to high oxygen concentration is responsible for broncho pulmonary dysplasia. Adult respiratory distress syndrome (ARDS) is characterized by pulmonary edema. ARDS is produced when neutrophils are recruited to lungs which subsequently release free radicals. Cigarette smoking enhances the emphysema inalpha-1 protease inhibitor deficiency. Cigarette smoke contains free radicals. Soot attracts neutrophils to the site which releases more free radicals. Thus, there is more elastase and less protease inhibitor, leading to lung damage.
4. **Diseases of the Eye:** Retrolental fibroplasia or retinopathy of prematurity is a condition seen in premature infants treated with pure oxygen for a long time. It is caused by free radicals, causing thromboxane release, sustained vascular contracture and cellular injury. Cataract formation is related with ageing process. Cataract is partly due to photochemical generation of free radicals. Tissues of the eye, including the lens, have high concentration of free radical scavenging enzymes.
5. **Shock Related Injury:** Release of free radicals from phagocytes damage membranes by lipid per oxidation. They release leucotrienes from platelets and proteases from macrophages. All these factors cause increased vascular permeability, resulting in tissue edema. Anti-oxidants have a protective effect.
6. **Arthrosclerosis and Myocardial Infarction:** Low density lipoproteins (LDL) promote atherosclerosis. They are deposited under the endothelial cells, which undergo oxidation by free radicals released from endothelial cells. This attracts macrophages. Macrophages are them converted into foam cells. This initiates the atherosclerotic plaque formation. Alpha tocopheroloffers some protective effect.
7. **Peptic Ulcer:** Peptic ulcer is produced by erosion of gastric mucosa by hydrochloric acid. It is shown that superoxide anions are involved in the formation of ulcer. Helicobacter pylori infection perpetuates the disease. This infection potentiates the macrophage oxidative burst leading to tissue destruction.
8. **Skin Diseases:** due to inborn defects, porphyrins accumulate in the skin. Exposure of sunlight will lead to erythema and eruptions in the patients. Sunlight acting on porphyrins produces singlet oxygen, which trigger inflammatory reaction, leading to the above symptoms. Certain plant products, called psoralens are administered in the treatment of

psoriasis and leukoderma. When the drugs is applied over the affected skin and then irradiated by UV light, singlet oxygen produced with clinical benefit.

9. **Cancer Treatment :** Free radicals contribute to cancer development because of their mutagenic property. Free radicals produce DNA damage, and accumulated damages lead to somatic mutations and malignancy. Cancer is treated by radiotherapy. Irrational produces reactive oxygen species in the cells which trigger the cell death. To increase the therapeutic effect of radiation, radio-sensitizers are administered, which increase the production of ROS.

1.5.4 .Evaluation of antioxidant activity

Antioxidant activity of different extracts was evaluated depending on their free radical scavenging activity; Antioxidants that scavenge free radicals are known to posses an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and diseases.

1.5.5 .Antioxidant protection system

To protect the cells and organ systems of the body against reactive oxygen species (ROS), humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Table 1.1) (Mark Percival, 1998).

These components include:

a. Endogenous Antioxidants

- Bilirubin
- Thiols, e.g., glutathione, lipoic acid, N-acetyl cysteine
- NADPH and NADH
- Ubiquinone (coenzyme Q10)
- Uric acid
- Enzymes:
- copper/zinc and manganese-dependent superoxide dismutase
- iron-dependent catalase
- selenium-dependent glutathione peroxidase

b. Dietary Antioxidants

- Vitamin C
- Vitamin E
- Beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein
- Polyphenols, e.g., flavonoids, flavones, flavonol's, and Proanthocyanidins

c. Metal Binding Proteins
- Albumin (copper)
- Ceruloplasmin (copper)
- Metallothionein (copper)
- Ferritin (iron)
- Myoglobin (iron)
- Transferrin (iron)

ROS	NEUTRALIZING ANTIOXIDANTS
Hydroxyl radical	Vitamin C, Glutathione Flavonoids, Lipoic acid
Superoxide radical	Vitamin C, Glutathione, Flavonoids, SOD
Hydrogen peroxide	Vitamin C, Glutathione, beta carotene, Vitamin-E, flavonoids, lipoic acid
Lipid peroxides	Beta-carotene, Vitamin-E, Ubiquinone, flavonoids, Glutathione peroxidase

Table 1.2: Various ROS and corresponding neutralizing antioxidants

Defense mechanisms against free radical-induced oxidative damage include the following:

i. Catalytic removal of free radicals and reactive species by factors such as CAT, SOD, GPx and thiol-specific antioxidants;
ii. Binding of proteins (e.g., transferrin, metallothionein, haptoglobins, caeroplasmin) to pro-oxidant metal ions, such as iron and copper;
iii. Protection against macromolecular damage by proteins such as stress or heat shock proteins; and
iv. Reduction of free radicals by electron donors, such as GSH, vitamin E (α- tocopherol), vitamin C (ascorbic acid), bilirubin, and uric acid (Halliwell and Gutteridge, 1999).

Animal CAT is heme-containing enzymes that convert hydrogen peroxide (H_2O_2) to water and O_2, and they are largely localized in sub cellular organelles such as peroxisomes. Mitochondria and the endoplasmic reticulum contain little CAT. Thus, intracellular H_2O_2cannot be eliminated unless it diffuses to the peroxisomes (Halliwell and Gutteridge, 1999). GSH-Px removes H_2O_2 by coupling its reduction with the oxidation of GSH. GSH-Px can also reduce other peroxides, such as fatty acid hydro peroxides. These enzymes are present in the cytoplasm at millimolar concentrations and also present in the mitochondrial matrix. Most animal tissues contain both CAT and GSH-Px activity. SODs are metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation. Three isoforms have been identified, and they all are present in all eukaryotic cells. The copper-zinc SOD isoform is present in the cytoplasm, nucleus, and plasma. On the other hand, the manganese SOD isoform is primarily located in mitochondria.

Dietary micronutrients also contribute to the antioxidant defense system. These include β- carotene, vitamin C, and vitamin E (the vitamin E family comprises both tocopherols and tocotrienols, with α-

tocopherol being the predominant and most active form). Water-soluble molecules, such as vitamin C, are potent radical scavenging agents in the aqueous phase of the cytoplasm, whereas lipid soluble forms, such as vitamin E and β- carotene, act as antioxidants within lipid environments. Selenium, copper, zinc, and manganese are also important elements, since they act as cofactors for antioxidant enzymes. Selenium is considered particularly important in protecting the lipid environment against oxidative injury, as it serves as a cofactor for GSH-Px.

The most abundant cellular antioxidant is the tripeptide, GSH (l-L-γ-glutamyl-l-cysteinyl glycine). GSH is synthesized in two steps. First, γ-glutamyl cysteine synthetase (γ-GCS) forms a γ-peptide bond between glutamic acid and cysteine, and then GSH synthetase adds glycine. GSH prevents the oxidation of protein thiol groups, either directly by reacting with reactive species or indirectly through glutathione transferases (Lauterburg et al., 1984)

1.5.6. Rationale and Objective

There are recent evidence that free radical induce oxidative damage to biomolecules. This damage causes cancer, aging, neurodegenerate diseases, atherosclerosis, malaria and several other pathogenic events in living organism. Antioxidant which scavenge free radicals are known to possess an important role in preventing these free radical induces disease. There is an increasing interest in the antioxidant effects of compound derived from plant, which could be relevant in relations to their nutritional incidence and their roles in health and disease. A number of report on the isolation and testing of plant derived antioxidant have been described during the past decade. Natural antioxidants constitute a broad range of substance including phenolic and nitrogen containing compounds carotenoids.

Lipid peroxidant is one of the main reasons for determination of food products during food processing and storage. Synthetic antioxidant such as tert-butyl-1-hydroxytoluene (BHT), Butylated hydroxianisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase shelf life, specially lipid and lipid containing product by regarding the process of lipid peroxdation. However, BHT and BHA are known to have not toxic carcinogenic effects on enzyme systems. Therefore the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.

1.6. The plant family:

Leguminosae or Fabaceae

The plant family under investigation- Trigonella foenum-graecum includes to the family Leguminosae

1.6.1. Taxonomic

Legumes (les légumineuses in french) are plants of the pea or bean family, the Leguminosae (Fabaceae in the USA).The Leguminosae is one of the largest families of flowering plants with 18,000 species classified into around 650 genera (Polhill & Raven, 1981). This is just under a twelfth of all known flowering plants.The Leguminosae is an extremely diverse family. The major characteristics of the family are given below.The Leguminosae constitute one of humanity's most important groups of plants. Legumes are used as crops, forages and green manures. They also synthesise a wide range of natural products such as flavours, drugs, poisons and dyes. Further information on the economic importance of the family is given below. (http://www.ildis.org/Leguminosae/) The Fabaceae or Leguminosae commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants.it includes trees, shrubs, and herbaceous plantsperennials or annuals, which are easily recognized by their fruit (legume) and their compound, stipulated leaves.(http://en.wikipedia.org/wiki/Fenugreek)

1.6.2. Some of Genera of Leguminosae:

- *Abrus*
- *Acacia*
- *Acaciella*
- *Achyronia*
- *Acmispon*
- *Acosmium*
- *Acrocarpus*
- *Adenanthera*
- *Adenocarpus*
- *Adenodolichos*
- *Adenolobus*
- *Adenopodia*
- *Adesmia*
- *Aenictophyton*
- *Aeschynomene*
- *Affonsea*
- *Afgekia*

1.7. Introduction Of Trigonella foenum-graecum:

Trigonella foenum-graecum is a medicinal plant that originates from the areas from North Africa to India. Fenugreek seeds have been used in traditional cuisines and traditional medicines of many nations of the Mediterranean region and the Middle East. Known also as Greek Hay, fanugreek has plenty of therapeutic values, and benefits of fenugreek have been known since the times of Ancient Egypt where it had been used to stimulate male libido as well as boost energy and athletic performance of ancient warriors. Numerous records of fenugreek seed uses and fenugreek side

effects were found in historic documents from Ancient Greece and the Roman Empire. In modern times, a great deal of studies and researches are being conducted to learn more about fenugreek benefits and healing powers. Trigonella foenum- graecum is a member of the bean family used to make the body stronger and increase vitality. Both the leaf and the seed have factored into stay well regimes since the day of the Bible and backwards. This one seems especially good for woman, as it packs hormones that seem to mimic female hormones.

1.7.1. Synonyms of Trigonella foenum-graecum:

Botanical Name :Trigonella Foenum-Graecum

Sanskrit Name : Methi

English Name : Fenugreek

France: Fenugrec

Italy: Fieno greco

Spain: Feno-greco

Arabic: Helba

Others name:

- Methika,
- Methini,
- Star fenugreek
- Trigonella,
- Trigonella balansae,
- Trigonella caerulea,
- Trigonella semen,
- Trigonella stellata,
- Trigonella,
- Trigonelline

1.7.2. Taxonomic hierarchy of the investigated plant:

Scientific classification

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants

Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae／Leguminosae – Pea family
Genus	*Trigonella* L. – fenugreek
Species	*Trigonella foenum-graecum* L. –sickle fruit fenugreek

1.7.3. Nutrition profile:

Fenugreek leaves contain these nutrients per 100 g of edible portion

- Carbohydrates: 6.0 g
- Protein: 4.4 g
- Fat: 0.9 g
- Calcium: 395 mg
- Phosphorus: 51 mg
- Iron: 1.93 mg
- Total energy: 49 kcal

1.7.4. Plant description:

An erect annual to 50 cm. which may be branched, the leaves are trifoliate and the leaflets oblong-lanceolate, to 5 cm. Its yellowish flowers 12 – 18 mm long (1 – 2) are in the leaf axils. The fruits are almost straight and flattened with a pronounced beak ;they are 50 – 110 mm long excluding the beak of 10 – 35 mm. Chromosome number 2n=16. The seeds are brownish, about 1/8 inch long, oblong, rhomboidal, with a deep furrow dividing them into two unequal lobes; they are contained, ten to twenty together, in long, narrow, sickle-like pods. There are about 50 000 seeds per kilogramme.

Fenugreek is a fodder of very ancient cultivation in Mediterranean countries. A highly aromatic plant which is used as a pot-herb, spice and fodder. It is widely grown in India and neighbouring countries as a flavouring and fodder, and in North Africa and Western Asia as a fodder and spice. The dried leaves of a small, clover-like, rosette forming, small-seeded type (Kasuri methi) var. *corniculata* (or sometimes *Trigonella corniculata*) is grown in Punjab and its foliage marketed dried as a flavouring

Water

It is cultivated both under irrigation and as a rain fed crop.

Soil

It will grow on a wide range of well drained soils.

Distribution

Fenugreek is grown as a cool season crop in India and the Mediterranean region both irrigated and as a rain fed crop; it will grow on a wide range of well drained soils. In cooler areas it may be grown in summer – the seed crop requires warm dry weather for ripening and harvest.

Crop management

It is either sown in spring or autumn according to climate. In Punjab it has been used as a summer catch-crop fodder or green manure, ripening in 2.5 - 3 months. Seed rates vary widely from 10 to 40 kg/ha, the lower rates being for rainfed crops. As a forage it is broadcast or drilled at 20 - 30 kg ha in pure stand or often mixed with oats. As a forage it is often fed as hay. Mixtures with small cereals are best for haymaking; it should be mown when the pods are well formed. The hay is nutritious, but highly aromatic and may flavour milk. Hard-seededness is not a problem, nor is seed production since fenugreek has been grown for its seeds as well as fodder since antiquity.

Seed production

When grown for seed it is usually grown in rows about 50 centimeters apart and thinned to 5-10 cm. The ripe crop is hand cut, and is often dried off the field before threshing.

Crop use and grazing management

It has long been used as hay or green feed and is reputed to be highly nutritious. It is not suited to grazing. As a hay crop it is best mixed with a white-straw cereal.

Conservation

Mixtures with small cereals are best for haymaking; it should be mown when the pods are well formed. The hay is nutritious, but highly aromatic, with a high content of coumarone, it may flavour milk and this must be taken into account in the timing of its use.

1.7.5. Use of *Trigonella foenum-graecum*

- Fenugreek is used as an herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell.
- Cuboid-shaped, yellow- to amber-colored fenugreek seeds are frequently encountered in the cuisines of the Indian Subcontinent, used both whole and powdered in the preparation

of pickles, vegetable dishes, daals, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhance flavor.

- Fresh fenugreek leaves are an ingredient in some Indian curries. Sprouted seeds and micro greens are used in salads. When harvested as microgreens, fenugreek is known assamudra methi in Maharashtra, especially in and around Mumbai, where it is often grown near the sea in the sandy tracts, hence the name samudra, "ocean" in Sanskrit. Samudra methi is also grown in dry river beds in the Gangetic plains. When sold as a vegetable in India, the young plants are harvested with their roots still attached and sold in small bundles in the markets and bazaars. Any remaining soil is washed off to extend their shelf life.
- In Turkish cuisine, fenugreek is used for making a paste known as çemen. Cumin, black pepper, and other spices are added into it, especially to make pastırma.
- In Persian cuisine, fenugreek leaves are called (shanbalile). They are the key ingredient and one of several greens incorporated into ghormeh sabzi and eshkeneh, often said to be the Iranian national dishes.
- In Egyptian cuisine, peasants in Upper Egypt add fenugreek seeds and maize to their pita bread to produce aish merahrah, a staple of their diet.
- Fenugreek is used in Eritrean and Ethiopian cuisine. The word for fenugreek in Amharic is abesh (or abish), and the seed is used in Ethiopia as a natural herbal medicine in the treatment of diabetes.
- Yemenite Jews following the interpretation of Rabbi Salomon Isaacides, Rashi, believe fenugreek, which they call hilbeh, hilba, helba, or halba is the Talmudic rubia. They use it to produce a sauce also called hilbeh, reminiscent of curry.
- It is advised by lactation advisers and used by breastfeeding mothers to help stimulate milk production and supply and it is also supposed to help either gain or reduce weight. However there is not enough evidence to support these claims.

1.7.6. Photograph of plant :

Figure 1.3.Trigonella foenum-graecum

CHAPTER 2
EXPERIMENTAL

2.1. Experimental Plant

Trigonella foenum-graecum included in Leguminosae was investigated in this study.

Plant Name	Family	Plant part used
Trigonella foenum-graecum	Leguminosae	Whole plant

2.2 Preparation of the Plant Extracts for Experiments

2.2.1 Collection and Identification

For this present investigation whole plant of *Trigonella foenum-graecum* were collected from the local market. The whole plant parts *Trigonella foenum-graecum* were then sent to National Herbarium Mirpur, Dhaka. Expert of National Herbarium identified as where a voucher specimen has been deposited (accession no.: DACB 37694).

2.2.2 Drying of the Samples

After collection of the plant, adulterants were carefully removed to get fresh sample. Then the collected samples were dried for few days in the laboratory under room temperature until proper drying of the sample. After drying the whole plant was preserved in air tight container until their extraction.

2.2.3 Extraction of the Dried leaves

The whole plant of *Trigonella foenum-graecum* was taken in an extraction vessel of the Soxhlet apparatus. About 800 ml methanols was added in the vessel and extracted by a Soxhlet apparatus at 70^0C. Then the methanol containing extracted constituents were filtered through cotton. This process was repeated at least three times in order to maximum extraction of the chemical constituents from the sample under investigation. Finally, total filtrate was completely dried using a rotary evaporator in vacuum at a temperature of 40^0C and obtained dried crude extract which were used for investigation. The crude extract was preserved in the refrigerator until their experiment.

CHAPTER 3
EXPERIMENTAL DESIGN

3.1.Material and Methods:

3.1.1. Materials:

The general laboratory equipment is given in the following lists Table

SN	Equipment
1	Electronic balance
2	Soxhlet apparatus
3	Rotary evaporator
4	Refrigerator
5	Heating Mentile
6	UV-Visible Spectrophotometer

Table (3.1): List of general laboratory equipment

Figure 3.1: Electronic balance

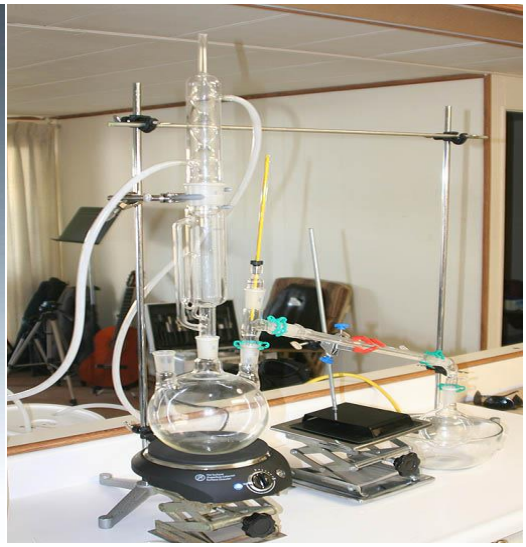


Figure 3.2: Soxhlet apparatus

Figure 3.3: Rotary evaporator

Antioxidant evaluation by various methods:

3.1.2. Methods:

3.1.2.1. Determination of Total Phenolic Content

Principle:

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu Reagent (FCR) (Velioglu et al., 1998). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$. In essence, it is believed that

the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI):

$$Mo(VI) + e \quad Mo(V)$$

Reagents:

Name of the Reagents	**Source**
Folin – ciocalteu reagent	Merck specialities private limited, India
Sodium carbonate (Na_2CO_3)	E. Merck (India) limited
Ethanol or Methanol	Merck, Germany
Galic acid (Analytical or Reagent grade)	Sigma Chemicals, USA

Table 3.2: List of the reagents used in the test and their source

Experimental procedure:

1. 0.5 ml of 1 mg/ml plant extract or standard of different concentration solution in a test tube was taken.
2. 0.5 ml of Folin – ciocalteu reagent solution into the test tube was taken.
3. After 5 minutes added5 ml of Sodium carbonate (7% w/v) solution into the test tube followed by 6.5 ml deionized distilled water and mixed thoroughly.
4. Incubated the test tube for 90 minutes at 230^0C to complete the reaction.
5. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.
6. A typical blank solution contained ethanol.
7. The Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula equation,

$C = (c \times V)/m$

Where:

C = total content of phenolic compounds, mg/g plant extract, in GAE;

c = the concentration of gallic acid established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant methanolic extract, g.

3.1.2.2. Determination Of Flavonoid Contents(Wang et al., 2000)

Reagents:

Name of the Reagents	Source
Aluminium Chloride ($AlCl_3$)	Fnie Chemicals, India
Potassium Acetate	E. Merck (India) limited
Ethanol or Methanol	Merck, Germany
Quercetin (Analytical or Reagent grade)	Sigma Chemicals, USA

Table 3.3: List of the reagents used in the test and their source

Experimental procedure:

1.1 ml of plant extract or standard of different concentration solution in a test tube was taken.
2. 3 ml of methanol into the test tube was added.
3. 200µl of 10% aluminium chloride solution into the test tube was added.
4. 200µl of 1M potassium acetate solution into the test tube was added.
5. 5.6 ml of distilled water into the test tube was added.
6. Incubated the test tube for 30 minutes at room temperature to complete the reaction.
7. Then the absorbance of the solution was measured at 415 nm using a spectrophotometer against blank.
8. A typical blank solution contained methanol.
9. The Total content of flavonoid compounds in plant methanol extracts in quercetine quivalents was calculated by the following formula equation

$C = (c \times V)/m$

Where:
C = total content of flavonoid compounds, mg/g plant extract, in quercetin;
c = the concentration of quercetin established from the calibration curve, mg/ml;
V = the volume of extract, ml;
m = the weight of pure plant methanolic extract, g.

3.1.2.3 .DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY

Principle:

The phosphor molybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The phosphor molybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acid pH. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reduction's and Mo(VI) and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm (Prieto et al., 1999).

$$Mo(VI) + e \rightarrow Mo\ (V)$$

Reagents used:

Name of the Reagents	**Source**
Concentrated H_2SO_4 (98%)	E. Merck (India) limited
Sodium Phosphate (Na_3PO_4)	Merck, Germany
Ammonium Molybdate	Merck, Germany
Ascorbic acid (Analytical or Reagent grade)	Merck, Germany

Table 3.4: List of the reagents used in the test and their source

Experimental procedure:

1. Take 300µl of plant extract or standard of different concentration solution in a test tube.
2. Add 3 ml of reagent solution into the test tube.
3. Incubate the test tube at 95^0C for 90 minutes to complete the reaction.
4. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature.
5. A typical blank solution contained 3 ml of reagent solution and the appropriate volume (300µl) of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples solution.
6. The antioxidant activity is expressed as the number of equivalents of ascorbic acid .and was calculated by the following formula equation

$A = (c \times V)/m$

where:

A = total content of Antioxidant compounds, mg/g plant extract, in Ascorbic acid;

c = the concentration of Ascorbic acid established from the calibration curve, mg/ml;

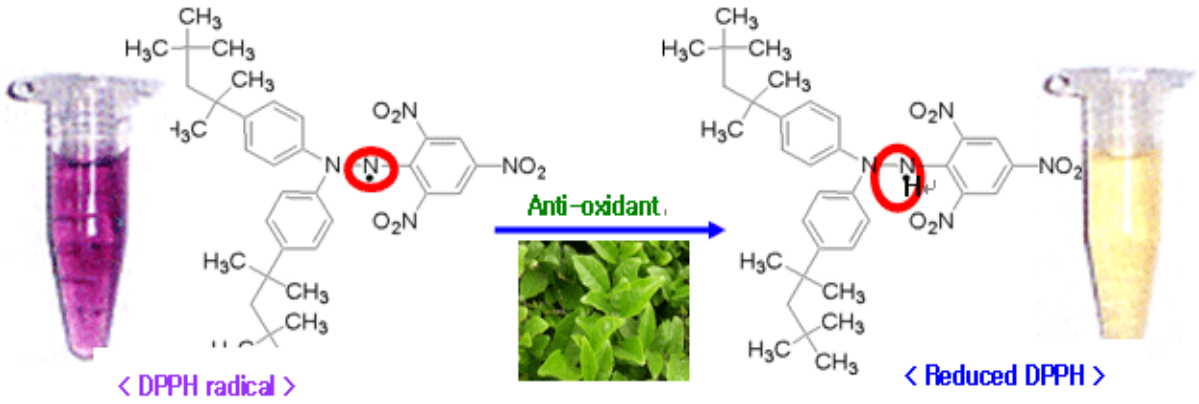
V = the volume of extract, ml;

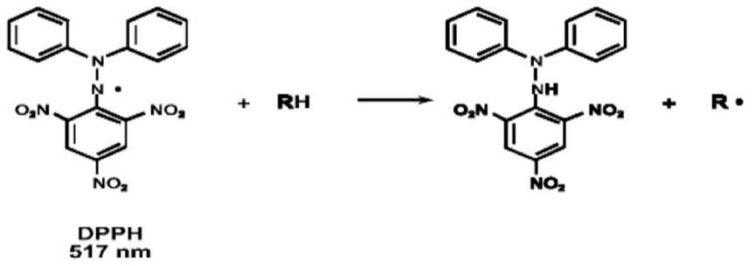
m = the weight of pure plant methanolic extract, g.

3.1.2.4. DPPH free radical scavenging Assay

Principle:

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Brand-Willams et al., 1995).





Materials and methods:

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

Reagents:

Name of the Reagents	Source
DPPH (1,1-diphenyl-2-picrylhydrazyl)	Sigma Chemicals, USA
Ethanol or Methanol	Merck, Germany
Ascorbic acid (Analytical or Reagent grade)	Merck, Germany

Table 3.5: List of the reagents used in the test and their source

Materials:

UV-spectrophotometer
Beaker (100 & 200 ml)
Test tube
Light-proof box
Pipette (5 ml)
Micropipette (50-200 µl)
Amber reagent bottle

Methods:

Inhibition free radical DPPH in percent *(I%)* was calculated as follows:

(I%) = (1 – Asample/Ablank) X 100

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition *(IC_{50})* was calculated from the graph plotted inhibition percentage against extract concentration.Ascorbic acid was used as positive control.Tests carried out in triplicate and average value was taken.

Experimental procedure:

1. 400 µl of plant extract or standard of different concentration solution in a test tube was taken.
2. 1.6 ml of reagent solution into the test tube was added.
3. Incubated the test tube for 30 minutes to complete the reaction.
4. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against blank.
5. A typical blank solution contained ethanol..
6. The percentage (%) inhibition activity was calculated from the following equation

$$\{(Ao - A1)/Ao\} \times 100$$

Where,

A0 is the absorbance of the control, and

A1 is the absorbance of the extract/standard.

7. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated.

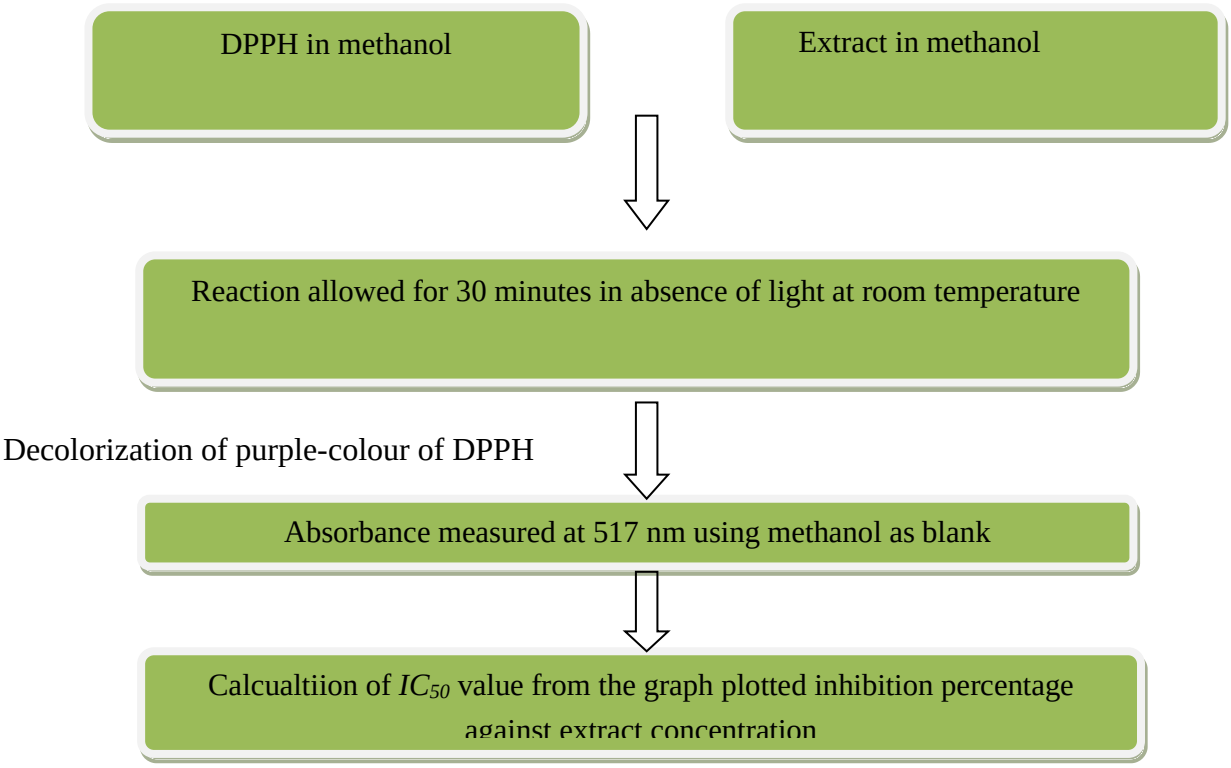


Figure 3.4.: Schematic representation of the method of assaying free radical scavenging activity

3.1.2.5. Reducing Power Capacity Assessment

Principle:

In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the

Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Makoto, 1986).

Reagents:

Name of the Reagents	Source
Potassium ferricyanide [$K_3Fe(CN)_6$]	Merck (India) Limited
Trichloro Acetic acid	Fine Chemicals, India
Ferric Chloride ($FeCl_3$)	Fine Chemicals, India
Ascorbic acid (Analytical or Reagent grade)	Merck, Germany

Table 3.6.: List of the reagents used in the test and their source

Experimental procedure:

1. 400 µl of plant extract or standard of different concentration solution was taken in a test tube.
2. 500 µl of Potassium ferricyanide [$K3Fe(CN)_6$], 1% solution added into the test tube.
3. Incubated the test tube for 10 minutes at 500C to complete the reaction.
4. 500 µl of Trichloro Acetic acid, 10% solution added into the test tube.
5. Centrifuged the total mixture at 3000 rpm for 10 min.
6. 1ml supernatant solution was withdrawn from the mixture and mix with 2.5 ml of distilled water.
7. 200 µl of Ferric chloride (FeCl3), 0.1% solution was added.
8. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against blank.
9. A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the samples solution.
10. Also take the absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation.
11. Increased absorbance of the reaction mixture indicated increase reducing power. The percentage (%) Reducing capacity was calculated from the following equation.

$$\{(Am - Ab)/Ab\} \times 100$$

Where,

Am is the absorbance of the reaction mixture, and

Ab is the absorbance of the blank.

CHAPTER 4
RESULT AND DISCUSSION

4.1 Yield of extract

% Yield of the methanol extracts as well as its different solvent soluble fractions of the seeds of *Trigonella foenum-graecum*is given bellow:

Extracts	Weight	% of the dried seeds
Methanol extract	8 g	4

4.2 Total Phenolic Content

Total phenolic contents were determined by using the Folin-Ciocalteu reagent and expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the methanol extracts of plants was calculated using the standard curve of Gallic acid (y = 0.0039x- 0.0579; R2 = 0.9868). The total phenolic was found to be 226.66±5.23 µg expressed as Gallic acid equivalent (GAE).

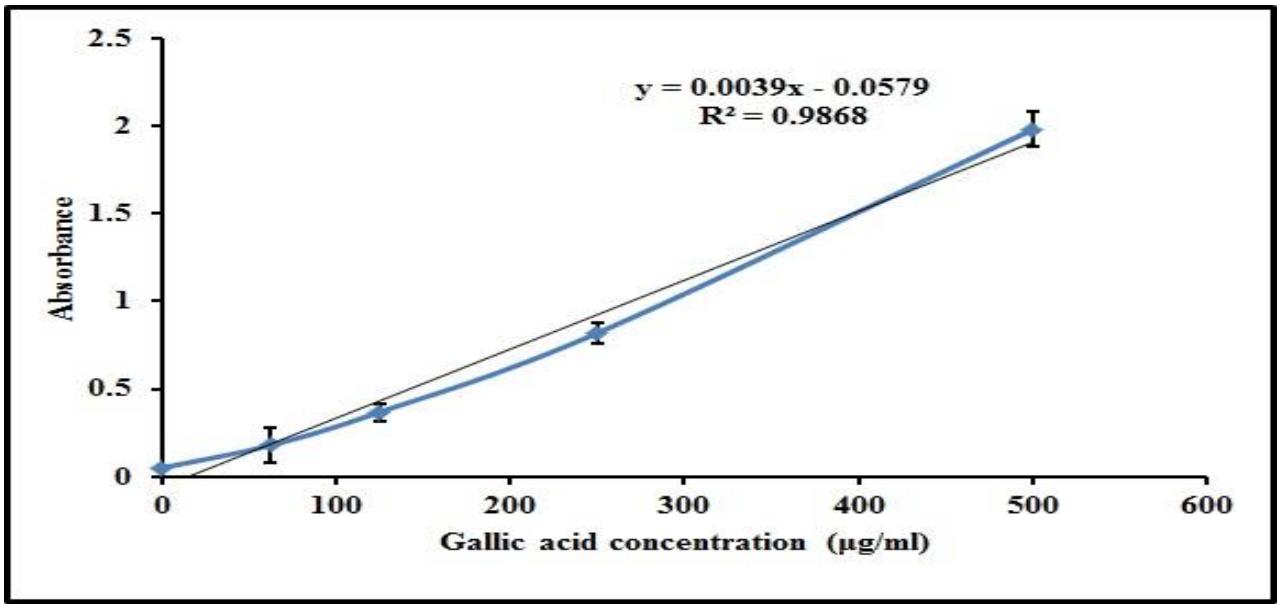


Figure 4.1: Standard curve using Gallic acid for the measurement of total phenolic contents in the methanol extract of *Trigonella foenum-graecum*

Plant parts	Total phenol mg/g plant extract (in GAE)
Methanol extract	226.66±5.23µg

Results are expressed as mean ± standard deviation of the duplicate experiments (n=2)

4.3. Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine the total flavonoids content in the methanol extract of*Trigonella foenum-graecum.* The total flavonoids contents of the methanol extract and two fractions of *Trigonella foenum-graecum* were calculated using the standard curve of quercetin (y = 0.0033x - 0.1183; R2 = 0.9969) and expressed as quercetin equivalents (QAE) per gram of the plant extract. The total flavonoids content was found to be 237.66±6.21µg expressed as quercetin equivalent (QE).

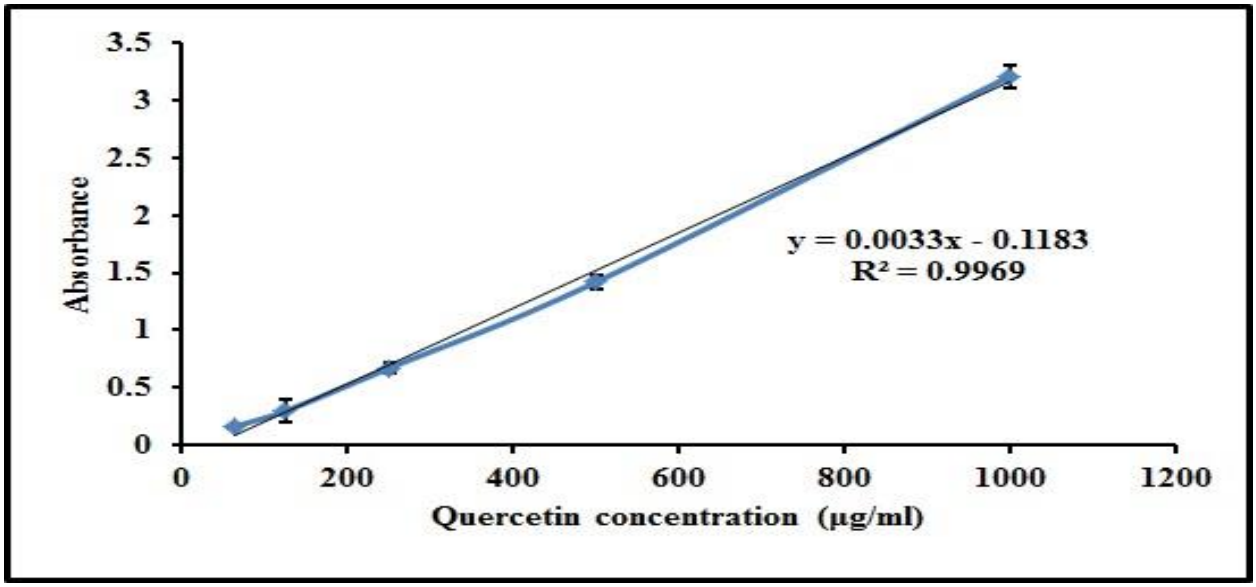


Figure 4.2: Standard curve using quercetin for the measurement of total flavonoid contents in the methanol extract of *Trigonella foenum-graecum.*

Plant parts	**Total flavonoids mg/g plant extract (in QE)**
Methanol extract	**237.6667µg**

Results are expressed as mean ± standard deviation of the duplicate experiments (n=2)

4.4. Total Antioxidant Capacity

Total antioxidant activity of the methanolic extracts of *Malva verticillata* was evaluated by the phosphor molybdenum method and expressed as ascorbic acid equivalent (AAE) per gram of plant extract. Total antioxidant contents were calculated using the standard curve of ascorbic acid (y = $7X10^{-05}$x - 0.0025; R^2 = 0.9987). The total antioxidant contents were found in the following order: 188.57±6.47 µg expressed as ascorbic acid equivalent (AAE).

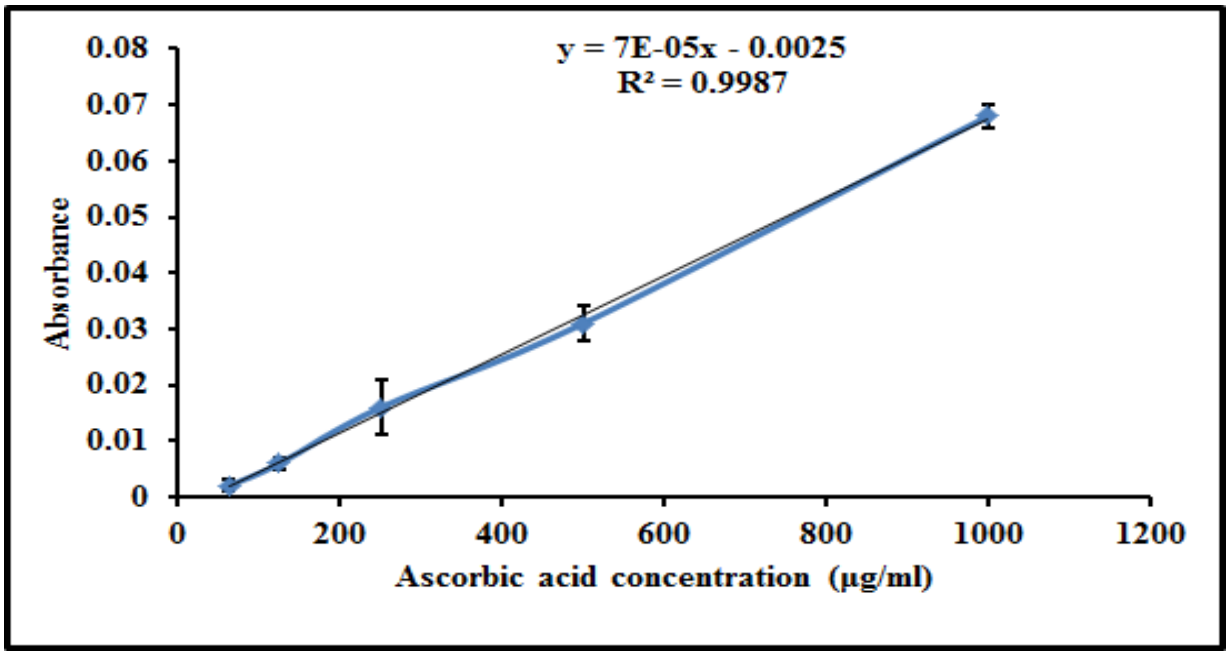


Figure 4.3: Standard curve using ascorbic acid for the measurement of total antioxidant in the methanol extract of*Trigonella foenum-graecum.*

4.5. DPPH Free Radical Scavenging Activity

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Practically, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine, which is manifested by a color change from violet to yellow, which is monitored spectrophotometrically. It is evident 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 IC_{50} value is 129.71 ± 3.95 µg/ml. On the other hand, the methanol extract showed promising DPPH free radical scavenging activity with IC_{50} value 281.875 ± 7.32 µg/ml.

IC_{50} value of ascorbic acid

SL.	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.066	1000	0.011	98.94	**129.71**
2		500	0.123	88.48	
3		250	0.256	75.40	
4		125	0.530	49.08	

IC_{50} value of *Trigonella foenum-graecum*

SL.	Absorbance of blank	Concentration (µg/ml)	Absorbance of extract	% Inhibition	IC_{50} (µg/ml)
1	0.066	1000	0.130	87.51	**281.871**
2		500	0.226	78.29	
3		250	0.561	46.10	
4		125	0.679	34.77	

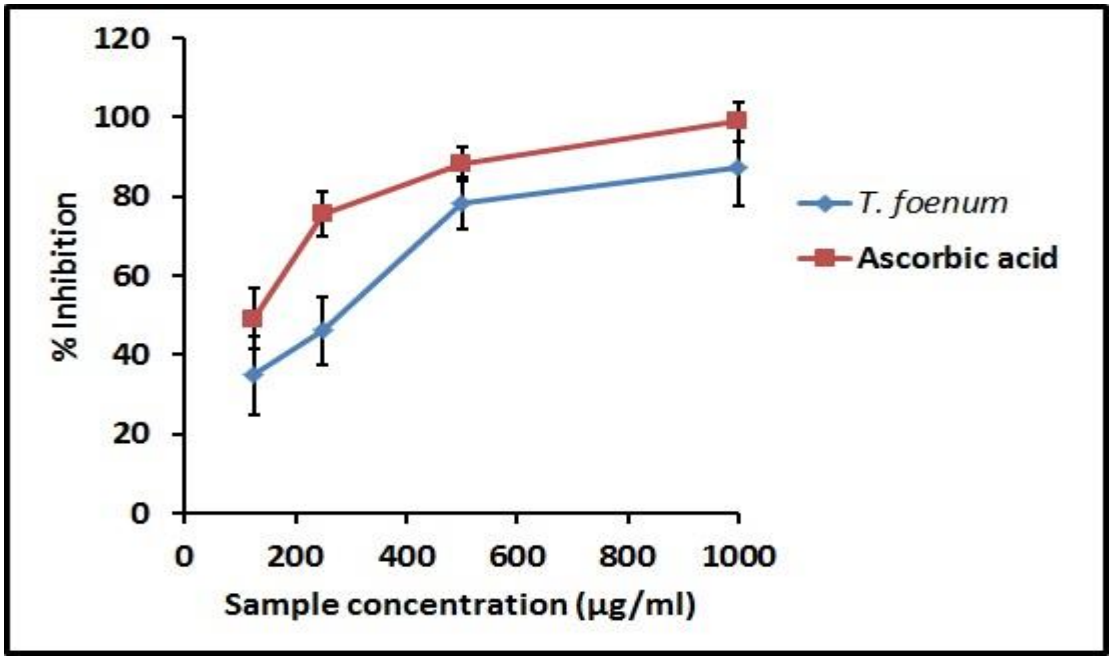


Figure 4.4: DPPH free radical scavenging activity of the methanol extract of*Trigonella foenum-graecum* at different concentration.

All values were expressed as the mean ± standard deviation of the duplicate experiments. Ascorbic acid was used as a positive control.

4.6. Reducing Power Assessment

The reductive capacity of the extracts were assessed using ferric to ferrous reductive activity as determined spectrophotometrically from the formation of Perl's Prussian blue colored complex (Yildirim and Mavi,2000). As shown in the figure 6.4, the absorbance value was increased with the increase of both sample and ascorbic acid concentration which implies the strength of ferric reducing power of the sample. Reducing power was found to increase with the increasing concentration of the methanol extract. Therefore, the methanol extract showed strong ferric reducing activity.

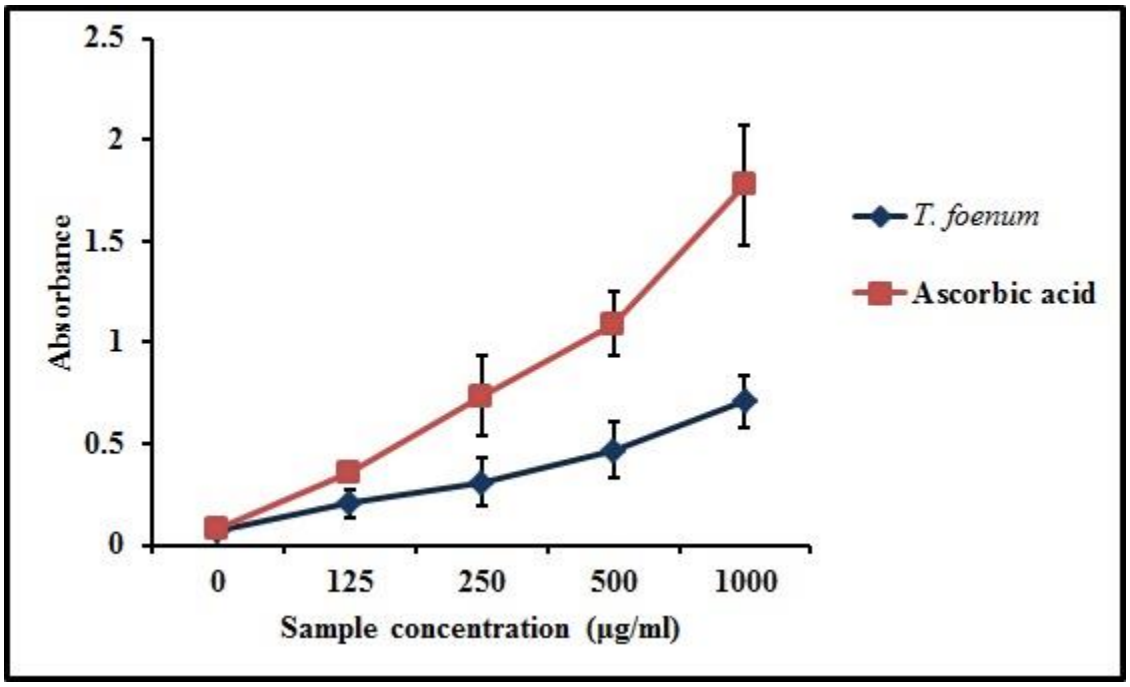


Figure 4.5: Reducing power of the methanol extract of *Trigonella foenum-graecum*at different concentration. All values were expressed as the mean ± standard deviation of the duplicate experiments. Ascorbic acid was used as positive control.

4.7. DISCUSSION

It has been recognized that plant contains many natural substances. The phenolic compounds are widely distributed, sometimes present surprisingly high concentration, in plants and have an antioxidant activity (Laporinic et al., 2005). The number of antioxidant compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defense mechanisms to counteract ROS in order to survive, is currently estimated to be between 4000 and 6000 (Havsteen, 2002; Robards et al., 1999; Wollgast and Anklam, 2000). They have the ability to scavenge free radicals such reactive oxygen species (ROS) which are determined by their reactivity as hydrogen or electron donating agents (Fermandez Pachon et al., 2006). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Rice et al., 1995). It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003, Cook and Samman, 1996).

Trigonella foenum-graecum , a member of the family Leguminosae, is an edible vegetable and commonly distributed all over the different geographical regions of Bangladesh.

Despite the antioxidant activity of the plants of Trigonella foenum-graecum importance, only a few studies have been conducted on the plant. I have also investigated total phenolic contents and flavonoid contents of the methanol extract. Total phenolic contents of the methanol extract of *Trigonella foenum-graecum*were found 226.66 ± 5.23 µg GAE/mg of the dried extract. On the other hand, the total flavonoid contents in the methanol extract was 237.6667 ± 6.21 µg QE/mg of the dried extract. The methanol extract of *Trigonella foenum-graecum* displayed very strong antioxidant activity by scavenging DPPH free radical with an IC_{50} value of 281.875 ± 7.32 µg/ml compared to the positive control ascorbic acid of which IC_{50} value is 129.7143 ± 3.95 µg/ml. On the other hand, *Trigonella foenum-graceum* methanolic extract also showed strong ferric reducing power compared with the ascorbic Acid. Moreover, the total antioxidant capacity of the methabnol extract of *Trigonella foenum-graceum* was found to be 188.57 ± 6.47 µg AAE/mg of the dried extract. From the above results, it can be concluded that the methanol extract of *Trigonella foenum-graceum*has very strong antioxidant potential which might be associated with the high level of phenolic and flavonoids type compounds present in the extract. Therefore, *Trigonella foenum-graceum* could be used as a source of naturally occurring potent antioxidants that might ameliorate oxidative stress and related disorders. However, this plant needs further investigation in order to find out the responsible chemical compounds associated with the antioxidant activity of *Trigonella foenum-graceum.*

CONCLUSION

In recent time natural antioxidants have attracted considerable attention to the nutritionist, food manufacturer and consumers due to their presumed safety and high therapeutic efficacy. All these activities may be attributed to the presence of poly phenolic compounds at high concentration in the plants. Therefore, *Trigonella foenum-graecum* could be used as a source of naturally occurring potent antioxidants. However, it is very important to find out the specific chemical constituents responsible for potent antioxidant activity of *Trigonella foenum-graecum* .Therefore, *Trigonella foenum-graecum* could be used as a source of naturally occurring potent antioxidants. The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. The results of *Trigonella foenum-graecum* in vitro studies suggest that the methanol extract of *Trigonella foenum-graceum* has very strong antioxidant potential which might be associated with the high level of phenolic and flavonoids type compounds present in the extract. Therefore, *Trigonella foenum-graceum* could be used as a source of naturally occurring potent antioxidants that might ameliorate oxidative stress and related disorders. However, this plant needs further investigation in order to find out the responsible chemical compounds associated with the antioxidant activity of *Trigonella foenum-graceum.*

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