Study of Antioxidant Activities On Plant Extracts Of

Solanum virginianum,

Drynaria quercifolia.

Submitted By

Tahmina Amin

ID No. 2013-1-70-003

Department of Pharmacy

East West University

Research Supervisor

Dr. Shamsun Nahar khan

Associate professor

Department of Pharmacy

East West University

This Thesis Paper is submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy.

CERTIFICATE

This is to certify that, the research work on 'Study Of Antioxidant Activities On Plant Extracts Of *Solanum virginianum, Drynaria quercifolia*.' submitted to Department of Pharmacy, East West University, Jahurul Islam city, Aftabnagar, Dhaka-1212, in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (B.Pharm) was carried out by Tahmina Amin. (ID No.2013-1-70-003) under the guidance and supervision and that not part of thesis has been submitted for any other degree. We further certify that all the sources of information of this connection are duly acknowledged.

....

Dr. Shamsun Nahar Khan
Associate Professor
Department of Pharmacy
East West University,
Aftabnagar, Dhaka-1212

ACKNOWLEDGEMENT

Above all, I express my gratitude to Almighty Allah for giving me the strength, energy and patience to carry out this research work. I would like to express my gratitude and admiration to **Dr. Shamsun Nahar Khan**, Associate Professor, Department of Pharmacy, East West University, for her suggestion, careful guidance, sincere help, constructive criticism and valuable time without which I would not have been able to complete this work. She had been very enthusiastic and supportive in my research. I would like to convey deepest love and obedience to my parents for their support, inspiration and guiding me all through my life until today, which keeps me strong and firm to do the things I needed to do. I would also like to take the opportunity to express my whole hearted gratitude to my fellow researcher friends specially Marita Fyroz Ahmed, Mahfuzur Rahman and near and dear ones who offered encouragement, information, inspiration and assistance during the period of constructing the research report .I am thankful to the laboratory instructors for their kind support during the laboratory work.

Dedication

This research work is dedicated to my beloved parents, honorable faculties and loving friend.

Contents

Chapter One: Introduction

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Contemporary science has acknowledged their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life. (Kelly K., 2009)

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences. (Ahvazi *et al*, 2012)

Medicinal plants, therefore, play an important role in health care systems of developing countries. In many countries there is an increasing emphasis on primary health care: basic health care which is not only effective, but affordable by underequipped and underfinanced countries, and by poor communities within those countries. Many governments have adopted policies of greater self-reliance in essential drugs, and traditional medicines are often cheap, readily accepted by consumers and locally available. For example, both China and Mongolia are pursuing health care systems founded on the practice of traditional medicine. (Rifkin et al, 1986)

A number of reviews relating to approaches for selecting plants as candidates for drug discovery programs have been published and these approaches can be briefly outlined as follows,

- Random selection followed by chemical screening
- Random selection followed by one or more biologic assays
- Follow-up of biologic activity reports

• Follow-up of ethnomedical (traditional medicine) uses of plants

• Use of databases Challenges and opportunity (Fabricant *et al*, 2001)

Medicinal plant

Any plant whose roots, leaves, seeds, bark, or plant part is used for therapeutic, tonic, purgative, or other health-promoting purposes.Medicinal plants are plats having recognized medicinal use. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. "Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes" (Ghani A.,1998)

Phytochemistry

Phyrochemistry is the study of phytochemicals, these are secondary metabolicsubstances found in plants. Many of these are known to provide protection againstinsectattacks and plant diseases. They also exhibit a number of protective functionsfor human consumers.Phytochemistry is widely used in the field of herbal medicine. (Saxena *et al*, 2013)

Primary metabolites

primary metabolites are directly involved in primary growth development and reproduction. Primary metabolites comprise many different types of organic compounds, including, but not limited to, carbohydrates, lipids, proteins, and nucleic acids. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle. Because of the importance of these and other primary pathways in enabling a plant to synthesize, assimilate, and degrade organic compounds, primary metabolites are essential. Examples of primary metabolites include energy rich fuel molecules, such as sucrose and starch, structural components such as cellulose,

informational molecules such as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and pigments, such as chlorophyll. In addition to having fundamental roles in plant growth and development, some primary metabolites are precursors (starting materials) for the synthesis of secondary metabolites. (Croteau *et al*, 2000)

Secondary Metabolites

Secondary metabolites largely fall into three classes of compounds: alkaloids, terpenoids, and phenolics. However, these classes of compounds also include primary metabolites, so whether a compound is a primary or secondary metabolite is a distinction based not only on its chemical structure but also on its function and distribution within the plant kingdom. (Wink, 2008)

Many thousands of secondary metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are used asmedicines. It is only since the late twentieth century that secondary metabolites have been clearly recognized as having important functions in plants. Research has focused on the role of secondary metabolites in plant defense. This is discussed below with reference to alkaloids, though it is relevant to many types of secondary metabolites.

Phytochemistry and medicinal plants

Phytochemicals from medicinal plants are receiving ever greater attention in the scientific literature, in medicine, and in the world economy in general. For example, the global value of plant-derived pharmaceuticals will reach $500 billion in the year 2000 in the OECD countries. In the developing countries, over-the-counter remedies and "ethical phytomedicines," which are standardized toxicologically and clinically defined crude drugs, are seen as a promising low cost alternatives in primary health care. The field also has benefited greatly in recent years from the interaction of the study of traditional ethnobotanical knowledge and the application of modem phytochemical analysis and biological activity studies to medicinal plants. (Kaufman *et al*, 1999)

Concept of free radicals, antioxidants in disease and health

Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. They are produced either from normal cell metabolisms in situ or from external sources (pollution, cigarette smoke, radiation, medication). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. This mini-review deals with the taxonomy, the mechanisms of formation and catabolism of the free radicals, it examines their beneficial and deleterious effects on cellular activities, it highlights the potential role of the antioxidants in preventing and repairing damages caused by oxidative stress, and it discusses the antioxidant supplementation in health maintenance. (Mukherjee *et al*, 2011)

Antioxidants and Free radicals

Antioxidants are intimately involved in the prevention of cellular damage -- the common pathway for cancer, aging, and a variety of diseases. The scientific community has begun to unveil some of the mysteries surrounding this topic, and the media has begun whetting our thirst for knowledge. Athletes have a keen interest because of health concerns and the prospect of enhanced performance and/or recovery from exercise. The purpose of this article is to serve as a beginners guide to what antioxidants are and to briefly review their role in exercise and general health. What follows is only the tip of the iceberg in this dynamic and interesting subject. (Freeman *et al*, 1982)

Production of free radicals in the human body

Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in

prostaglandin synthesis, and in the cytochrome P-450 system. Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions. (Halliwell, 1994)

Some internally generated sources of free radicals are

- Mitochondria
- Xanthine oxidase
- Peroxisomes
- Inflammation
- Phagocytosis
- Arachidonate pathways
- Exercise
- Ischemia/reperfusion injury
- Some externally generated sources of free radicals are:
- Cigarette smoke
- Environmental pollutants
- Radiation
- Certain drugs, pesticides
- Industrial solvents
- Ozone

Oxidative stress

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, is discussed in relation to its possible role in the production of tissue damage in diabetes mellitus. Important free radicals are described and biological sources of origin discussed, together with the major antioxidant defense mechanisms. Examples of the possible consequences of free radical damage are provided with special emphasis on lipid peroxidation. Finally, the question of whether oxidative stress is increased in diabetes mellitus is discussed. (Mohanta M.C. *et al*, 2013)

Mechanisms of oxidative stress

Free radicals are species containing one or more unpaired electrons in their outer atomic orbital. This electron imbalance renders them highly reactive and capable of widespread oxidation of lipids, proteins, DNA and carbohydrates. This eventually causes disruption of cell membranes, leading to release of cell contents and death (Halliwell B, Gutteridge JMC, 1989). Free radicals are formed by several exogenous processes such as radiation and tobacco smoke, and are the endogenous natural by-products of cellular metabolism.

When oxygen is reduced in the electron transport chain, oxygen-derived free-radical intermediates are formed. The superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) intermediates can escape from the system, and in the presence of transition metal ions (e.g. Fe^{2+}, Cu^{2+}) form the far more damaging hydroxyl radical (OH^-).

Figure: Mechanisms of oxidative stress. (Xu *et al, 2014)*

One example of this oxidative damage is lipid peroxidation. Free radicals may attack polyunsaturated fatty acids within membranes, forming peroxyl radicals. These newly-formed free radicals can then attack adjacent fatty acids within membranes causing a chain reaction of lipid peroxidation. The lipid hydroperoxide end-products are also harmful and may be responsible for some of the overall effect, which can lead to tissue and organ damage.

Mechanism of action of antioxidants

Two principle mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive oitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation. (Krinsky NI., 1992)

Types of antioxidants

Enzymatic

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. These are: Superoxide dismutase, Catalase, Glutathione systems.

Non-Enzymatic

Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are: Ascorbic acid, Glutathione, Melatonin, Tocopherols and tocotrienols (Vitamin E), Uric acid.

Plants as a source of antioxidants

Synthetic and natural food antioxidants are used routinely in foods and medicine especially those containing oils and fats to protect the food against oxidation. There are a number of synthetic phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) being prominent examples. These compounds have been widely uses as antioxidants in food industry, cosmetics and therapeutic industry. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated temperature, strict legislation on the use of synthetic food additives, carcinogenic nature of some synthetic antioxidants, and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants (Papas AM., 1999). In view of increasing risk factors of human to various deadly diseases, there has been a global trend toward the use of natural substance present in medicinal

plants and dietary plats as therapeutic antioxidants. It has been reported that there is an inverse relationship between the dietary intake of antioxidant-rich food and medicinal plants and incidence of human diseases. The use of natural antioxidants in food, cosmetic and therapeutic industry would be promising alternative for synthetic antioxidants in respect of low cost, highly compatible with dietary intake and no harmful effects inside the human body. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers (Brown JE, 1998).

Attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, and legumes (Furuta S, 1997). There are several reports showing antioxidant potential of fruits (Wang H., 1996).

Strong antioxidants activities have been found in berries, cherries, citrus, prunes, and olives. Green and black teas have been extensively studied in the recent past for antioxidant properties since they contain up to 30% of the dry weight as phenolic compounds (Lin JK. et al., 1998).

Chapter Two: Objective of Study

The knowledge of drugs goes back to prehistoric times. Man as savage known by experience how to relieve his sufferings by the use of herbs growing him. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. This century has witnessed many great achievements in the field of medicine. One of the great advancement in medical care in this century has been the introduction of a number of drugs which are having more toxic side effects.

The approach for drug development from plant resources depends on the aim. Different strategies will result in a herbal medicine or in an isolated active compound. However, apart from this consideration, the selection of a suitable plant for a pharmacological study is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria. How the plant is used by an ethnic group is a very important consideration. Keeping these selection criteria in mind, I selected four medicinally important plants for my research work.

Solanum virginianum, Drynaria quercifolia are plants that are very commonly distributed in Bangladesh. Ethno pharmacological data reveals that these plants are very widely used by various ethnic groups to treat various health problems. So these plants could be very good source of discovering novel compounds with medicinal properties.

Encouraged by these ethnobotanical data on the traditional uses of these plants, I wanted to explore some pharmacological effects of various plant samples which were previously collected as a part of the on-going research project conducted and supervised by Dr. Shamsun Nahar Khan. In our study we mainly focus to determine the antioxidant activity.

Chapter three:Introduction to plants

Solanum virginianum

Solanum virginianum is an erect or creeping herbaceous perennial plant that sometimes becomes woody at the base; it can grow 50 - 70cm tall. The plant is copiously armed with sturdy, needle-like prickles 5 - 20mm long. The plant is harvested from the wild for local use as a food and medicine.The fruits are harvested for commercial extraction of solasodine, which is used in the synthesis of hormones.(Fern *et al*, 2014)

Fig: Solanum virginianum

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Solanales
Family	Solanaceae – Potato family
Genus	Solanum L. – nightshade
Species	Solanum virginianum L. – Surattense nightshade

Description of the Plant:

A very prickly, diffuse herb, somewhat woody at the base; stem somewhat zigzag. Prickles compressed, straight, yellow, often exceeding 1.3 cm long. Leaves 5-10 cm long, ovate or elliptic, sinuate or subpinnatifid, obtuse or subacute, armed on the midrib and nerves with long yellow sharp prickls. Flowers in extra axillary few-flowered cymes; corolla purple, 2 cm long. Berry 1.3-2 cm diam., yellow or white with green veins, surrounded by the enlarged calyx.

Using Information:

Roots are diuretic and expectorant; employed in cough, asthma, chest pain and catarrhal fever. Fruit juice is useful in sore throat and rheumatism. Stem, flowers and fruits are carminative. Paste of the leaves is applied on painful joints to relieve pains. Seeds are given as an expectorant in asthma and cough. Decoction of the plant is useful in gonorrhoea. The plant also possesses cardioactive and antipyretic acivities.

Crude plant extract caused hypotension which has been attributed to release of histamine by some constituents (Rastogi & Mehrotra, 1990).

Chemical Constituents:

The plant contains sterols, alkaloids and glycosides. The plant also contains quercetin glycoside, apigenin, sitosterol and carpesterol. Fruits contain steroidal glycoalkaloids, solasonine, solamargine, solasurine, solanocarpine, solanine-S and alkaloidal bases, solanidine-S and solasodine. Seeds contain solanocarpine. Dry fruits contain traces of isochlorogenic, neochlorogenic, chlorogenic and, caffeic acid. Quercetin-3-O-β-D-glucopyranosyl-(1→ 4)-mannopyranoside, apigenin and sitosterol have also been isolated from dry fruits.

Distribution:

Chuadanga, Khulna, Kustia, Nawabganj, Rajshahi, in fallow lands. (Ghani, A., 1998)

Drynaria quercifolia

Drynaria quercifolia is a robust, epiphytic fern with a long, creeping rhizome, growing up to 1 metre tall. It produces two types of annual frond - short, sterile ones up to 40cm tall that remain on the plant for several years and have a main purpose of trapping organic material to provide nutrient for the plant, and taller fertile shoots that produce spores. The root is sometimes gathered from the wild for local medicinal use. (Merrill, 1995)

Fig: Drynaria quercifolia

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Division	Pteridophyta – Ferns
Class	Filicopsida
Order	Polypodiales
Family	Polypodiaceae – Polypody family
Genus	Drynaria (Bory) J. Sm. – basket fern
Species	Drynaria quercifolia (L.) J. Sm. – basket fern

Using information:

Whole plant is anthelmintic, pectoral, expectorant and tonic; used in the treatment of phthisis, hectic fever, dyspepsia, skin diseases and cough. The fronds are used for cough and poulticing

swellings. Rhizome is tonic and astringent to the bowels; used in typhoid; extract is taken by Chakma as a remedy for jaundice and dysentery. Peeled rhizome is chewed or its juice mixed with sugar is taken in scanty urination and spermatorrhoea by the Marma tribe.

Aquous extracts possesses antibacterial properties. (Ramesh et al, 2001)

Distribution:

All over the country on tree trunk and shady old walls.

Constituents

- Phytochemical screening yielded phenols, tannins, alkaloids, proteins, xanthoproteins, carboxylic acid, coumarins and saponins.
- Yields catechin, coumarins, flavonoids, phenolics, saponin, steroids, tannins, and triterpenes.
- Study of dried rhizomes yielded friedelin, epifriedelinol, beta-amyrin, beta-sitosterol 3-beta-D-glucopyranoside and naringin.
- Phytochemical analysis of fertile fronds yielded the presence of coumarins, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and terpenoids. Total phenolic content was 186 mg/g equivalent of gallic acid. (Mithraja et al, 2012)

Chapter four:Literature Review

Literature Review

Antioxidant activity of Solanum virginianum

Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves.

OBJECTIVE:

To investigate antidiabetic efficacy of the extract of field grown and in vitro raised leaves of Solanum xanthocarpum (S. xanthocarpum) against alloxan induced diabetic rats.

METHODS:

The antidiabetic activity of the crude methanol extracts of the field grown and in vitro raised leaves of S. xanthocarpum at different concentrations (100-200 mg/kg bw) was tested against alloxan induced diabetic rats. The antidiabetic efficacy was validated through various biochemical parameters and the antioxidant effect was also determined. The phytochemical analyses of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves were done by estimating their chlorophyll, carotenoids, total sugar, protein, amino acid and minerals contents.

RESULTS:

The results revealed that the methanol extracts of both the leaves (field grown and in vitro raised) of S. xanthocarpum was efficient anti hyperglycemic agents at a concentration of 200 mg/kg bw and posses potent antioxidant activity. However, the extracts of in vitro rasied S. xanthocarpum raised leaves exhibit higher efficacy than the field grown leaves in all tested concentrations. Proximal composition and mineral analysis of S. xanthocarpum revealed higher concentration of contents in in vitro rasied S. xanthocarpum than field grown S. xanthocarpum.

CONCLUSIONS:

From the results it can be concluded that the leaves extracts of S. xanthocarpum can be a potential candidate in treating the hyperglycemic conditions and suits to be an agent to reduce oxidative stress. (Poongothai *et al*, 2011)

ANTIOXIDANT EFFECT OF THE STEM AND LEAVES OF SOLANUM XANTHOCARPUM

Solanum xanthocarpum L is used in the traditional medicine as diuretic. In the present study, the antioxidant activity of ethanol, chloroform and ethyl acetate extract of leaves and stem of Solanum xanthocarpum Lam was studied and the activity was compared with DPPH and BHT as standard. By employing DPPH (2,2-diphenyl-1picrylhydrazyl radical) scavenging assays, it was shown that all the ethanol extracts of leaves and stem show antioxidant activity. (Gupta *et al, 2006)*

Evaluation of Antioxidant Activity of Spilanthes calva, Solanum virginianum, Stevia rebaudiana, Ruellia tuberosa and Phytochemical Investigation of Mikania cordata

Antioxidant assay is widely used by the pharmaceutical industries to screen for antioxidant activity in compound libraries. In present study five plant samples were selected to conduct the antioxidant activity; these are Spilanthes calva, Solanum virginianum, Stevia rebaudiana, Ruellia tuberosa and Mikania cordata. In total phenolic content assay Stevia rebaudiana has the highest amount of phenolic content than other extracts. Spilanthes calva has the least amount of phenolic content among all extracts. DPPH radical activity of methanolic extract of Stevia rebaudiana is good after which is methanolic fraction of Mikania cordata. In Nitric oxide scavenging assay Solanum virginianum is good after which is methanolic extract of Stevia rebaudiana. In reducing power assay Solanum virginianum showed highest level of reducing power where as Stevia rebaudiana and Ruellia tuberosa showed moderate reducing power activity. Among all different fractions of Mikania cordata methanolic fraction showed the highest antioxidant activity. In Column chromatography, I used an open column to purify individual chemical compounds from mixtures of compounds. The methanolic extract of Mikania cordata was subjected to open column and different fractions were collected using four different solvents. Lastly I experimented these fractions to identify their antioxidant property. (Rahman *et al, 2016)*

PHYTOCHEMICAL INVESTIGATION AND ANTI-OXIDANT STUDIES ON THE ROOTS OF SOLANUM XANTHOCARPUM LINN

Chemical and physical investigation of methanolic extract of roots of Solanum xanthocarpum was carried out. By phytochemical screening the presence of alkaloid, triterpenoid, phenols, tannins, flavanoids, carbohydrates, phytosterols, fats and fixed oil were confirmed. Column chromatography was performed and a compound was isolated. The extract was estimated for their total phenolic content. Total phenolic content was found to be 76µg. Total flavonoid content was found to be 73µg. Antioxidant study was carried out on methanolic extract. It showed good % DPPH radical scavenging activity. IC50 value of the compound was found to be 52µg/ml. (Sen *et al,* 2006)

Antioxidant and hepatoprotective effects of Solanum xanthocarpum leaf extracts

Solanum xanthocarpum Schard. and Wendl. (Solanaceae) has been used in traditional Indian medicines for its antioxidant, anti-inflammatory, and antiasthmatic properties. Objective: The present study demonstrates the antioxidant and hepatoprotective effects of S. xanthocarpum. On the basis of in vitro antioxidant properties, the active fraction from column chromatography of the methanol extract of S. xanthocarpum leaves (SXAF) was chosen as the potent fraction and used for hepatoprotective studies in rats. Materials and methods: The antioxidant activity was evaluated by 2,20 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and reducing power assays. Rats were pre-treated with 100 and 200 mg/kg b.w. of SXAF for 14 d with a single dose of CCl4 in the last day. Hepatoprotective properties were determined by serum biochemical enzymes, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), antioxidant enzymes (SOD, CAT, GSH, and GST), and histopathology studies. Results: SXAF exhibited significant antioxidant activity in scavenging free radicals with IC50 values of 11.72 mg (DPPH) and 17.99 mg (ABTS). Rats pre-treated with SXAF demonstrated significantly reduced levels of serum LDH (1.7-fold), ALP (1.6-fold), and AST (1.8-fold). Similarly, multiple dose SXAF administration at 200 mg/kg b.w. demonstrated significantly enhanced levels of SOD (1.78 ± 0.13), CAT (34.63 ± 1.98), GST (231.64 ± 14.28), and GSH (8.23 ± 0.48) in liver

homogenates. Histopathological examination showed lowered liver damage in SXAF-treated groups. Discussion and conclusion: These results demonstrate that SXAF possesses potent antioxidant properties as well as hepatoprotective effects against CCl4-induced hepatotoxicity. (Jalali *et al*, 2014)

Antioxidant activity of Drynaria quercifolia

IN VITRO ANTIOXIDANT ACTIVITY OF *DRYNARIA QUERCIFOLIA* L. RHIZOME

Antioxidant activity of methanolic extract of *Drynaria quercifolia* rhizome at different concentrations (100, 200 and 300µg/ml) were studied in various *in vitro* models. Results were compared with standard ascorbic acid. Antioxidant activity of extract was increased with the increasing concentration. The order of antioxidant potential according to models were found to be highest in nitric oxide scavenging activity followed by total antioxidant activity, reducing power assay and hydrogen peroxide scavenging activity. IC50 values were found to be 180, 230, 230 and 240µg/ml respectively in nitric oxide scavenging activity, total antioxidant assay, reducing power assay, and hydrogen peroxide scavenging activity. In conclusion, the results of present analysis demonstrated that *Drynaria quercifolia* rhizome possess potential antioxidant activity, and could be used as a viable source of natural antioxidants. (Prasanna *et al*, 2015)

Phytochemical investigation and antioxidant activity study of Drynaria quercifolia Linn rhizome

In the present research investigation we extracted the powdered rhizome of Drynaria quercifolia l inn by soxhlation method using different solvents .Then extracts were subjected to preliminary p hyto-chemical investigation followed by evaluation of anti-oxidant activity by DPPH assay meth od. It was observed that, the methanolic extract was found to be effective anti-oxidant on compar ision to the other extracts and has significant activity compared to the standard drug. (Beknal *et al, 2010)*

Antioxidant and anti-inflammatory activity of aqueous and methanolic extracts of rhizome part of drynaria quercifolia (L.) J. Smith

The present investigation was aimed to justify the scientific basis in traditional use of Drynaria quercifolia as anti-inflammatory agent in North-East India. Methods: In-vitro antioxidant activities were evaluated by Reducing power method, Superoxide scavenging method and DPPH radical scavenging method. In-vitro and in vivo anti-inflammatory activity were evaluated using albumin denaturation and membrane stabilizing method and carrageenan induced inflammation method. In-vitro cyclooxygenase inhibition was also done to investigate the pathway of anti-inflammatory action. Result: Both methanol (MEDQ) and aqueous (AEDQ) extracts showed significant (**$p<0.01$) inhibition of rat paw edema in dose dependent manner and the MEDQ was the most active. The MEDQ exhibited highest inhibition of COX-1 and COX-2, protein denaturation and haemolysis at 100µg/ml. The extracts also exhibited antioxidant property against DPPH, super oxide radicals and reducing power activity. The maximum 95.972µg Gallic acid equivalents total phenolic content and 81.03µg Quercetin equivalent flavonoids content in MEDQ were estimated that was 20 fold (phenolic content) and 3 fold (flavonoids) more than AEDQ. Conclusion: These observations established the traditional claim of usefulness of D. quercifolia rhizome against inflammation, which could be due to cyclooxygenase enzyme inhibition and free radical scavenging activities of the extracts. (Das *et al,* 2014)

Chapter Five:Materials and Method

Materials and method

Four plant samples were selected to conduct this thesis work; these are *Stephania japonica, Solanum virginianum, Micania cordata* and *Drynaria quercifolia*. All four sample plants are subjected to phytochemicals, antioxidant and cytotoxic test. Among all sample only the crude extract of *Spilanthes acmella* is subjected to solvent-solvent extraction by four different solvent systems.

Materials

Lists of glass wares

Glass rod, pipette, pasteur pipette, test tube, vial, conical flask, separating funnel, beaker (large, medium and small), round bottomed flask, flat bottomed flask, volumetric flask, funnels, reagent bottle, measuring cylinders, water tank/aquarium, watch glass, magnifying glass, petri dish.

Lists of other material

Aluminium foil paper, spatula, pipette pumper, micropipette tip, cotton wool cloth, gas burner, forceps and tongs, labels and masking tape, filter paper, permanent marking pen, scissors, pH meter, pencil and pen, scale, tissue paper.

Lists of equipments

Rotary vaccum evaporator, UV-Visible Spectrophotometer, hot air oven, centrifuge machine, lamp with bulb, oxygen blower device, electric balance, rough balance, distilled water plant.

Lists of Solvents

Methanol, ethanol, di-chloro-methane, n-hexane, ethyl acetate, dimethyl sulfoxide (DMSO)

Lists of Reagents

Folin-Ciocalteu reagent, salicylic acid, sodium carbonate, DPPH (2,2-diphenyl-2-picrylhydrazyl), ascorbic acid, monobasic sodium phosphate, dibasic sodium phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, potassium dichromate, tamoxifen

Lists of plant sample

1. *Solanum virginianum*
2. *Drynaria quercifolia*.

List of organism

Artemia salina leach (brine shrimp eggs)

Study Protocol

Our present study was designed to evaluate phytochemical, antioxidant and cytitoxic property of crude extract *Solanum virginianum, Drynaria quercifolia*. The study protocol consisted of the following steps ---

- Cold extraction of the powdered plant with methanol and ethanol at room temperature.
- Filtration and solvent evaporation of the methanolic and ethanolic crude extract.
- Partitioning of methanolic crude extract of *Spilanthes acmella* with n-hexane, dicholoromethane and ethyl acetate.
- Performing Biochemical investigation and phenolic content test of crude extract.
- Performing DPPH radical scavenging assay and reducing power assay of crude extract.
- Brine shrimp lethality bioassay and determination of LC_{50} value for crude extract and different fractions.

Phytochemical Investigation

The Phytochemical investigation of a plant can be divided roughly into the following major steps:

- Collection and proper identification of the plant material
- Preparation of plant sample
- Extraction of the plant material

Collection of plant

The plant was collected from different places of Bangladesh. By using plant sample herbarium sheet was prepared and identified by Bangladesh National Herbarium. A voucher specimen had been deposited at the Bangladesh National Herbarium. The proper time of harvesting or collecting is particularly important because the nature and the quantity of constituents very gently in some species according to the season.

Cleaning and Drying

The collected plant was subjected to clean properly, separated from unwanted plant part and other unwanted material. The plant material is subjected to cut into small pieces to facilitate drying and grinding into coarse powder. Drying is the most common and fundamental method for post- harvest preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of the plants material in an uncomplicated manner. The plant was dried in room temperature and drying was completed within 2 to 3 weeks. By drying the plant materials it removes sufficient moisture to ensure good keeping qualities and to prevent molding the action of enzyme, the action of bacteria and chemical or other possible changes. Proper and successful drying involved two main principles: control of temperature and regulation of air flow. The plant material can be dried by room temperature.

Grinding and Sieving

After complete drying the dried leaves were then ground in coarse powder using high capacity grinding machine. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use.

Extraction of the plant material

About 500 gm of powdered plant material was taken in clean, round bottomed flask (2.5 liters) and macerated at room temperature in 2 liters of methanol (*Spilanthes acmella, Mimosa pudica),* and 2 litres of ethanol *(Micania cordata, Drynaria quercifolia*) for 3 days and everyday it was

used to shake properly to ensure the maximum amount of constituents present in the grinded plant become soluble into ethanol. After 3 days later, the mixture was filtered. For filtration, white cotton cloth was used. After filtration two parts were obtained.

- The residue portion over the filter
- The filtered part

The filtrated part, which contains the substance soluble in ethanol, poured into a 1000 round bottle flask, and then the flask was placed in a rotary evaporator. The evaporation was done at 40-50 degree Celsius temperature. The number of rotation per minute was selected as 125 RPM. The pressure of vacuum pump machine was 6 bars. The water flow through the distillation chamber was also provided in a satisfactory flow rate. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper. The concentrated extract was then air dried to solid residue.

Total phenolic content assay

Introduction

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, It has been reported that there is an inverse relationship between the antioxidative status occurrence of human diseases.
In addition, antioxidant compounds which are responsible For Such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders.

Principle

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The intensity of the color change is measured in a spectrophotometer at 700 nm. The absorbance value will reflect the total phenolic content of the compound.

Materials & Methods

Total phenolic content of crude extracts was measured employing the method as described by Arpona Hira et al. (2013) involving Folin-Ciocalteu reagent as oxidizing agent and salicylic acid as standard.

Tab 5.4.1: Composition of Folin-Ciocalteu Reagent

Water	57.5ml
Lithium Sulfate	15.0mg
Sodium Tungstate Dihydrate	10.0mg
Hydrochloric Acid 25%	10.0mg
Phosphoric Acid 85 % solution in water	5.0mg
Molybdic Acid Sodium Dihydrate	2.5mg

Standard curve preparation

Salicylic acid was used here as standard. Different concentration of Salicylic acid solution were prepared having a concentration ranging from 10 mg/ml to 0.625 mg/ml. 5.0 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 4.0 ml of Na_2CO_3 (7.5 % w/v) solution was added to 100µl of Salicylic acid solution. The mixture was incubated for 1 hour at room temperature. After 1 hour the absorbance was measured at 700 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

Sample preparation

10 mg of crude extract was taken and dissolved in 1ml of methanol (*Spilanthes acmella, Mimosa pudica)* and 1ml of ethanol (*Micania cordata, Drynaria quercifolia)* to get a sample concentration of 10mg/ml in every case.

Determination of phenolic content of samples

100 µl solution of crude extract mixed with 5.0 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 4.0 ml of Na_2CO_3 (7.5 % w/v) solution. The mixture was incubated for 1 hour at

room temperature. After 1 hour the absorbance was measured at 765 nm. Using the absorbance of the sample, total phenolic content is measured by using following equation ---

$$T = \frac{C \times V}{M} \text{ mg/g}$$

Where,

T = Total phenolic content

C = x (Concentration from linear regresson equation)

V = Volume of sample

M = Mass of sample

DPPH radical-scavenging activities

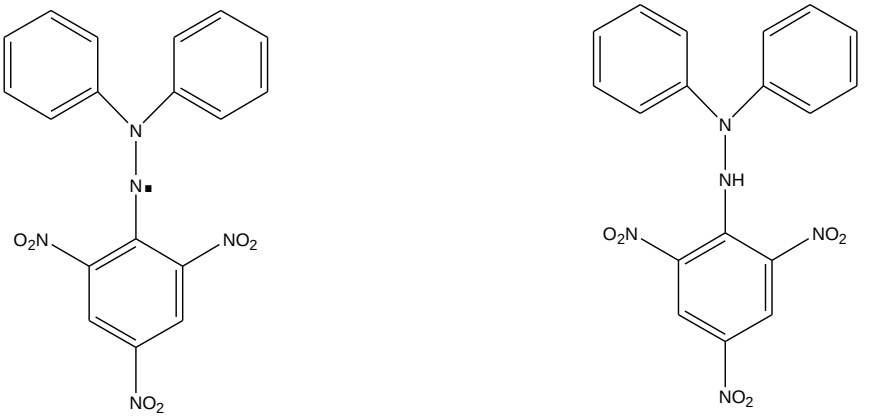
Introduction

There is considerable recent evidence that free radical induce oxidative damage to biomolecules. This damage causes cancer, aging, neurodegenerative diseases, atherosclerosis, malaria and several other pathological events in living organisms. Antioxidants which 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. A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids.

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidant such as *tert*-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase self life, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, BHT and BHA are known to have not only toxic and carcinogenic effects and humans, but abnormal effects on enzyme systems. Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.

Principle

The free radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it was reduced. The changes in color (from deep violet to light yellow) were measured at 517 nm on a UV-visible light spectrophotometer.



*DPPH (oxidized form)

DPPH (reduced form)

Figure: Reaction of DPPH with antioxidant.

Material and Method

DPPH was used to evaluate the free radical scavenging activity of crude extracts was measured employing the slightly method described by Arpona Hira et al. (2013) involving DPPH as oxidizing agent and Ascorbic acid as standard.

Preparation of DPPH solution

A dry 250ml conical flask was cleaned and covered with an aluminium foil protect its contents from light. Accurately weighted 1mg of DPPH placed in conical flask and 50ml methanol was added to prepare 0.1mmol/L or 40µg/ml DPPH solution.

Preparation of sample solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10µg/µl ten test tube were taken and covered with an aluminium foil protect its contents from light and these test tubes were marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 to 500 µg/ml. The test was done three times.

Preparation of standard solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10µg/µl ten test tube were taken and covered with an aluminium foil protect its contents from light and these test tubes were marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 to 500 µg/ml. The test was done three times.

Measurement of DPPH radical scavenging activity

2ml of a methanol solution of the extract at different concentration were mixed with 2ml of a DPPH methanol solution and this mixture was vigorously shacked and left at 25^0C for 60 minutes in the dark. After 60 minutes reaction period at room temperature in dark place the absorption was measured at 517nm of methanol as blank by UV spectrophotometer.

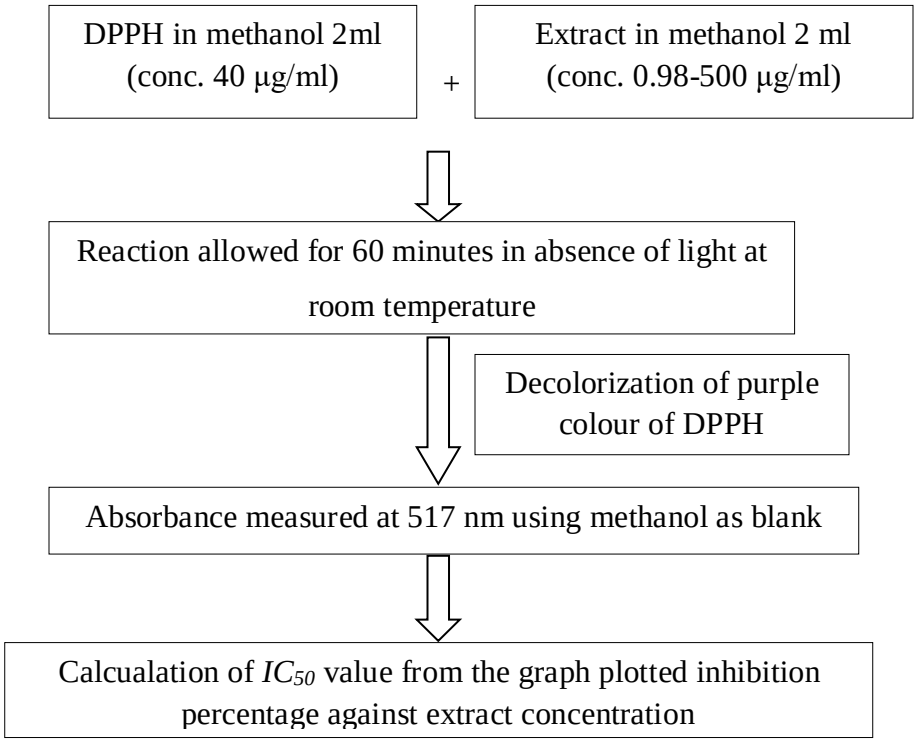


Figure: Schematic representation of the method of assaying free radical scavenging activity.

Reducing Power Assay

Introduction

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the super oxi de anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, an d various lipid eroxides. These free radicals may either be produced by physiological or biochem ical processes or by pollution and other endogenous sources. All these free radicals are capable o f reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage

Antioxidants prevent the human system by neutralizing the free radicals interactively and synergi stically. Plants arerich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, bet alains and other metabolites which are rich in antioxidant activity.

Principle

Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Antioxidant

Potassium ferricyanide + Ferric chloride ⟶ Potassium ferrocyanide + ferrous chloride

Material and Method

Reducing power assay of crude extracts was measured employing the method as described by Arpona Hira et al. (2013) involving Phosphate Buffer (2.5ml, 0.2M, pH 6.6), 1% Potassium Ferricyanide [$K_3Fe(CN)_6$], 10% Trichloroacetic acid 0.1% $FeCl_3$and Ascorbic acid as standard.

Preparation of sample solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10µg/µl ten test tubes were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 to 500 µg/ml. The test was done triplicate.

Preparation of standard solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10µg/µl ten test tube were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 to 500 µg/ml. The test was done triplicate.

Procedure

1ml of stock mixture (concentration 0.98µg/ml to 500µg/ml) is mixed with 1ml of distilled water added with 2.5ml of Phosphate Buffer and 2.5ml of 1% Potassium Ferricyanide. The reaction mixture is incubated at 50^{o}C for 20minute. After incubation 10% Trichloroacetic acid is added. The mixture is Centrifuged for 10min at 3000rpm. After centrifugation Upper layer was taken (2.5ml) dissolved with 2.5ml distilled water and 0.5ml of $Fecl_{3.}$ Absorbance was measured at 700nm.

Chapter Six: Reasult and Discussion

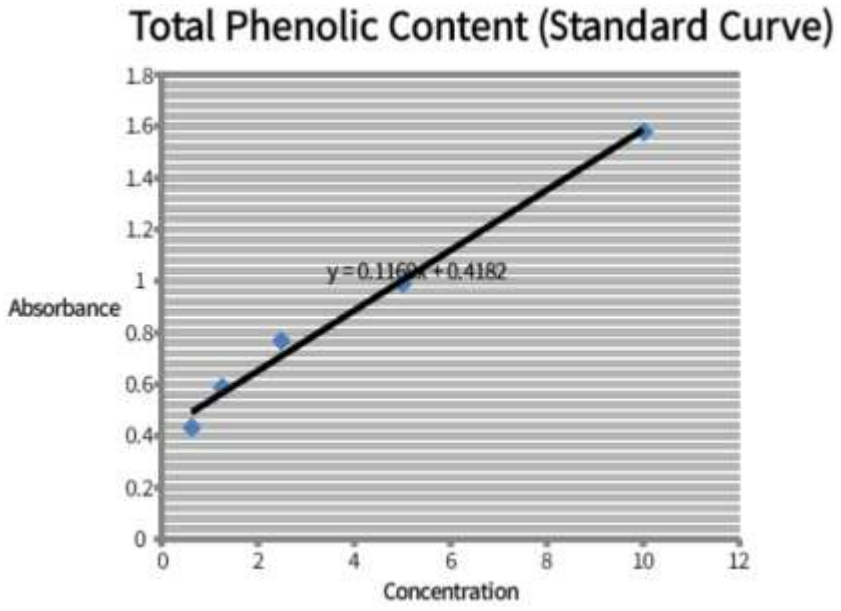
Total Phenolic Content Assay

The Methanolic extract of Solarium verganium and Ethanolic extract of Drynaria quercifolia were subjected to Total Phenolic Content Assay by the method described by (Arpona et al., 2013). Here, Total Phenolic Content was measured as Salicylic acid equivalence.

Standard Curve Preparation by Using Salicylic Acid

SL	Concentration mg/ml	Absorbance	Regression Equation	R^2
1	0.625	0.433		
2	1.25	0.586		
3	2.5	0.766	Y = 0.116x + 0.418	0.991
4	5	0.990		
5	10	1.580		

Total Phenolic Content (Standard Curve)



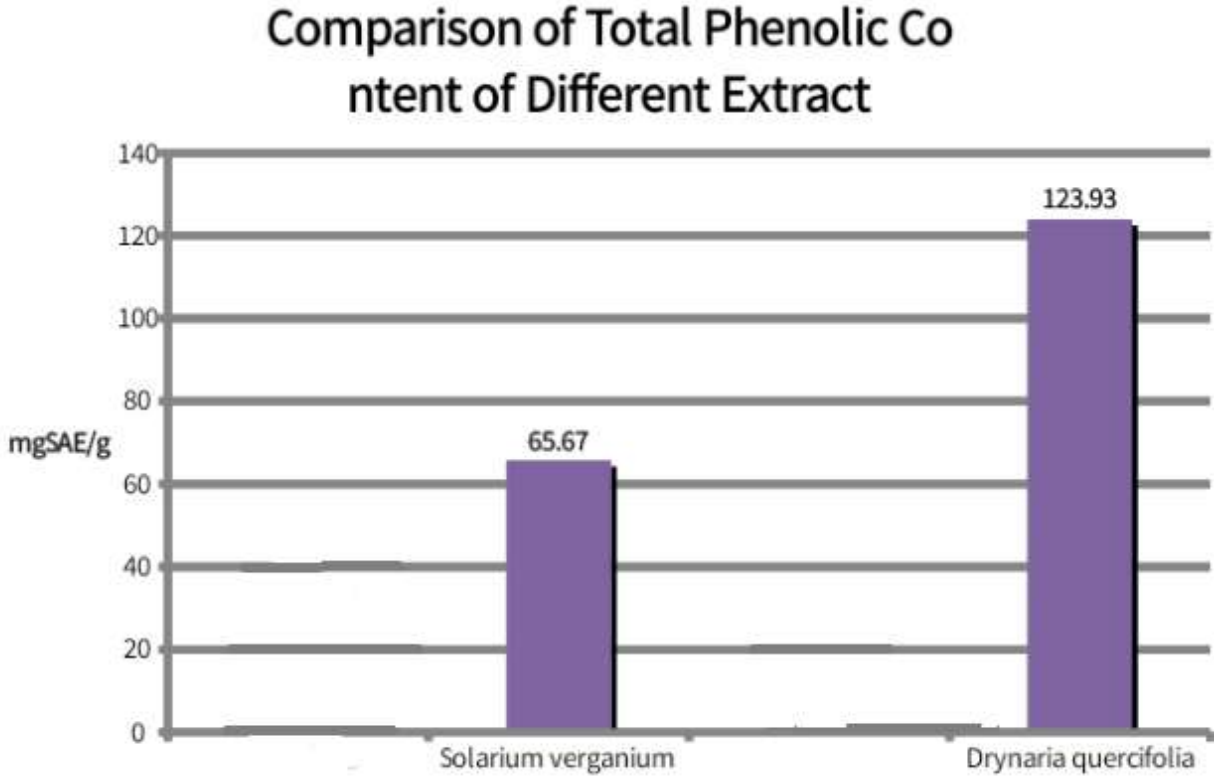
Total Phenolic Content of Solarium verganium

Absorbance	mgSAE/g	Mean±SD
1.185	66.12	65.67±0.43
1.175	65.26	
1.179	65.60	

Total Phenolic Content of Drynaria quercifolia

Absorbance	mgSAE/g	Mean±SD
1.851	123.53	123.93±0.361
1.857	124.05	
1.859	124.22	

Comparison of Total Phenolic Content of Different Extracts



Discussion:

From the above result we can see that among four experiment extracts, ethanolic extract of Drynaria quercifolia has the highest amount of phenolic content (123.93±0.43) followed by methanolic extract of Solanum verginianum (65.67±0.43 mgSAE/g). So from the above data it can be said that, ethanolic extract of Drynaria quercifolia have highest amount of phenolic content (123.93±0.43 mgSAE/g) thus it can be a good source of phenol.

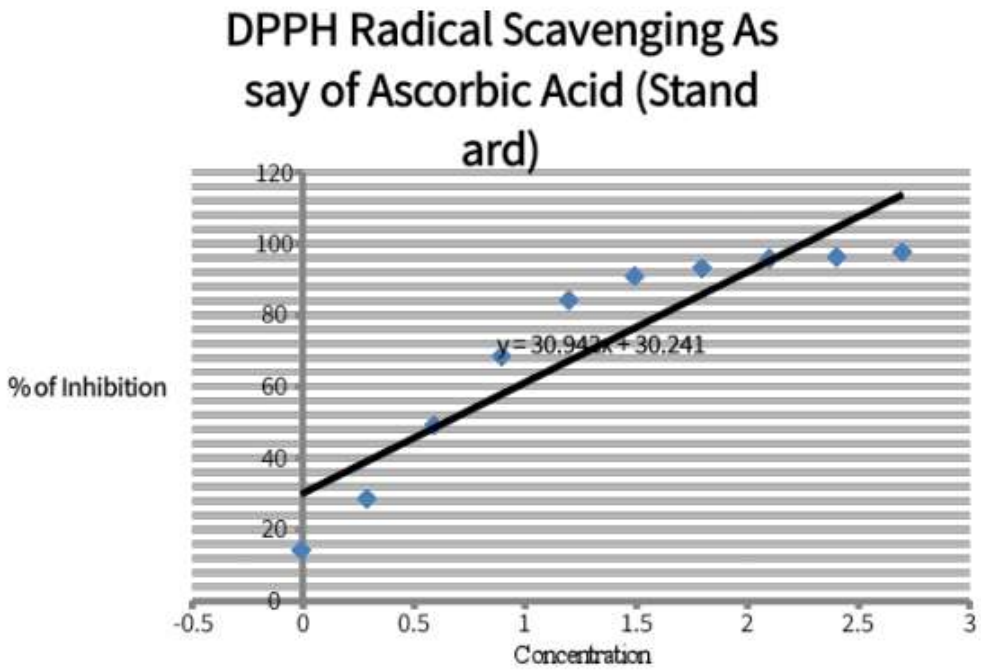
DPPH Radical Scavenging Assay

The methanolic extract of Solarium verganium and ethanolic extract of Drynaria quercifolia were subjected to DPPH Radical Scavenging Assay according to method described by (Arpona et al., 2013) and ascorbic acid was used as reference standard in this experiment.

DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

Absorbance of Blank	Concentration µg/ml	Log Concentration	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.625	0.98	- 0.009	0.536	14.24	4.35
	1.95	0.290	0.508	18.72	
	3.91	0.592	0.365	41.60	
	7.81	0.893	0.198	68.32	
	15.63	1.194	0.098	84.32	
	31.25	1.495	0.058	90.72	
	62.5	1.796	0.042	93.25	
	125	2.097	0.027	95.68	
	250	2.398	0.021	96.32	
	500	2.699	0.015	97.60	

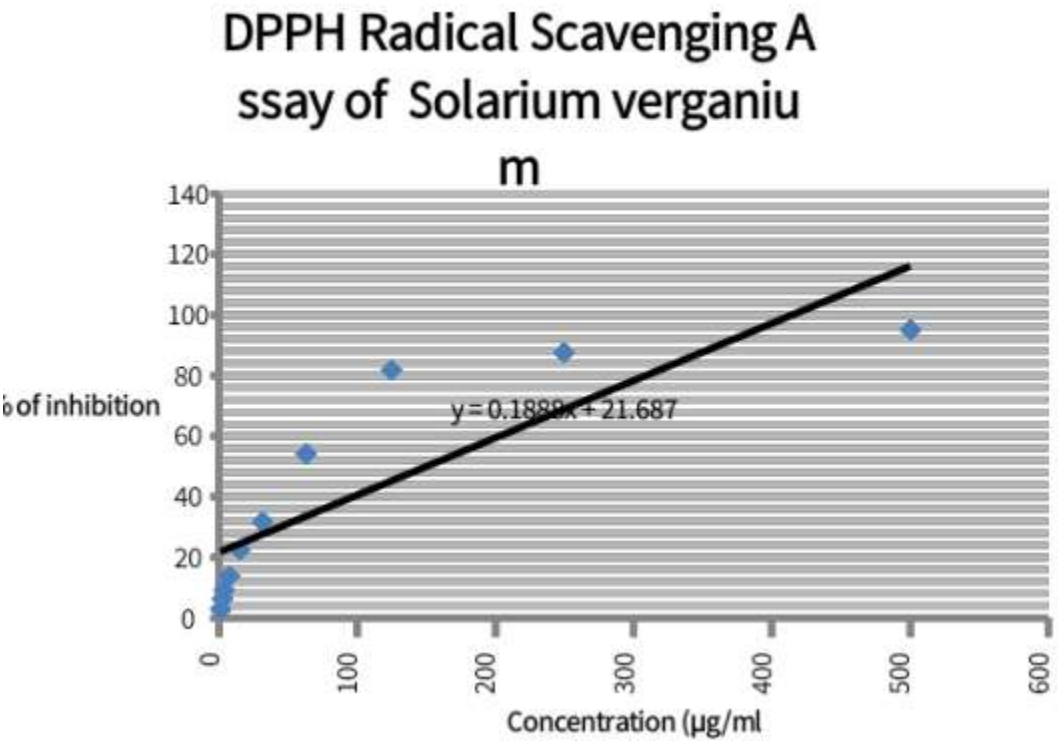
DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)



DPPH Radical Scavenging Assay of Solarium virginianum

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.675	0.98	0.655	2.96	150.64
	1.95	0.633	6.22	
	3.91	0.613	9.19	
	7.81	0.580	14.08	
	15.63	0.523	22.52	
	31.25	0.460	31.85	
	62.5	0.311	53.93	
	125	0.122	81.93	
	250	0.083	87.70	
	500	0.033	95.11	

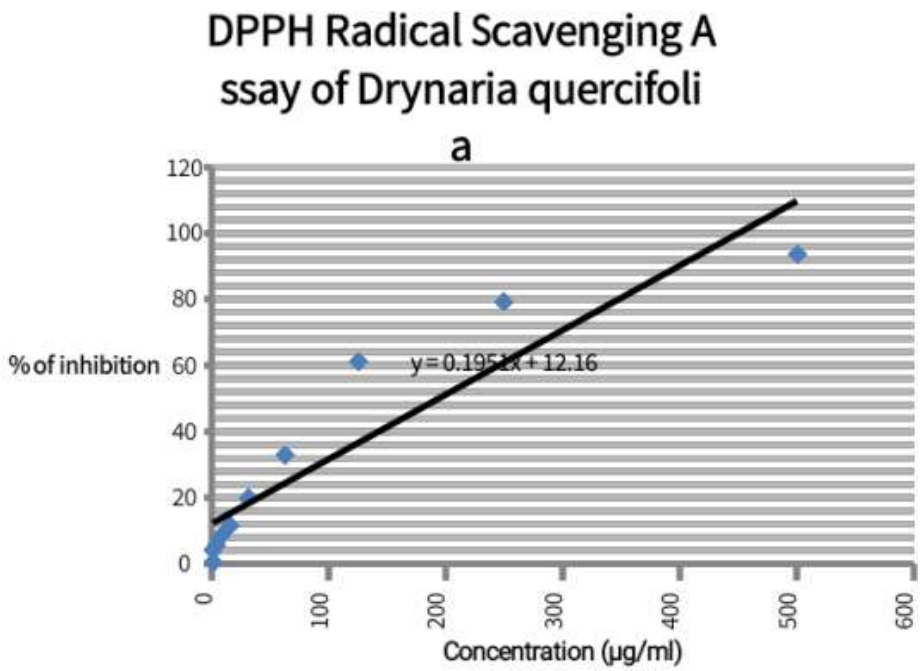
DPPH Radical Scavenging Assay of Solanum virginianum



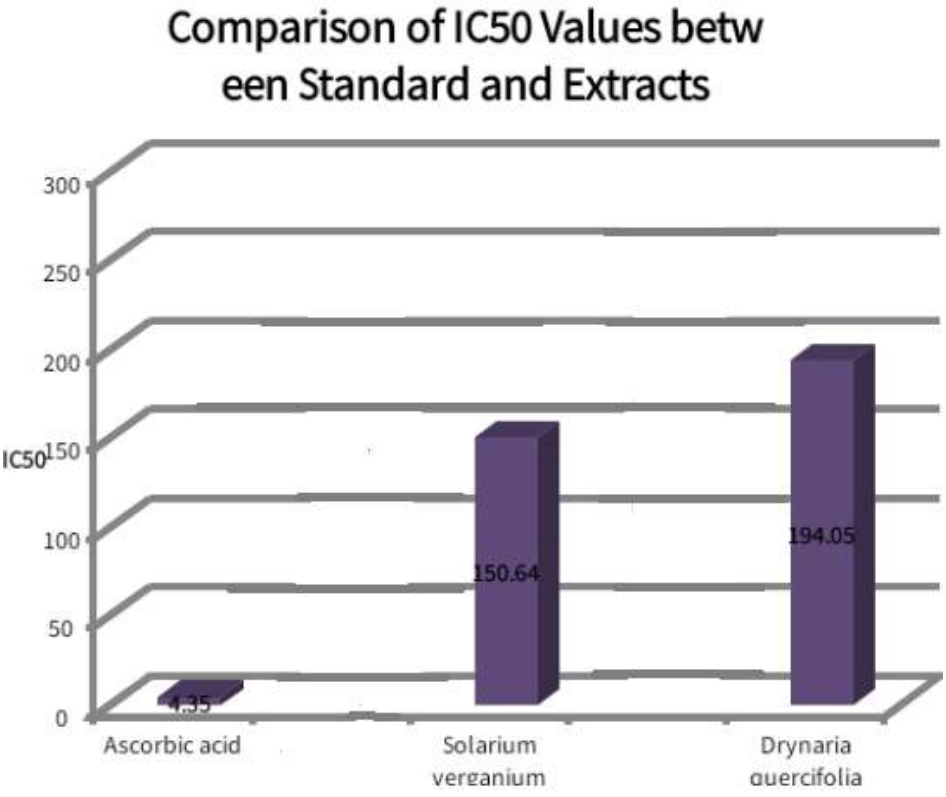
DPPH Radical Scavenging Assay of Drynaria quercifolia

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.553	0.98	0.550	0.54	194.05
	1.95	0.530	4.16	
	3.91	0.523	5.42	
	7.81	0.508	8.137	
	15.63	0.488	11.75	
	31.25	0.446	19.34	
	62.5	0.370	33.09	
	125	0.216	60.94	
	250	0.116	79.02	
	500	0.035	93.67	

DPPH Radical Scavenging Assay of Drynaria quercifolia



Comparison of IC_{50} between Standard and Extract



Discussion:

The result of the tests is present in the following figure. The extract demonstrated an antioxidant activity by using DPPH where DPPH radical activity of methanolic extract of Solarium verganium is good (IC_{50} 150.64µg/ml) followed by ethanolic extract of Drynaria quercifolia (IC_{50} 194.05µg/ml).

Reducing Power Assay

The Methanolic extract of Solarium verganium and Ethanolic extract of Drynaria quercifolia were subjected to Reducing Power Assay according to method described by (Arpona et al., 2013). Here, ascorbic acid was used as reference standard.

Reducing Power Assay of Ascorbic acid

SL.	Concentration µg/ml	Absorbance			Mean±SD
		1	2	3	
1	0.98	0.100	0.098	0.097	0.098±0.002
2	1.95	0.103	0.105	0.104	0.103±0.003
3	3.91	0.136	0.137	0.135	0.136±0.001
4	7.81	0.213	0.212	0.215	0.213±0.002
5	15.63	0.276	0.274	0.276	0.275±0.001
6	31.25	0.476	0.478	0.474	0.476±0.002
7	62.5	0.852	0.855	0.853	0.855±0.002
8	125	1.333	1.339	1.337	1.336±0.003
9	250	2.419	2.421	2.418	1.895±0.002
10	500	3.077	3.075	3.074	2.503±0.002

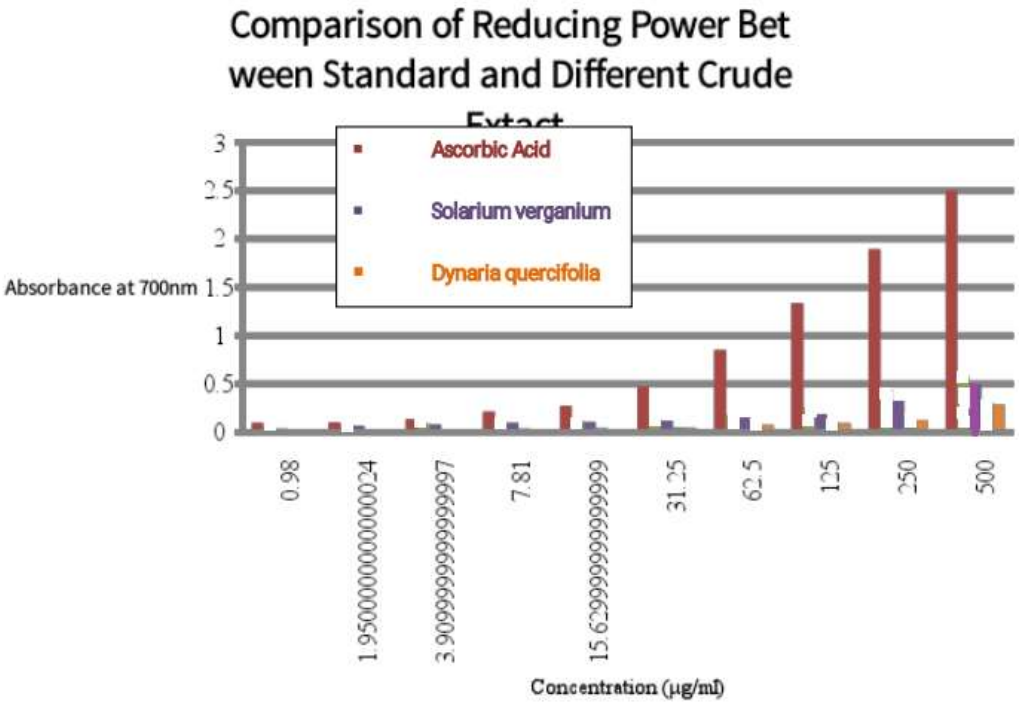
Reducing Power Assay of Solarium virginianum

SL.	Concentration µg/ml	Absorbance			Mean±SD
		1	2	3	
1	0.98	0.040	0.039	0.039	0.039±0.001
2	1.95	0.074	0.071	0.073	0.068±0.002
3	3.91	0.092	0.092	0.091	0.083±0.001
4	7.81	0.099	0.097	0.095	0.097±0.002
5	15.63	0.111	0.112	0.110	0.109±0.002
6	31.25	0.123	0.120	0.118	0.120±0.003
7	62.5	0.155	0.153	0.152	0.153±0.002
8	125	0.188	0.194	0.182	0.188±0.006
9	250	0.326	0.322	0.329	0.326±0.004
10	500	0.502	0.501	0.505	0.503±0.002

Reducing Power Assay of Drynaria quercifolia

SL.	Concentration µg/ml	Absorbance			Mean±SD
		1	2	3	
1	0.98	0.075	0.076	0.076	0.009±0.001
2	1.95	0.079	0.077	0.078	0.012±0.002
3	3.91	0.082	0.085	0.081	0.021±0.003
4	7.81	0.091	0.092	0.091	0.029±0.001
5	15.63	0.093	0.093	0.095	0.036±0.002
6	31.25	0.097	0.096	0.097	0.049±0.001
7	62.5	0.126	0.126	0.127	0.079±0.001
8	125	0.115	0.117	0.115	0.093±0.001
9	250	0.249	0.248	0.249	0.125±0.001
10	500	0.286	0.286	0.288	0.286±0.001

Comparison of Reducing Power between Standard and Extract



Discussion:

In reducing power assay higher absorbance of the reaction mixture indicates higher reductive potential. In the graph above we can see that with the increase of concentration absorbance is also increased. From the above graph we can conclude that among four different extracts Solanum virginianum is showing highest level of reducing power followed by Drynaria quercifolia. The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity. Further studies will help in identifying the individual compounds that aids in the reducing power and to identify the synergistic effect. Also a correlation between the reducing power and antioxidant activity can be derived. In the present investigation, we have warranted the concentration dependent reducing ability of Ascorbic Acid, Solanum virginianum, Drynaria quercifolia, the methanolic extracts of Solarium virinianum and ethanolic extracts of Drynaria quercifolia.

Chapter Seven:Conclusion

Plants are the basic source of knowledge of modern medicine. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs. Nowa-days herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness and no side effect in clinical experience. Large number of plants belonging to different families have been studied for their therapeutic properties.

Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract, and cancer. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Synthetic antioxidants are recently reported to be dangerous to human health. Thus the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. In addition to endogenous antioxidant defense systems, consumption of dietary and plant-derived antioxidants appears to be a suitable alternative. Dietary and other components of plants form a major source of antioxidants. The traditional Indian diet, spices, and medicinal plants are rich sources of natural antioxidants; higher intake of foods with functional attributes including high level of antioxidants in antioxidants in functional foods is one strategy that is gaining importance.

Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principles will yield dividends in near future in improving health, especially among people who do not have access to the use of costlier western systems of medicine.

Chapter Eight:References

References:

Al Nayeem, A., Khatun, A., Rahman, M.S. and Rahman, M., 2013. Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves. Journal of Pharmacognosy and Phytotherapy, 3(8), pp.118-123.

Ahmed, N.U., Akter, R., Satter, M.A., Khan, M.S., Islam, F. and Abdullah, A.M., 2011. Anti-Inflammatory, Antioxidant and Anti-Diarrheal Effects of Ethanol Extract of Stephania Japonica. Bangladesh Journal of Scientific and Industrial Research, 46(4), pp.437-442.

Ahvazi, M., Khalighi-Sigaroodi, F., Charkhchiyan, M.M., Mojab, F., Mozaffarian, V.A. and Zakeri, H., 2012. Introduction of medicinal plants species with the most traditional usage in Alamut region. *Iranian journal of pharmaceutical research*: IJPR, 11(1), p.185.

Alam, M.S., Hassan, M.A. and Uddin, M.Z., 2006. A preliminary checklist of the Angiospermic flora of Ghagotia Union under Kapasia Upazila in Gazipur District, Bangladesh. Bangladesh Journal of Plant Taxonomy, 13(2), pp.155-170.

Baral, B., Bhattarai, N. and Vaidya, G.S., 2012. Pharmacological and Antagonistic Potentials of Mikania micrantha. Nepal Journal of Science and Technology, 12, pp.75-84.

Beknal, A.K., Korwar, P.G., Halkai, M., Kulkarni, U., Patil, B.S. and Soodam, S.R., 2010. Phytochemical investigation and antioxidant activity study of Drynaria quercifolia Linn. rhizome. Int J Curr Pharmaceut Res, 4, pp.36-9.

Brown JE, Rice-Evan CA 2000. Luteolin-rich Artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radical Research, Vol. 29, pp. 247–255.

Croteau, R., Kutchan, T.M. and Lewis, N.G., 2000. Natural products (secondary metabolites). Biochemistry and molecular biology of plants, 24, pp.1250-1319.

Das, B., Choudhury, M.D., Dey, A., Talukdar, A.D., Nongalleima, K.H. and Deb, L., 2014. Antioxidant and anti-inflammatory activity of aqueous and methanolic extracts of rhizome part of Drynaria quercifolia (L.) J. Smith. Int J Pharm Pharm Sci, 6(6), pp.43-49.

Desmet, P. and Brouillet, L., 2013. Database of Vascular Plants of Canada (VASCAN): a community contributed taxonomic checklist of all vascular plants of Canada, Saint Pierre and Miquelon, and Greenland. PhytoKeys, (25), p.55.

Dong, L.M., Jia, X.C., Luo, Q.W., Zhang, Q., Luo, B., Liu, W.B., Zhang, X., Xu, Q.L. and Tan, J.W., 2017. Phenolics from Mikania micrantha and Their Antioxidant Activity. Molecules, 22(7), p.1140.

Fabricant, D.S. and Farnsworth, N.R., 2001. The value of plants used in traditional medicine for drug discovery. Environmental health perspectives, 1(9), p.69.

Fern, K., 1997. Plants for a future: edible & useful plants for a healthier world. Permanent Publications, 34, pp. 321-354.

Freeman, B.A. and Crapo, J.D., 2003. Biology of disease: free radicals and tissue injury. Laboratory investigation; a journal of technical methods and pathology, 47(5), pp.412-426.

Furuta S, Nishiba Y, Suda I 2010 Fluorometric assay for screening antioxidative activities of vegetables. Journal of Food Science, 62, pp. 526–8.

Ghani, A., 2003. Medicinal plants of Bangladesh: chemical constituents and uses. Asiatic society of Bangladesh, 26, pp. 231-243

Gupta, V.K. and Sharma, S.K., 2006. Plants as natural antioxidants 9(12), pp. 589-603

Halliwell, B., 2005. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?. The lancet, 344(8924), pp.721-724.

Hyland, B.P.M., Whiffin, T., Christophel, D.C., Gray, B. and Elick, R.W., 2003. Australian tropical rainforest plants. Trees, Shrubs and Vines. CSIRO, Melbourne.

Jalali Ghassam, B., Ghaffari, H., Prakash, H.S. and Kini, K.R., 2014. Antioxidant and hepatoprotective effects of Solanum xanthocarpum leaf extracts against CCl4-induced liver injury in rats. Pharmaceutical biology, 52(8), pp.1060-1068.

Kaufman PB, Cseke LJ, Warber S, Duke JA, Brielmann HL 2003 Natural Products from Plantns (CRC Press, Boca Raton, FL), pp.263-319

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

Krinsky NI 2002 Mechanism of action of biological antioxidants. Procceding for the Society of Experimental Biology and Medicine, 200, pp. 248–54.

Lin JK, Lin CH, Ling YC, Lin-Shian SY, Juan IM 2011 Survey of catechins, gallic acid and methylxantines in green, oolong, puerh and black teas. Journal of Agriculture and Food Chemistry, 46, pp. 3635–42.

M. Habibur Rahman, M Badrul Alam et al / International Journal of Pharmacology, 2011, 7(2), pp 257 – 262

Mahanta M, Mukherjee AK., 2001, Neutralization of lethality, myotoxicity and toxic enzymes of Naja kaouthia venom by Mimosa pudica root extracts, Journal of Ethnopharmacol; 75, pp. 55-60

Merrill, E.D., 1995. Plant life of the Pacific world. Tuttle Publishing, pp. 134-142

Mithraja, M.J., Irudayaraj, V., Kiruba, S. and Jeeva, S., 2012. Antibacterial efficacy of Drynaria quercifolia (L.) J. Smith (Polypodiaceae) against clinically isolated urinary tract pathogens. Asian Pacific Journal of Tropical Biomedicine, 2(1), pp.S131-S135.

Mukherjee, S. and B Gogoi, J., 2011. Free Radicals in Diseases and Potential Role of Phytoconstituents-A Cause with Emerging Importance. Current Chemical Biology, 5(3), pp.197-212.

Papas AM 2012 Diet and antioxidant status. Food and Chemical Toxicology, Vol. 37, pp. 999–1007.

Paul, R.K., Jabbar, A. and Rashid, M.A., 2000. Antiulcer activity of Mikania cordata. Fitoterapia, 71(6), pp.701-703.

Poongothai, K., Ponmurugan, P., Ahmed, K.S.Z., Kumar, B.S. and Sheriff, S.A., 2011. Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine, 4(10), pp.778-785.

Prasanna, G. and Chitra, M., 2015. In vitro antioxidant activity of Drynaria quercifolia L. rhizome. International Journal of Pharmaceutical Sciences and Research, 6(7), p.3061.

Rahman, Md & Alam, MD Badrul & Chowdhury, Nargis & Jha, Mithilesh & M, Hasan & Khan, Md & Rahman, Md. Saifur & Ekramul Haque, M. 2011. Antioxidant, Analgesic and Toxic Potentiality of Stephania Japonica (Thunb.) Miers. Leaf. International Journal of Pharmacology.

Rahman, M., 2016. Evaluation of Antioxidant Activity of Spilanthes calva, Solanum virginianum, Stevia rebaudiana, Ruellia tuberosa and Phytochemical Investigation of Mikania cordata (Doctoral dissertation, East West University).

Rahman, M.H., Alam, M.B., Hossain, M.S., Jha, M.K. and Islam, A., 2011. Antioxidant, analgesic and toxic potentiality of methanolic extract of stephania japonica (thunb.) miers. leaf. International Journal of Pharmaceutical Sciences and Research, 2(6), p.1588.

Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P. and Lakshmanaperumalsamy, P., 2001. Phytochemical and antimicrobial studies on Drynaria quercifolia. Fitoterapia, 72(8), pp.934-936.

Rao AL, Bharani M, Pallavi V 2006. Role of antioxidants and free radicals in health and disease. Advances in Pharmacology and Toxicology, 7, pp. 29–38.

Rifkin, S.B. and Walt, G., 2015. Why health improves: defining the issues concerning 'comprehensive primary health care'and 'selective primary health care'. Social Science & Medicine, 23(6), pp.559-566.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A., 2013. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry, 1(6), pp. 235-249

Sen, S., Chakraborty, R., De, B. and Mazumder, J., 2009. Plants and phytochemicals for peptic ulcer: An overview. Pharmacognosy Reviews, 3(6), p.270.

Smith, F., 2014. Annals and Magazine of Natural History, Including Zoology, Botany, and Geology (Vol. 9, No. 53). Taylor & Francis.

Talalay, P. and Talalay, P., 2001. The importance of using scientific principles in the development of medicinal agents from plants. Academic Medicine, 76(3), pp.238-247.

Uddin, M.N., Ahmed, N.U., Rahman, M.A., Akter, R. and Akter, R., 2016. Antioxidative Potential of the Polyphenolics of Stephania japonica var. Discolor (Blume) Forman: A Chromatographic (High-Performance Liquid Chromatography) and Spectrophotometric Measure. International journal of food properties, 19(4), pp.911-928.

Uddin, S.N., Amin, M.N., Shahid-Ud-Daula, A.F.M., Hossain, H., Haque, M.M., Rahman, M.S. and Kader, M.A., 2014. Phytochemical screening and study of antioxidant and analgesic

potentials of ethanolic extract of Stephania japonica Linn. Journal of Medicinal Plants Research, 8(37), pp.1127-1133.

Wang H, Cao G, Prior RL 2008 Total antioxidant capacity of fruits. Journal of Agriculture and Food Chemistry, 44, pp. 701–5.

Wink, M., 2008. Ecological roles of alkaloids. Modern alkaloids: structure, isolation, synthesis and biology. Wiley-VCH, Verlag GmbH & Co, pp. 3-24.

Xu, Y., Wang, C., J Klabnik, J. and M O'Donnell, J., 2014. Novel therapeutic targets in depression and anxiety: antioxidants as a candidate treatment. Current neuropharmacology, 12(2), pp.108-119.