Evaluation of Antioxidant activity of *Stephania japonica* and *Mikania cordata*

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CERTIFICATE

This is to certify that, the research work on 'Evaluation of Antioxidant activity of *Stephania japonica* and *Mikania cordata*' submitted to Department of Pharmacy, East West University, Jahurul Islam city, Aftabnagar, Dhaka-1212, in partial fulfilment of therequirements for the degree of Bachelor of Pharmacy (B.Pharm) was carried out by Marita Fyroz (ID No: 2013-1-70-079) under the guidance and supervision and that no 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.

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Dedication

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

Contents

Introduction

1. Introduction

The study of disease and their treatment have existed since the beginning of human civilization. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki R. et al., 1975). From that point of traditional medicine systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. However the value of these systems is much more than a significant anthropologic or archeologic fact. Their value is as a methodology of medicinal agents, which according to the World Health Organization (WHO), almost 65% of the world's population have incorporated into their primary modality of health care (Farnsworth NR. et al., 1985).

Since the ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants' use were instinctive, as is the case with animals (Stojanoski N., 1999). In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. Norman R. Farnsworth of the University of Illinois declared that, for every disease that affect mankind there is a treatment and cure occurring naturally on the earth. Plant kingdom is one of the major search areas for effective works of recent days. The goals of using plants as sources of therapeutic agent are –

1. To isolate bioactive compounds for direct use of drugs, e.g. Digoxin, Digitoxin, Morphine, Reserpine, Taxol, Vinblastin, Vincristine.
2. To produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g. Metformin, Nabilone, Oxycodon (and other narcotic analgesic), Taxotere, Teniposide, Verapamil, and Amiodarone, which are based, respectively, on galegine, Δ^9-tetrahydrocannabinol, Morphine, Taxol, Podophyllotoxin, Khellin, and Khellin.
3. To use agents as pharmacological tools, e.g. lysergic acid diethylamide, mescaline, yohimbina, and,
4. To use the whole plant or part of it as a herbal remedy, e.g. cranberry, echinaceae, feverfew, garlinc, ginko biloba, St. John's wort, saw palmetto.

The importance of plants in search of new drugs is increasing with the advancements of medical sciences.

In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry (i.e. Chemical Medicine) in 16th century, plants had been the source of treatment and prophylaxis (Kelly K., 2009). Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again.

Medicines, the core of health care, cure diseases (such as antibiotics), relieve symptoms (such as analgesics), are preventive (such as anti-hypertension drugs) or substitute for endogenous compounds (such as insulin). The search for medicines, which undoubtedly began in prehistorical times, has led to compounds such as morphine, atropine, tubocurarine, quinine and digoxin. Indeed, many of the present day medicines in the western world have been developed on the basis of traditional medicines with receptors and mechanisms of action identified only recently. This identification of receptors has opened ways of screening for novel, bioactive compounds and to design and subsequently synthesize similar structures (R.J. Bogers et al.)
Ayurvedic medicine is a system of healing that relies heavily on herbs and other plants including oils and common spices. Currently, more than 600 herbal formulas and 250 single plant drugs are included in the "pharmacy" of Ayurvedic treatments. Historically, Ayurvedic medicine has grouped plant compounds into categories according to their effects (for example, healing, promoting vitality, or relieving pain). Ayurvedic medicine (also called Ayurveda) is one of the world's oldest medical systems. It originated in India and has evolved there over thousands of years. In the United States, Ayurvedic medicine is considered complementary and alternative medicine (CAM) more specifically, a CAM whole medical system. Many therapies used in Ayurvedic medicine are also used on their own as CAM for example, herbs, massage, and specialized diets.

Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses. In every period, every successive century from the development of humankind and advanced civilizations, the healing properties of certain medicinal plants were identified, noted, and conveyed to the successive generations. The benefits of one society were passed on to another, which upgraded the old properties, discovered new ones, till present days. The

continuous and perpetual people's interest in medicinal plants has brought about today's modern and sophisticated fashion of their processing and usage (Biljana Bauer Petrovska, 2012)

1.1. Medicinal plants

A considerable number of definitions have been proposed for medicinal plants. 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)

1.2. Phytochemistry

Phytochemistry can be defined as the branch of biochemistry dealing with plants and plant processes. These are chemicals derived from plants. In a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant disease. They also exhibit a number of protective functions for human consumers. Phytochemistry also deals with the identification, biosynthesis and metabolism of chemical constituents of plants. It is the early class of organic chemistry.

1.3. Primary metabolites

Primary metabolites are involved in growth, development and reproduction of the organism. The primary metabolite is typically a key component in maintaining normal physiological processes; thus, it is often referred to as a central metabolite. Primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are deemed essential for proper growth. Primary metabolites consist of various kinds of organic compounds, like carbohydrates, lipids, proteins and nucleic acids. Without primary metabolites key cellular cycles such as glycolysis, the Krebs cycle and the Calvin cycle is not possible, for that every plant kingdom contained those substances. This substance helps plants to take part in synthesis, assimilation and

degradation of organic substances, sucrose and starch, structural components such as cellulose, information molecules such as DNA and RNA and pigments, such as chlorophyll are the main primary metabolite contained by the plants. Although these substances are key for the plant survival they also acts as precursors for the synthesis of secondary metabolites sometimes.

1.4. Secondary metabolites

Secondary metabolites are substances which are produced by plants as defense chemicals. Their absence does not cause bad effects to the plants. They include alkaloids, phenolics, steroids, essential oils, lignins, resins and tannina etc.

Secondary metabolites are compounds bio synthetically derived from primary metabolites. Secondary metabolites or Secondary compounds are compounds that are not required for normal growth and development and are not made through metabolic pathways common to all plants. In plant kingdom they are limited to occurrence and may be restricted to a particular taxonomic group genus, species or family. Secondary metabolites are accumulated by plant cells in smaller qualities than primary metabolites. Secondary metabolites are synthesized in specialized cells at particular developmental stages making extraction and purification difficult.

1.5. Phytochemistry and medicinal plants

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). This is in contrast to primary products, such as carbohydrates, lipids, proteins, heme, chlorophyll, and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman et al., 1999; Wink, 1999).

1.6. Concept of Free radicals and Disease

The knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health and disease management (Aruoma OI., 2003). It is ironic that oxygen, an element indispensable for life, (Mohammed AA., Ibrahim AA., 2004) under certain situations has deleterious effects on the human body (Bagchi K., Puri S., 1998). Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as ROS, which have a tendency to donate oxygen to other substances. Free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms (Aruoma OI., 2003).

1.7. Free Radicals

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants (Cheeseman KH., Slater TF., 1993). The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids (Young IS., Woodside JV., 2001). Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets.

1.8. 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 (Bagchi K., Puri S., 1998). 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 (Liu T. et al., 1999). Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.

Some internally generated sources of free radicals are (Ebadi M., 2001): 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.

1.9. Oxidative Stress

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. It is associated with damage to a wide range of molecular species including lipids, proteins and nucleic acids (Mc Cord JM., 2000). Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g. xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation, producing excess ROS.

The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease (Rao AL. et al., 2006)

1.10. Mechanism 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 (Halliwell B., 1984).

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^-) (Halliwell B, Gutteridge JMC, 1989).

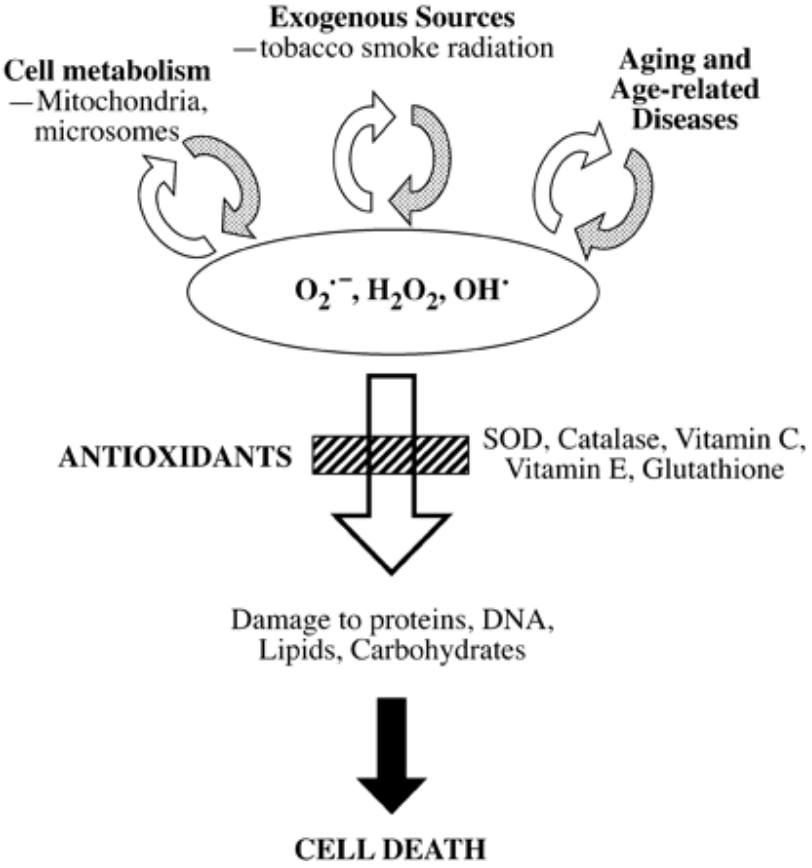


Figure 1.1: Mechanisms of oxidative stress.

One example of this oxidative damage is lipid peroxidation (Gutteridge JMC., 1995). 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.

1.11. Oxidative stress and human diseases

A role of oxidative stress has been postulated in many conditions, including anthersclerosis, inflammatory condition, certain cancers, and the process of aging. Oxidative stress is now

thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematous, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischema), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases and many others. An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and functions.

1.12. Antioxidant

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell B., 1995).

These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the body (Shi HL., 1999). Other lighter antioxidants are found in the diet. Although there is several enzymes system within the body that scavenges free radicals, the principle micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid) and B-carotene (Levine M. et al., 1991). The body cannot manufacture these micronutrients, so they must be supplied in the diet.

1.13. Antioxidant defense system

Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist and metal-chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS (Frie B. et al., 1988).

1.14. Mechanism of action of antioxidants

Two principle mechanisms of action have been proposed for antioxidants (Rice-Evans CA, Diplock AT., 1993). 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 nitrogen 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).

1.15. 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

1.16. 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).

Objective of Study

2. Objective of Study

Since the ancient times, in search for rescue for their disease, the people looked for drugs in nature. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered. Plants had been the source of treatment and prophylaxis. Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again.

The selection of a suitable plant for a pharmacological study is very essential and decisive step. There are different ways to select a suitable plant, including traditional use, chemical content, toxicity, randomized selection or a combination of several criteria. The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; which is usually known as ethnobotany or ethnopharmacology. 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.

Stephania japonica and *Micania cordata* are two plants that are very commonly distributed in Bangladesh. Ethnopharmacological 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 of these two plant samples.

Introduction to Plant

3. Introduction to plant

3.1. *Stephania japonica*

Stephania japonica, known as snake vine, is a common scrambler often seen in sheltered areas near the sea. It is a dioecious vine without prickles. Greenish small flowers form on compound umbels, growing from the leaf axils in the warmer months. Inflorescencesare 4 to 8 cm long. The fruit is an oval shaped, orange or red drupe, 2 to 5 mm long. A feature of this plant is the peltate leaves, (the stem is attached to the leaf, away from the leaf edge).

Figure 2.1: *Stephania japonica***.**

The slender stems can become slightly woody when old, from a woody rootstock. The leaves are sometimes harvested from the wild for medicinal purposes and to make a jelly. Extracts from the leaves have shown mild insecticidal properties against fruit flies in Thailand. The bitter-tasting root is very poisonous due to its picrotoxin content.

Roots, tubers and leaves of the plant contain alkaloids, steroids and fats. Stems contain bis-benzylisoquinoline alkaloids, stephasubine and 3´, 4´-dihydro-stephasubine, saponins, steroids and fats. Roots contain the alkaloids, fangchinoline, dl-tetrandrine, d-tetrandrine and d-isochondrodendrine . Aknadinine, epistephanine, hernandifoline and magnoflorine have been

isolated from aerial parts. Roots and tubers contain alkaloids - aknadinine, aknadine and aknadicine. A new alkaloid-3-O-dimethylhernandifoline also isolated from the plant.

3.1.2. Distribution

A widespread vine has been seen as far south as Eden, New South Wales, north through Queensland. It is also seen in Japan, India Nepal and many other areas of south-east Asia and the Pacific region. The original specimen was collected in Japan, hence the specific epithet "japonica". The variety in New South Wales is known as bicolor, as the under-side of the leaf is somewhat paler than above.

3.1.3. Taxonomy

Domain	: Eukaryota
Kingdom	: Plantae
Subphylum	: Euphyllophytina
Superorder	: Ranunculanae
Infraphylum	: Radiatopses
Class	: Magnoliopsida
Subclass	: Magnoliidae
Order	: Ranunculales
Family	: Menispermaceae
Genus	: *Stephania*
Species	: *Stephania japonica*

Scientific Name: *Stephania japonica.*

3.1.4. Traditional uses

Leaves and roots are bitter and astringent; used in fever, diarrhoea, urinary diseases and dyspepsia. Leaves are mounted on abscess and kept for bursting. Leaves are mecerated in a glass of water and are taken after mixing with molasses to cure urethritis. Leaves are also given for gastritis in Khagrachari. Root paste is taken for vertigo and dysentery; root tuber mixed with root juice of Flemingia stricta is taken for asthma; root paste is warmed and rubbed in hydrocele.

Ethanolic extract of the leaf possesses wide range of good antibacterial and antifungal properties (Dutta, 2006).

3.3. *Mikania cordata*

Mikania cordata is a creeping woody perennial from the family Asteracea. A climber to 8 m or more of regeneration on old farms and in secondary jungle and waste places generally, widespread throughout the Region; probably of S American origin, but now pantropical.

Figure 2.3: *Mikania cordata.*

A fast growing, creeping or twining, perennial vine; stems branched, pubescent to glabrous, ribbed, from 3 to 6 m long; leaves opposite, cordate or triangular-ovate, blade 3 to 12 cm long, 2 to 6 cm wide, on a slender petiole 1 to 8 cm long, base broadly cordate, tip acuminate, margins crenate, dentate, or entire, surfaces nearly glabrous, three- to seven-veined from base; flowers in small heads in open, nearly flat-topped (corymbose) panicles; axillary and terminal heads 6 to 9 mm long, four-flowered; involucral bracts four, obtuse or acute, 5 to 6 mm long, glabrous or subglabrous with one additional smaller bract about 3 mm long; corolla white or yellowish white, about 5 mm long; anthers bluish gray or grayish black; style white; fruit an achene, linear-oblong, 2 to 3 mm long, five angled, blackish brown, glandular; pappus of 40 to 45 bristles, about 4 mm long, white at first, reddish afterwards. It may be distinguished by the following characteristics: 40 to 45 reddish pappus bristles, corollas white, and heads 7 to 7.5 mm long.

3.3.1. Distribution

This plant is widely distributed in tropical region including Southeast Asia and Eastern Africa, but currently invasive in many parts of the world.

3.3.2. Taxonomy

Domain	: Eukaryota
Kingdom	: Plantae
Subkingdom	: Trachoeobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Asteales
Family	: Asteriaceae
Genus	: *Mikania*
Species	: *Mikania cordata*

Scientific Name: *Mikania cordata*

3.3.3. Characteristics

The plant is a notorious invasive vine occurring up to 2000 m elevation. Grows most frequently in places receiving high rainfall, probably 1,500 mm or more; prefers rich, damp soil; rarely grows in dry areas; and thrives in open, disturbed places. For that reason it is common in young secondary forests, in forest clearings, in plantation tree crops, fallow or neglected lands and along rivers and streams, waste areas, steep hillsides and even mountainsides from whence winds probably spread the seeds to new areas. The species will grow in partial shade, but cannot tolerate dense shade. Large amounts of seed transported by the wind or by adhering to human clothing or the hair of animals. Vegetative reproduction can occur from broken stem fragments that may be dislodged and transported by machinery or by rainfall run-off.

3.3.4. Used part

Whole plant, leaves, roots.

3.3.5. Traditional uses

The plant is used as a cover crop to prevent erosion and the leaves are used in some places as a soup vegetable, and can be used as cattle fodder. In southern Nigeria a decoction is given for coughs, and the leaf-juice is a remedy for sore eyes. In Portuguese East Africa, the Tongas use the plant as a remedy for snake and scorpion bite. An infusion of the plant is given in affections of the stomach and intestines. In Malaysia the leaves are used for rubbing on the body against itches. In Java they are used for poulticing the wound of circumcision and other wounds.

Literature Review

4. Literature Review

4.1. *Stephania japonica*

4.1.1. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of Stephania japonica Linn.

The present study was conducted to evaluate the possible phytochemicals present, antioxidant activity and analgesic potential of ethanolic extract of leaves of Stephania japonica (Linn.). For investigating the antioxidant activity, four complementary test systems, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, reducing power assay, Fe++ ion chelating ability and total phenolic content were used. Analgesic activity of the extract was evaluated using acetic acid-induced writhing model of pain in mice. In DPPH free radical scavenging test, IC50 value for ethanolic crude extract was found moderate (18.57 ± 0.079 μg/ml) while compared to the IC50 values of the reference standards ascorbic acid and BHA (1.93 ± 0.027 and 4.10 ± 0.035 μg/ml), respectively. In reducing power assay, the maximum absorbance for ethanolic crude extract was found to be 2.013 ± 0.024 at 100 μg/ml, compared to 2.811 ± 0.013 and 2.031 ± 0.019 for standard ascorbic acid and butylated hydroxyanisole (BHA), respectively. The IC50 value of the extract as % Fe++ ion chelating ability was determined as 18.68 ± 0.029, where ethylenediaminetetraacetic acid (EDTA) showed 8.87 ± 0.035. The total phenolic amount was also calculated as moderate in ethanolic crude extract (237.71 ± 0.57 mg/g of gallic acid equivalent). At the dose of 500 mg/kg body weight, the extract showed significant analgesic potential in acetic acid induced writhing in mice, showing 41.47% inhibition ($P < 0.001$) comparable to that produced by diclofenac Na (45.02%) used as standard drug. These results show that ethanolic extract of leaves of S. japonica (Linn.) has moderate antioxidant and potent analgesic activity. These activities increase with the increase of concentrations. The potency of the extract may be due to the presence of phytochemicals like tannins, flavonoids, phenolics etc.

4.1.2. Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, Stephania japonica and Smilax australis, growing in the understory of an open Eucalyptus forest

Leaves of Stephania japonica and Smilax australis were characterized in situ on the coast of north-eastern New South Wales, Australia, where they were growing naturally in three different

light environments: deep shade, in the understory of an open Eucalyptus forest where they received frequent sunflecks of high intensity, and in an exposed site receiving full sunlight. In deep shade the xanthophyll cycle remained epoxidized during the day and the vast majority of absorbed light was utilized for photosynthesis. In the exposed site both deepoxidation and epoxidation of the xanthophyll cycle and changes in the level of xanthophyll-dependent thermal energy dissipation largely tracked the diurnal changes in photon flux density (PFD). In the understory the xanthophyll cycle became largely deepoxidized to zeaxanthin and antheraxanthin upon exposure of the leaves to the first high intensity sunfleck and this high level of deepoxidation was maintained throughout the day both during and between subsequent sunflecks. In contrast, thermal energy dissipation activity, and the efficiency of photosystem II, fluctuated rapidly in response to the changes in incident PFD. These findings suggest a fine level of control over the engagement of zeaxanthin and antheraxanthin in energy dissipation activity, presumably through rapid changes in thylakoid acidification, such that they became rapidly engaged for photoprotection during the sunflecks and rapidly disengaged upon return to low light when continued engagement might limit carbon gain.

4.1.3. A Survey of Medicinal Plant Usage by Folk Medicinal Practitioners in Seven Villages Of Ishwardi Upazilla, Pabna District, Bangladesh

Folk medicinal practitioners (Kavirajes) are the primary health-care providers to substantial segments of the rural population as well as the urban population of Bangladesh. Every village of Bangladesh has at least one practicing Kaviraj. The Kavirajes rely primarily on simple formulations of medicinal plants for treatment of ailments. While overall, simple ailments are treated by the Kavirajes, occasionally complicated ailments, which are hard to cure with allopathic medicine, are also treated by them. In previous ethnomedicinal surveys, we have observed considerable variation in the use of medicinal plants by the Kavirajes of different regions of Bangladesh, which extended to Kavirajes of even the same village or adjoining villages. To get a comprehensive picture of the medicinal plants used by the Kavirajes, it is therefore necessary to conduct surveys of individual villages. The objective of the present study was to conduct a survey among Kavirajes of seven villages in Ishwardi Upazilla (sub-district), which is in Pabna district of Bangladesh. A total of 80 plants distributed into 45 families were observed to be used by the Kavirajes. The Euphorbiaceae and the the Lamiaceae family

contributed 7 plants per family, followed by the Apocynaceae family with 5 plants, and the Araceae, Asteraceae, Combretaceae, Menispermaceae, and Solanaceae family with 3 plants each. The Kavirajes used both whole plant as well as plant parts for treatment of ailments. Leaves constituted 35.1% of the total uses, followed by roots at 17.5%, and barks and fruits at 11.4% each. Twenty one plants were used for treatment of gastrointestinal disorders like constipation, dysentery, loss of appetite, and acidity. Thirteen plants were used to treat skin disorders like eczema, pimples, and itches, while twelve plants were used for treatment of respiratory tract disorders like asthma, coughs, and colds. The Kavirajes also treated hepatic disorders (e.g. jaundice), sexual disorders, pain, fever, bleeding from cuts and wounds, bone fractures, eye disorders, ear problems, toothache, loss of hair, hemorrhoids, gonorrhea, infections, physical weakness, helminthiasis, leprosy, vomiting, snake bite, gall bladder stones, burns, chicken pox, malaria, rheumatic fever, diphtheria, anemia, rheumatism, menstrual problems, urinary problems, and physical weakness. Other complicated diseases treated by the Kavirajes, included diabetes, hypertension, heart disorders, tumors, malnutrition of fetus, and leukemia. The medicinal plants used by the Kavirajes can form a rich source of plants for further scientific studies leading to discovery of novel therapeutic compounds.

4.1.4. Evaluation of antinociceptive activity of methanolic extract of leaves of Stephania japonica Linn

Stephania japonica is a common plant, widely distributed in all over Bangladesh. Traditionally, this plant is considered as one of the important ingredients in treatment of a variety of ailments including inflammation, pain, rheumatism, cancer, bone fracture, fever etc. However, the scientific reports regarding the antinociceptive effect of this plant are very limited. This study evaluated the antinociceptive effect of methanolic extract of S. japonica (MESJ) leaves.

4.1.5. Anti-Inflammatory, Antioxidant and Anti-Diarrheal Effects of Ethanol Extract of Stephania Japonica

The present study was carried out to investigate anti-inflammatory, antioxidant and anti-diarrheal effect of ethanol extract of Stephania japonica. This study showed that the plant extract has significant ($p<0.05$) anti-inflammatory effect at all phases of carrageenan induced inflammation at a dose level 2g/kg. The DPPH free radical scavenging effect of the extract was compared with

standard antioxidant ascorbic acid. IC50 values were found 33.57 µg/ml for the extract and 15.57 µg/ml for ascorbic acid. S. japonica extract at dosage level 2g/kg and 1g/kg decreased the gastrointestinal motility 36.56 and 21.53 %, respectively, in rats. The ethanol extract of the plant also reduced the total number of feces as well as wet feces of rats in castor oil-induced diarrheal model. The results revealed that the extract possesses promising anti-inflammatory, antioxidant and antidiarrheal activity.

4.1.6. Antioxidative Potential of the Polyphenolics of Stephania japonica var. Discolor (Blume) Forman: A Chromatographic (High-Performance Liquid Chromatography) and Spectrophotometric Measure

This study investigated the quantitative phytochemical contents, total phenolics, total flavonoids, total carotenoids, antioxidative capacity, tannin, proanthocyanidins, lycopene, chlorophyll a, and chlorophyll b of the Stephania japonica extract. Comprehensive antioxidative effects of the extract were also investigated. Quantitative assays were conducted through both spectrophotometric and high-performance liquid chromatographic methods. Antioxidative effects were measured through FeCl3 reducing power, metal chelating power, reducing power, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging activity, N, N-dimethyl-1,4-diaminobenzene free radical scavenging activity, superoxide radical scavenging activity, and nitric oxide scavenging effect. The contents of total phenolics, total flavonoids, total carotenoids, total antioxidative capacity, tannin, proanthocyanidins, lycopene, chlorophyll a, and chlorophyll b were found to be 47.32 ± 0.75 mg tannic acid equivalent, 61.41 ± 1.58 mg catechin equivalent, 63.29 ± 2.21 mg, 22.85 ± 0.70 mg ascorbic acid equivalent, 76.17 ± 0.97 mg tannic acid equivalent, 94.96 ± 4.49 mg catechin equivalent, 22.19 ± 0.79 µg, 22.19 ± 0.79 µg, 7.52 ± 1.24 mg, and 10.43 ± 2.11 mg, respectively, in 1 g of ethanol extract. A high concentration of epicatechin, p-coumaric acid, and rutin hydrate and moderate concentration of caffeic acid and quercetin was detected in the extract. The IC50 value for ferric reducing power assay, metal chelating assay, reducing power assay, ABTS scavenging assay, N, N-dimethyl-1,4-diaminobenzene scavenging assay, superoxide scavenging assay and nitric oxide (NO) assay were 465.06 ± 7.32 µmol ascorbic acid/g, 1656.52, 270.55, 457.27, 632.74, 217.5, and 464.00 µg/mL, respectively. No beta carotene was detected in the extract. The extract was demonstrated to be a very potential source of antioxidative metabolites.

4.1.7. A Randomized Survey of Medicinal Plants used by Folk Medicinal Healers of Sylhet Division, Bangladesh

Sylhet division lies in the north-eastern corner of Bangladesh and comprises of four districts – Sylhet, Habiganj, Sunamganj, and Moulvibazar. The division contains a diversity of floral species, some of which are quite distinct from the rest of the country. A randomized ethnomedicinal survey was conducted among the folk medicinal practitioners of Komolganj in Moulvibazar district, Gulapganj of Sylhet district, and Chunarughat of Habiganj district. Informed consent was obtained from the healers and the survey was conducted with the help of a semi-structured questionnaire. In the present survey, the methodology employed was that of the guided field-walk, where the healers took the interviewers to localities from where they collected their medicinal plants and pointed out the plants besides describing the plant parts used and the ailments that they were used for. Plant specimens were collected from the field, dried in situ and identification completed at the Bangladesh National Herbarium. Information on 107 plant species distributed into 53 families was obtained. The Asteraceae family contributed the largest number of plant species (seven) followed by the Euphorbiaceae, Fabaceae and Rutaceae families (six each). Leaves comprised the major plant part used for the treatment of different ailments (48.3%) followed by fruit (15.9%) and bark (10.3%). Most plants were used to treat common ailments like gastrointestinal disorders, helminthiasis, debility, pain, skin problems, respiratory problems, fever, bleeding from cuts and wounds, urinary tract problems and sexual disorders. However, a number of plants were also used to treat more complicated ailments like cardiovascular disorders, hepatic disorders, epilepsy and cancer or tumors. In the majority of cases, a single plant part was used for treatment of any given ailment. Folk medicine in Bangladesh has a history of usage going back thousands of years. The medicinal plants used by the folk medicinal healers thus possess considerable potential for discovery of lead compounds or novel compounds that may serve as the source of effective modern drugs.

4.1.8. SCREENING OF PHYTOCHEMICALS WITH RESPECT TO ANTIOXIDANT PROPERTIES OF CERTAIN ETHNOMEDICINALLY IMPORTANT PLANTS FROM N.E. INDIA.

The demand of herb base pharmaceuticals is increasing day-by-day and hence herbs can be a better option for the replacement of synthetic drugs. Worldwide trend towards the use of herbs for remedies has created tremendous opportunities for pharmacological application. Medicinal plant, as natural remedies against different physiological ailment, plays a vital role with their active novel compounds in pharmaceutical industries. The ethnic people use a wide variety of wild plants and plant products as their food and to cure different ailment in their traditional health care system. India has one of the largest concentrations of tribal population in the world and North East India, especially Assam is a treasure of different ethnic groups with their own unique system of indigenous knowledge, most of which has remained unexplored. Traditionally used plant species, for healing different physiological ailment, like Cajanus cajan, Costus speciosus and Stephania japonica were screened for their phto-chemical investigation in the present study. Traditionally, Cajanus cajan (L.) Millsp., family Fabaceae, leaves have been used by Rabha tribe to cure Jaundice and also described as useful for the treatment of small pox, chicken pox, diuretic, haemostatic, astringent, measles and mouth wash by local people. Costus speciosus (Koenig) Sm., family Costaceae, is also used by tribal people to treat against Jaundice as it has hepatoprotective properties. Stephania japonica L., family Menispermeaceae, is traditionally used as medicinal herbs for the treatment of asthma, tuberculosis, dysentery, hyperglycaemia, fever, intestinal complaints, sleep disturbance, inflammation etc. in the present investigation, total phenolic compound, ascorbic acid, flavannol content and antioxidant activity were studied for these three herbs. It was observed that total phenolic content and antioxidant activity and ascorbic acid content were higher in C. specious than rest two species. However, flavannol content was higher in C. cajan than the rest.

4.1.9. ANTIOXIDANT, ANALGESIC AND TOXIC POTENTIALITY OF METHANOLIC EXTRACT OF STEPHANIA JAPONICA (THUNB.) MIERS. LEAF

In the present study crude methanolic extract of Stephania japonica leaf was investigated for possible antioxidant, analgesic and cytotoxic activity. The extract showed antioxidant activity in DPPH radical scavenging activity, nitric oxide scavenging activity and reducing power assays. In both DPPH radical and NO scavenging assay, the extract exhibited moderate antioxidant activity and the IC50 values in DPPH radical scavenging and NO scavenging assays were found to be 105.55 ± 1.06 μg/ml and 129.12 ± 0.15 μg/ml, respectively while the IC50 values of ascorbic

acid were 12.30 ± 0.11 μg/ml and 18.64 ± 0.22 μg/ml, respectively. Reducing power activity of the extract increased in a dose dependent manner. Analgesic activity of the crude extract was evaluated using acetic acid-induced writhing model of pain in mice. The crude extract at 200 mg/kg and 400 mg/kg b.w. doses displayed significant ($p < 0.001$) reduction in acetic acid induced writhing in mice with a maximum effect of 75.89 % reduction at 400 mg/kg b.w. which is comparable to the standard, diclofenac sodium (86.52 %). The extract was also investigated for toxic potentiality using Brine Shrimp lethality bioassay. In this bioassay the extract showed significant toxicity to Brine Shrimp nauplii with the LC50 value of 25.19 ± 0.98 μg/ml.

4.1.10. Antioxidant, Analgesic and Toxic Potentiality of Stephania Japonica (Thunb.) Miers. Leaf

In the present study crude methanolic extract of Stephania japonica leaf was investigated for possible antioxidant, analgesic and toxic activity. The extract showed antioxidant activity in DPPH radical scavenging activity, nitric oxide scavenging activity and reducing power assays. In both DPPH radical and NO scavenging assay, the extract exhibited moderate antioxidant activity and the IC50 values in DPPH radical scavenging and NO scavenging assays were found to be 105.55±1.06 and 129.12±0.15 μg mL-1, respectively while the IC50 values of ascorbic acid were 12.30±0.11 and 18.64±0.22 μg mL-1, respectively. Reducing power activity of the extract increased in a dose dependent manner. Analgesic activity of the crude extract was evaluated using acetic acid-induced writhing model of pain in mice. The crude extract at 200 and 400 mg kg-1 b.wt. doses displayed significant ($p<0.001$) reduction in acetic acid induced writhing in mice with a maximum effect of 75.89% reduction at 400 mg kg-1 b.wt. which is comparable to the standard, diclofenac sodium (86.52%). The extract was also investigated for toxic potentiality using Brine Shrimp lethality bioassay. In this bioassay the extract showed significant toxicity to Brine Shrimp nauplii with the LC50 value of 25.19±0.98 μg mL-1. The study clearly indicates that the extract possesses good analgesic and cytotoxic activity along with moderate antioxidant potential.

3.3 *Mikania cordata*

Gastroprotective effects of flavonoids in plant extracts

The purpose of this paper is to overview the relations between plant-originated substances and their bioactivity measured in terms of antioxidant, cytoprotective and antiulcer activities. In addition, we assessed whether these compounds are capable of affecting the gastric mucosal lesions induced by absolute ethanol applied intragastrically (i.g.). The following plant-originated flavonoid substances were considered; Solon (Sophoradin extract), Amaranth seed extract, grapefruit-seed extract (GSE) and capsaicin (extract of chilly pepper). The area of gastric mucosa lesions and gastric blood flow were measured in rats with ethanol-induced lesions without (control) and with one of the tested substances without and with capsaicin denervation of afferent nerves or administration of L-nitro-arginine (L-NNA), an inhibitor of nitric oxide synthase (NOS). Male Wistar rats, weighing 180-220 g fasted for 24 h before the study where used 100% ethanol was applied i.g. to induce gastric lesions, whose area was determined by planimetry. Gastric blood flow was assessed using electrolytic regional blood flowmeter. All tested plant-originated substances afforded gastroprotection against ethanol-induced damage and this was accompanied by increase in gastric microcirculation, both changes being reversed by pretreatment with neurotoxic dose of capsaicin or by pretreatment with L-NNA. We conclude that plant-originated flavonoid substances are highly gastroprotective probably due to enhancement of the expression of constitutive NOS and release of NO and neuropeptides such as calcitonin gene related peptide (CGRP) released from sensory afferent nerves increasing gastric microcirculation.

Full Length Research Paper

Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves

The ethanol extract of the dried leaves of Mikania cordata (Family-Asteraceae) was investigated for its possible bioactive chemical groups and antinociceptive, cytotoxic and antibacterial activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 125 and 250 mg/kg body weight ($p<0.001$) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The

crude extract produced the moderate cytotoxic activity against brine shrimp Artemia salina(LC50=90 and LC90=166 µg/ml). The extract showed antibacterial activity against some types of microorganisms upon which the extract was employed. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

A Survey of Medicinal Plants Used by Kavirajes of Barisal Town in Barisal District, Bangladesh

A substantial section of the population of Bangladesh is poor and more than a third of the total population of 150 million people lives below the poverty line (i.e. having a daily income of less than US$ 1 per day). The poorer section of the population resides mostly in the rural areas and the urban slums. The rural population and population in small towns in addition suffer from proper access to health-care facilities and are not always in a position to afford the costs of allopathic treatment. They therefore rely on folk medicinal practitioners otherwise known as Kavirajes for treatment of their various ailments. The Kavirajes rely on administration of medicinal plants either orally or topically for treatment of diseases. Each Kaviraj has his unique repertoire of medicinal plants, which is closely guarded and usually passed onto an immediate member of the family in the successive generation. The objective of the present study was to conduct a survey on medicinal plant usage among selected Kavirajes of Barisal town in Barisal district, Bangladesh. Interviews were conducted with the help of a semistructured questionnaire and the guided field-walk method. Information was obtained as to the local name of plants, parts used, formulations and dosages. It was found that the interviewed Kavirajes used 49 plants distributed into 28 families in their treatment of various ailments. The Asteraceae, Fabaceae, Lamiaceae, and Moraceae families contributed 4 plans each, while the Solanaceae and the Verbenaceae family contributed 3 plants per family. The various plant parts used by the Kavirajes in their formulations included whole plant, leaf, stem, root, bark, flower, fruit, seed, and sap. Leaves constituted the major plant part used (34.8% of total uses), followed by roots (15.2%). Gastrointestinal disorders (stomach ache, constipation, dysentery, and diarrhea) formed the major group of ailments treated by the Kavirajes and a total of 11 plants were used to treat these ailments. Eight plants were used to treat skin disorders, 7 plants for pain relief, and 6 plants for respiratory tract disorders like coughs and mucus. Other ailments treated by the Kavirajes included urinary tract problems, cuts and wounds, meho (a term used by the Kavirajes to indicate urinary problem arising from endocrinological disorders or diabetes), fever, skin disorders,

malaria, rheumatism, dog and snake bites, hepatic disorders (jaundice, enlarged liver), tooth infections, eye problems, heart disorders, diabetes, hydrocele, goiter, helminthiasis, menstrual problems, and fractures. Plants have always formed a rich source of modern drugs. The medicinal plants used by the Kavirajes need to be scientifically studied for phytochemical constituents and pharmacological activities towards discovery of lead compounds and more efficacious newer drugs.

Materials and Method

5. Materials and method

Four plant samples were selected to conduct this thesis work; these are *Solanum virginianum*, *Stephania japonica*, *Micania cordata* and *Drynaria quercifolia*. All four sample plants are subjected to antioxidant test.

5.1 Materials

5.1.1. 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 and measuring cylinders.

5.1.2. Lists of other material

Aluminium foil paper, spatula, pipette pumper, micropipette tip, labels and masking tape, permanent marking pen, tissue paper.

5.1.3. Lists of equipments

UV-Visible Spectrophotometer, hot air oven, centrifuge machine, electric balance, rough balance and sonicator.

5.1.4 Lists of Solvents

Methanol and ethanol

5.1.5 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.

5.1.6. Lists of plant sample

1. *Solanum virginianum*
2. *Stephania japonica*
3. *Micania cordata*
4. *Drynaria quercifolia*.

5.2. Study Protocol

Our present study was designed to evaluate antioxidant property of different extracts of Stephania *japonica, Solanum virginianum, Micania cordata* and *Drynaria quercifolia*. The study protocol consisted of the following steps:

- Collection of different extracts of the four plant samples.
- Performing phenolic content test of the extracts.
- Performing DPPH radical scavenging assay of the extracts
- Performing reducing power assay of the extracts.

5.4. Total phenolic content assay

5.4.1. 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 occurrences of human diseases.
In addition, antioxidant compounds which are responsible for such antioxidant activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders.

5.4.2. 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.

5.4.3. 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

5.4.4. 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.

5.4.5. 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.

5.4.6. 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

5.5. DPPH radical-scavenging activities

5.5.1. 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.

5.5.2. 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.

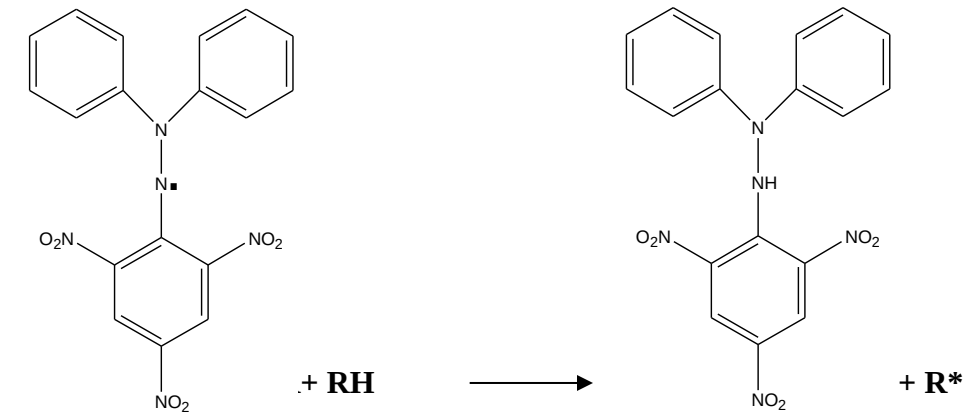


Figure 5.5.1.: Reaction of DPPH with antioxidant.

5.5.3. 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.

5.5.3.1. 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.

5.5.3.2. 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.

5.5.3.3. 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.

5.5.3.4. 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.

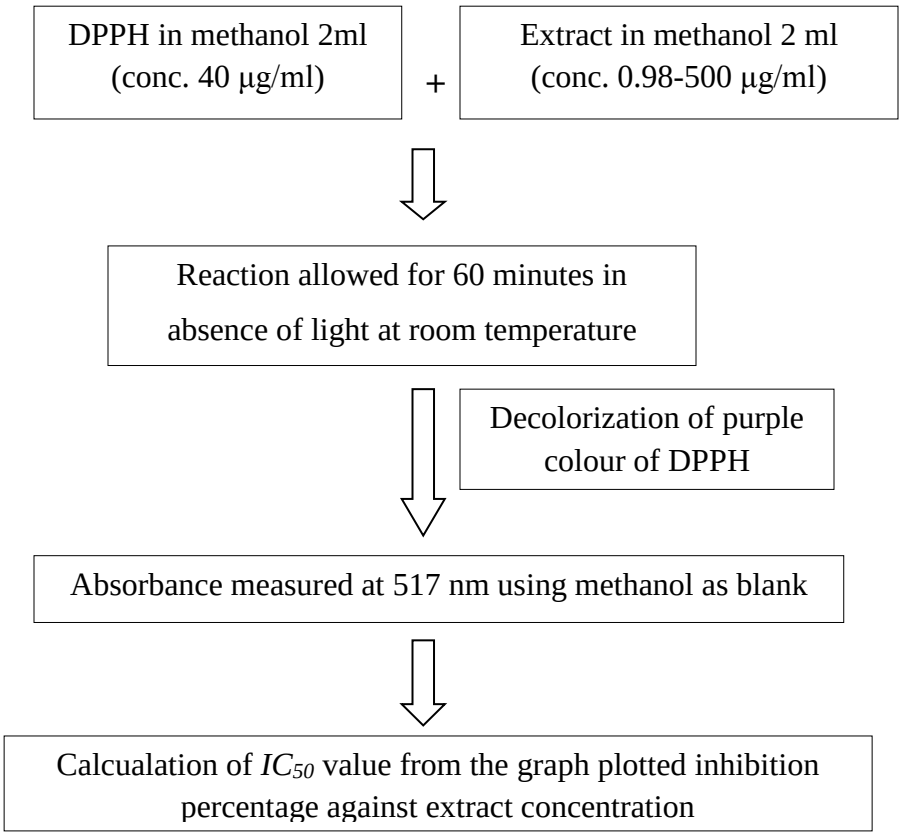


Figure 5.5.2.: Schematic representation of the method of assaying free radical scavenging activity.

5.6. Reducing Power Assay

5.6.1. Introduction

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reacti ve, oxygen-
containing molecules. Types of ROS include the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid er oxides. These free radicals may either be produced by physiological or biochemical processes or by pollution and other endogenous sources. All these free radicals are capable of reacting with m embrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellul ar 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.

5.6.2. 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.

$$\text{Potassium ferricyanide} + \text{Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide} + \text{ferrous chloride}$$

5.6.3. 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.

5.6.3.1. 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.

5.6.3.2. 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.

5.6.3.3. 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.

Results and Discussion

Total Phenolic Content Assay

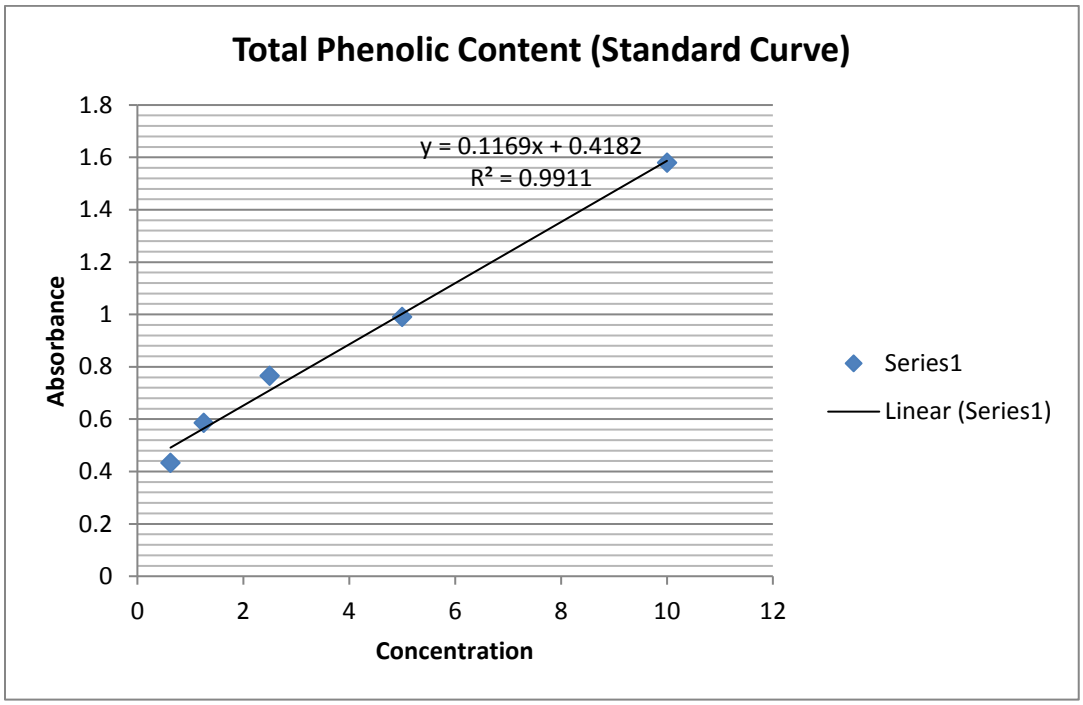
The Methanolic extract of *Stephania japonica* and Ethanolic extract of *Mikania cordata* were subjected to Total Phenolic Content Assay by the method described by (Arpona et al., 2013). Here, Total Phenolic Content was measured as Salicylic acid equivalence.

Standard Curve Preparation by Using Salicylic Acid

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

]

Total Phenolic Content (Standard Curve)



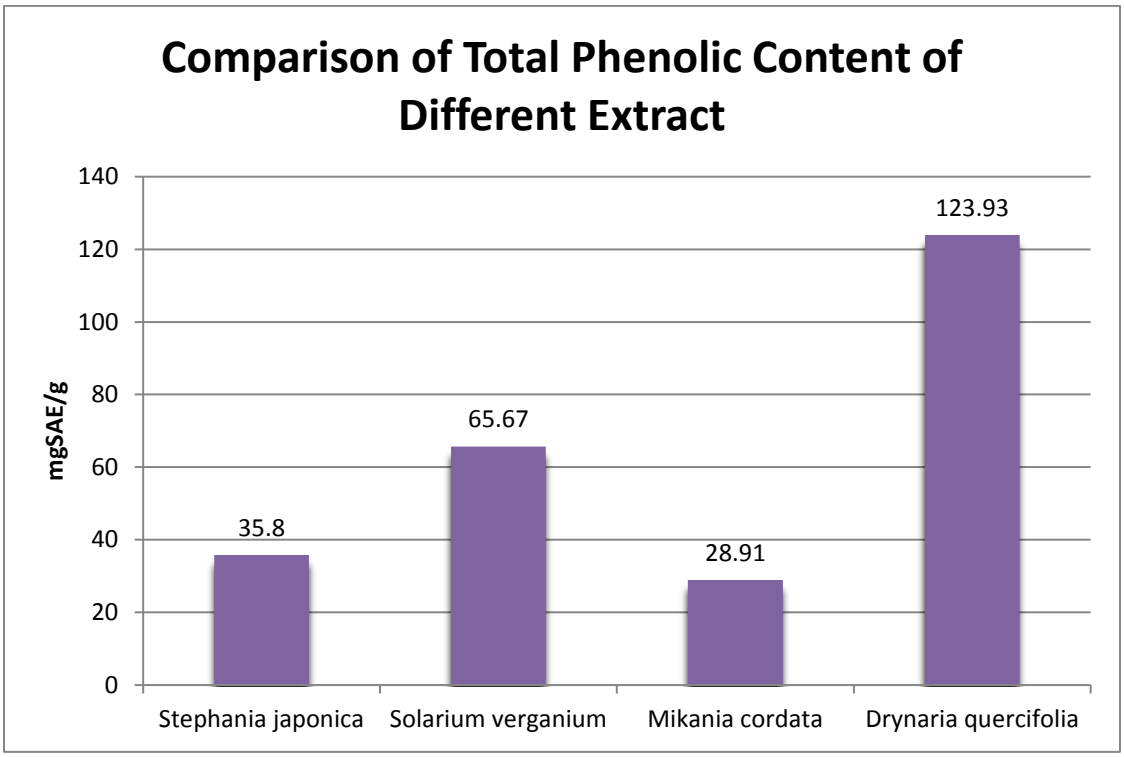
Total Phenolic Content of *Stephania japonica*

Absorbance	mgSAE/g	Mean±SD
0.834	35.86	35.80±0.18
0.835	35.94	
0.831	35.60	

Total Phenolic Content of *Mikania cordata*

Absorbance	mgSAE/g	Mean±SD
0.752	28.79	28.91±0.27
0.757	29.22	
0.751	28.71	

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 *Solarium verganium* (65.67±0.43 mgSAE/g). Methanolic extract of *Stephania japonica* have less amount of phenolic content (35.80±0.18 mgSAE/g) than *Solarium verganium* (65.67±0.43 mgSAE/g). Ethanolic extract of *Mikania cordata* (28.91±0.27 mgSAE/g) have the least amount of phenolic content among all four extracts.

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.

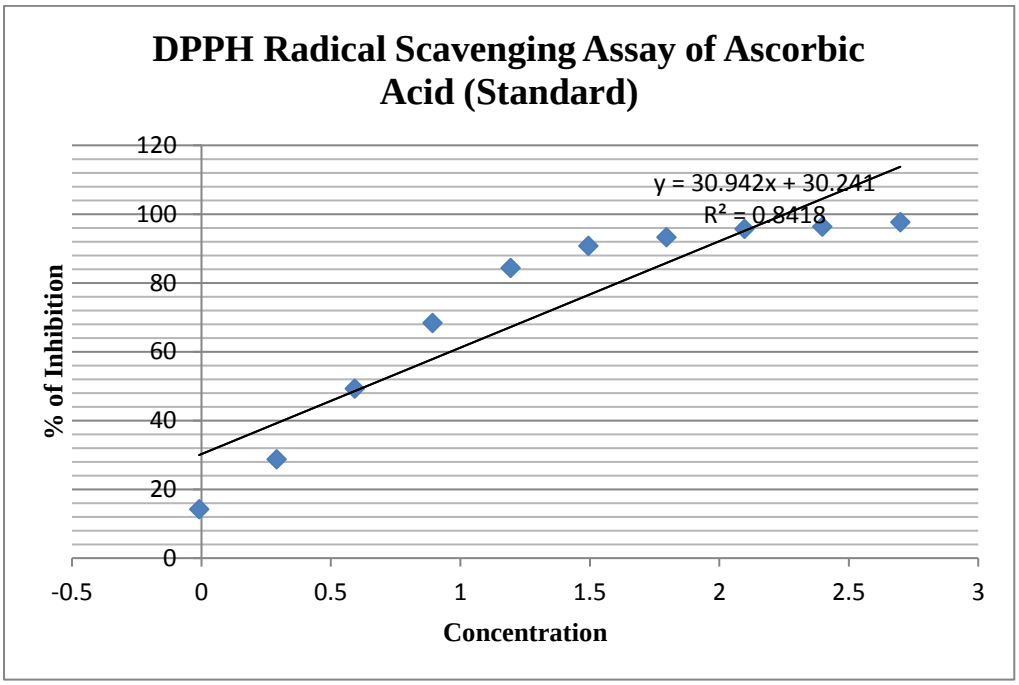
DPPH Radical Scavenging Assay

The methanolic extract of *Stephania japonica and* ethanolic extract of *Mikania cordata* 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)
	0.98	- 0.009	0.536	14.24	
	1.95	0.290	0.508	18.72	
	3.91	0.592	0.365	41.60	
	7.81	0.893	0.198	68.32	
0.625	15.63	1.194	0.098	84.32	4.35
	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	

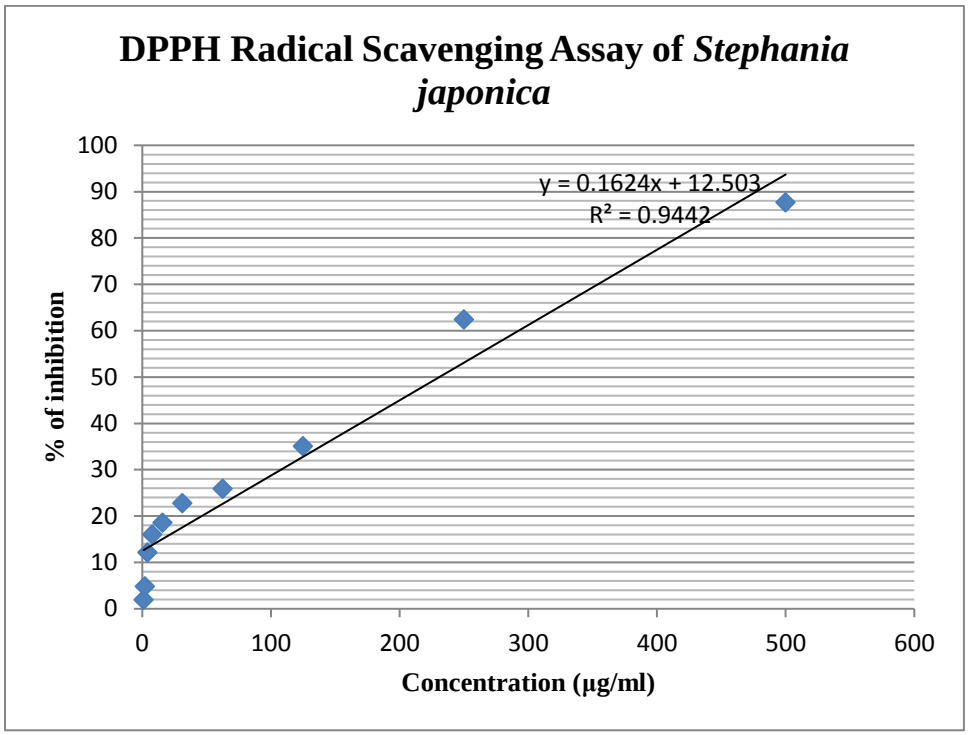
DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

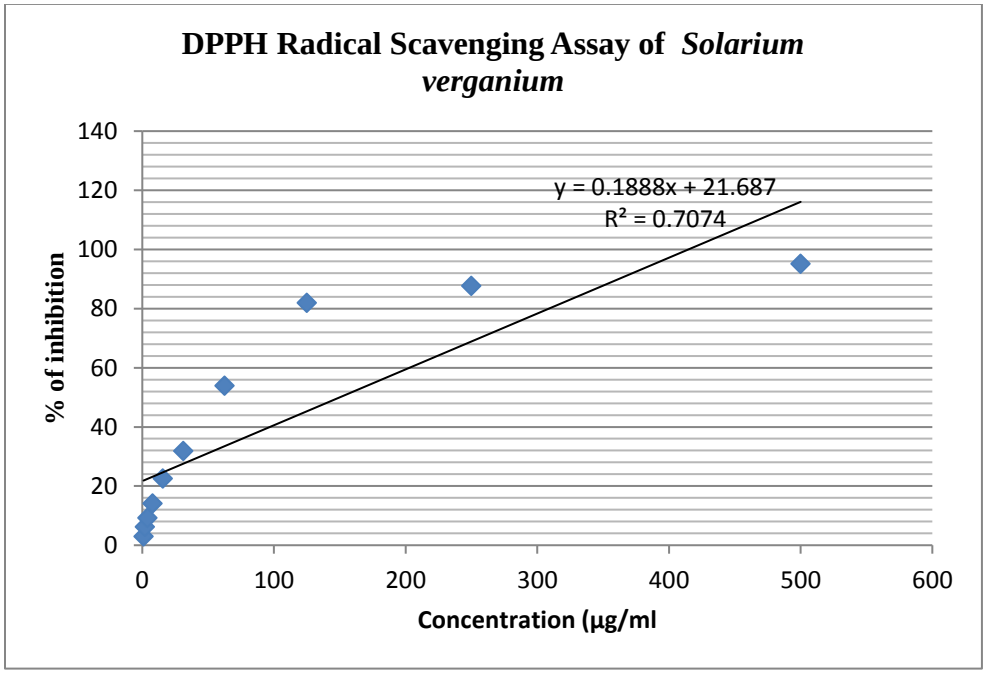


DPPH Radical Scavenging Assay of *Stephania japonica*

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.625	0.98	0.613	1.92	231.48
	1.95	0.595	4.80	
	3.91	0.549	12.16	
	7.81	0.525	16.00	
	15.63	0.509	18.56	
	31.25	0.470	24.80	
	62.5	0.463	25.92	
	125	0.406	35.04	
	250	0.235	62.40	
	500	0.077	87.68	

DPPH Radical Scavenging Assay of *Stephania japonica*

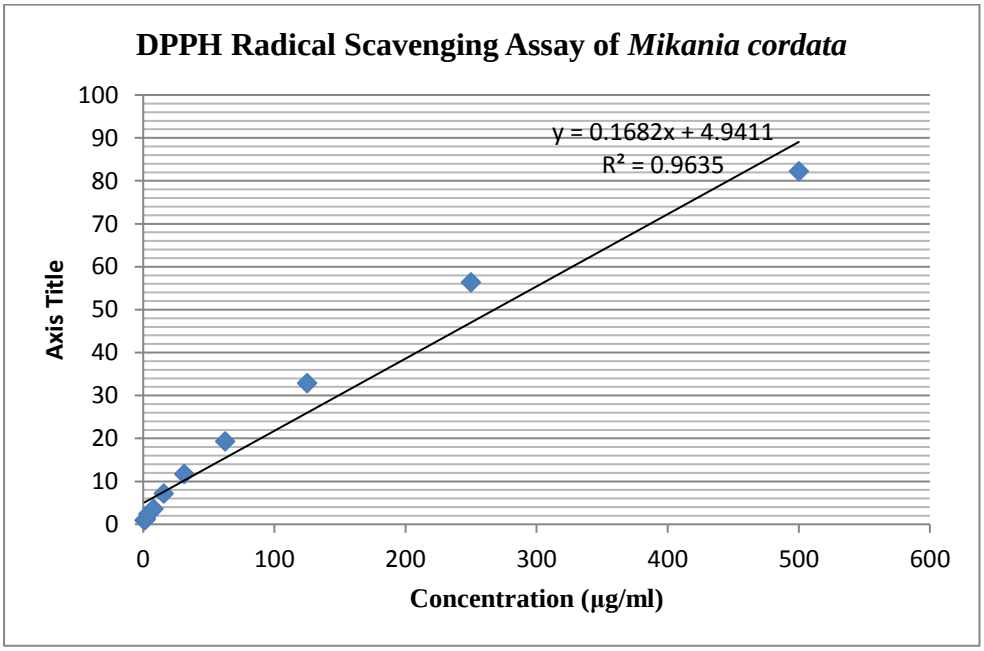




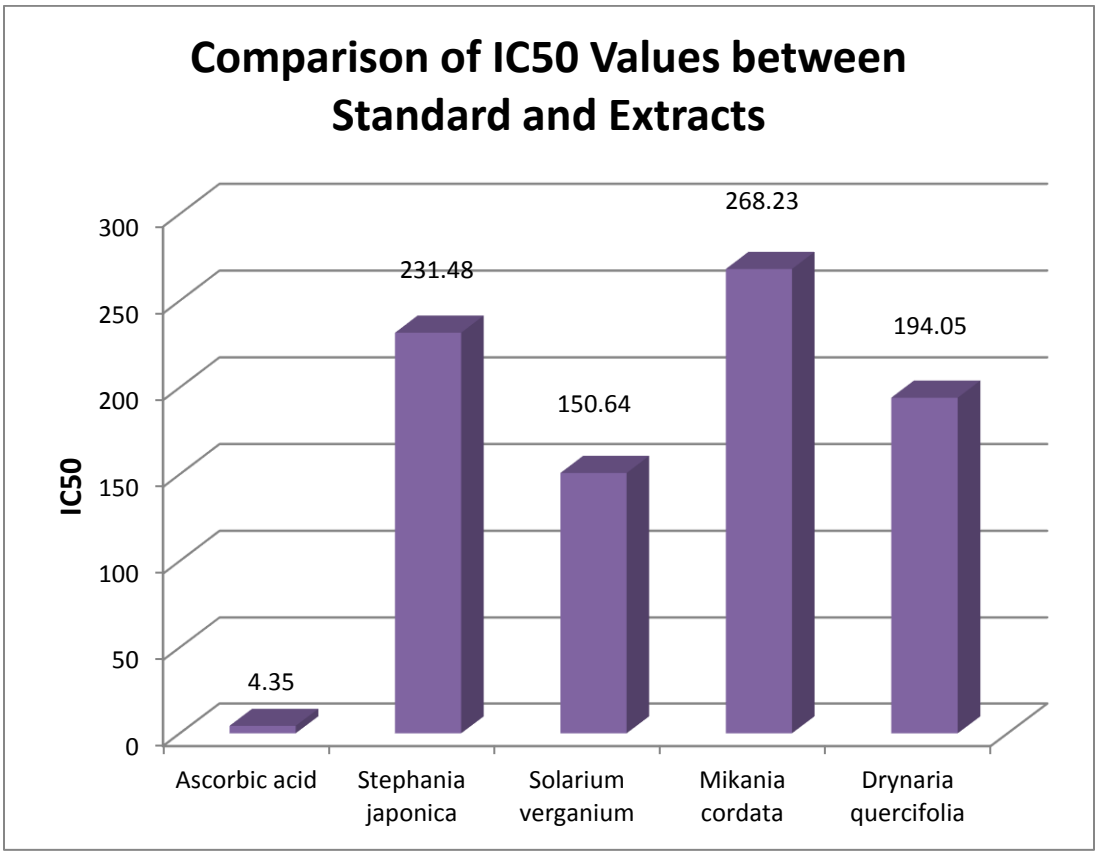
DPPH Radical Scavenging Assay of *Mikania cordata.*

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.675	0.98	0.669	0.89	268.23
	1.95	0.667	1.19	
	3.91	0.659	2.37	
	7.81	0.651	3.56	
	15.63	0.627	7.11	
	31.25	0.596	11.70	
	62.5	0.545	19.26	
	125	0.453	32.89	
	250	0.295	56.30	
	500	0.120	82.22	

DPPH Radical Scavenging Assay of *Mikania cordata*



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). DPPH radical activity of methanolic extract of *Stephania japonica* (IC_{50} 231.48µg/ml) is moderate and highest activity is observed for ethanolic extract of *Mikania cordata* (IC_{50} 268.23µg/ml).

Reducing Power Assay

The Methanolic extract of *Stephania japonica*, *Solarium verganium* and Ethanolic extract of *Mikania cordata*, *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**	**Absorbance**			**Mean±SD**
	µg/ml	**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

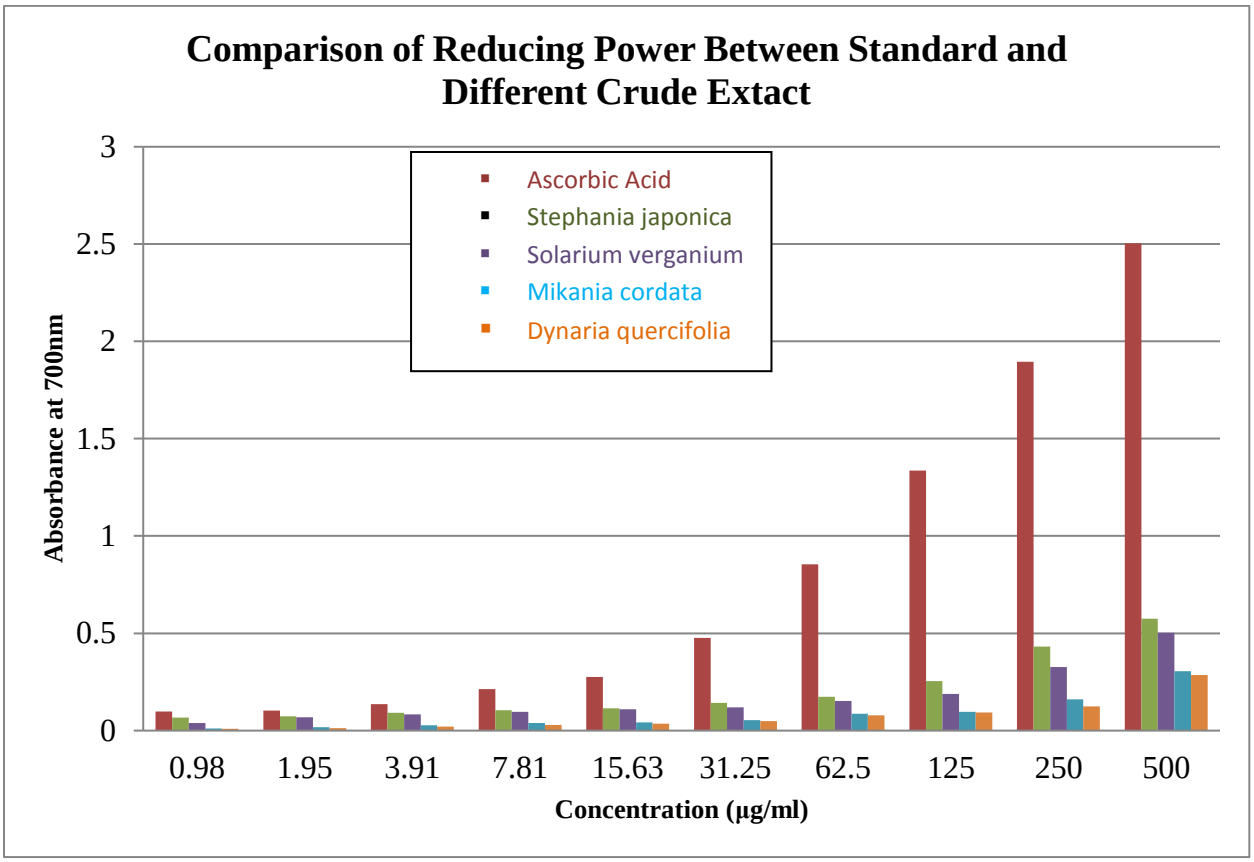
Reducing Power Assay of *Stephania japonica*

SL.	Concentration µg/ml	Absorbance			Mean±SD
		1	**2**	**3**	
1	0.98	0.067	0.065	0.068	0.067±0.002
2	1.95	0.074	0.075	0.073	0.074±0.001
3	3.91	0.090	0.087	0.091	0.092±0.002
4	7.81	0.101	0.105	0.107	0.104±0.003
5	15.63	0.115	0.113	0.119	0.115±0.003
6	31.25	0.145	0.143	0.139	0.142±0.003
7	62.5	0.174	0.174	0.173	0.174±0.001
8	125	0.253	0.255	0.257	0.255±0.002
9	250	0.429	0.435	0.431	0.432±0.003
10	500	0.578	0.575	0.573	0.575±0.003

Reducing Power Assay of *Mikania cordata*

SL.	Concentration µg/ml	Absorbance			Mean±SD
		1	**2**	**3**	
1	0.98	0.011	0.010	0.011	0.011±0.001
2	1.95	0.019	0.018	0.017	0.018±0.002
3	3.91	0.027	0.025	0.031	0.028±0.003
4	7.81	0.041	0.037	0.039	0.039±0.002
5	15.63	0.042	0.043	0.045	0.043±0.002
6	31.25	0.054	0.053	0.052	0.053±0.001
7	62.5	0.086	0.087	0.085	0.086±0.001
8	125	0.097	0.097	0.095	0.097±0.001
9	250	0.141	0.142	0.141	0.161±0.001
10	500	0.306	0.306	0.305	0.306±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 *Stephania japonica* is showing highest level of reducing power followed by *Solarium verganium, Mikania cordata and Drynaria quercfolia*.

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 the methanolic extracts of *Stephania japonica, Solarium verganium* and ethanolic extracts of *Mikania cordata, Drynaria quercifolia.*

Conclusion

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.

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