ANTIOXIDANT ACTIVITY OF SELECTED FRESH GREEN LEAFY VEGETABLES CULTIVATED IN BASANTATAR, DHARAN

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Antioxidant Activity of Selected Fresh Green Leafy Vegetables Cultivated in Basantatar, Dharan

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in the partial fulfillment of the requirements for the Bachelor degree in Food Technology

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Approval Letter

This *dissertation* entitled *Antioxidant Activity of Selected Fresh Green Leafy Vegetables Cultivated in Basantatar, Dharan* presented by Sudip Bhattarai has been accepted as the partial fulfillment of the requirements for the degree of Bachelor of Food Technology.

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Abstract

Fresh green leafy vegetables *Brassica juncea* (Broad leaf Mustard), *Chinopodium album* (Lamb's quarter), *Trigonella foenum graecum* (Fenugreek), *Anethum sowa* (Dill greens) *and Amaranthus tricolor* (Red Amaranth) were collected from Basantatar, Dharan, Sunsari district Nepal and were washed with distilled water, fresh leaves of plant were extracted in 99% methanol to carry out the antioxidant assays. The antioxidant assay was determined by three different parameters namely total antioxidant capacity, reducing power and DPPH scavenging assay.

The total antioxidant capacity (TAC) of *B. juncea, C. album, T. foenum graecum, A. sowa* and *A. tricolor* were found to be 33.55±0.65, 38.78±0.35, 40.41±0.32, 50.87±0.28 and 36.53±0.73 mg AAE/100 g Fresh weight respectively. Similarly, the reducing power of *B. juncea, C. album, T. foenum graecum, A. sowa* and *A. tricolor* were found to be 19.38±0.05, 18.28±0.08, 18.06±0.12, 41.02±0.65 and 19.06±0.13 mg AAE/100 g respectively. Finally, the DPPH scavenging activity of *B. juncea, C. album, T. foenum graecum, A. sowa* and *A. tricolor* were found to be 21.85±0.61%, 26.97±0.4%, 31.55±1.22%, 58.45±2.22% and 41.38±1.12% respectively. Overall the study shows that the methanolic extract of fresh *A. sowa* possessed higher antioxidant activity in all three antioxidant assays among the vegetables selected in this assay.

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List of Abbreviations

Abbreviation	Full Form
AAE	Ascorbic Acid Equivalent
DPPH	2,2 -Diphenyl-1- picrylhydrazyl
FW	Fresh Weight
NCDs	Non Communicable Diseases
RONS	Reactive Oxygen Nitrogen Species
ROS	Reactive Oxygen Species
TAC	Total Antioxidant Capacity

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Part I

Introduction

1.1 Background

Green leafy vegetables are a blessing for a safe and healthier life and have been in use for centuries. They are considered as an essential part of the diet to meet the daily nutrient requirements. They are the most readily available sources of carbohydrates, fats, important protein (Bhat and Al-Daihan, 2014). Green leafy vegetables can be used fresh as a salad or can be cooked/processed as per the interest of the consumer (Sharma and Kumar, 2013). These are becoming more popular for the masses day by day due to the increased awareness of consumers about natural and organic foods.

Their bioactive substances have a wide range of biological functions, including antioxidant and antimicrobial activities (Burt, 2004) and can be helpful in management of oxidative stress and age related human aliments (Gacche *et al.*, 2010). They are rich source of carotene, ascorbic acid, riboflavin, folic acids and minerals like calcium, iron and phosphorus (Fasuyi, 2006). Being a photosynthetic tissue, leafy vegetables have higher levels of vitamin K when compared with other fruits and vegetables due to direct involvement of vitamin K (phylloquinone) in photosynthesis process. Vegetables as medicinal plants contain none or less toxic effects (Evarando *et al.*, 2005), and have the ability to synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activities (Dhiman *et al.*, 2012). Green leafy vegetables are also rich in compounds having antidiabetic, anti-histaminic, anti-carcinogenic and hypolipidemic properties and possess preventive or curative properties against cardiovascular disease, ageing, obesity, hypertension, insomnia and ageing. Leafy vegetables are natural source of antioxidants and rich in phytochemicals (Bhat and Al-Daihan, 2014). The burden over synthetic chemicals can be reduced by encouraging the use of green leafy vegetables in food and food products.

1.2 Statement of the problem

Non-communicable diseases (NCDs), such as cardiovascular diseases, cancer, diabetes and chronic respiratory diseases, are the leading global cause of death and are responsible for 70% of deaths worldwide (WHO, 2017). Premature death from non-communicable diseases (NCDs) continues to be one of the major development challenges in the 21st century. NCDs account death of 15 million women and men between the ages of 30 and 70 each year, and leave no country untouched. In Nepal, 65% of death occurs from NCDs where as 22% of population has risk of premature death from NCDs (WHO, 2017).

During the fiscal year 2014/15 the production of vegetables was 3580085 metric tons (MT). The productivity of vegetables has increased from 9.5 to 12.2 MT/hector between 1998 and 2007. Due to health awareness and important nutritive values of vegetables and fruits, the per capita consumption of vegetables in Nepal has increased from 49 kg/person/year to 60 kg/person/year, but remains still below the human vegetable nutritional requirement i.e., 104 kg/person/year (Shrestha and Rai, 2012).

Many chronic diseases such as cancer and cardiovascular diseases represent an increasing proportion of morbidity and mortality in the developing countries. Various research findings have demonstrated that changes in oxygen utilization in the body and increased formation of reactive oxygen species (ROS) contribute to many chronic diseases. Although an organism is naturally equipped with antioxidant protection systems to cope with the harmful effects of ROS, the endogenous antioxidant defence system is not totally adequate to counteract the oxidative stress (Houston, 2010). Therefore, protection against oxidative stress depends partly on the adequacy of dietary antioxidants (Kaliora *et al*., 2006). Evidence suggests that phytochemicals from fruits and vegetables, including leafy vegetables, are capable of providing protection against free radicals. Thus the present study takes into consideration the antioxidant activities of fresh green leafy vegetables since it is considered as to have plenty of antioxidant functions.

1.3 Objectives

1.3.1 General objective

The general objective of this work was to analyze the antioxidant activity of five different fresh green leafy vegetables cultivated in Basantatar, Dharan.

1.3.2 Specific objectives

To fulfill the general objective, the following specific objectives were proposed.

- To prepare methanolic (99%) extract of fresh leaves of *Brassica juncea*, *Chinopodium album*, *Trigonella foenum graecum*, *Amaranthus tricolor* and *Anethum sowa*
- To determine the total antioxidant capacity of the above fresh green leafy vegetables
- To determine reducing power assay of the above fresh green leafy vegetables
- To find out DPPH (2,2 -Diphenyl-1- picrylhydrazyl) radical scavenging activity of the above fresh green leafy vegetables

1.4 Significance of the study

Green leafy vegetables are used since ancient periods as source of food as they contain many nutrients and minerals which are helpful in maintaining human health. The health and nutrition of expanding world populations are major upcoming challenges especially in developing countries. Plant foods are sources of energy, micronutrients and nutrients essential to health, in addition to phytochemicals with further health benefits including glycemic control, immuno-stimulation or antioxidant activity.

Due to availability of junk foods, the demand of fresh vegetables and fruits are declining day by day in today's busy lives. On the contrary, vegetables and fruits are the only two food constituents that when taken on a regular basis can keep all kinds of diseases away (Shrestha and Rai, 2012). The increased consumption of vegetables and herbs containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in the human body. Endogenous antioxidant defense mechanisms may be insufficient and hence dietary intake of antioxidant compounds is essential. The intake of natural antioxidants has been associated with reduced risk for cancer, cardiovascular disease, diabetes, and diseases associated with aging (Sun *et al.*, 2002).

Man has tremendous knowledge on edible plants since before civilization. Traditional vegetables are valuable sources of nutrition in rural areas where exotic species are not available. Leafy vegetables hold an important place in well-balanced diets. Green leafy vegetables are the cheapest of all the vegetables within the reach of poor man, being richest in their nutritional value. The lack of knowledge especially on the nutritive value of these green leafy vegetables among the public in general is the main drawback in their lower consumption. Ethno botanists elucidate the overlapping roles of plants used have both nutritional and therapeutic context to promote health and respond to disease. The ingestion of phytochemicals found in traditional foods has direct implications for the well-being of people. Plants used for their medicinal attributes may contain phytochemicals with pharmacological and physiological activities. Green leafy vegetables, represent an important proportion of foods with medicinal value (Kumar *et al.*, 2013). Limited information is available on the medicinal properties associated with leafy vegetable consumption in Nepal. Here is, however, no detailed report on evaluation of the antioxidant potential of green leaves, which are the most consumed type of vegetables. The goal of this study was to

evaluate the antioxidative activity of green leafy vegetables and to find a new potential source of natural antioxidants. Therefore, methanol extracts of green leaves were prepared, and their total antioxidant capacity, free radical scavenging activity, reducing power ability, were determined.

1.5 Limitation of the study

Although there are many vegetables are cultivated and consumed in Dharan due to time limit, this study had the following limitations.

a. Only five species of green leafy vegetables were used for research work.
b. Phytochemical analysis was not carried out.

Part II

Literature review

2.1 Introduction

2.1.1 Broad Leaf Mustard

The leaf mustard plant or mustard green scientifically known as *Brassica juncea* belongs to the family Cruciferae. They are widely used as vegetable throughout the world. Cruciferous vegetables are high nutritive value and a good source of natural antioxidants due to high level of carotenoids, tocopherols and ascorbic acid (Sarangthem *et al.*, 2011). Its primary center of origin is central Asia (northwest India), with secondary centers in central and western China, eastern India, Burma, and through Iran to the Near East. The principle growing countries are Bangladesh, Central Africa, China, India, Japan, and Pakistan, as well as southern Russia north of the Caspian Sea (Kumar *et al.*, 2011). Its local name is *Rayo*.

B. juncea is an annual herbaceous plant. The plants are tall (90-200 cm), erect and heavily branched. The leaves are dilated at the base, are stalked, broad and pinnatified. The fruits (siliquae) are slender and only 2 to 6.5 cm long, strongly ascending or erect with short and stout beaks (Kumar *et al.*, 2011). It is not only rich in vitamins, minerals, and dietary fibers, but it also contains a high amount of flavonoids. It has been reported to contain antioxidants like flavonoids, carotenes, lutein, indoles, and zeaxanthin (Kapoor *et al.*, 2014). Leaf mustard possessed antioxidant activity (Huang *et al.*, 2012). It is widely used as a spice throughout the world as well as in folk medicines such as diuretics, expectorants, and stimulants It is consumed in cooked, raw, salt-preserved, and pickled forms. It is a rich source of vitamin A, vitamin C, and phenolic compounds, which are protective against oxidative damages and carcinogenesis (Park *et al.*, 2017).

2.1.1.1 Plant description

The genus Brassica contains over 150 species that are cultivated worldwide as oilseed crops and/or vegetables. *Brassica juncea* is one such economically important plant well known in India for centuries for its nutritive and medicinal values (Manohar *et al.*, 2009) . The leaves as well as the seeds of this mustard variety are edible, and diverse medicinal uses of its seeds

are also well known in other countries. During more recent years it has also been cultured to produce a greater variety of benefits, including selenium, chromium, iron, and zinc food supplements. In general, the plant is taxonomically defined as follows:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Capparales

Family: Brassicaceae

Genus: *Brassica*

Species: *juncea*

2.1.1.2 Potential bioactive constituents of *Brassica juncea*

Together with glucosinolates, numerous polyphenolic secondary metabolites of Brassica juncea are often considered to be its major therapy relevant bioactive components (Cartea *et al.*, 2010). However, medicinal phytochemistry and structure activity relationships of these and other extractable components of the herb still remain to be properly defined. Table 2.1 shows the pharmacological activities of some constituent of *Brassica juncea.*

Table 2.1 Isolated constituent of *Brassica juncea* and their pharmacological activities

No.	Constituents	Activities
1.	Glucosinolates	Goitrogenic
2.	Isothiocyanates	Fungicidal activity, antitumor activity, Antimicrobial, Anti-tumour and antioxidant activity
	I. Allyl isothiocyanate	
	II. Phenyl isothiocyanate	
3.	Phenolic compounds (Sinapic Acid, Sinapine)	Anxiolytic activity, antioxidant, Cognition improving activity
4.	Fatty Acids (α-linolenic acid)	Astrocyte developing activity and other health benefits
5.	Kaempferol glycosides	Antioxidant activity
6.	Proteins	
	I. Napins	Antifungal, Allergenicity
	II. Juncin	Antifungal

Source: Kumar (2011)

2.1.1.2.1 Glucosinolates

Glucosinolates belong to the class of organic compounds which are characterized by a glucose-derived functional group attached to a sulphonated oxime through a side chain which may be either aliphatic, aromatic or heterocyclic. More than 200 individual glucosinolates have already been identified in diverse Brassicaceae plants, and many of them are also known to be present in *Brassica juncea*. In general, glucosinolates are water-soluble anions, which in the presence of the enzyme myrosinase and water generate isothiocyanates, thiocyanates or nitriles (Kumar *et al.*, 2011).

2.1.1.2.2 Flavonoids and their glycosides

The most abundant polyphenols in Brassica species are the flavonoids (mainly flavonols, but also anthocyanins) and the hydroxycinnamic acids. Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge. Flavonols are often the most widespread flavonoids of mustard greens. Quercetin, Kaempferol and Isorhamnetin, the main flavonols in Brassica crops, are most commonly found as O-glycosides. Within the colored flavonoids, anthocyanins are the most important group of plant pigments, also considered as multifunctional components of food due to their antioxidant activities and other beneficial biological properties (McDougall *et al.*, 2007).

2.1.1.2.3 Phenolic compounds

More than a dozen other phenolic acid conjugates have also been encountered in *Brassica juncea* leaves (Cartea *et al.*, 2010). Sinapic acid, the main phenolic compound in mustard meal**.**

2.1.2 Fenugreek leaves

Fenugreek (*Trigonella foenum graecum*) constitues a self-pollinated annual herbaceous legume, belongs to Fabaceae family and popularly known as Greek hay, bird's foot (Mashkor, 2014) and *Methi*. It is one of the well documented and most ancient recorded medicinal herbs (Wani and Kumar, 2018) used as major culinary ingredient since ancient in India. Fenugreek is supposed to be originated from southeastern Europe and western Asia. Presently, it is extensively cultivated in many parts of the world, including India, northern Africa and United States (Altuntas *et al.*, 2005). Fenugreek leaves and seeds have been used extensively to prepare powders and extracts for medicinal uses (Bensch *et al.*, 2003). This plant used for blood lipids and sugar decreasing in diabetic and non-diabetic peoples and have antioxidant and antibacterial activity. The plant contains active constituents such as alkaloids, flavonoids, steroids, Saponins etc. It is an old medicinal plant. It has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolaemic, effects, anti-inflammatory effects (Subhashini *et al.*, 2011). According to Bukhari *et al.* (2008) the *T. foenum graecum* consist of volatile oil, phenolic acids and flavonoids.

2.1.2.1 Plant description

It is an erect hairy annual of the bean family, reaching 30-60 cm. The plant grows to a height of about three feet, has three part leaves, the long slender stems bear tripartite, toothed, grey-green obovate leaves, 20-25 mm (3/4-1 in) long. *Trigonella foenum graecum* has long stalked leaves up to 5 cm long stipules triangular, lanceolate, leaflets about 2.5 cms long, obovate to obanceolate. The root is a mass of fingery structures. The sissile axillary flowers are white or pale yellow. The thin, sword-shaped pods are 10-15 cm (4-6 in), with a curved beak-like tip, each carrying 10-20 seeds. The plant radiates a spicy odour which persists on the hands after touching. Wild and cultivated varieties exist. Flowers are 1-2, axillary, sessile, racemed, whitish or lemon yellow that bloom from June to July. Pod 5.7 cm long with a persistent beak, hairy with 10-20 seeds. Mild mediterranean climates are most suitable. Plants mature in about four months. The flowering season for the herb fenugreek is generally midsummer (Kor *et al.*, 2013).

2.1.2.2 Scientific classifications

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Super-kingdom: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub-class: Rosidae

Order: Fabales

Family: Leguminosae/Fabaceae

Genus: *Trigonella*

Species: *graecum*

The young leaves and seedling sprouts of fenugreek are eaten as greens. The fresh or dried leaves have a bitter taste and a strong unique odor and are used to flavor other dishes. Green fenugreek leaves are one of the most ancient medicinal herbs. It was found that fresh fenugreek leaves are rich source of ascorbic acid and β-carotene. The green fenugreek leaves (fresh or dried) are used as herb. The fresh leaves are used in the vegetables as green leafy vegetable in the diets (Meghwal and Goswami, 2012).

2.1.3 Lamb's quarters

Chenopodium album (Chenopodiaceae) is an annual shrub used as folk medicine and widely grown in Europe, North America, Asia, and Africa. As therapeutic agents, it is used as laxative, anthelmintic against round and hook worms, as blood purifier in hepatic disorders, spleen enlargement, intestinal ulcers and burns. Various bioactivities such as antifungal, antipruritic, antinociceptive and hypotensive properties of crude and isolated compounds from the plant justified its uses in traditional medicine. The plant is very nutritious and rich in protein, vitamin A, vitamin C, calcium, phosphorus, iron and potassium content. It has been found to have flavonoid as phenolic amide, saponin, cinnamic acid amide, alkaloid chinoalbicin, apocortinoid, xyloside, phenols and lignans as active phytoconstituents (Agrawal *et al.*, 2014). Local name of *Chinopodium album* is *Bethu*. It is a fast-growing weedy annual plant in the genus Chenopodium.

Though cultivated in some regions, the plant is elsewhere considered a weed. Common names include lamb's quarters, melde, goosefoot, manure weed, and fat-hen, though the latter two are also applied to other species of the genus Chenopodium, for which reason it is often distinguished as white goosefoot (Flowersofindia.net, 2013). It is sometimes also called as pigweed. *C. album* is extensively cultivated and consumed in Northern India as a food crop

2.1.3.1 Taxonomic classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Chenopodiaceae

Genus: *Chenopodium*

Species: *album*

2.1.3.2 Plant description

Stems are rarely slender, angled, often striped green, red or purple. Leaves are simple, rhomboid, deltoid to lanceolate, upper entire, lower toothed or irregularly lobed, extremely variable in cultivated forms, 10-15 cm long, petioles often as long as thick blade, 1 to 1.3 cm in length. The opposite leaves can be very varied in appearance. The first leaves, near the base of the plant, are toothed and roughly diamond-shaped, 3-7 cm long and 3-6 cm broad. It has been found in dark green colour with smooth undersurface. The leaves are waxy-coated, unwettable and mealy in appearance, with a whitish coat on the underside. Flowers are radial, symmetrical and grow in small cymes on a dense branched inflorescence, 10-40 cm long, contains shining black seeds (Pande and Anupam, 2010).

The leaves and young shoots may be eaten as a leaf vegetable, either steamed in its entirety, or cooked like spinach, but should be eaten in moderation due to high levels of oxalic acid. Each plant produces tens of thousands of black seeds. These are high in protein, vitamin A, calcium, phosphorus, and potassium (Johnson *et al.*, 1995).

2.1.3.3 Phytoconstituents

The phytoconstituents reported were b-sitosterol, lupeol, 3 hydroxy nonadecyl henicosanoate, ascorbic acid, b-carotene, catechin, gallocatechin, caffeic acid, p-coumaric acid, ferulic acid, campesterol, xanthotoxin, stigmasterol, imperatorin, ecdysteroid, cinnamic acid, amide alkaloid, phenol, saponin, apocarotenoids, crytomeridiol, n-trans-feruloyl-4-O-methyl dopamine and syringaresinol (Usman *et al.*, 2010).

The antioxidant activity of *C. album*, like other natural phenolic antioxidants, e.g. flavonoids, is a consequence of the presence of the phenolic moieties in the structures (Mandindi, 2015). The antioxidant activity of phenolic natural products is predominantly owing to their redox properties, i.e. the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential (Nahar and Sarker, 2014).

2.1.4 *Amaranthus*

Amaranthus, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants. Some amaranth species are cultivated as leaf vegetables, pseudocereals, and ornamental plants. Most of the *Amaranthus* species are summer annual weeds and are commonly referred to as pigweed. It is known as *Latte* in local area. Catkin-like cymes of densely packed flowers grow in summer or autumn. Approximately 60 species are recognized, with inflorescences and foliage ranging from purple, through red and green to gold (Bensch *et al.*, 2003).

Amaranthus belonging to the family of Amaranthaceae, distributed throughout the tropical and subtropical countries. Both leaves and seeds contain protein of an unusually high quality. Different edible species of *Amaranthus* are being consumed widely as leafy vegetable across the world due mainly to its lower price and rich source of protein, carotenoids, vitamin C, dietary fiber (Shukla *et al.*, 2006) and minerals such as calcium, iron, zinc and magnesium (Kadoshnikov *et al.*, 2008). Several species of amaranths contain phytochemicals including flavonoids and antioxidants that help protect cells and tissues from damaging effects of free radicals and oxidative stress (Akin-Idowu *et al.*, 2017). *Amaranthus* has well been documented to possess important pharmacological properties including

anticancer (Baskar *et al.*, 2012), anti-inflammatory (Kabiri *et al.*, 2011)and antioxidant activity (Amin *et al.*, 2006).

2.1.4.1 Red Amaranth

Main vegetable type of leaf amaranth is *Amaranthus tricolor*, originated in south East Asia, particularly in India (Shukla *et al.*, 2006). Tender stems and leaves contains protein, fat, carbohydrates , calcium, iron, phosphorus, vitamin A and vitamin C. Due to their nutritional superiority, amaranths have been suggested as alternative source of rich protein leafy vegetables feeding those overpopulated and undernourished areas (Tejaswini *et al.*, 2017).

A. tricolor has various leaf colors such as white (light green), dark green, red, purple and variegated (Khandaker *et al.*, 2008). Red amaranth is a wonderful vegetable with reddish veined dark green leaves or fully red to purple leaves, suitable for growing in warm weather, in which young leaves and stems can be harvested periodically. Red amaranth is also especially nutritious, rich in easily digestible minerals i.e., iron and calcium, as well as protein, vitamin C, and beta-carotene (Islam *et al.*, 2003).

2.1.4.1.1 Plant description

An annual, ascending or erect herb, attaining 1.2 m high or more in cultivation, stem stout, usually much branched, branches angular, glabrous. Inflorescence a head, axillary and terminal. Leaf colour dark green, light green or red. Terminal leaves may be red, purple, yellow or variegated. Fruit circumscissile below the middle, ovoid, 1.5 mm long. Seeds ovoid, 1.5 mm in diameter, shining, brown, smooth, lenticular (Rahman and Gulshana, 2014)**.**

2.1.4.1.2 Scientific classification

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Order: Caryophyllales

Family: Amaranthaceae

Genus: *Amaranthus*

Species: *tricolor*

2.1.4.1.3 Bioactive components

Mature leaves of *A. tricolor* and *A. caudatus* contain red-violet pigments, the betacyanins amaranthin and isoamaranthin. They are derivatives of betanidin, which is formed from 3,4-dihydroxyphenylalanine (Rao *et al.*, 2010). Betalain pigments are water-soluble red-violet (betacyanins) and yellow (betaxanthins) pigments. These pigments replace anthocyanins in most plant families of the order *Caryophyllales* (Cai *et al.*, 2005). Betalains occur only in the plants of the order *Caryophyllales* (Old name: *Centrospermae*), such as the family *Amaranthaceae* which includes several important genera, i.e. *Amaranthus, Celosia, Gomphrena* and *Iresine* (Biswas *et al.*, 2013). Several betalains (16 red-violet betacyanins and 3 yellow betaxanthins) were reported to be isolated and identified in different portions (viz. stems, leaves, and inflorescences) from plants of the Amaranthaceae family (Schliemann *et al.*, 2001). Betalain pigments are of particular interest because of their limited biological distribution, occurring only in those plant species confined to the order Caryophyllales, notably the red beet (Chenopodiaceae) and certain fungi such as the fly-agaric mushroom (*Amanita muscaria*) (Cai *et al.*, 2005). Betalains are attracting increasing attention because of their use for food coloring and their antioxidant and radical scavenging properties against certain oxidative stress-related disorders, anticancer, antiviral and antiparasitosis properties (Zou *et al.*, 2005). Glycosides, phenolic compounds, flavonoids and saponins are the phytochemicals present in *A. tricolor* (Baang *et al.*, 2015).

2.1.5 Dill (*Anethum sowa*)

Anethum sowa belongs to family Apiaceae, commonly known as sowa. The genus name Anethum is derived from Greek word *aneeson* or *aneeton*, which means strong smelling. Its common use in ayurvedic medicine is in abdominal discomfort, colic and for promoting digestion (Mathur, 2012). In recent years the scientific literature reports pharmacological effects of dill such as antibacterial (Singh *et al.*, 2001), antimycobacterial (Stavri and Gibbons, 2005), antioxidant (Satyanarayana *et al.*, 2004), cancer chemopreventive (Amin and Sleem, 2007). The well-known properties of dill from the traditional medicine, such as carminative, stomachic, diuretic have been reported (Hosseinzadeh *et al.*, 2002). Dill include essential oils, fatty oil, proteins, carbohydrates, fibre, and mineral elements such as calcium, potassium, magnesium, phosphorous, sodium, vitamin A and niacin (Kaur and Arora, 2010).

This aromatic herb was commonly used for flavoring and seasoning of various foods such as pickles, salads, sauces, and soups. The fresh dill greens are called as *Sounp ko Saag* in local language. Dill fruits have a strong aromatic smell and taste but they lose these properties on cooking because of the loss of essential oils. Dill oil is extracted from the seeds, leaves, and stems of the plant, and is an essential oil used as flavoring in the food industry (Ramadana *et al.*, 2013). Dill seeds have been used as household remedy to relief digestive problems such as stomachache, indigestion and flatulence. Dill water is believed to have a soothing effect and is given to babies to treat gripe, relieve hiccups and colic. The aroma composition of the dill has been investigated in numerous studies and was reported that the main components were calvone, limonene, and dill adipole. Dill is also a galactagogue that is known to increase the flow of milk in nursing mothers and will then be taken by the baby in the milk to help prevent colic. Chewing the seeds reduce bad breath (Kaur and Arora, 2010).

2.1.5.1 Classification

Kingdome: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Apiales

Family: Apiaceae

Genus: *Anethum*

Species: *sowa*

2.1.5.2 Plant description

Anethum grows up to 90 cm tall, with slender stems and alternate leaves finally divided three or four times into pinnate sections slightly broader than similar leaves of fennel. The yellow flower develops into umbels (Jana and Shekhawat, 2010). The seeds are not true seeds. They are the halves of very small, dry fruits called schizocarps. Dill fruits are oval, compressed, winged about one-tenth inch wide, with three longitudinal ridges on the back and three dark lines or oil cells (vittae) between them and two on the flat surface. The taste of the fruits somewhat resembles caraway. The seeds are smaller, flatter and lighter than caraway and have a pleasant aromatic odor.

2.1.5.3 Potential bioactive compounds

Anethum sowa has been reported to contain flavonoids, phenolic, and essential oil. The excellent antioxidant activity of *A. sowa* is attributed not only to the presence of a high content of polyphenols but also to that of volatile constituents that are present in dill (Ramadana *et al.*, 2013). The essential oils of *A. sowa* has high total phenolic content (Gumus *et al.*, 2016).

2.2 General method of extraction of phytochemicals (Antioxidants)

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures (Handa *et al.*, 2008). There are different methods of extraction, the purpose of which is to separate the soluble plant metabolites, leaving behind the insoluble cellular residues. The commonly used extraction methods are maceration, infusion, decoction, soxhlet extraction, supercritical fluid extraction etc. The crude extract obtained through these methods contains complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids. Some of the crude extracts obtained may be ready for use as medicinal agents in the form of tinctures and as fluid extracts where as some need further processing (Azwanida, 2015) .

2.2.1 Maceration

Maceration is a technique used in wine making and has been adopted and widely used in medicinal plants research. Maceration involves soaking plant materials (coarse or powdered) in a stoppered container with a polar solvent and allowed to stand at chilled temperature for a period of two to three days (Handa *et al.*, 2008). This process is intended to soften and break the plant cell wall to release the soluble phytochemicals. After two to three days, the mixture is pressed or strained by filtration. The choice of solvents will determine the type of compound extracted from the samples (Azwanida, 2015).

Significant advances have been made in the processing of medicinal plants such as the modern extraction methods; microwave-assisted (MAE), sonication and supercritical fluid extraction (SFE). With such variety of methods present, selection of proper extraction method needs meticulous evaluation. However, maceration have been suggested by Vongsak *et al.* (2013), as more applicable, convenient and less costly method compared to other modern extraction methods since all these extraction methods resulted in crude extracts containing a mixture of metabolites having almost similar recovery of phytochemicals. This particular fact suggests that preparation of crude extract through modern technology, which is rather complex and time consuming is not necessary if proper preparation and extraction are done (Azwanida, 2015). Suitable conditions for each extraction methods are also important.

2.3 Different solvents used in extraction

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted (Tiwari *et al.*, 2011). Table 2.2 shows different solvents used for active component extraction.

Table 2.2 Solvents used for active component extraction

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoid	Flavonol
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptide	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

Source: Tiwari *et al*. (2011)

2.4 Antioxidants

An antioxidant can be defined as: “any substance that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate” (Halliwell and Gutteridge, 1995) but later the word “oxidation” was altered to “oxidative damage” that suggests an in vivo biological process: “any substance that delays, prevents or removes oxidative damage to a target molecule” (Halliwell, 2007). Most recently, Apak *et al*. (2016) gave a more specific definition: “natural or synthetic substances that may prevent or delay oxidative cell damage caused by physiological oxidants having distinctly positive reduction potentials, covering reactive oxygen species (ROS)/reactive nitrogen species (RNS) and free radicals (i.e. unstable molecules or ions having unpaired electrons)”. These definitions demonstrate the roles of antioxidants at cellular levels in humans as they are related to oxidative stress and free radicals and further to potential health effects in human.

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Young and Woodside, 2001). Occurrence of chronic diseases were recognized to be associated with the oxidative stress, where the reactive oxygen species (ROS) and reactive nitrogen species (RNS) including free radicals were continuously produced in human cells and led to oxidative damage to cell components (Bagchi and Puri, 1998). In concern by that, there is an increasing interest in antioxidants; chemical compounds that possess the ability to neutralize these free radicals in the body by reducing or scavenging its activities (Pisoschi and Negulescu, 2011). The antioxidant properties are the basis of preventive function towards many chronic diseases, including neurodegenerative diseases: stroke, Alzheimer’s disease and Parkinson’s disease, cardiovascular diseases: atherosclerosis and hypertension, diabetes, cancer and osteoporosis etc. (Kaczora *et al.*, 2016). Leafy vegetables contain significant amount of health promoting phytochemicals such as polyphenols. They have been reported to exhibit antioxidant activity which allows them to scavenge free radicals in the body (Meena *et al.*,2016).

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 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 (Lobo *et al.*, 2010)

Some of the most widely used *in vitro* antioxidant methods as described by Mermlestein (2008) are Oxygen Radical Absorbance Capacity method (ORAC), Hydroxyl Radical Antioxidant Capacity (HORAC) assay, Trolox Equivalent Antioxidant Capacity (TEAC) method, Ferric Reducing/Antioxidant Power (FRAP), metho Peroxyl Radical Scavenging Capacity (PSC) method, Total Oxyradical Scavenging Capacity (TOSC) method, The DPPH method, Total Radical-Trapping Antioxidant Parameter (TRAP) method.

2.4.1 Total antioxidant capacity (TAC)

It is also known as total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant status (TAS), total antioxidant response, or other synonyms (Erel, 2004). One major advantage of TAC assays is that, by definition, estimate the antioxidant components of a sample in a global way. Total antioxidant activity by phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) complex at acidic pH (Venkatachalam *et al.*, 2014). Measuring each antioxidant component individually is labor-intensive and time-consuming, requiring complex and costly techniques (Erel, 2004). Other advantages of using TAC assays include simplicity of the techniques, low cost per sample, speed of reactions and possibility to be performed using automated, semi-automated, or manual methods (Marques *et al.*, 2014). Table 2.3 shows the total antioxidant capacity of selected fruits and vegetables from South India.

Table 2.3 Total antioxidant capacity of fruits and vegetables

Plant extract	Total antioxidant capacity (mg AAE/100 g fresh weight) *
Mulberries	45.78±1.70[d]
Papaya	40.05±2.50[c]
Mango	31.21±1.70[a]
Guava	41.11±1.50[c]
Tomato	34.57±3.00[b]
Red onion	48.78±2.40[f]
Red cauliflower	41.29±1.80[c]
Red grapes	48.13±1.20[e]
Carrot	47.65±1.20[e]
Beetroot	61.11±2.10[g]

Source: Venkatachalam (2014)

*The values are expressed in mean ± standard deviation. The superscript alphabets in the column shows the significant difference ($P < 0.05$).

2.4.2 DPPH radical scavenging assay

DPPH assay measures the ability of a substance to scavenge the DPPH radical, reducing it to hydrazine. When a substance that acts as a donor of hydrogen atoms is added to a solution of DPPH, hydrazine is obtained, with a change in color from violet to pale yellow (Formagio *et al.*, 2014). The DPPH• test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors. The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolorized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme:

$DPPH^{\bullet} + AH \rightarrow DPPH^{\bullet} - H + A^{\bullet}$,

$DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH^{\bullet} - R$

The $DPPH^{\bullet}$ radical is one of the few stable organic nitrogen radicals, which bears a deep purple color. It is commercially available and does not have to be generated before assay. This assay is based on the measurement of the reducing ability of antioxidants toward $DPPH^{\bullet}$. The DPPH assay is considered to be mainly based on an electron transfer (ET) reaction, and hydrogen-atom abstraction is a marginal reaction pathway. The test is simple and rapid and needs only a UV-vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening (Prior *et al.*, 2005).

2.4.3 Reducing power

The reducing power assay is a relatively simple, quick, and inexpensive direct method of measuring the combined ("total") antioxidant activity of reductive (electron donating) antioxidants in a test sample. The assay uses the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) as the signal, or indicator, reaction, and this is tied to a color change (Benzie and Devaki, 2018). In this assay, a key oxidant (in the form of a ferric salt in aqueous solution) is reduced by the electron donating (reductive) antioxidants in the reaction mixture that have a redox potential, under the reaction conditions employed, lower than that of the half reaction.

The flavonoids and phenolic acids are present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of increase in the absorbance of the reaction mixtures, the absorbance increases the antioxidant activity increases. The antioxidant compound present in the samples forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm by UV-Spectrophotometer (Vijayalakshmi and Ruckmani, 2016). The study done by Meena *et al.* (2016) on the reducing power activity of some fresh green leafy vegetables are shown in the Table 2.4.

Table 2.4 Reducing power assay of some green leafy vegetables

Name of vegetable	Reducing Power (mg AAE /100 g fresh weight)
Carrot leaves	43.5 ± 3.18
Chenopodium leaves	55.4 ± 1.21
Coriander leaves	69.3 ± 1.15
Mint	77.8 ± 2.49
Mustard leaves	46.5 ± 3.34
Onion leaves	53.9 ± 2.07
Fenugreek leaves	48.3 ± 2.91
Spinach	18.3 ± 0.76

Source: Meena *et al.* (2016)

Values are expressed in mean ± standard deviation.

Part III

Materials and methods

3.1 Raw materials

The plants under the study were *Trigonella foenum graecum* (Fenugreek Leaves), *Anethum sowa* (Fresh Dill Greens), *Brassica juncea* (Broad Leaf Mustard), *Chinopodium album* (Lamb's Quarter), *Amaranthus tricolor* (Red Amaranth) were collected from Basantatar, Dharan, Sunsari, Nepal.

3.2 Identification of the plant

The plant specimen was taxonomically identified from the Department of Biology of Central Campus of Technology as shown in the Table 3.1.

Table 3.1 Taxonomic identification of green leafy vegetables

English name	Local name	Scientific name
Fenugreek Leaves	*Methi ko Saag*	*Trigonella foenum graecum*
Fresh Dill Greens	*Soup ko Saag*	*Anethum sowa*
Broad Leaf Mustard	*Rayo ko Saag*	*Brassica juncea*
Lamb's Quarter	*Bethu ko Saag*	*Chinopodium album*
Red Amaranth	*Latte ko Saag*	*Amaranthus tricolor*

3.3 Chemicals required

The required chemicals were obtained from Central Campus of Technology laboratory, Dharan.

List of chemicals

1) Ammonium molybdate (Thermo Fisher Scientific India Pvt. Ltd.)
2) Ascorbic acid (Thermo Fisher Scientific India Pvt. Ltd.)
3) Disodium hydrogen orthophosphate (Thermo Fisher Scientific India Pvt. Ltd.)
4) DPPH (Thermo Fisher Scientific India Pvt. Ltd.)
5) Ferric chloride (Thermo Fisher Scientific India Pvt. Ltd.)
6) Methanol (Merck (India) Ltd.)
7) Sodium dihydrogen orthophosphate (Thermo Fisher Scientific India Pvt. Ltd.
8) Sulphuric acid (Thermo Fisher Scientific India Pvt. Ltd)
9) Trichloroacetic acid (Thermo Fisher Scientific India Pvt. Ltd.)
10) Potassium ferricyanide (Thermo Fisher Scientific India Pvt. Ltd.)

3.4 Apparatus required

The required apparatus were obtained from Central Campus of Technology laboratory, Dharan.

List of equipments used

1) Centrifuge (Victolab, India)
2) Electric balance (Phoenix instrument)
3) Spectrophotometer (Labtronics, India)
4) Soxhlet apparatus (Y.P. Scientific industries)
5) Hot air oven (Victolab, India)
6) Incubator (Victolab, India)
7) Muffle furnace (Accumax, India)
8) Rotatory Vacuum Evaporator (IKA RV 10)
9) Refrigerator (Victolab, India)

3.5 Collection and preparation of sample

The plant specimen under study were collected in April 2018 from Basantatar, Dharan, Sunsari District of Nepal. The basic flow diagram of methodology is made by modifications from the methodologies described by Jaradat *et al.* (2015) and is shown in Fig. 3.1.

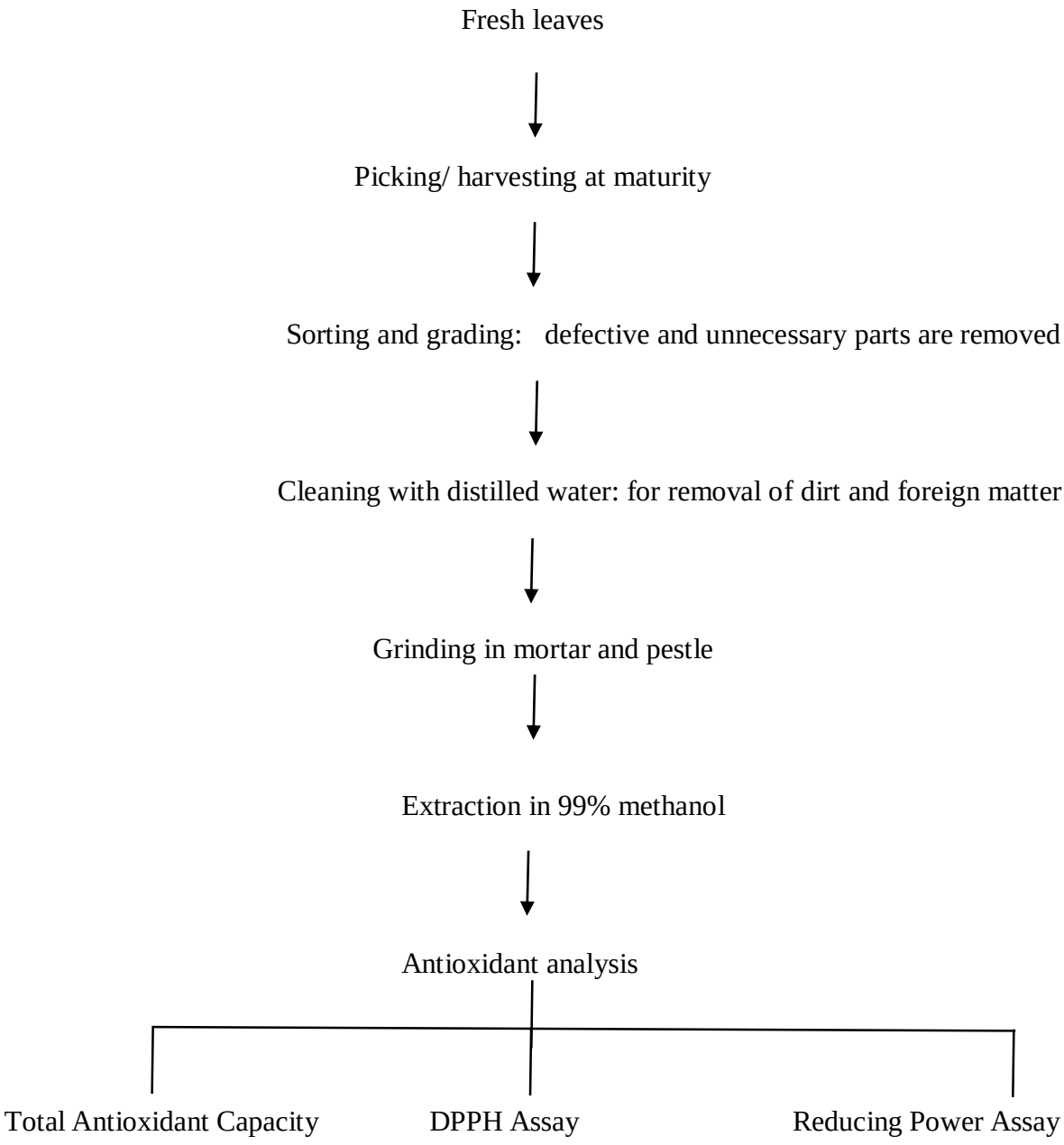


Fig. 3.1 Flow diagram of methodology

3.6 Preparation of plant extracts for antioxidant evaluation

About 10 g of the grounded plant were soaked in 1 Liter of methanol (99%) and shaken by hand at 30 min interval for 8 h per day at room temperature for three days and stored in refrigerator for 4 days. The extracts were then filtered using filter papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4ºC and the antioxidant test was done directly within five minutes (Jaradat *et al.,* 2015).

3.6.1 Total antioxidant capacity

The total antioxidant capacity of leaf extracts was analyzed by the phosphomolybdenum reduction assay method according to the procedure described by (Prieto *et al.,* 1999) . The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract of different vegetables, and subsequent formation of green phosphate/Mo (V) complex at acid pH (R. Kumar *et al.,* 2018). The tubes containing leaf extract (0.3 ml) and 3 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95ºC for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm spectrophotometrically against a blank. The antioxidant capacity was expressed as ascorbic acid equivalents (AAE).

The antioxidative potential of plant extracts can be measured using various *in vitro* assays and each assay is based on at least one feature of antioxidant activity. However, total antioxidant properties of plants cannot be evaluated by any single method because of their complex nature of phytochemicals. Therefore, two or more methods should always be employed in order to evaluate the total antioxidative effects of plant extracts (Gunathilake and Ranaweera, 2016).

3.6.2 DPPH radical scavenging assay

The capacity of prepared extracts to scavenge the ‘stable’free radical DPPH was monitored according to the method of (Hatano *et al.,* 1988) with slight modifications. Extracts (100 µl) were dissolved in 3.9 ml freshly prepared methanolic solution of DPPH (1 mM). The mixture was vortexed for 15 s and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/VIS spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula.

% inhibition = $(A_{control} - A_{sample})/A_{control} \times 100$

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao *et al.*, 2008). Environmental temperature plays a significant role on antioxidant activity evaluation and it is more pronounced in cold weather (Iqbal and Bhanger, 2006).

3.6.3 Reducing power assay

The reducing power of the prepared extracts was determined according to the method of (Oyaizu, 1986). Briefly, each extract (1 ml) was mixed with 2.5 ml of a 0.2M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50ºC for 20 min and then 2.5 ml of 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged for 10 min at 3000rpm. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride solution. Absorbance of the reaction mixture was read using UV/VIS spectrometer (SP-3000) at 700 nm. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

The various biological and environmental factors in which the plant grew also contribute to the plant antioxidant power (Dimcheva and Karsheva, 2018). The reduction of Fe3+ has been described as an indicator of electron donating activity which can demonstrate the antioxidant potential of different phenolic compound of phyto origin (Gunathilake and Ranaweera, 2016). The reducing power is generally associated with the presence of reductones (Saritha *et al*., 2010), which has been shown to exhibit antioxidant potential by splitting the free radical chains by donating hydrogen atoms. Reductones can prevent the peroxide formation by reacting with the precursors of peroxides.

3.7 Statistical analysis

Analysis was carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2010.

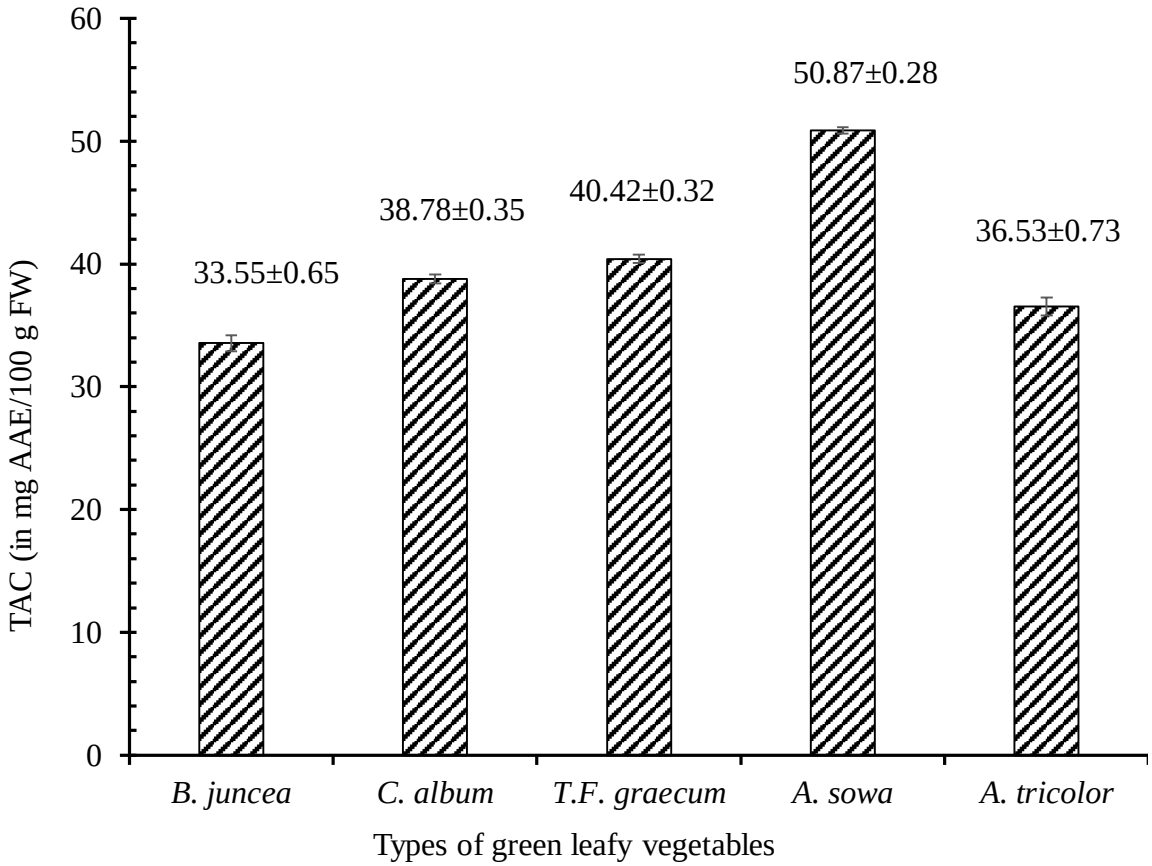
Part IV

Results and discussion

Five different fresh green leafy vegetables were collected from Basantatar, Dharan, Sunsari district of Nepal and bioactive components were extracted by soaking in 99% methanol for 72 h at room temperature and then concentrated in a rotary vaccum evaporator. After that each sample extracts were analyzed for TAC, reducing power assay and DPPH scavenging assay.

4.1 Total antioxidant capacity (TAC)

In the study, TAC of 99% methanolic extract of different vegetable extract are shown in Fig. 4.1.



The values are expressed in mean ± standard deviation**.**

Fig. 4.1 Total antioxidant capacity of green leafy vegetables

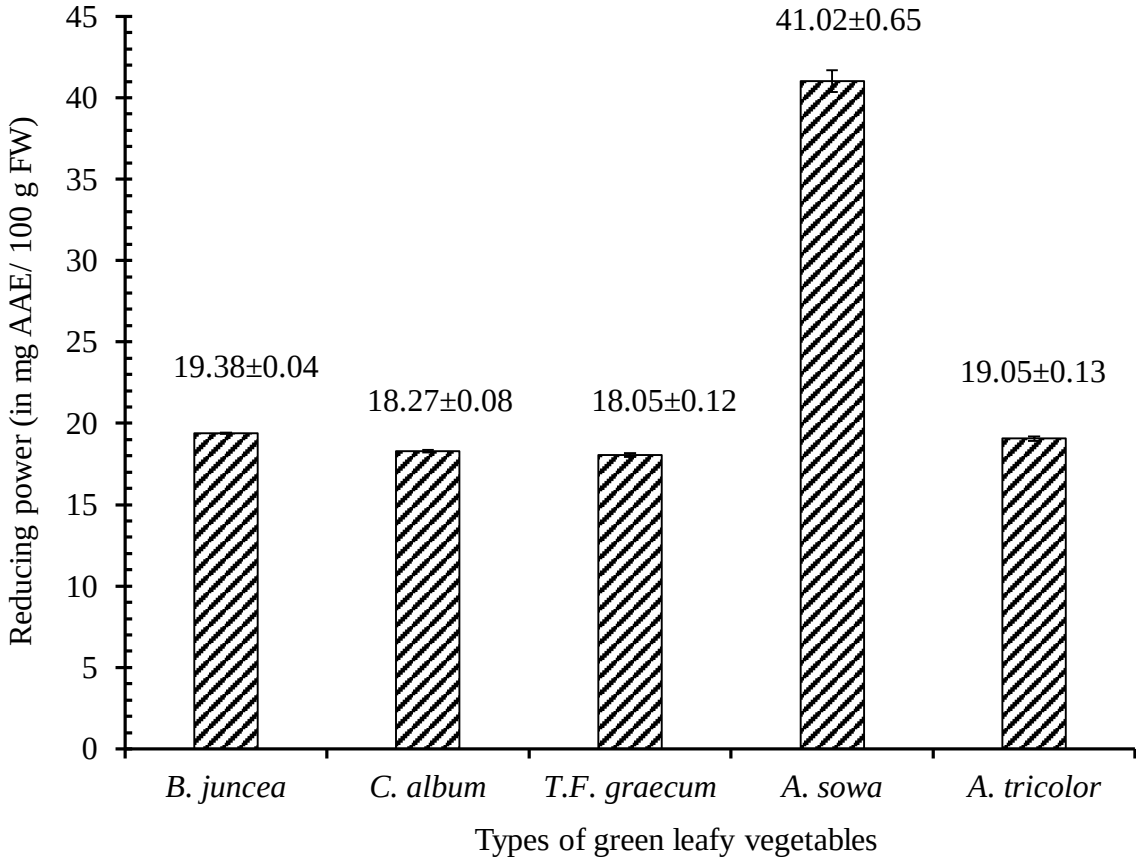
TAC of 99% methanolic extract of *Brassica juncea, Chinopodium album, Trigonella foenum graecum, Anethum Sowa* and *Amaranthus tricolor* leaves were found to be 33.55±0.65, 38.78±0.35, 40.42±0.32, 50.78±0.28 and 36.53±0.73 mg AAE/100 g fresh wt. (FW) respectively.

The study done Kapoor *et al.* (2014) on *B. juncea* was found to be 46.2±3.25 mg AAE/100 g which is close to the current findings. *C. album* leaves TAC was found to be 38.78±0.35 mg AAE/100 g. A study done in India where the TAC value of beetroot (which also belongs to the Chenopodiaceae family) was found to be 61.1±2.10 mg AAE/100 g fresh weight. This value is higher than the obtained value due to the polyphenol and flavonoid content of beetroot higher than that of *C. album* (Venkatachalam *et al.*, 2014). The TAC of *T. foenum graecum* and *A. sowa* was found to be 40.42±0.32 and 50.78±0.28 mg AAE/100 g. The study done in Indian selected fruits and vegetables was in the range of 31.2 to 61.1 mg AAE/100 g. From the above results, it can be concluded that the methanol extract of *T. foenum graecum* leaves has very strong antioxidant potential which might be associated with the high level of phenolic and flavonoids type compounds present in the extract (Bukhari *et al.*, 2008). The value of TAC of *A. sowa* extract can be correlated with total phenolic content found in the essential oils of it (Gumus *et al.*, 2016).

The 99% methanolic extract of *Amaranthus tricolor*'s TAC was found to be 36.53±0.73 mg AAE/100 g. The study done by Akin-Idowu *et al.* (2017) in *A. caudatus* and *A. Cruentus* was found to be 14.02±4.92 and 14.5±8.40 mg AAE/100 g respectively. This variation is due to the compositional variation of phytochemicals, methods of extraction, etc. The antioxidative potential of plant extracts can be measured using various *in vitro* assays and each assay is based on at least one feature of antioxidant activity. However, total antioxidant properties of plants cannot be evaluated by any single method because of their complex nature of phytochemicals. Therefore, two or more methods should always be employed in order to evaluate the total antioxidative effects of plant extracts (Gunathilake and Ranaweera, 2016). Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Young and Woodside, 2001).

4.2 Reducing power assay

The reducing power of 99% methanolic extract of fresh green leafy vegetables is shown in Fig. 4.2.



The values are expressed in mean ± standard deviation.

Fig. 4.2 Reducing power of green leafy vegetables

Reducing power of 99% methanolic extract of *B. juncea*, *C. album*, *T. foenum-graecum*, *A. Sowa* and *A. tricolor* were found to be 19.38±0.04, 18.27±0.08, 18.05±0.12, 41.02±0.65 and 19.05±0.13 mg AAE/100 g fresh weight (FW) respectively.

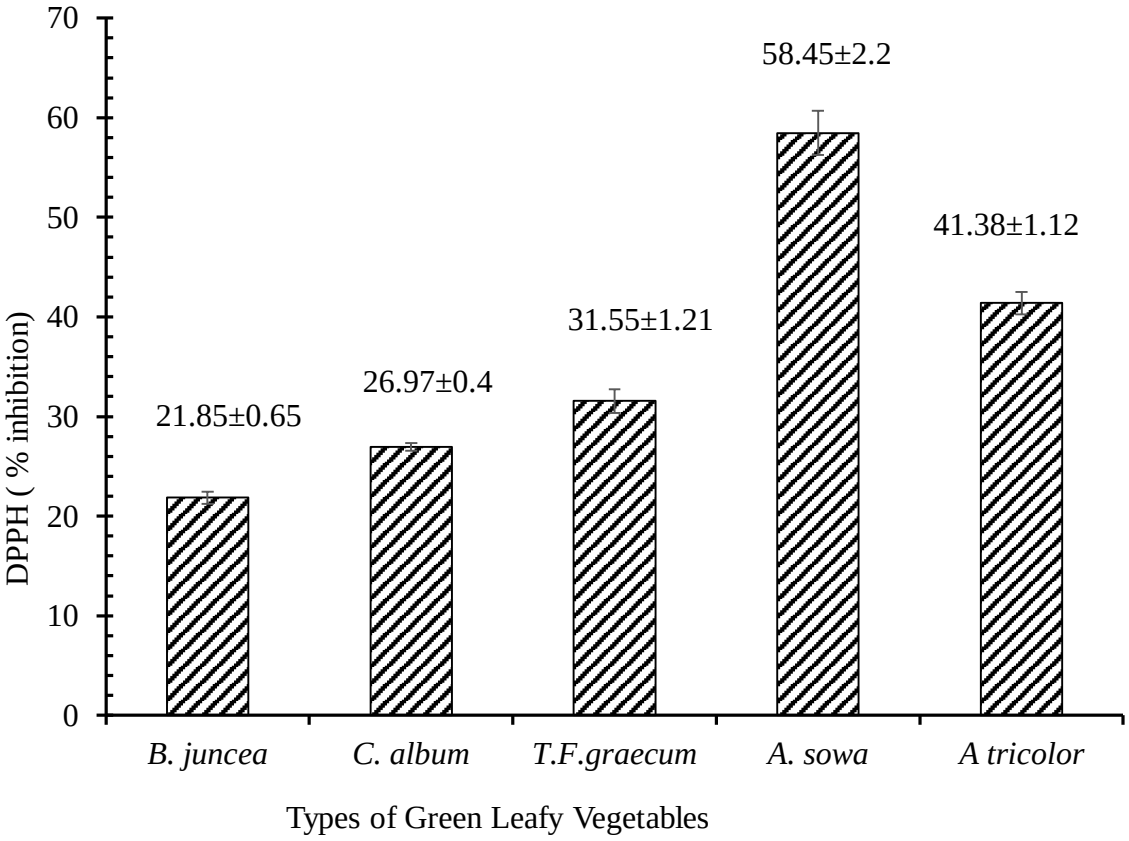
The similar study done on reducing power of *B. juncea*, *C. album* and *T. foenum graecum* leaves extract by Meena *et al.* (2016) was found to be 46.5±3.34, 55.4 ± 1.21 and 48.3±2.91 mg AAE/100 g fresh weight. This difference in value may be due to the different varieties used, stage of harvesting, environmental factors, etc. According to Bukhari *et al.* (2008) the *T. foenum graecum* has volatile oil, phenolic acids and flavonoids.

The reducing power of 99% methanolic extract of *A. sowa* was observed to be 41.02±0.65 mg AAE/100 g and this observation is similar to the result obtained by Meena *et al.* (2016) where reducing power of carrot leaves was 43.5 ± 3.18 mg AAE/100 g. *A. sowa* has been reported to contain flavonoids, phenolic, and essential oil. The excellent antioxidant activity of *A. sowa* is attributed not only to the presence of a high content of polyphenols but also to that of volatile constituents that are present in dill (Ramadana *et al.*, 2013). *A. tricolor* showed the reducing power to be 19.05±0.13 mg AAE/100 g. One research done by Akin-Idowu *et al.* (2017) showed that the reducing power of other variety of amaranth such as *A. caudatus* and *A. Cruentus* extract was found to be 16.8±0.16 and 19.2±0.12 mg AAE/100 g.

The various biological and environmental factors in which the plant grew also contribute to the plant antioxidant power (Dimcheva and Karsheva, 2018). The reduction of Fe^{3+} has been described as an indicator of electron donating activity which can demonstrate the antioxidant potential of different phenolic compound of phyto origin (Gunathilake and Ranaweera, 2016). The reducing power is generally associated with the presence of reductones (Saritha *et al.*, 2010), which has been shown to exhibit antioxidant potential by splitting the free radical chains by donating hydrogen atoms. Reductones can prevent the peroxide formation by reacting with the precursors of peroxides.

4.3 DPPH scavenging assay

The DPPH scavenging activity of 99% methanolic extract of fresh green leafy vegetables are shown in Fig. 4.3.



The values are expressed in mean ± standard deviation.

Fig. 4.3 DPPH scavenging activity of green leafy vegetables

The DPPH radical scavenging activity of 99% methanolic extract of *T. foenum graecum* and *A. sowa* were found to be 31.55±1.21% and 58.45±2.22% respectively. According to the one study found that the scavenging activity of 50% ethanolic soxhlet extract of *T. foenum graecum* and *A. sowa* were 25.7±0.34% and 44.6±0.42% (Gacche *et al.*, 2010). The higher activity of methanol extract of *A. sowa* can be related to its high phenolic and flavonoid content (Kaur *et al.*, 2018). Similarly, the scavenging activity of *B. juncea* extract was observed to be 21.85±0.61%. However, the study done in Indian mustard ranged from 30.87% to 66.30% (Sarangthem *et al.*, 2011). *A. tricolor*'s % inhibition of DPPH radical was 41.38±1.12%. But the research done in *Chinese* variety of *A. tricolor* extract showed the

result of 17.32±3.0% inhibition. This may be due to the fact that the extract was prepared several times and was mixed up at last which decreased the concentration of antioxidant per volume. That's why the % inhibition was lower than the current value and the % inhibition increases with the increase in extract concentration per volume (Baang *et al.*, 2015). The DPPH radical scavenging activity of 99% methanolic extract of *C. album* was found to be 26.97±0.4%. According to the Mandindi (2015) % inhibition of DPPH of *Chinopodium* extract was about 45%.

This variation in result from other workers might be due to different varieties which lead to genetic variability, time of harvest, stage of harvest, analytical procedure applied, and climatic conditions (Kumar *et al.*, 2018). It may be also due to the variation in solvent used during extraction, extraction temperature etc. On the other hand, environment condition such as temperature, light, water or soil may concern on composition of compounds. Environmental temperature plays a significant role on antioxidant activity evaluation and it is more pronounced in cold weather (Iqbal and Bhanger, 2006).

Part V

Conclusions and recommendations

5.1 Conclusions

Antioxidant activity of five different fresh green leafy vegetables were assessed by extracting in 99 % methanol for total antioxidant capacity, reducing power assay and DPPH scavenging assay. Within the scope of the present work done, following conclusions were drawn.

- The total antioxidant capacity (TAC) of 99% methanolic extract for *B. juncea, C. album, T. foenum graecum, A. sowa* and *A. tricolor* were found to be 33.55±0.65, 38.78±0.35, 40.41±0.32, 50.87±0.28 and 36.53±0.73 mg AAE/100 g fresh weight respectively.
- The reducing power of *B. juncea*, *C. album*, *T. foenum-graecum, A. sowa* and *A. tricolor* were found to be 19.38±0.04, 18.28±0.08, 18.06±0.12, 41.02±0.65 and 19.06±0.13 mg AAE/100 g respectively.
- The DPPH scavenging activity of *B. juncea, C. album*, *T. foenum-graecum, A. sowa* and *A. tricolor* were found to be 21.85±0.61%, 26.97±0.4%, 31.55±1.22%, 58.45±2.22% and 41.38±1.12% respectively.
- From the above observation it is clear that the methanolic extract of fresh *Anethum sowa* have the highest antioxidant activity among other fresh green leafy vegetables selected for this study.

5.2 Recommendation

The following recommendation can be drawn from conclusion.

- Since *Anethum sowa* is good source antioxidants, its cultivation and utilization must be promoted in both local levels and national level.
- Phytochemical analysis can be carried out for different vegetables consumed in Nepal.
- Effect of cooking on nutrients and antioxidant property can be carried out.

Part VI

Summary

Brassica juncea, Chinopodium album, Trigonella foenum graecum, Anethum sowa and *Amaranthus tricolor* are some green leafy vegetables found in the market of Dharan. It is not only consumed in Dharan but throughout the country and the whole world. These types of vegetables are mostly cultivated and consumed in south Asia and South East Asia. The green leafy vegetables are considered as the rich source of micro nutrients and bioactive compunds (antioxidants). Green leafy vegetables are also rich in compounds having antidiabetic, anti-histaminic, anti-carcinogenic and hypolipidemic properties and possess preventive or curative properties against cardiovascular disease, ageing, obesity, hypertension, insomnia and ageing. Leafy vegetables are natural source of antioxidants and rich in phytochemicals. The sample required for analysis is collected from Basantatar, Dharan, Sunsari, Nepal. Data was analyzed using Microsoft Excel 2010.

The fresh leaves collected from market was subjected for antioxidant analysis. Fresh leaves of plant were extracted in 99% methanol to carry out the antioxidant assays. The antioxidant assay was determined by three different parameters namely total antioxidant capacity, reducing power and DPPH scavenging assay. The total antioxidant capacity (TAC) for *B. juncea, C. album, T. foenum graecum, A. sowa* and *A. tricolor* was found to be 33.55±0.65, 38.78±0.35, 40.41±0.32, 50.87±0.28 and 36.53±0.73 mg/100 g fresh weight in terms of ascorbic acid equivalent respectively. Similarly, the reducing power of *B. juncea, C. album, T. foenum graecum, A. sowa* and *A. tricolor* was found to be 19.38±0.04, 18.28±0.08, 18.06±0.12, 41.02±0.65 and 19.06±0.13 mg AAE/100 g respectively. Finally, the DPPH scavenging activity of *B. juncea, C. album, T. foenum-graecum, A. sowa* and *A. tricolor* was found to be 21.85±0.61%, 26.97±0.4%, 31.55±1.22%, 58.45±2.22% and 41.38±1.12% respectively. The results obtained in this work are noteworthy, with respect to the antioxidant activities of the methanol extracts. So the promotion of cultivation and utilization of this plant should be carried out from the local levels. Furthermore, studies in isolation and quantification of the phytochemicals and other compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, and their effects through in vivo studies are needed to evaluate their natural biological function.

References

Agrawal, Y. M., Agrawal, P. Y. and Shamkuwar, B. P. (2014). Phytochemical and biological activities of C*henopodium album*. *Int. J. Pharm. Tech Res.* **6** (1), 383-391.

Akin-Idowu, P. E., Ademoyegun, O. T., Olagunju, Y. O., Aduloju, A. O. and Adebo, A. G. (2017). Phytochemical content and antioxidant activity of five grain Amaranth species *Am. J. Food Sci. Technol.* **5** (6), 249-255 [doi:10.12691/ajfst-5-6-5].

Alam, M. T., Hasan, I., Perveen, A., Nazamuddin, M. and Perveen, S. (2014). Hubla (*Trigonella foenum graecum*): The common Indian spice full of medicinal values. *Int. J. Preclin. Pharma. Res.* **5** (1), 41-46.

Altuntas, E., Ozgo, E. and Taser, F. (2005). Some physical properties of fenugreek (*Trigonella foenum-graceum L*.) seeds. *J. Food Eng.* **71**, 37–43.

Amin, I., Norazaidah, Y. and Emmy Hainida, K. I. (2006). Antioxidant activity and phenolic content of raw and blanched amaranthus species. *Food Chem.* (94), 47–52.

Amin, W. M. and Sleem, A. A. (2007). Chemical and biological study of aerial parts of dill (*Anethum graveolens*). *Egyptian J. Biomed. Sci.* **23** (1), 73-90.

Ashok kumar, C. K., Divya Sree, M. S., Joshna, A., Mohana Lakshmi, S. and Satheesh Kumar, D. (2013). A review on south Indian edible leafy vegetables. *J. Global Trends Pharma. Sci.* **4** (4), 1248-1256.

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med. Aromat. Plants*. **4** (3), 2-6.

Baang, R. P., Del Rosario, R. M. and Palmes, N. D. (2015). Phytochemical profiles and antioxidant activity of selected indigenous vegetables in northern Mindanao, Philippines. *Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng.* **9** (8), 769-774.

Bagchi, K. and Puri, S. (1998). Free radicals and antioxidants in health and disease. *EMHJ*. **4**, 350-360.

Baskar, A. A., Al Numair, K. S., Alsaif, M. A. and Ignacimuthu, S. (2012). *In vitro* antioxidant and antiproliferative potential of medicinal plants used in traditional Indian medicine to treat cancer. *Redox Rep*. **17**, 145–156.

Bensch, N., Horak, M. J. and Peterson, D. (2003). Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Sci.* **51**, 37-43.

Benzie, I. F. F. and Devaki, M. (2018). The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. *In:* "Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications" (1st ed.). (R. Apak, E. Capanoglu and F. Shahidi, Eds.). pp. 77-104. USA. John Wiley & Sons Ltd.

Bhat, R. S. and Al-Daihan, S. (2014). Phytochemical constituents and antibacterial activity of some green leafy vegetables. *Asian Pac. J. Trop. Biomed*. **4** (3), 189-193.

Biswas, M., Dey, S. and Sen, R. (2013). Betalains from *Amaranthus tricolor* L. *J. Pharmacogn. Phytochem.* **1** (5), 87-95.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods. A review. *Int. J. Food Microbiol.* **94** (3), 223-253.

Cai, Y. Z., Sun, M. and Corke, H. (2005). Characterization and application of betalain pigments from plants of the amaranthaceae. *Trends Food Sci. Technol*. **16**, 370-376.

Cartea, M. E., Francisco, M., Soengas, P. and Velasco, P. (2010). Phenolic compounds in brassica vegetables. *Molecules.* **16** (1), 251-280.

Dhiman, K., Gupta, A., Sharma, D. K., Gill, N. S. and Goyal, A. (2012). A review on the medicinal important plants of the family of Cucurbitaceae. *Asian J. Clin. Nutr*. **4**, 16-26.

Dimcheva, V. and Karsheva, M. (2018). *Cistus incanus* from strandja mountain as a source of bioactive antioxidants. *Plants.* **7** (1), 8.

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.* **37**, 277-285.

Evarando, L. S., Oliveira, L. E., Freire, L. K. R. and Sousa, P. C. (2005). Inhibitory action of some essential oils and phytochemicals on growth of various moulds isolated from foods. *Braz. Arch. Biol. Technol*. **48**, 234-241.

Fasuyi, A. O. (2006). Nutritional potentials of some tropical vegetable meals, chemical characterization and functional properties. *Afr. J. Biotechnol*. **5** (1), 49-53.

Flowersofindia.net. (2013). *Chenopodium album* - Bathua. Retrieved from http://www.flowersofindia.net/catalog/slides/Bathua.html. [Accessed 15 August 2018].

Formagio, A. S. N., Volobuff, C. R. F., Santiago, M., Cardoso, C. A. L., Vieira, M. C. and Pereira, Z. V. (2014). Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in *Psychotria* leaf extracts. *Antioxidants*. **3**, 745-757.

Gacche, R. N., Kabaliye, V. N., Dhole, N. A. and Jadhav, A. D. (2010). Antioxidant potential of selected vegetables commonly used in diet in Asian subcontinent. *Indian J. Nat. Prod. Resour*. **1** (3), 306-313.

Gumus, T., Demirci, A. S., Sonuc, M. N., Demirok, N. T., Tulukcu, E. and Gulcu, M. (2016). Investigation of antimicrobial and antioxidant activities of essential oils extracted from medicinal plants. *J. Food Safety Food Qual*. **67** (1), 1–28. [doi:10.2376/0003-925X-67-17].

Gunathilake, K. D. P. P. and Ranaweera, K. K. D. S. (2016). Antioxidative properties of 34 green leafy vegetables. *J. Func. Foods*. **26**, 176-186.

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochem. Soc. Transact*. **35**, 1147-1150.

Halliwell, B. and Gutteridge, J. C. (1995). The definition and measurementof antioxidants in biological systems. *Free Radic. Biol. Med.* **18**, 125-126.

Handa, S., Khanija, S., Longo, G. and Rakesh, D. (2008). "Extraction Technologies for Medicinal and Aromatic plants " (1st ed.). United Nations Industrial Development Organization. Italy.

Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in liquorice root: Their relative astringency and radical scavenging effects. *Chem. Pharmaceut. Bull*. **36**, 1090-2097.

Hosseinzadeh, H., Karemi, G. R. and Ameri, M. (2002). Effects of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice. *J. BMC Pharmacol*. **2** (1), 21-30.

Huang, S., Huang, M. and Feng, B. (2012). Antioxidant activity of extracts produced from pickled and dried mustard (*Brassica juncea* Coss. Var. foliosa Bailey). *Int. J. Food Prop*. **15** (2), 374-384. [doi: 10.1080/10942912.2010.487628].

Iqbal, S. and Bhanger, M. I. (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J. Food Compos. Anal*. **19**, 544-541.

Islam, M. S., Khatoon, J. A., Alamgir, M. and Hossain, M. A. (2003). Nutritional status of red amaranth as influenced by selected pesticides. *Pak. J. Biol. Sci*. **6**, 2044–2049.

Jana, S. and Shekhawat, G. S. (2010). *Anethum graveolens*: An Indian traditional medicinal herb and spice. *Pharmacogn. Rev*. **4** (8), 179–184. [doi: 10.4103/0973-7847.70915].

Jaradat, N., Hussen, F. and Ali, A. A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. *J. Mater. Environ. Sci.* **6** (6), 1771-1778.

Johnson, D., Kershaw, L., MacKinnon, A. and Pojar, J. (1995). "Plants of the Western Boreal Forest and Aspen Parkland". Lone Pine Publishing. [ISBN 978-1-55105-058-4].

Juntachole, T. and Berghofer, E. (2005). Antioxidative properties and stability of ethanolic extracts of holy basil and galangal. *Food Chem.* **92**, 193-202.

Kabiri, N., Asgary, S. and Setorki, M. (2011). Lipid lowering by hydroalcoholic extracts of *Amaranthus Caudatus* L. induces regression of rabbits atherosclerotic lesions. *Pharmacologyonline*. **1**, 84-91.

Kaczora, A., Baranskaa, M. and Czamaraa, K. (2016). Carotenoids: Overview of nomenclature, structures, occurrence, and functions. *In:* "Carotenoids: Nutrition, Analysis and Technology". (A. Kaczor and M. Baranska, Eds.). pp. 19-34. UK. John Wiley & Sons, Ltd.

Kadoshnikov, S. I., Kadoshnikova, I. G., Kulikov, Y. A. and D.M., M. (2008). Researches of fractional composition of protein of amaranth. *Curr. Nutr. Food Sci*. **4**, 196-205.

Kapoor, D., Kaur, S. and Bhardwaj, R. (2014). Physiological and biochemical changes in *Brassica juncea* plants under Cd-induced stress. *BioMed. Res. Int.* 13. [doi:10.1155/2014/726070].

Kaur, G. J. and Arora, D. S. (2010). Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family umbelliferae - current status. *J. Med. Plant. Res*. **4** (2), 87-94.

Kaur, N., Chahal, K. K., Singh, R. and Urvashi. (2018). Phytochemical screening and antioxidant activity of *Anethum graveolens* L. seed extracts. *J. Pharma. Innov*. **7** (6), 324-329

Khandaker, L., Ali, M. B. and Oba, S. (2008). Total polyphenol and antioxidant activity of red amaranth (*Amaranthus tricolor* L.) as affected by different sunlight level. *J. Jpn. Soc. Hort. Sci*. **77** (4), 395–401.

Kor, N. M., Didarshetaban, B. M. and Pour, H. R. S. (2013). Fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant. *Int. J. Adv. Biol. Biom. Res.* **1** (8), 922-931.

Kumar, R., Kumar, D. and Patwa, R. (2018). Evaluation of phytochemical constituents and antioxidant activity of *Chenopodium album* of Bundelkhand region. *J. Pharm. Res*. **12** (1).

Kumar, V., Thakur, A. K., Barothia, N. D. and Chatterjee, S. S. (2011). Therapeutic potentials of *Brassica juncea*: An overview. *Int. J. Guniene Trad. Med.* **1** (1), e2. [doi: 10.5667/tang.2011.0005].

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmaco. Rev.* **4** (8), 118-126.

Mandindi, T. K. (2015). Phytochemical and antioxidant composition of selected local wild plants in South Africa: Consideration of alternative nutrients for health promotion IPCBEE 81 (17), 96-102. [doi:10.7763/IPCBEE].

Manohar, R. P., Pushpan, R. and Rohini, S. (2009). Mustard and its uses in ayurveda. *IJTK*. **8** (3), 400-404.

Marques, S. S., Magalhaes, L. M., Toth, I. V. and Segundo, M. A. (2014). Insights on antioxidant assays for biological samples based on the reduction of copper complexes-the importance of analytical conditions. *Int. J. Mol. Sci.* **15**, 11387-11402.

Mashkor, I. M. A. A. (2014). Phenolic content and antioxidant activity of fenugreek seeds extract. *IJPPR*. **6**(4), 841-844.

Mathur, R. (2012). Antimicrobial potential and phytochemical analysis of plant extracts of *Anethum sowa*. *Int. J. Cur. Res. Rev*. **4**(20), 55.

McDougall, G. J., Fyffe, S., Dobson, P. and Stewart, D. (2007). Anthocyanins from red cabbage - stability to simulated gastrointestinal digestion. *Phytochem*. **68** (9), 1285-1294.

Meena, S., Agrawal, M. and Agrawal, K. (2016). Effect of blanching and drying on antioxidants and antioxidant activity of selected green leafy vegetables. *IJSR*. **5** (10), 1811-1814.

Meghwal, M. and Goswami, T. K. (2012). A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. *J. Food Process. Technol.* **3** (9). [doi:10.4172/2157-7110.1000181].

Mermlestein, W. (2008). Determining antioxidant activity. *Food Technol*. **11**, 63-65.

Mukta, J. F. (2015). Evaluation for antioxidant activity of *Trigonella foenum-graecum* using several *in vitro* assay systems. B. of Pharmacy. Thesis. Daffodil International Univ. , Bangladesh.

Nahar, L. and Sarker, S. D. (2014). Chenoalbuside: an antioxidant phenolic glycoside from the seeds of *Chenopodium album* L. (Chenopodiaceae). *Braz. J. Pharmacogn*. **15** (4), 279-282.

Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucosamine. *Jpn. J. Nutr*. **44**, 307-315.

Pande, M. and Anupam, A. (2010). Preliminary pharmacognostic evaluations and phytochemical studies on leaf of *Chenopodium album* (*Bathua Sag*). *Asian J. Exp. Biol. Sci.* **1** (1), 91-95.

Park, S., Jang, H., Lee, J., Choi, Y., Kim, H., Hwang, J., Seo, D., Kim, S. and Nam, J. (2017). Changes in the phenolic compounds and antioxidant activities of mustard leaf (*Brassica juncea*) kimchi extracts during different fermentation periods. *Food Sci. Biotechnol.* **26** (1), 105-112. [doi:10.1007/s10068-017-0014-5].

Pathak, V., Dwivedi, R. and Shukla, P. (2014). Pharmacognostical study of *Anethum sowa* (Dill) seed. *Int. J. Rec. Biotech.* **2** (3), 6-14.

Pisoschi, A. M. and Negulescu, G. P. (2011). Methods for total antioxidant activity determination: A review. *Biochem. Anal. Biochem*. **1**, 106.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem*. **269**, 337-341.

Prior, R. L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **53**, 4290-4302.

Rahman, A. H. M. M. and Gulshana, M. I. A. (2014). Taxonomy and medicinal uses on amaranthaceae family of Rajshahi, Bangladesh. *Appl. Ecol. Environ. Sci.* **2** (2), 54-59 [doi:10.12691/aees-2-2-3].

Ramadana, M. M., Abd-Algaderd, N. N., El-kamalid, H. H., Ghanemb, K. Z. and Farragc, A. R. H. (2013). Volatile compounds and antioxidant activity of the aromatic herb *Anethum graveolens*. *J. Arab Soc. Med. Res.* **8**, 79–88.

Rao, K. N., Padhy, S. K., Dinakaran, S. K., Banji, D., Madireddy, S. and Avasarala, H. (2010). Study of pharmacognostic, phytochemical, antimicrobial and antioxidant activities of *Amaranthus tricolor* Linn. leaves extract. *Iranian J. Pharma. Sci. Autumn.* **6**(4), 289-299.

Sarangthem, Kananbala and Hoikhokim. (2011). Traditional fermentation of mustard green (*Brassica juncea*) enhances the nutritive value. *Adv. Res. J. Crop Improv.* **2** (2), 154-157.

Saritha, V., Anilakumar, K. R. and Farhath, K. (2010). Antioxidant and antibacterial activity of *Aloe vera* gel extracts. *J. Pharm. Biol. Arch. Int.* **1** (4), 376-384.

Satyanarayana, S., Sushruta, K., Sarma, G. S., Srinivas, N. and Raju, S. G. V. (2004). Antioxidant activity of the aqueous extracts of spicy food additives-evaluation and comparison with ascorbic acid in *in-vitro* systems. *J. Herb. Pharmacother*. **4** (2), 1-10.

Schliemann, W., Cai, Y., Degenkolb, T., Schmidt, J. and Corke, H. (2001). Betalains of *Celosia argentea*. *Phytochem*. **58**, 159-165.

Sharma, H. P. and Kumar, R. A. (2013). Health security in ethnic communities through nutraceutical leafy vegetables. *J. Environ. Res. Dev*. **7** (4), 1423-1429.

Shrestha, S. and Rai, S. K. (2012). Survey of marketable vegetables and edible fruits in Dharan, eastern Nepal. *Nep. J. Biosci*. **2**, 134-147.

Shukla, S., Bhargava, A., Chatterjee, A., Srivastava, J., Singh, N. and Singh, S. P. (2006). Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). *Plant Foods Hum. Nutr*. **61**, 23-28.

Singh, G., Kapoor, I. P. S., Pandey, S. K., Singh, U. K. and Singh, R. K. (2001). Studies on essential oils: part 10, antibacterial activity of volatile oils of some spices. *Phytother. Res*. **16**, 680-682.

Sochor, J., Ryvolova, M., Krystofova, O., Salas, P., Hubalek, J., Adam, V., Trnkova, L., Havel, L., Beklova, M., Zehnalek, J., Provaznik, I. and Kizek, R. (2010). Fully automated spectrometric protocols for determination of antioxidant activity: Advantages and disadvantages. *Molecules.* **15**, 8618-8640.

Stavri, M. and Gibbons, S. (2005). The antimycobacterial constituents of dill (*Anethum graveolens*). *Phytother. Res*. **19** (11), 938-941.

Subhashini, N., Thangathirupathi, A. and Lavanya, N. (2011). Antioxidant activity of *Trigonella foenum-graecum* using various *in vitro* and *ex vivo* models. *Int. J. Pharm. Sci.* **3**(2), 96-102.

Sun, J., Chu, Y. F., Wu, X. and Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem*. **50**, 7449–7454.

Tejaswini, N., Saidaiah, P., Reddy, R. K. and Ramesh, T. (2017). Evaluation of vegetable amaranth (*Amaranthus tricolor*. L) genotypes for yield and yield attributing traits. *J. Pharmacogn. Phytochem*. **6** (6), 2572-2578.

Tiwari, P., Kumar, B., Kaur, M., Gurpreet, K. and Kaur, H. (2011). Phytochemical screening and extraction: A Review *Internationale Pharmaceutica Sciencia*. **1** (1), 98-106.

Usman, L. A., Hamid, A. A., Muhammad, N. O., Olawore, N. O., Edewor, T. I. and Saliu, B. K. (2010). Chemical constituents and anti-inflammatory activity of leaf essential oil of nigerian grown *Chenopodium album* L. *EXCLI*. **9**, 181-186.

Valavanidis, A., Vlachogianni, T., Fiotakis, K. and Loridas, S. (2013). Pulmonary oxidative stress, inflammation and cancer: Respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *Int. J. Environ. Res. Public Health*. **10**, 3886-3907.

Venkatachalam, K., Rangasamy, R. and Krishnan, V. (2014). Total antioxidant activity and radical scavenging capacity of selected fruits and vegetables from South India. *IFRJ*. **21** (13), 1039-1043.

Vijayalakshmi, M. and Ruckmani, K. (2016). Ferric reducing anti-oxidant power assay in plant extract. *Bangladesh J. Pharmacol.* **11**, 570-572.

Wani, S. A. and Kumar, P. (2018). Nutraceutical properties and utilization of fenugreek in food product. *J. Saudi Soc. Agr. Sci.* **17**, 97 –106.

WHO. (2017). Noncommunicable Diseases Progress Monitor 2017 [Report]. Geneva,

Retrieved from http://apps.who.int/iris/bitstream/handle/10665/258940/9789241513029-eng.pdf;jsessionid=ECF10FFA023EDEA51CE4E831FAF30972?sequence=1. [Accessed 7 July, 2018].

Yadav, R. K., Kalia, P., Kumar, R. and V., J. (2013). Antioxidant and Nutritional Activity Studies of Green Leafy Vegetables. *IJFST*. **4** (7), 707-7012.

Young, I. S. and Woodside, J. V. (2001). Antioxidants in health and disease. *J. Clin. Pathol.* **54**, 176-186.

Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., Shan, L., Lin, Y. and Kong, W. (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chem.* **107**, 296-304.

Zou, D., Brewer, M.Garcia, F., Feugang, J. M., Wang, J. and Zang, R. (2005). Cactus pear: a natural product in cancer chemoprevention. *Nutr. J.* **4**, 25-36.

Appendices

Appendix A (Standard curves)

Appendix A.1

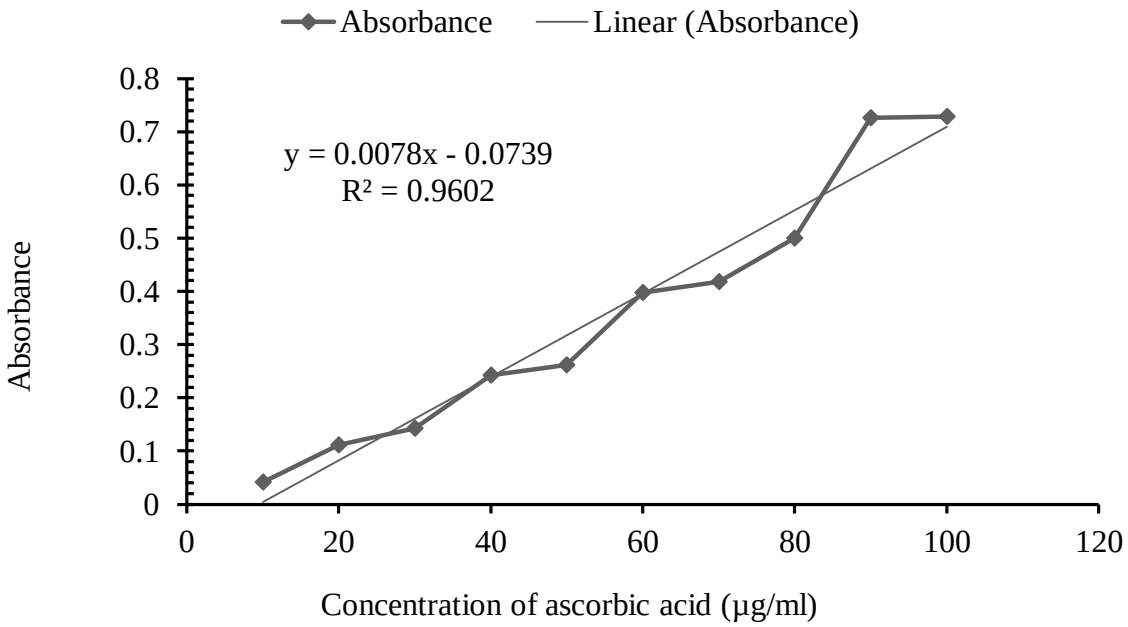


Fig. B.1 Ascorbic acid standard curve in methanol for TOAC determination

Appendix A.2

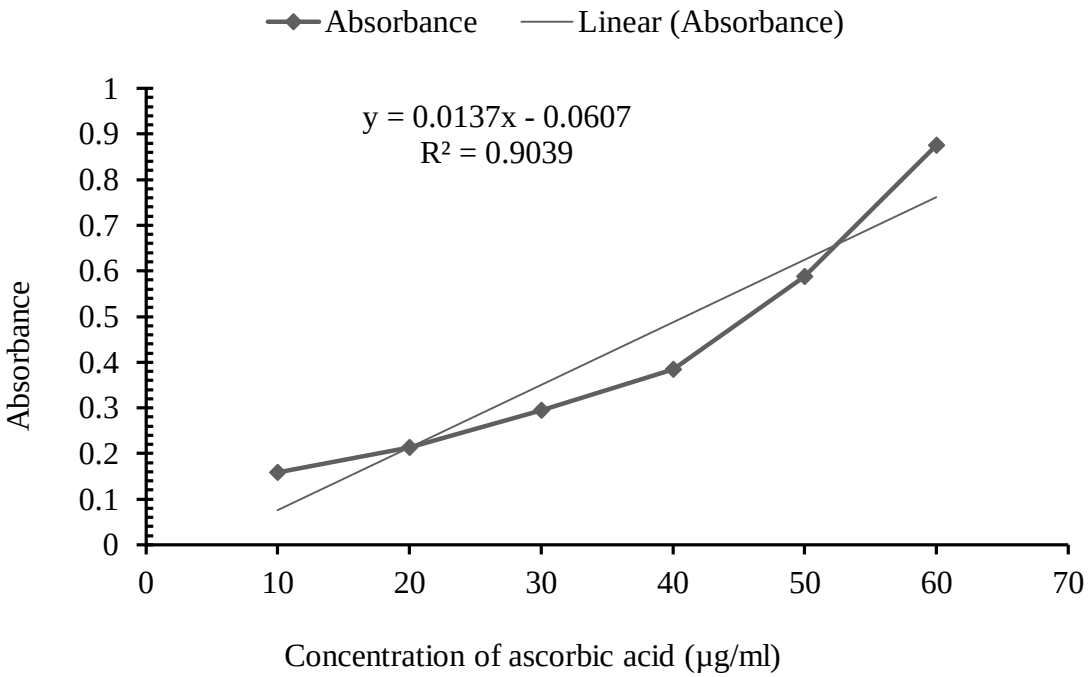


Fig. B.2 Ascorbic acid standard curve in methanol for reducing power determination

List of Plates

P1 Fresh Green leafy vegetables

P2 Extraction in methanol

P3 Rotary vaccum evaporator