Determination of Antioxidant Activity of Various Extracts of *Dracaena spicata*

A Dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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DECLARATION BY THE CANDIDATE

I, **Muhammedullah Rumi**, hereby declare that this dissertation, entitled '**Determination of Antioxidant Activity of Various Extracts of *Dracaena spicata'*** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) is a genuine & authentic research work carried out by me. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma or Fellowship.

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CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation, entitled '**Determination of Antioxidant Activity of Various Extracts of *Dracaena spicata'*** is a research work carried out by **Muhammedullah Rumi** (ID: 2013-1-70-046) in 2016, under the supervision and guidance of me, in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled '**Determination of Antioxidant Activity of Various Extracts of *Dracaena spicata'*** is a research work carried out by **Muhammedullah Rumi** (ID: 2013-1-70-046), under the supervision and guidance of **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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Dedication

This Research Paper is dedicated to

My beloved parents, they are my biggest inspirations.

Abstract:

Indigenous knowledge of herbal medicines for skin diseases like boils, eczema, scabies, septic abscess, itching and skin allergy, burns, chicken pox, warts and leucoderma, fungal and bacterial infections, including healing cuts and wounds has been documented by randomly interviewing Chakma, Marma and Tanchangya tribes of the hill tracts districts of Bangladesh since 1995. The plant *Dracaena spicata* has been used for the general promotion of health and longevity by Asian tribal (especially Chakma, Marma and Tanchangya). It is used as a traditional medicine for the treatment of various diseases cough, syphilis, conjunctivitis, constipation; pills prepared from the leaves are taken with warm water twice daily for the treatment of measles by the Chakma etc. The aim of the present study was to evaluate the antioxidant activity of petroleum Ether, Dichloromethane and Methanol extract of *Dracaena spicata*. The antioxidant activity was measured by DPPH, Total Phenol and Total Flavonoid tests. The IC_{50} values of DPPH test were 120.2492, 127.1228 and 48.8535 µg/ml for Petroleum Ether, Dichloromethane Methanol consecutively. The Total Flavonoid contents were 68, 56.6667 and 94.3334 in mg/g equivalent to Quercetin for Petroleum Ether, Dichloromethane Methanol consecutively. The Total Phenol contents were 216.2, 82, 58.6 mg/g equivalent to Gallic acid for Petroleum Ether, Dichloromethane Methanol consecutively. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity. In conclusion, further investigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported antimicrobial and antioxidant properties of *Dracaena spicata.*

Key Words: Medicinal Plant, *Dracaena spicata*, Antioxidant, DPPH, Total Phenol, Total Flavonoid

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Chapter 1

Introduction

1.1 General Introduction: Medicine is considered as one of the most important necessity to all of us. It is derived from the Latin words ars medicina meaning "the art of healing". It is a branch of the health sciences and is the sector of public life concerned with maintaining or restoring human health through the study, diagnosis, treatment and possible prevention of disease, injury and other damage to a body or mind.

It is both an area of knowledge, a science of body system and their diseases and treatment. This branch of science encompasses treatment by drugs, diet, exercise and other nonsurgical means. It is also used to maintain our health. An agent such as drug is used to treat disease or injury. There are different types of medicine, we have herbal medicine, which came from different kinds of plants, medicines treat in hospital and etc. Herbal medicine, also called botanical medicine or phytomedicine, refers to use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbalism is becoming more mainstreams as up-to-date analysis and research show their value in the treatment and prevention of disease. Some of us believe in herbal medicines, for it is pure came from plants and no other ingredients. Herbal medicine also uses for cough, fever, toothache and some other diseases that might catch from our environment. Herbalists treat many conditions such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, and an irritable bowel syndrome among others. Plants had been used for medicinal purposes long before recorded history. For most herbs, the specific ingredient that causes a therapeutic effect is not known. Whole herbs contain many ingredients and it is likely that they work together to produce the desired medicinal effect.

Some medicines may cause problems if you take them with other medicines. This is why it is important to tell your doctor and pharmacist about all the medicines you are taking. And some medicines can cause problems, even if you take them correctly. Call your doctor or pharmacist if you think your medicine is making you feel worse. We take medicine to make us feel better when we are sick. (Jennifer Alinio, 2007).

1.2 Medicinal Plants: Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. Roughly 50,000 species of higher plants (about 1 in 6 of all species) have been used medicinally. This represents by far the biggest use of the natural world in terms of number of species.

Most species are used only in folk medicine, traditional systems of formal medicine using relatively few (e.g. 500-600 commonly in Traditionally Chinese Medicine). Around 100 plant species have contributed significantly to modern drugs. The use of medicinal plants is increasing worldwide.

The medicinal uses of plants grade into their uses for other purposes, as for food, cleaning, personal care and perfumery. Plants are used in medicine to maintain and augment health - physically, mentally and spiritually - as well as to treat specific conditions and ailments.

World Health Organization (WHO) has defined medicinal plants as plants that contain properties or compounds that can be use for therapeutic purposes or those that synthesize metabolites to produce useful drugs. Medicinal plants constitute an important component of flora and are widely distributed in India. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can leads to the development of novel and safe medicinal agents. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures. A large proportion of such medicinal compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses. The rich knowledge base of countries like India and China in medicinal plants and health

care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs. India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. More over the agro climatical conditions are conducive for introducing and domesticating new exotic plant varieties. At present majority of the people are relying for their primary health care on traditional medicine.

The number of plants with medicinal properties far exceeds the number of plants used as food source. For instance, Chinese herbalists have identified more than 5,000 medicinally important indigenous plants and the Amazon, the Golden triangle region of northern Thailand, the tropics of the Venezuela-Guyana border, and the teeming forests of central Africa, all have native human populations using 10 indigenous plant resources for healing purposes. However, despite the huge biological potential of epiphytes and endophytes associated with these higher plants, these microorganisms have received little attention. The search for bio active natural products of endophytic fungi, isolated from higher plants, are attracting considerable attention from researchers worldwide, as indicated by the increase of work and publications on therapeutic potential during recent years.

Since the discovery of the world's first billion-dollar anticancer drug, paclitexel (Taxol) from *Pestalotiopsis microspora*, a fungus that colonizes the Himalayan yew tree *Taxus wallichiana*, without causing apparent injury to the host plant, interest is growing in symptomless parasitic fungi, termed the 'endophytes'. The term endophyte is applied to fungi (or bacteria) which live within plant tissues, for all or part of their life cycle and cause no apparent infections. (Plantlife, 2010)

1.3 Characteristics of Medicinal Plants:

Medicinal plants have many characteristics when used as a treatment, as follow:

1.3.1 Synergic Medicine: The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

1.3.2 Support of Official Medicine: In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

1.3.3 Preventive Medicine: It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. (Bassam Abdul Rasool Hassan, 2012)

1.4 Importance of Medicinal Plants: We have so much to benefit from by returning to plants in their most natural state. The famous father of medicine, Hippocrates was quoted as saying: *'Let thy food be thy medicine, and thy medicine shall be thy food'.* (Evita Ochel, 2010)

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs.

Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples. (Wordpress, 2010)

The efficacy of some herbal products is beyond doubt, the most recent examples being *Silybum marianum* (silymarin), *Artemisia annua* (artemesinin) and *Taxus baccata* (taxol). On the other hand, randomized, controlled trials have proved the efficacy of some established remedies, for instance, *Ginkgo biloba* for tinnitus, *Hypericum perforatum* is a reputed remedy for depression. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herb.

Recently research has supported biological activities of some medicinal herbs. Cancer is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease. *Allamanda cathratica* [allamandin], *Elephatopus elatus* [elephantpoin], *Helenium autmnale* [helenalin], *Vernonia hymenlepis*, *Heliotropium indicum* [Indicine-N-oxide], *Daphne mezereum* (mezerien) and *Stereospermum suaveolans* [laphacol] are medicinal plants that have shown significant tumor inhibiting effect.

Diabetes mellitus is another area where a lot of research is going on. *Ajuga reptens* (the active principle is said to potentiate effects of insulin), *Galagea officinalis* (galagine), *Bougainvillea spectabilis* (pinitol), *Momordica charantia* (chirantin), *Gymnema sylvestre* (gymnemic acid) are some medicinal herbs that have shown effectiveness in non-insulin dependent diabetes. Recently extract of *Tecoma stans* has shown potent anti diabetic activity. Alkaloid tecomonine is considered to be active principle of the herb.

Arthritis is another potential disease where no satisfactory answer is present in modern medicine. *Commiphora mukul* (guggulsterones), *Boswellia serrata* [boswellic acid], *Withania somnifera* (withanolides), *Ruscus acueleatus* (ruscogenin), *Harpagophytum procumbens* (harpagoside) are prominent plants with anti- arthritic activity. Harpagoside is a precious constituent as it has anti rheumatoid activity. Rest of all natural products has anti-inflammatory activity.

Chrysanthemum parthenium traditionally known as feverfew has shown promising results in migraine, a disease that has eluded the researchers from centuries. The herb contains sesquiterpenes lactones called parthenolides, which are the active principles of the herb.

Hepatoprotective action of certain botanicals deserves attention. *Sedum sarmentosum* (sarmentosin), *Schisandra chinensis* (waweizichun and schisantherin) have shown their ability to lower raised liver enzymes in viral hepatitis.

Croton sublyratus (plaunotol) has potent and wide spectrum anti peptic ulcer action. A number of plant derivatives have shown anti-Aids activity. *Ancistrocladus korupensis* (michellamine-b), *Caulophyllum langigerum* (calanolide-a), *Caulophyllum teymani* (costatolide-a), *Homalanthus nutans* (prostratin), *Conospermum* (concurvone) are the medicinal herbs from African countries that are being employed in research for finding a suitable cure for Aids.

The concept of antioxidants is fastly catching up and latest research has shown that a number of herbal derivatives have excellent antioxidant action. *Bacopa monnieri* contains bacosides A and B and bacoside A is a strong antioxidant, which reduces several steps of free radical damage. *Coleus forskohlii* (forskolin), *Camellia sinensis* (polyphenols), *Huperzia serrata* (huperzine), *Pinus maritima* (Pycnogenol), *Borago officinalis* (gamma linoleic acid) and *Vinca minor* (Vinpocetine) are potential antioxidants.

The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to phytochemical screening for the detection of various plant constituents.

With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases. (Pandey, R.P. & Dilwakar, P.G., 2008)

1.5 Uses of Some Medicinal Plants:

1. Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.

2. Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, and Variegated Sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.

3. Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.

4. Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.

5. To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as Chirayta, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.

6. Sandalwood and Cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.

Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf, they serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.

7. Indian sages were known to have remedies from plants which act against poisons from animals and snake bites.

8. Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.

9. Some herbs like aloe, sandalwood, turmeric, sheetraj hindi and khare khasak are commonly used as antiseptic and are very high in their medicinal values.

10. Ginger and cloves are used in certain cough syrups. They are known for their expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea and bronchi. Eucalyptus, Cardamom, Wild cherry and cloves are also expectorants.

11. Herbs such as Chamomile, Calamus, Ajwain, Basil, Cardamom, Chrysanthemum, Coriander, Fennel, Peppermint and Spearmint, Cinnamon, Ginger and Turmeric are helpful in promoting good blood circulation. Therefore, they are used as cardiac stimulants.

12. Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases.

13. Herbal medicine practitioners recommend calmative herbs, which provide a soothing effect to the body. They are often used as sedatives.

14. Certain aromatic plants such as Aloe, Golden seal; Barberry and Chirayta are used as mild tonics. The bitter taste of such plants reduces toxins in blood. They are helpful in destroying infection as well.

15. Certain herbs are used as stimulants to increase the activity of a system or an organ, for example herbs like Cayenne.

16. A wide variety of herbs including Giloe, Golden seal, Aloe and Barberry are used as tonics. They can also be nutritive and rejuvenate a healthy as well as diseased individual.

17. Honey, turmeric, marshmallow and liquorices can effectively treat a fresh cut and wound. They are termed as vulnerary herbs. (Zahid, 2016)

1.6 Classification of Medicinal Plants:

There are a large number of medicinal and aromatic plants in the nature which are used for medicinal and aromatic purposes. Moreover, medicinal plants are sometimes used for aromatic purposes similarly aromatic plants may also be used for medicinal purpose! Hence, classification of medicinal and aromatic plants is difficult. Since there are a large number of plants in these two groups an attempt has been made in this chapter to facilitate for further study. (My Agricultural Information Bank, 2015)

Medicinal plants are generally classified on the basis of their growth habit. It may be a tree, shrub, herb, annuals, biennial, tubers, rhizomes and climbers.

Table 1: Medicinal Trees

Sr. No.	Common Name	Botanical Name	Parts Used
1	Babul	*Acacia nilotice*	Pods, leaves, bark, gum
2	Bael	*Aegle marmelos*	Roots, leaves, fruit
3	Neerh	*Azaflirachta indica*	Bark leaves, flowers, seed, oil
4	Palas	*Butea monossperma*	Bark, leaves, flowers, seed, gum
5	Gugul	*Commiphora mukul*	Resinous gum
6	Olive	*Olea europeae*	Leaves, Oil
7	Arjun	*Terminalia arjuan*	Bark
8	Behela	*Terminalia bellirica Gaertu*	Bark, fruit
9	Hirda	*Terminalia bellirica Gaertu*	Fruits
10	Nagakesar	*Mesua ferrea*	Blowers, oil
11	Markingnut	*Semecarpus &anacardium*	Fruits

Table 2: Medicinal Shrubs

Sr. No.	Common Name	Botanical Name	Parts Used
1	Davana	*Artemisia nilagirica*	Leaves, flowering top
2	Safed musli	*Aparagus adscendens*	Tuberous roots
3	Belladonna	*Atropa belladonna*	Leaves and roots
4	Lavender	*Lavandula officinalis*	Flowers
5	Sarpagandha	*Rauvalfia serpentina*	Roots

6	Chitrak	*Plumbage zeylanica*	Leaves, roots

Table 3: Medicinal Herbs

Sr. No.	Common Name	Botanical Name	Parts Used
1.	Brahmi	*Bacopa monnieri*	Whole plant
2.	Am haldi	*Curcuma amada*	Rhizomes
3.	Haldi	*Curcuma domestica*	Rhizomes
4.	Datura	*Datura metel*	Leaves, flowers
5.	Kalazira	*Nigella sativa*	Seed
6.	Afim	*Papaver somniferum*	Latex, seed
7.	Pipli	*Piper Longum*	Fruits, roots
8.	Babchi	*Psoralea corylifolia*	Seed, Fruit

Table 4: Medicinal Annuals

Sr. No.	Common Name	Botamical Name	Parts Used
1.	Jangali muli	*Blumea lacera*	Whole plant
2.	Cockscomb	*Celosia cristala*	Inflorescence
3.	Red poppy	*Papaver rhoeas*	Flowers
4.	Bhui amla	*Phyllantius niruri*	Whole plant

Table 5: Biennial

Sr. No.	Common Name	Botanical Name	Parts Used
1	Bankultthi	*Cassia abus*	Leaves, seeds
2	Caper spurge	*Euphorbia lathyrus*	Seed latex

3	Catchfly	*Melandrium firmum*	Whole plant

Table 6: Tubers and Rhizomes

Sr. No.	Common Name	Botanical Name	Parts Used
1	Satavar	*Asparagus adscendens*	Tubers
2	Safed musli	*Chlorophytum borivilianum*	Tubers
3	Puskarmul	*Inula racemosa*	Roots
4	Sakarkhand	*Manihot esculenta*	Tubers

Table 7: Biennial

Sr. No.	Common Name	Botanical Name	Parts Used
1	Chocloate vine	*Akebia quinata Deene*	Stem, fruit
2	Malkunki	*Celustrus paniculatus Wild*	Bark, leaves, seed
3	Hajodi	*Cissus quadrangularis L.*	Whole plant
4	Khira	*Cucumis sativus L.*	Fruit, seed
5	Gudmar	*Gymnema sylvestre Retzx*	Whole plant, leves
6	Kali mirch	*Piper nigrum L.*	Fruit

(My Agricultural Information Bank, 2015)

1.7 Families of Medicinal Plants: Most of the medicinal plants belong to the following families:

1. Compositae
2. Labiatae
3. Umbelliferae
4. Boraginaceae
5. Cruciferae

1.7.1 Medicinal Plants of the Compositae Family: The Compositae family, also known as the Daisy family, contains the highest number of medicinal plants as compared to other families, all members being sun lovers. They have either a disk flower or a ray flower. Being dry and hard the fruits often have plumes of hairs to aid in wind dispersal. Medicinal plants belonging to this family include chamomile, field and pot marigolds, daisy, wormwood, chicory, thistles, ragwort and artichoke.

1.7.2 Medicinal Plants of the Labiatae Family: A very important medicinal plant family is the Labiatae family, also known as the mint family. Plants in this family are herbs or shrubs often with an aromatic smell. They are often met in the Mediterranean countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. The common characteristics are square stems and mostly irregular two-lipped flowers having four stamens. The fruit is small with four s (seeds). Some examples from this family include horehound, lavander, balm, micromeria, the mints, thyme and rosemary, basil, sage.

1.7.3 Medicinal Plants of the Umbelliferae Family: The Umbelliferae or parsley members often have hollow stems and flowers in clusters called umbels and a characteristic umbrella-arranged fruit. These plants usually produce an essential oil, an asset to survive during the hot summer days. Bullwort (*Ammi majus*), wild celery (*Apium graveolens*), wild carrot (*Daucus carota*), sea holly (*Eryngium maritima*), fennel (*Foeniculum vulgaris*), anise (*Pimpinella anisum*), wild parsley (*Petroselinium crispum*) are all parsley family members.

1.7.4 Medicinal Plants of the Boraginaceae Family: The Boraginaceae or borage family is made up of herbs or small shrubs with bristly stems and leaves. Members of the Boraginaceae all have tubular flowers mostly in curved racemes, five stamens being attached to the tube. The ovary is superior usually forming a fruit composed of four nutlets. Examples in this family include borage (*Borago officinalis*), common comfrey (*Symphytum officinale*), purple alkanet (*Anchusa asurea*), yellow gromwell (*Neatostema apulum*), viper's bugloss (*Echium vulgare*) and southern hound's tongue (*Cynoglosum creticum*).

1.7.5 Medicinal Plants of the Cruciferae Family: The Cruciferae or mustard (cress) family is characterised by plant that have flowers with cross-like petals. This family groups a large group of medicinal plants that include Wallflower (*Cheiranthus cheiri*), Bitter cress (*Cardamine hirsuta*), Black mustard (*Brassica nigra*), Horseradish (*Armoracia rusticana*), Hedge mustard (*Sisymbrium officinale*), White mustard (*Sinapis alba*), Wild radish (*Raphanus raphanistrum*), Watercress (*Nasturtium officinale*).

There are some other families of plant to which herbs belong such as Rosaceae family, Rutaceae and Solanaceae families, Malvaceae and other families. (B.C. Bennett, n.d.)

1.8 Traditional Medicine: Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. (Rumana Huque, 2014)

It is evident that the non-availability of drugs and commodities, poor access to services by the poor, imposition of unofficial fees, lack of trained providers, a rural-urban imbalance in health providers' distribution, weak referral mechanisms and unfavorable opening hours are contributing to low use of public facilities in Bangladesh. This indicates that though the health care seeking behavior is partly associated with the socio-economic status of the population, the supply side problems existing within the health system also influence service utilization.

(Dr Xiaorui Zhang, 2000)

Table 8: Selected Modern Drugs that Came from Traditional Medicine

Drug	What it is for	Derived from	Originally used in
Artemisinin	Antimalarial	Produced from the Chinese herb Qinghao or sweet wormwood	Traditional Chinese medicine for chills and fevers
Cromoglycate	Asthma	Based on the plant Khella,	Traditional Middle Eastern remedy

	prevention	whose active ingredient is khellin	for asthma. Also traditionally used in Egypt to treat kidney stones
Etoposide	Anticancer	Synthesized from podophyllotoxin, produced by the American mandrake plant	Various remedies in Chinese, Japanese and Eastern folk medicine
Hirudin	Anticoagulant	Salivary glands in leeches, now produced by genetic engineering	Traditional remedies across the globe, from Shui Zhi medicine in China to eighteenth and nineteenth century medicine in Europe
Lovastatin	Lowers cholesterol	Foods such as oyster mushrooms and red yeast rice, also used to synthesis other compounds such as pravastatin	Mushrooms are used to treat a wide range of illnesses in traditional medicine in China, Japan, Eastern Europe and Russia
Opiates	Painkilling	Unripe poppy seeds	Traditional Arab, Chinese, European, Indian and North African medicines as pain relief and to treat a range of illnesses including diarrhea, coughs and asthma
Quinine	Antimalarial	Bark of the cinchona tree	Traditional remedies to treat fevers and shivers in South America
Vinca alkaloids (vincristine, vinblastine)	Anticancer	Synthesized from indole alkaloids produced by the rosy periwinkle	Folk remedies across the world use periwinkle plants, including as an antidiabetic in Jamaica.

(Andrea Rinaldi, Priya Shetty, 2010)

1.9 Aim of This Experiment: Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers can satisfy their needs, utilize the indigenous medicinal plants or their semi processed products.

Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases. The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs, problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity.

The main objective of this study was to discovery of new medicinal compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Dracaena spicata is a medicinal plant used traditionally in Bangladesh. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities available for conducting the study, this research work was performed on this plant.

The principal aim of the present study was to investigate the scientific basis of the traditional uses of the Methanol, Dichloromethane (DCM) and Petroleum Ether extracts of *Dracaena spicata* to evaluate their *in-vitro* pharmacological (antioxidant) activities.

1.10 Study Area: The research was carried out in the Research Lab, Microbiology Lab and Pharmacognosy Lab of Department of Pharmacy, East West University, Dhaka.

1.11 Data Collection:

All the relevant data has been collected from two types of Sources:

1.11.1 Primary Sources: Direct personal contact and observations of the experiments carried out in the laboratory.

1.11.2 Secondary Sources: Various publications like journals, papers, documents and websites.

Figure 1: *Dracaena spicata (*eMonocot, 2010)

1.12 General Information:

Group: Monocot

Family: Agavaceae - Century-plant family

Duration: Annual

Growth Habit: Shrub

Bangla/Vernacular Name: Dracaena

Tribal Name: Kadorateng gaas (Chakma, Tanchangya) *(*eMonocot, 2010)

1.12.1 Taxonomy:

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Liliopsida

Order: Asparagales

Family: Asparagaceae

Subfamily: Nolinoideae

Genus: *Dracaena*

Species: *Dracaena spicata*

(Encyclopedia of Life, 2013)

1.12.2 Distribution: Forests of Chittagong, Chittagong Hill Tracts and Cox's Bazar, Andaman Islands and Myanmar.

1.12.3 Parts utilized: Rhizomes, flowers, seeds, leaves, roots, fruits.

1.12.4 Synonym:

Dracaena wallichii Kunth

Draco spicata (Roxb.) Kuntze

Pleomelespicata (Roxb.) N.E.Br

1.12.5 General Description: Caulescent. Leaves lanceolate, drooping. Spikes terminal, bractes many-flowered. Corol cylindric, at last becoming twisted. Stigma three-lobed. It is a native of Chittagong, Bangladesh and usually blossoms in April. Root fibrous. Stem erect, toward the top succulent, perennial, marked with the cicatrices of the fallen leaves, as in the other Dracaena. Leaves crowded about the extremity of the plant, sheathing, lanceolate, drooping, entire, pointed; smooth on both sides; from six to twelve inches long, and two or three broad. Spikes terminal, bent a little to one side; numerous pointed, recurved bractes surround the base, and a few shorter, appressed ones from thence to the flower-bearing position. Flowers numerous, sessile, collected in small fascicles, each fascicle having a small, cordate, pointed bracte immediately under it. Calyx none. Corol one-petalled, cylindric divided half way down into three exterior, and three interior slender, linear, equal, straight segments; color pale greenish yellow, as they advance in age the tube becomes twisted. Filaments inserted on the base of the segments of the corol, and of their length. Stigma three-lobed. Berry with from on to three, distinct, round, and smooth lobes; while immature, a deep olive green, when ripe, deep reddish orange; each lobe containing a single large, round, smooth, white, horny seed. *(*eMonocot, 2010)

1.13 Uses of *Dracaena spicata*:

- Leaf juice is used to cure long term fever, coughs and mucus in nose by traditional healers of the Marma tribe of Naikhongchhari, Bandarban District.
- The sap from the root is taken for conjunctivitis and constipation (Chakma).
- A leaf extract is taken and a paste of the leaves is applied to the whole body for the treatment of hysteria (Tanchangya).
- Pills prepared from the leaves are taken with warm water twice daily for the treatment of measles by the Chakma;

- A root extract of *Dracaena spicata* and *Pandanus foetidus* is taken together and administrated to healthy children during outbreaks of measles by the Tanchangya
- Rhizome juice is prescribed against leucorrhoea.
- Powdered rhizome with honey is given for piles, dysentery and dyspepsia.
- Root juice is drunk to keep stomach cool and to get relief from burning sensation during urination.
- Root paste of the red flowered plant is given for treating menorrhagia. *(*eMonocot, 2010)

1.14 Antioxidants: Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidants include-

- Beta-carotene
- Lutein
- Lycopene
- Selenium
- Vitamin A
- Vitamin C
- Vitamin E

Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors.

High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin E may increase risks of prostate cancer and one type of stroke. Antioxidant supplements may also interact with some medicines. To minimize risk, tell you of your health care providers about any antioxidants you use. (U.S. National Library of Medicine, 2017)

1.15 Classification of Antioxidants: Antioxidants can be classified into two major types based on their source, i.e. natural and synthetic antioxidants (schematic representation of the classification of antioxidants is shown in Figure 2).

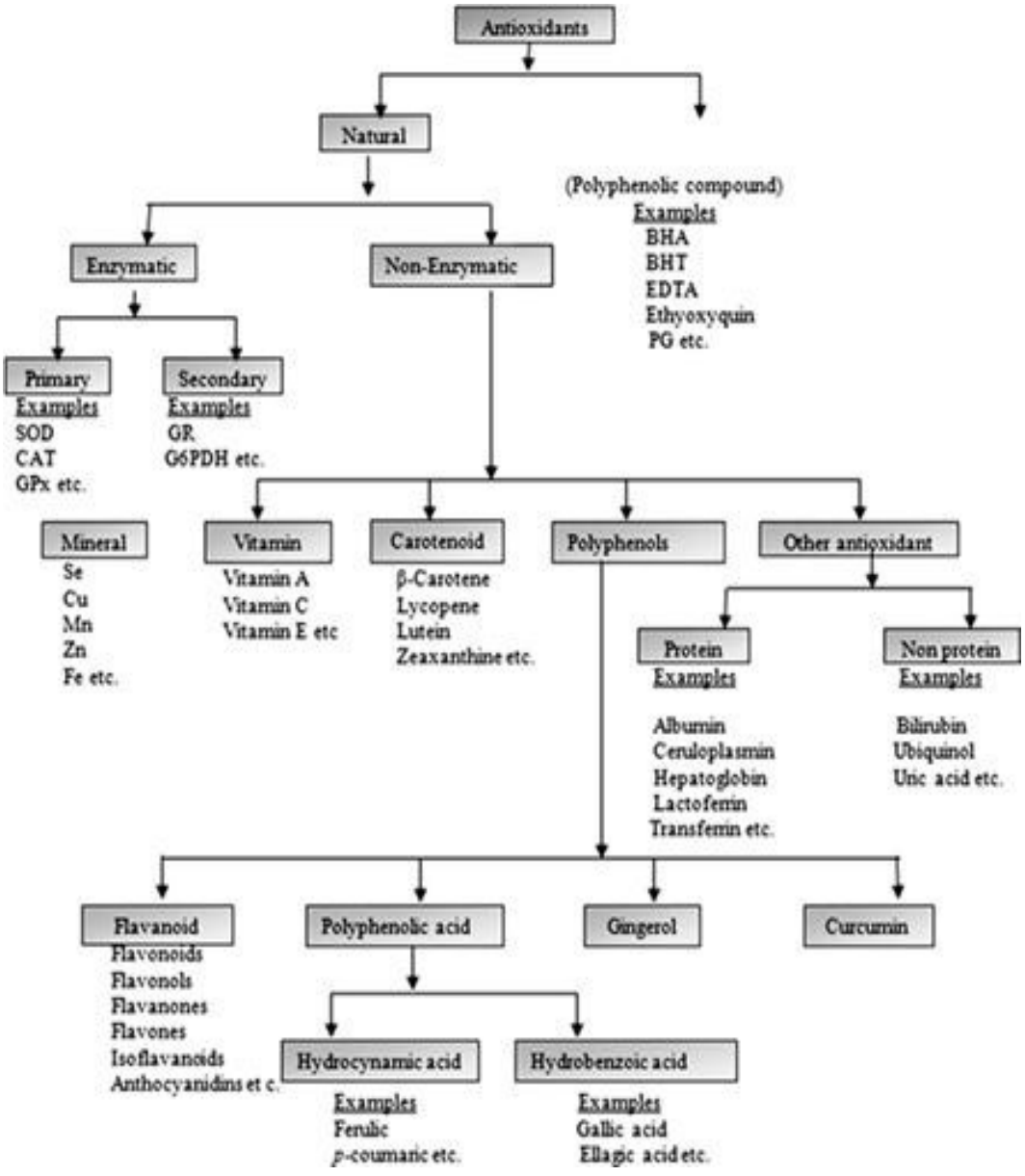

Figure 2: Schematic Representation of Classification of Antioxidants (Academic library, 2014)

1.15.1 Natural Antioxidants: Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories, i.e., enzymatic antioxidants and nonenzymatic antioxidants.

1.15.1.1 Enzymatic Antioxidants: Enzymatic antioxidants are uniquely produced in the human body and can be subdivided into primary and secondary antioxidant.

1.15.1.1.1 Primary Antioxidants: Primary antioxidants mainly include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx).

Superoxide dismutase (SOD) enzyme is found in both the dermis and the epidermis. It removes the superoxide radical (O^{-}) and repairs the body cells damaged by free radical. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide. SOD is also known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by scavenging superoxide anions, it promotes the activity of NO.

Catalase enzyme (CAT) is found in the blood and most of the living cells and decomposes H_2O_2 into water and oxygen. Catalase with glucose peroxidase is also used commercially for the preservation of the fruit juices, cream consisting of egg yolk, and salad by removing the oxygen.

Glutathione peroxidase (GPx) is a group of seleniumdependentenzymes, anditconsistsofcytosolic, plasma, phospholipidhydroperoxide, and gastrointestinal glutathione peroxidase. GPx (cellular and plasma) catalyzes the reaction of H_2O_2 by reduced glutathione (GSH); as a result, oxidized glutathione (GSSG) is produced and it is again recycled to its reduced form by glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

1.15.1.1.2 Secondary Antioxidant: Secondary antioxidant includes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH). G6PDH generates NADPH. GR is required to recycle the reduced glutathione (GSH) using secondary enzyme GR and NADPH.

Glutathione is a cysteine containing peptide-type antioxidant and is synthesized in the body cells. The thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and

reduced. A high level of glutathione is found in the cells (~3,100 μg/g of tissue), maintained in the reduced form (GSH) by the enzyme GR, and in turn reduces other metabolites and enzyme systems, such as ascorbate. Due to its high concentration and its role in maintaining redox state in the cells, it is considered one of the most important cellular antioxidants.

1.15.1.2 Nonenzymatic Antioxidants: They are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism. Some of the known nonenzymatic antioxidants are minerals, vitamins, carotenoids, polyphenols, and other antioxidants as listed below.

1.15.1.2.1 Minerals: Minerals are required in the body cells for the proper functioning of the enzymes. Their absence is known to affect the metabolism of many macromolecules. They include selenium, copper, iron, zinc, and manganese. They act as cofactors for the enzymatic antioxidants.

1.15.1.2.1.1 Iron (Fe): Iron is the most abundant trace metal found to bound with protein in the biological system. Normally the concentration of free iron is very low and the low concentrations of iron-binding proteins promote ROS production, lipid peroxidation and oxidative stress. Hence iron supplementation helps in reducing the oxidative stress.

1.15.1.2.1.2 Magnesium (Mg): Magnesium is a cofactor for glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) involved in pentose cycle which catalyzes the production of NADPH from NADP during the glucose metabolism and hence maintains the normal ratio of GSH to GSSG and a normal redox state in cells. Deficiency of magnesium reduces GR activity and GSSG does not reduce to GSH, hence causing oxidative damage to the cells.

1.15.1.2.1.3 Selenium (Se): Selenium is also a very important component of enzymatic antioxidant. In the presence of selenium (Se), glutathione peroxidase (GPx) plays a protective role against oxidation of lipid and protects the cell membrane and takes part in H_2O_2 and lipids' hydroxyperoxide metabolism. Hence, Se behaves like vitamin E and can be substituted in place of vitamin E and is used to prevent the risk of cancer and cardiovascular diseases.

1.15.1.2.1.4 Copper (Cu), Zinc (Zn), and Manganese (Mn): SOD is a class of enzyme that consists of different types of SODs, depending upon their metal cofactor such as Cu–Zn and Mn.

Cu–Zn SOD is found in the cytosol having Cu and Zn at their active sites which helps in proton conduction, whereas Mn-SOD is found in mitochondria and has Mn at its active site. These metals are responsible for SOD's antioxidant activities.

1.15.1.2.2 Vitamins: Vitamins form the class of micronutrients required for the proper functioning of the body's antioxidant enzyme system, such as vitamin A, vitamin C, vitamin E, and vitamin B. They cannot be synthesized in our body and hence need to be supplemented in the diet.

1.15.1.2.2.1 Vitamin A: Vitamin A is helpful in night vision and in maintenance of epithelial cells in mucus membranes and skin. Because of its antioxidant properties, it assists immune system also and is found in three main forms: retinol, 3,4-didehydroretinol, and 3-hydroxyretinol. The main sources of this include sweet potatoes, carrots, milk, egg yolks, and mozzarella cheese.

1.15.1.2.2.2 Vitamin C: Vitamin C is water soluble and is also called as ascorbic acid. It is found in fruits (mainly citrus), vegetables, cereals, beef, poultry, fish, etc. It is helpful in preventing some of the DNA damage caused by free radicals, which may contribute to the aging process and the development of diseases, such as cancer, heart disease, and arthritis.

1.15.1.2.2.3 Vitamin E: Vitamin E is a lipid-soluble vitamin. This consists of eight different forms such as α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Most abundantly found in almonds, safflower oil, soybean oils, oil of wheat germs, nuts, broccoli, fish oil, etc., α-tocopherol possesses highest bioavailability and is the most important lipid-soluble antioxidant which reacts with the lipid radical and protects the membranes from lipid peroxidation; as a result, oxidized α-tocopheroxyl radicals are produced that can be recycled to the reduced form through reduction by other antioxidants, such as ascorbate and retinol.

1.15.1.2.3 Carotenoid: Carotenoid consists of β-carotene, lycopene, lutein, and zeaxanthin. They are fatsoluble colored compounds found in fruits and vegetables. β-Carotene is found mostly in radish-orange-green color food items including carrots, sweet potatoes, apricots, pumpkin, mangoes, and cantaloupe along with some green and leafy vegetables, including collard greens, spinach, and kale. Lutein is abundant in green leafy vegetables such as collard

greens, spinach, and kale. Lutein is best known for its role in protection of retina against harmful action of free radicals and also prevents atherosclerosis.

Although lycopene, lutein, canthaxanthin, and zeaxanthin do not possess provitamin A activity, β-carotene is known as a precursor for vitamin A. Tomato is a good source of lycopene and spinach is a good source of zeaxanthin. It has been shown that lycopene is a potent antioxidant and is the most effective compound in removing singlet oxygen found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, and other foods.

1.15.1.2.4 Polyphenols: Polyphenols is a class of the phytochemicals that possess marked antioxidant activities. Their antioxidant activities depend on their chemical and physical properties which in turn regulates the metabolism depending on their molecular structures. These consist of phenolic acids, flavonoids, gingerol, curcumin etc.

Flavonoid is a major class of polyphenolic compound and is mostly found in vegetables, fruits, grains, seeds, leaves, flower, bark, etc. Some of the spices, such as ginger and turmeric, are also good sources of polyphenolic compound, e.g., gingerol is obtained from the rhizomes of ginger, whereas curcumin (diferuloylmethane) is the main bioactive component of turmeric and is known to possess good antioxidant activity. Curcumin is an excellent scavenger of ROS, such as O_2 radicals, lipid peroxyl radicals (LO_2), OH radicals, and nitrogen dioxide (NO_2) radicals, which induced oxidative stress. Curcumin has been shown to inhibit lipid peroxidation and has been shown to increase GSH levels also in epithelial cells which lead to lower ROS production

1.15.1.2.5 Other Antioxidants:

1.15.1.2.5.1 Transition Metal-Binding Proteins: Albumin, ceruloplasmin, hepatoglobin, and transferrin are the transition metal-binding proteins found in human plasma, bind with transition metals, and control the production of metal catalyzed free radicals. Albumin and ceruloplasmin are the copper ion sequesters, hepatoglobin is hemoglobin sequester, and transferrin acts as free iron sequester.

1.15.1.2.5.2 Nonprotein Antioxidants: Bilirubin, uric acids, and ubiquinol are nonprotein antioxidants which inhibit the oxidation processes by scavenging free radicals.

Bilirubin Bilirubin is an end product of heme catabolism. It is a lipid-soluble cytotoxic product that needs to be excreted. However, bilirubin efficiently scavenges peroxyl radical at micromolar

concentrations in in vitro model and is regarded as the best antioxidant against lipid peroxidation.

1.15.1.2.5.3 Uric Acid: Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals. Urate reduces the oxo-heme oxidant formed by peroxide reaction with hemoglobin and protects erythrocytes from peroxidative damage. The plasma-urate levels in humans are about 300 μM, making it one of the major antioxidants in humans.

1.15.1.2.5.4 Coenzyme Q: Coenzyme Q is also known as ubiquinol (Co Q) and is an oil-soluble antioxidant. This is produced in the body through monovalent pathway, in heart, liver, kidney, pancreas, etc.

1.15.2 Polyphenolic Compunds: Such as BHA, BHT, EDTA etc. (Academic library, 2014)

1.16 Techniques for Measurement of Antioxidant Activity:

There are three major techniques mostly used for the measurement of antioxidant activity in various samples-

1.16.1 Chemical Assays for Antioxidant Activity: There are many chemical assays used for the assessment of antioxidant activity in the products (herbal, nutraceuticals, and food items). Some of the well-documented and most practiced methods are described below-

1.16.1.1 Oxygen Radical Absorption Capacity: Oxygen radical absorption capacity (ORAC) method uses dichlorofluorescein as the fluorescent probe and an azo-compounds, such as 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) as the radical generator. It measures the inhibition of the peroxyl radical induced oxidation initiated by thermal decomposition of AAPH. Over time, the free radical generated from the thermal decomposition of AAPH quenches the signal from the fluorescent probe fluorescein. The subsequent addition of an antioxidant produces a more stable fluorescence signal due to the inhibition of fluorescein decay by single antioxidant and/or complex mixture. Rate of decay of fluorescence measures the antioxidant's capacity.

1.16.1.2 Determination of Total Phenolic Content (TPC): Total phenolic content of the extracts are determined using Folin–Ciocalteu (FC) reagent using spectrophotometer, measured

at 725 nm. This method is based on reduction ability of phenolic functional group. Oxidation and reduction reaction of phenolate ion takes place at base condition. The reduction of phosphotungstate– phosphomolybdenum complex (Folin–Ciocalteu reagent) by phenolat ion will change its color to blue. The reduction of complex will increase when the extract contains more phenolic compounds. Thus the color will be darker and the absorbance will be higher, showing higher antioxidant activity.

1.16.1.3 1,1′-Diphenyl-2-Picrylhydrazyl: DPPH (1,1′-diphenyl-2-picrylhydrazyl) assay is carried out as per the reported method of Brand-Williams et al. (1995). DPPH− free radical is obtained by dissolving DPPH in methanol and is stable when placed under the dark at −20 °C until used. As DPPH− reacts with antioxidants present in the sample, color changes from violet to yellow and absorbance of the solution so obtained is measured spectrophotometrically at 515 nm.

1.16.1.4 Trolox Equivalent Antioxidant Capacity: In this assay, ABTS {2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)} is used to measure the antioxidant capacity of the substance (food stuffs). Trolox equivalent antioxidant capacity (TEAC) is also known as ABTS assay and the procedure is based on the reported method of Arnao et al. (2001). When ABTS reacts with potassium persulfate, it becomes a free radical (ABTS+) which gives blue color to the solution. The phenolics, thiols, or vitamin C present in the food stuffs scavenge this ABTS+ free radical and convert it into its neutral colorless form which is measured spectrophotometrically. ABTS+ absorbs light at 734 nm.

1.16.1.5 Ferric Reducing Antioxidant Power: Ferric reducing antioxidant power (FRAP) assay is carried out using the earlier reported method as described by Benzie and Strain (1996). When ferric chloride reacts with 2,4,6-tripyridyl-*s*-triazine (TPTZ) at low pH, ferric is converted into ferrous causing formation of ferrous tripyridyl triazine complex. FRAP values are obtained by comparing the absorbance change at 593 nm in reaction mixture with those containing ferrous ions in known concentration.

1.16.1.6 Determination of Total Reducing Power (TRP): TRP is determined following the method of Negi et al. (2005). It is measured spectrophotometrically in terms of their capacity to reduce the potassium ferricyanide (Fe^{3+}) to the potassium ferrocyanide (Fe^{2+}), depending upon the concentration of the antioxidant compounds present in the sample, which in turn reacts with

ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. (Academic library, 2014)

1.16.2 Biochemical Assays for Antioxidant Activity Assessment: Antioxidant activity may also be measured in biological system, i.e., in vivo and in vitro models. These include measurement of oxidative stress marker of adduct or end product of ROS with the molecules, such as lipid, protein, DNA, and other molecules. These methods include thiobarbituric acid reactive substances (TBARS), SOD, CAT, GPx, GSH, and ferrous oxidation-xylenol orange (FOX) assay. These assays may be carried out in blood, urine, breath and tissues. Some of the examples are described below:

1.16.2.1 TBARS: TBARS method determines the extent of lipid peroxydation in sample. TBARS is the reaction product of thiobarbituric acid (TBA) and malondialdehyde (MDA) which results from the decomposition of lipid hydroperoxide in the sample which is read spectrophotometrically at 532 nm.

1.16.2.2 Protein Carbonyl: Protein carbonyl content results from the oxidative cleavage of protein. In this case, 2,4-dinitrophenylhydrazine (DNPH) reacts with protein carbonyl and forms a Schiff base to produce corresponding hydrazone. The amount of protein hydrazone produced is quantified spectrophotometrically at an absorbance between 360 and 380 nm.

1.16.2.3 FOX: Hydroperoxide content of the lipid can be determined from its ability to oxidize ferric (Fe^{2+}) to ferrous (Fe^{3+}). Ferrous (Fe^{3+}) formed a complex with xylenol orange reagent (bluish-purple color) which is measured at 560 nm.

1.16.2.4 CAT: Catalase activity can be measured by using H_2O_2 as a substrate according to the method of Aebi (1984).

1.16.2.5 SOD: SOD is measured using the method of Kakkar et al. (1984) where nicotinamide adenine dinucleotide (NADH) is used as a substrate. The color intensity of the chromogen (purple color) in butanol layer is measured against butanol (blank) on spectrophotometer at 560 nm.

1.16.2.6 ROS: In this assay, 2′,7′-dichlorofluorescein diacetate (DCFDA) is used to measure ROS level. It undergoes cellular oxidation by ROS and gets converted into fluorescent dichlorofluorescein (DCF) which is highly fluorescent at 530 nm.

1.16.3 Instrumental Technique (Antioxidant Analyzer): Recently, an instrument named PHOTOCHEM Antioxidant Analyzer developed by Analytik Jena UK is being used for the measurement of antioxidant property of different products. It is capable of measuring both water-soluble and lipid-soluble antioxidants in a single system. It is based on the principle of photochemiluminescence with luminometric detection. It can measure the antioxidant capacity of lyophilized vegetables, fruits juices, beer, and water; lipid-soluble antioxidative capacity in baker's yeast, cheese, tea, and coffee; and lipid-soluble antioxidative capacity in edible oilandsalamiextracts. (Academic library, 2014)

Chapter 2

Literature Review

2.1 Literature Review on *Dracaena spicata*:

2.1.1 Evaluation of Antimicrobial Activities of Some Bangladeshi Medicinal Plants: The crude methanol extracts of aerial parts of *Abrus precatorius L*., leaf of *Magnolia pterocarpa Roxb., Dracaena spicata Roxb*. and *Ravenala madagascariensis Sonn*. as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for disc diffusion assay. Among the test samples of *A. precatorius*, the highest zone of inhibition (15.0mm) was exhibited by the carbon tetrachloride soluble fraction against *Pseudomonas aeruginosa*. The *M. pterocarpa* extractives exhibited significant zone of inhibition ranging from 7.0 to 23.0mm against the test organisms. The highest zone of inhibition (23.0mm) was demonstrated by the carbon tetrachloride soluble fraction against *Pseudomonas aeruginosa*. This fraction also exhibited 20.0mm zone of inhibition against the gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Vibrio parahemolyticus*. Among the test samples of *D. spicata*, the highest (18.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against *Pseudomonas aeruginosa*. The test samples of *R. madagascariensis* exhibited weak antimicrobial activity with zone of inhibition ranging from 2.0 to 9.0mm (Sharmin *et al.*, 2014).

2.1.2 Evaluation of Thrombolytic and Membrane Stabilizing Activities of Four Medicinal Plants of Bangladesh: The crude methanol extracts of aerial parts of *Abrus precatorius L*., leaf of *Magnolia pterocarpa Roxb.* and *Dracaena spicata Roxb.* and leaf and bark of *Ravenala madagascariensis Sonn.* as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing activities. Among the extractives of *A. precatorius*, the crude methanol extract exhibited the highest thrombolytic activity (34.92±0.54 %) while the carbon tetrachloride soluble fraction of *M. pterocarpa* exhibited 22.59±0.88 % clot lysis. *D. spicata* extractives showed mild thrombolytic activity. The methanolic crude extract of *R. madagascariensis* leaf and the aqueous soluble fraction of *R. madagascariensis* bark extract showed 45.32±0.82 % and 32.67±0.74% clot lysis, respectively. In hypotonic solution and heat induced conditions, the crude methanol extract of *A. precatorius* and the hexane soluble fraction of crude methanol extract of *M. pterocarpa* inhibited 63.46±0.84 % & 36.54±0.21 % and 66.12±0.66 % & 40.54±0.02 %

haemolysis of RBCs, respectively as compared to 71.90 % and 42.12 % inhibition by acetyl salicylic acid (0.10 mg/ml), respectively. The crude methanol extract of *D. spicata* demonstrated 64.44±0.68 % and 36.52±0.19 % inhibition of hypotonic solution and heat induced hemolysis, respectively. The chloroform soluble fraction of *R. madagascariensis* leaf extract demonstrated 28.72±0.61 % & 39.97±0.39 % and the hexane soluble fraction of *R. madagascariensis* bark extract revealed 53.78±0.17 % & 41.83±0.61 % inhibition of hypotonic solution and heat induced hemolysis of RBCs, respectively (Chowdhury *et al*., 2013).

2.1.3 Antimicrobial Activity Test: Antimicrobial screening: Antimicrobial activity of the extractives was determined against gram positive and gram negative bacteria and fungi by the disc diffusion method Measured amount of the test samples were dissolved in definite volume of solvent (chloroform or methanol) and applied to sterile discs and carefully dried to evaporate the residual solvent. In this investigation, ciprofloxacin (30µg/disc) disc was used as the reference.

Result: The test samples of *D. Spicata* exhibited zone of inhibition ranging from 7.0 to 18.0mm against the test organisms. The highest (18.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against *Pseudomonas aeruginosa*. Against gram positive bacteria *Staphylococcus aureus*, the carbon tetrachloride and aqueous soluble extractives revealed 15.0mm zone of inhibition.

2.1.4 Thrombolytic Activity: The thrombolytic activity was evaluated by the method developed by using streptokinase as positive control.

Result: The crude methanol extracts of aerial parts of leaf of *Dracaena spicata* as well as its hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing potentials. In order to identify the drugs with the ability to promote lysis of blood clot from natural resources, the extractives D*racaena spicata* were assessed for thrombolytic activity. Addition of 100 µl streptokinase, a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37°C showed 66.77 % lysis of clot. On the other hand, distilled water, treated as negative

control, revealed a negligible lysis of clot (3.79 %). *Dracaena spicata* extractives showed mild thrombolytic activity and the highest thrombolytic activity was demonstrated by the carbon tetrachloride soluble fraction (21.05±0.23 %).

2.1.5 Membrane Stabilizing Activity: The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale *et al.,* 2008.

Result: The membrane stabilizing activity of *Dracaena spicata* extractives was also determined. The hexane soluble fraction of crude methanol extract of *Dracaena spicata* demonstrated 64.44±0.68 % & 36.52 % inhibition of hypotonic solution and heat induced hemolysis, respectively. *Dracaena spicata* exhibited significant membrane stabilizing activity 2.4.Antipyretic activity: Root extract of the plant possesses antipyretic activity mild.

Chapter 3

Methodology

3.1 Preparation of Plant Extracts for Experiments:

3.1.1 Materials:

3.1.1.1 Reagents:

1. Methanol

2. Petroleum Ether

3. Dichloromethane

5.1.1.2 Equipments:

1. Beaker

2. Funnel

3. Glass rod

4. Grinding machine

5. Filter paper

6. Cotton

7. Separating funnel

8. Measuring cylinder

9. Cotton cloth

3.1.2 Collection: *Dracaena spicata* is not so available throughout the country. The plant was collected from Chittagong hill tract area. The plant was taxonomically identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen (Accession No. 40633) has been deposited for future reference.

3.1.3 Process of Powdering: At first the plants were cleaned to remove dust, soil etc within them. After this the whole amount of plant was dried. The dried plants were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy

penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. The amount of powder was 800g. During powdering of sample, the blander was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the blander.

3.1.4 Extraction: The fine powder of plants was dissolved in 3.5 liter methanol, Petroleum Ether and Dichloromethane (DCM) and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 5 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.1.5 Filtration: After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a conical flask and covered with aluminum foil paper was prepared for rotary evaporation.

Figure 3: Rotary Evaporator

3.1.6 Evaporation and Extract preparation: For evaporating the solvent and collect for reuse I have used rotary evaporator machine with a vacuum pump which helped to reduce the pressure of the inside of glass tube coil, as well as the whole system. Reduction of pressure causes quick evaporation. On the other part condenser recommenced the solvent so that I could reused it. For this solvent almost 70% solvent get back into liquid form. The extraction

was collected from the evaporating flask and the solvent is collected from the receiving flask. Extract transferred into a 50 ml beaker and covered with aluminum foil.

3.2 Antioxidant Activity Tests:

3.2.1 Apparatus

•Test tubes
•Beakers
•Vials
•Thermometer
•Micropipette
•UV spectrophotometer
•Electronic balance
•Magnetic stirrer

3.2.2 Reagents

•Methanol
•0.02 mg/ml methanolic solution of DPPH
•Ascorbic acid (ASA) which is used as positive control.
•Distilled water

3.2.3 Control Preparation of Antioxidant Activity Measurement: Ascorbic acid (ASA) was used as positive control. Calculated amount of ASA was dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration from 200.0 to 0.7825 µg/ml.

3.2.4 Test Sample Preparation: Petroleum Ether, Dichloromethane, Methanol extract of the bark and leaf were used as test samples. Calculated amount of different extracts were measured and were dissolved in methanol to get a mother solutions having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration from 200.0 to 0.78125 µg/ml.

3.3 DPPH Solution Preparation:

3.8 mg of DPPH was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light-proof box.

3.3.1 Assay of Free Radical Scavenging Activity:

2.0 ml of a methanol solution of the samples (extractives/control) at different concentration (200 µg/ml to 0.78125 µg/ml) were mixed with 2.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spetrophotometer.

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

$$(I\%) = (1 - A_{sample}/A_{control}) \; X \; 100$$

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

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

3.4 Determination of Total Flavonoid Content:

Total flavonoid contents of the plant fractions were determined according to the method described by Kumaran and Karunakaran (2007).

3.4.1 Reagents and Chemicals:

List of reagents used in total flavonoid content determination test

1. Aluminium Chloride.

2. Potassium Acetate.

3. Methanol.

4. Quercetin.

3.4.2 Experimental Procedure:

1. 1.0 ml of each fraction (200 µg/ml) and standard (quercetin) in different concentrations were taken in test tubes.

2. 3 ml of methanol was added into the test tubes.

3. 200 µl of 10% aluminium chloride solution was added.

4. 200 µl of 1 M potassium acetate solution was added to the mixtures in the test tubes.

5. Then 5.6 ml of distilled water was added into the test tubes.

6. The test tubes were incubated for 30 minutes at room temperature to complete reaction.

7. Then the absorbance of the solution was measured at 415 nm using a spectrophotometer against blank.

8. Methanol was used as the blank.

9. Total flavonoid contents of the fractions were expressed as quercetin equivalents (QE) after calculation using the following equation:

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

Where:

C = total flavonoid contents, mg/g plant extract in QE,

c = concentration of quercetin obtained from calibration curve (mg/ml),

3.5 Total Phenol Content Determination:

Total phenolic contents of the plant fractions were determined according to the method described by Yu et al. (2002).

3.5.1 Reagents and Chemicals:

1. Folin – ciocalteu reagent Merck, Germany
2. Sodium carbonate E. Merck (India) limited
    - Methanol Merck, Germany
    - Gallic acid Sigma Chemicals, USA

3.5.2 Principle:

Total phenolic contents of the fractions were determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates–molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$.It is believed that the molybdenum is easier to be reduced in the complex and electrontransfer reaction occurs between reductants and Mo (VI):

Mo (VI) + e ® Mo (V)

3.5.3 Experimental Procedure:

1. 1.0 ml of each fraction (200 µg/ml) and standard (gallic acid) in different concentrations were taken in test tubes.

2. 5 ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tubes.

3. 4 ml of Sodium carbonate solution was added into the test tubes.

4. In case of standard, the test tubes were incubated for 30 minutes at 20.0ºC while the test tubes containing samples (four fractions) were incubated for 1 hour at the same temperature (20.0ºC) for completion of reaction.

5. The absorbance was read at 765 nm using a UV-VIS spetrophotometer against blank.

6. Blank solution contained methanol.

7. The total phenolic contents were determined from a standard curve prepared with Gallic acid.

(Kumaran A, Karunakaran AJ, 2007)

Chapter 4

Results and Discussion

4.1 DPPH Test:

4.1.1 Preparation of DPPH Scavenging Activity Curve:

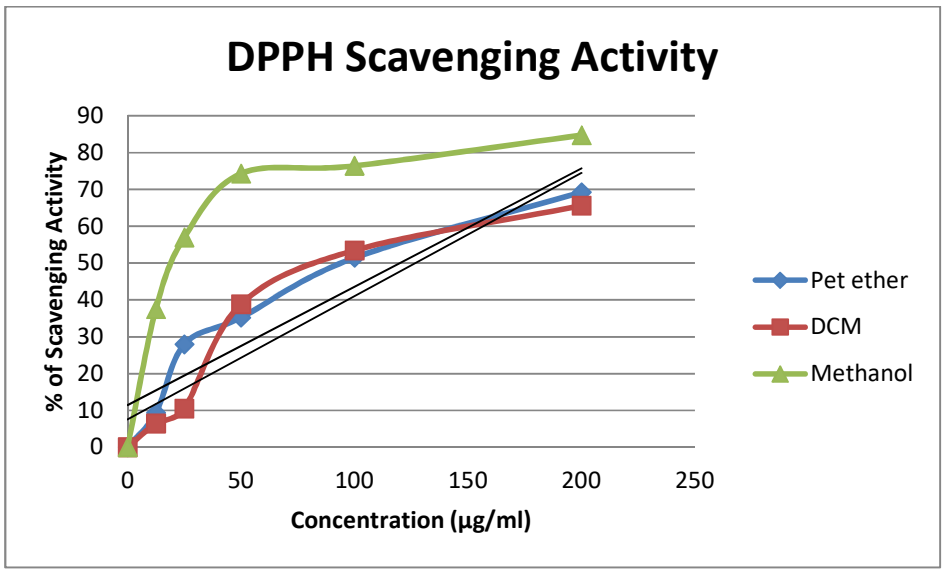

Figure 4: DPPH Scavenging Activity of Petroleum Ether, Dichloromethane and Methanol Extract of *Dracaena spicata*

4.1.2 Results of DPPH Test:

Table 9: Result of DPPH Test

Extract/Standard	IC_{50} Value (µg/ml)	Regression Line	R^2 Line
Petroleum Ether	120.2492	y = 0.321x+11.40	R^2 = 0.878
Dichloromethane	127.1228	y = 0.314x+34.66	R^2 = 0.553
Methanol	48.8535	y = 0.334x+7.541	R^2 = 0.849

4.2 Total Flavonoid Test:

4.2.1 Preparation of Standard Curve for Quercetin:

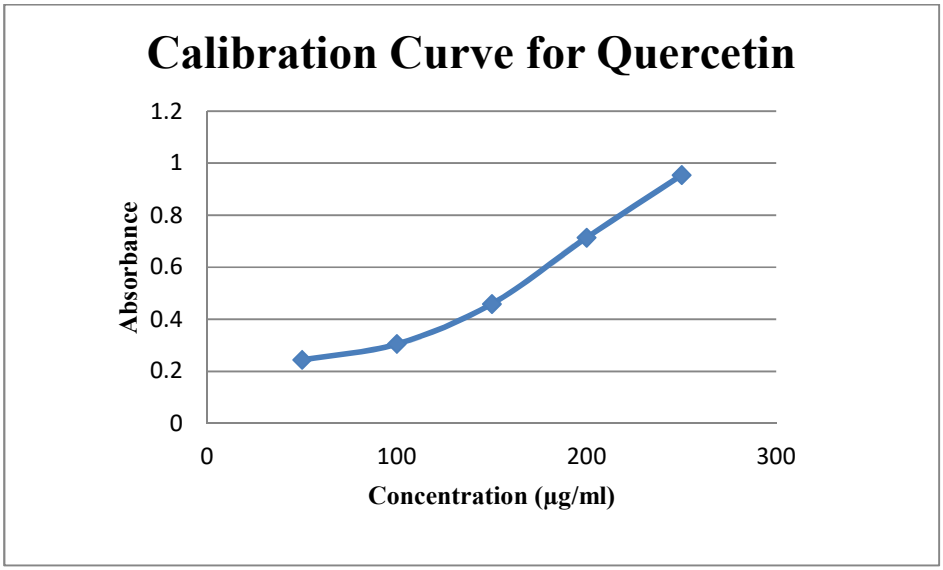

Figure 5: Standard Curve of Quercetin

4.2.2 Results of Total Flavonoid Content:

Table 10: Result of Total Flavonoid Content

Extracts	Total Flavonoid Content (in mg/g, QE)
Petroleum Ether	68
Dichloromethane	56.6667
Methanol	94.3334

4.3 Total Phenol Test:

4.3.1 Preparation of Standard Curve for Gallic Acid:

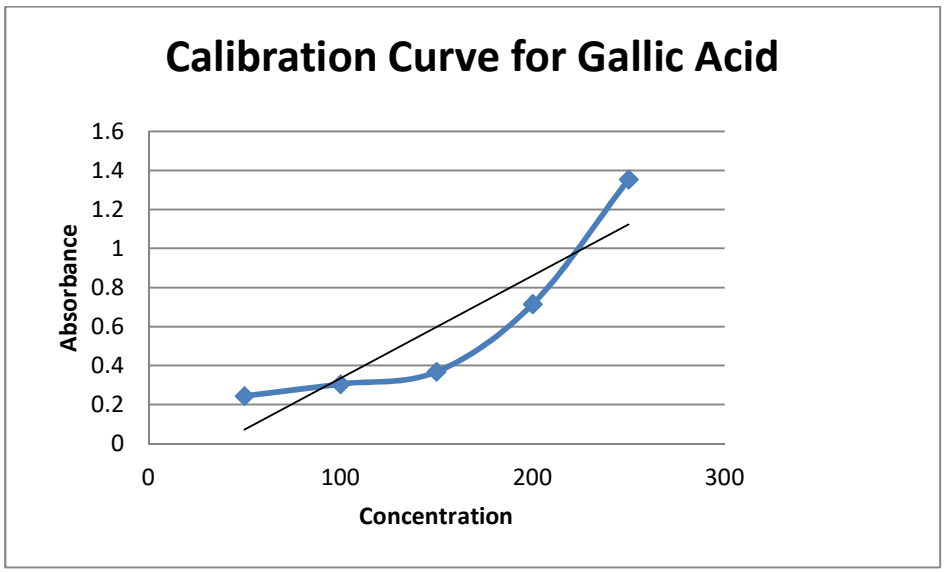

Figure 6: Standard Curve of Gallic Acid

4.3.2 Results of Total Phenol Test:

Table 11: Result of Total Phenol Test

Extract	Total Phenol Content(in mg/g, GAE)
Petroleum Ether	216.2
Dichloromethane	82
Methane	58.6

4.4 Discussion:

The plant *Dracaena spicata* has been used for the general promotion of health and longevity by Asian tribal (especially Chakma, Marma and Tanchangya). It is used as a traditional medicine for the treatment of various diseases cough, syphilis, conjunctivitis, constipation; pills prepared from the leaves are taken with warm water twice daily for the treatment of measles by the Chakma etc. The aim of the present study was to evaluate antioxidant activity of Petroleum Ether, Dichloromethane and Methanol extracts of *Dracaena spicata.* Due to its huge therapeutic use by the tribal I get interested to do experiment on this plant. The therapeutic value of medicinal plants lies in the various chemical constituents in it.

In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect. In oxygen involving metabolism, Reactive Oxygen Species (ROS) are natural byproducts. Such typical ROSs is superoxides, hydroxyl, peroxyl, and alkoxy free radicals. Under relaxed condition, production and scavenging of ROS is in equilibrium. However, different environmental stresses like pollution, drought, temperature, excessive light intensities and nutritional limitations are able to increase ROS production. Such stressful conditions are called oxidative stress. Oxidative stress can contribute to diseases such as cancer and cardiovascular disease. So, it a dying need to find out the antioxidant potential of the compounds found from the natural sources.

The antioxidant activity was measured by DPPH, Total Phenol and Total Flavonoid tests. The IC_{50} values of DPPH tests were 120.2492, 127.1228 and 48.8535 µg/ml for Petroleum Ether, Dichloromethane Methanol consecutively. The Total Flavonoid contents ware 68, 56.6667 and 94.3334 in mg/g equivalent to Quercetin for Petroleum Ether, Dichloromethane Methanol consecutively. The Total Phenol contents were 216.2, 82, 58.6 mg/g equivalent to Gallic Acid for Petroleum Ether, Dichloromethane Methanol consecutively. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity. It becomes difficult to describe the all properties selectively to any one group of constituents without further studies, which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active principles present in these plants.

Chapter 5

Conclusion

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. . In my experiment it shows very positive result for anti-oxidant activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

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