Phytochemical and Pharmacological Investigation on Petroleum Ether Extract of *Murraya koenigii* Leaf

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

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Declaration by the Candidate

I, **Nusrat Jahan**, hereby declare that this dissertation, entitled **"Phytochemical and Pharmacological Investigation on Petroleum Ether Extract of *Murrya koenigii* Leaf"** which is submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy, is a genuine & authentic research work carried out by me under the supervision and guidance of **M. Saleh Yunus**, Lecturer, Department of Pharmacy, East West University, Dhaka. The contents of this thesis paper, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree / Diploma / Fellowship.

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This is to certify that the dissertation entitled **"Phytochemical and Pharmacological Investigation on Petroleum Ether Extract of *Murrya koenigii* Leaf"** is a genuine research work carried out by **Nusrat Jahan**, ID: 2014-1-70-001, under the supervision of **M. Saleh Yunus** (Lecturer, Department of Pharmacy, East West University) in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy. I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in this connection are duly acknowledged.

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Dedication

This research paper is dedicated to

my beloved Parents

and my family members

Abstract

Murraya koenigii (curry leaves) is an abundant source of bioactive phytochemicals which has been used for not only the general promotion of health and longevity but also as a traditional medicine for the treatment of various diseases. The aim of this study was to determine antioxidant activity, antibacterial activity and cytotoxic property of the Petroleum ether extract of *Murraya koenigii* leaf. After conducting antioxidant tests by Total Phenolic Content and Total Flavanoid Content, it revealed the presence of 341mg of AAE/gm of phenolic components and 75.717mg of Quercetin/gm flavanoid components respectively. The Brine Shrimp Lethality Bioassay study clearly indicates the presence of cytotoxic properties by LC50 effect of petroleum ether extract of leaf of *Murraya koenigii*. The Zone of inhibition also showed low to moderate antibacterial activities which justify its use in traditional medicine.

Key Words: *Murraya koenigii*, Phytochemical, Petroleum ether, Total Phenolic Content and Total Flavanoid Content, Brine Shrimp Lethality Bioassay, LC50, Zone of inhibition.

Aims of the Present Study

Present Study is conducted for the evaluating the cytotoxic, antibacterial and antioxidant properties from the Petroleum ether fraction of *Murraya koenigii* (curry leaves) leaves extract. To conduct the cytotoxic activity of *Murraya koenigii*, investigation is done by brine shrimp lethality bioassay. To investigate in vitro antioxidant property of aqueous extract by total phenolic content and total flavonoid content. *Murraya koenigii* is a very common plant which is used in our country as well as in world by a lot of people for several purposes. All the parts of this plant are used for medicinal activity.

To achieve this objective, the whole work was designed in the following way:

- ✓ Cytotoxic study with Petroleum ether fraction.
- ✓ Antioxidant study with Petroleum ether fraction.
- ✓ Observation of antimicrobial action with Petroleum ether fraction.

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List of Abbreviations

Abbreviated Form	Meaning
DMSO	Dimethyl Sulfoxide
gm	Gram
Hr	Hour
LC50	Lethal concentration required to kill 50% of the sample population
µ g	Micro gram
ml	Milliliter
µl	Microliter
UV	Ultraviolet
WHO	World Health Organization

Chapter One
Introduction

1.1 Nature, as a primary source of drugs

Our lifestyle is now getting techno-savvy, we are moving away from nature. But we cannot escape from nature because we are part of nature. Nature is the master of craftsman of all molecules which is created almost all infinite arrangement of molecular entities. Form millennia the plants, animals, rocks and trees were the only pharmaceutical source. All living substance on Earth, every one of us is still a depositor in Nature as the supreme pharmacy on Earth. Nature stands as an immeasurable resource for drug development, novel chemotypes and pharmacophores and scaffolds, for strengthen them into effective drugs. These drugs are effective against a wide range of disease indications and use for other valuable bioactive agents. (Veeresham, 2012)

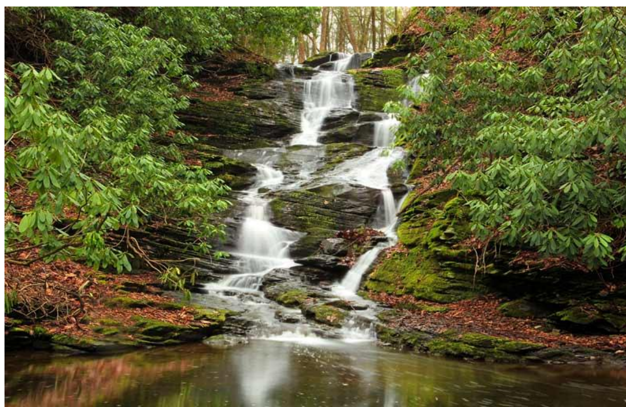


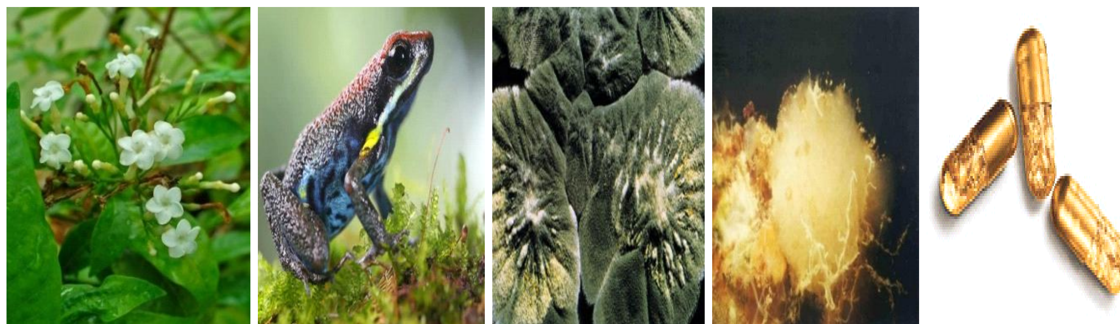
Figure1.1: Nature

From ancient time, natural products have been use as backbone of traditional system for healing purpose throughout the world and have also been a vital part of their history and culture.

Hippocrates was quoted as saying:

'Let thy food be thy medicine, and thy medicine shall be thy food'.

Drugs are mainly obtained from five major natural sources. Different sources of drugs are Plant sources, Animal sources, Microbiological sources, Marine sources and Mineral sources. (howMed, 2015)



Plant Source:	Animal Source:	Microbial Source:	Marine Source:	Microbial Source:
Rauvolfia serpentina plant contains around 200 alkaloids. The major alkaloid reserpine is used to treat high blood pressure and mental disorders	Ecuadorian poison frog is a source of Epibatidine, which is ten time more potent than morphine. Ecuadorian poison frog's skin is the main source of drug.	Penicillin elucidated from the filamentous fungus Penicillium notatum. Penicillin is use as antibiotic agent.	From *Mediterranean Tunicat Aplidium albicans*, Aplidin was isolated which is effective against various cancers.	Penicillin elucidated from the filamentous fungus Penicillium notatum. Penicillin is use as antibiotic agent.

Figure1.2 : Different Sources of Drugs

In modern drug development, natural products play important roles. Especially, the drug development of antibacterial, antiviral, anticancer and antitumor agents from natural sources. Multitude of natural product-derived compounds in various stages of clinical development decorated the existing viability and significance of the use of natural products as sources of new drug candidates.

So, it is seen that-

"Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine." (Kumar, 2009)

1.2 Plant, as a vital source of drugs

Plant is a living organism of like- trees, shrubs, flower, vegetable, weed, herbs, grasses, ferns, and mosses which are naturally growing in a permanent site, absorbing water and inorganic substances through its roots, and synthesizing nutrients in its leaves by photosynthesis using the green pigment chlorophyll. (Encyclopedia of Life, 2012)

From the dawn of civilization, plant are mainly used to treat many conditions, such as allergies, headache, antiseptic, premenstrual syndrome, rheumatoid arthritis, edema, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and among others.

Over 3,000 million years ago, the first living-organism which resembled a plant appeared. It was blue-green algae which lived in the sea and can still found in the water today. When the plants made their first appearance on Earth the atmosphere was unlivable for all oxygen breathing creatures. The air was made out of carbon dioxide, a gas which is deadly for human. Then photosynthetic plants came along and slowly over several million years, cleaned the atmosphere and filled it with oxygen. From the past, ample evidence from various sources: written documents, preserved monuments and even original plant medicines. (Odec,2002)

1.3 Phytochemistry

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. In the study of phytochemistry, it describe the structures of the large number of secondary metabolic compounds found in plants, the functions of these compounds in human and plant biology, and the biosynthesis of these compounds. Phytochemistry is widely used in the field of herbal medicine.

Phytochemical technique mainly applies to the quality control of herbal medicine of various chemical components, such assaponins, alkaloids, volatile oils, flavonoids and anthra-quinones etc. In most cases, biologically active compounds in herbal medicine have not been determined. It is important for the phytochemical methods screening and analyzing bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. The study paves the way to discovery of new therapeutic compounds as needed by the modern scientific approach of producing medicines. (Saxena,*et al.*, 2013)

1.3.1 Plant's Constituents Screening

Phytochemical analysis refers to the extraction, analysis and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

Alkaloids: This group mainly comprised of a wide variety of plants which contain nitrogen-bearing molecules that make them very active. Many of these plants have been used to create well-known drugs used for medicinal purposes. One such component is, vincristine, is used to treat some types of cancer.

Flavonoids: Flavonoids are found widely in the plant and have a wide range of medicinal uses and actions. They often act as pigments by giving a yellow or white color to flowers and fruits. Some flavonoids have anti-viral and anti-inflammatory properties.

Phenols: Phenols are plant compounds that are thought to be produced to protect against infection, have anti-inflammatory and antiseptic and anti-viral properties. Phenols vary in structure and range from salicylic acid (similar to aspirin) to complex sugar-containing phenolic acids.

Cardiac Glycosides: These compounds are found in various medicinal plants and have strong direct action on the heart. Cardiac glycosides such as digitoxin, digoxin, and convallotoxin support heart strength and rates of contraction when failing.

Proanthocyanins: These compounds are pigments, which give fruits and flowers red, purple, or blue hues and are closely related to tannins and flavonoids. These compounds have been documented to be valuable in protection of circulation specifically in the heart, eyes, and feet.

Saponins: This group of active compounds obtains its name from the fact that like soap, they produce lather when placed in water. There are two main forms of saponins: steroidal and triterpenoid.

Tannins: Most plants produce tannins. Tannins serve as a deterrent to herbivory by insects and grazing animals given that that they provide a harsh unpalatable flavor. Tannins are also

useful in curing leather because of their tendency to contract and astringe tissues by binding with precipitating proteins.

Minerals: Many plants have high levels of minerals because they can draw minerals from the soil and can convert them into a form that is more easily used by the human body. Mineral content is often the key factor in a plant‟ s effectiveness as a medicine.

Polysaccharides: Polysaccharides are found in all plants and comprised of multiple units of sugar molecules linked together. For medicinal purposes, the "sticky" polysaccharides produce mucilage or gums that are commonly found in bark, roots, leaves, and seeds. These sticky polysaccharides are able to soak up large quantities of water and form jelly like masses that can be used to treat dry or irritated tissues such as skin and mucous membranes.

Vitamins: Many plants contain high levels of useful vitamins. Many well-known fruits and vegetables have high levels of vitamin C and beta-carotene. Lesser-known vitamin containing plants like watercress, rose hips, and sea buckthorn have high levels of vitamins B, C, and E.

Volatile oils: Volatile oils are extracted from plants and are used to produce essential oils that play a very important role in medicinal botany. These oils are often very complex and can be comprised of 100 or more compounds. These oils have many uses. For example, tea tree oil is a strong antiseptic. (Chemical Book, 2016)

1.4 Taxonomy and Terminology

Botany is the study of plants which is one of the major fields of biology, together with zoology and microbiology. Specializations in this field of botany consist of the study of mosses, algae, lichens, ferns, and fungi. Other specialties in botany contain plant physiology, the study of the vital processes of plants, such as photosynthesis, respiration, and plant nutrition.

Biochemists study the effects of soil, temperature, and light on plants, while plant morphologists study of the evolution and development of leaves, roots, and stems with a focus on the tissues at the tips of stems where the cells have the ability to divide.

Taxonomy is the field of biology which deals with the nomenclature, identification and classification of organisms. There are over one million known species on Earth and probably more than several million are not even identified. Taxonomists are responsible for identifying, naming, and classifying all these different species.

Systematic is a discipline of biology that clearly examines the natural variation and relationships of organisms, and which includes the field of taxonomy and also deals with the relationships of different groups of organisms, as most of them are strive to construct natural classification systems reflecting evolutionary relationships. (Science Encyclopedia-JRank Articles, 2017)

By DNA characterizations and other modern analysis, fungi and bacteria have been detached to separate kingdoms. However, a plant cell contains cellulose. Particularly, fungi have cell walls which contain chitin. In the purest sense of taxonomy, Lichens are not considered plants.

Viruses are also not considered as plants because, they are acellular, but can inhabit a host cell of another organism. Additionally, in many classifications they are not considered a living organism at all.

Myxomycetes or slime molds are also not considered plants but slightly are heterotrophs which can swallow bacteria, fungal spores and other items.

The botany is the scientific study of plants. It has identified around 350,000 extant taxa of plants, by distinct as seed plants, bryophytes, ferns and fern allies. About 400,000 plant species have been described as flowering plants.

Vascular plants (Tracheophyta) have lignified tissue and specialized structures termed as xylem and phloem. They transport water, minerals, and nutrients upward from the roots and return sugars and other photosynthetic products into vascular plants. Vascular plants are ferns, club mosses, flowering plants and other gymnosperms.

Different species structure is consider as common and have inherited genetic pathways, that structures are state as homologous. For example, cacti spines share the same basic structure and development as leaves of other vascular plants, so cactus spines are homologous to leaves. (Idc-online, 2017)

1.5 Kingdom Plantae

From Aristotle to Linnaeus and into the 20th century, throughout the scientific history, the species were divided into two kingdoms: animals and plants. This kingdom division was done according their physical and biological characteristics. By DNA characterizations and other modern analysis, fungi and bacteria have been detached to separate kingdoms. (Idc-online, 2017)

R.H. Whittaker prepared the organisms into five kingdoms and classified these organisms on the basis of cellular structure, mode and source of nutrition and body design. The five kingdoms proposed by Whittaker are Monera, Protista, Fungi, Plantae, and Animalia. (BYJU’S, 2016)

In biological Kingdom Plantae, predominantly eukaryotes included familiar organisms’ like- trees, forbs, shrubs, grasses and ferns etc. The Kingdom Plantae contains about 300,000 different species of plants. Modern classification schemes are determined by strict categorizations according to inherent in DNA and common origin. (Idc-online, 2017)

1.5.1 Divisions of Kingdom Plantae

The Kingdom plantae is also called as kingdom Metaphyta. The Kingdom plantae includes all types of eukaryotic, multicellular, photosynthetic plants found in biosphere. Most of the organism in this kingdom is autotrophs, which synthesis their own food with the help of solar energy. There are very few species, which are both autotrophs and heterotrophs. The history of life on earth and the success of many organisms literally depend on the success of plants. This classification is mainly based on their similarities and differences within plants. (TutorVista, 2017)

Based on “The presence and absence of vascular tissue, and also the presence and absence of seeds”- these criteria’s, Plant kingdom has been divided into the four main groups. (Hunker, 2017)

They are as follows:

- ✓ Phyllum Bryophyta
- ✓ Phyllum Pteridophyta
- ✓ Phyllum Gymnosperms
- ✓ Phyllum Angiosperms (Horticulture and Soil Science Wiki, 2017)

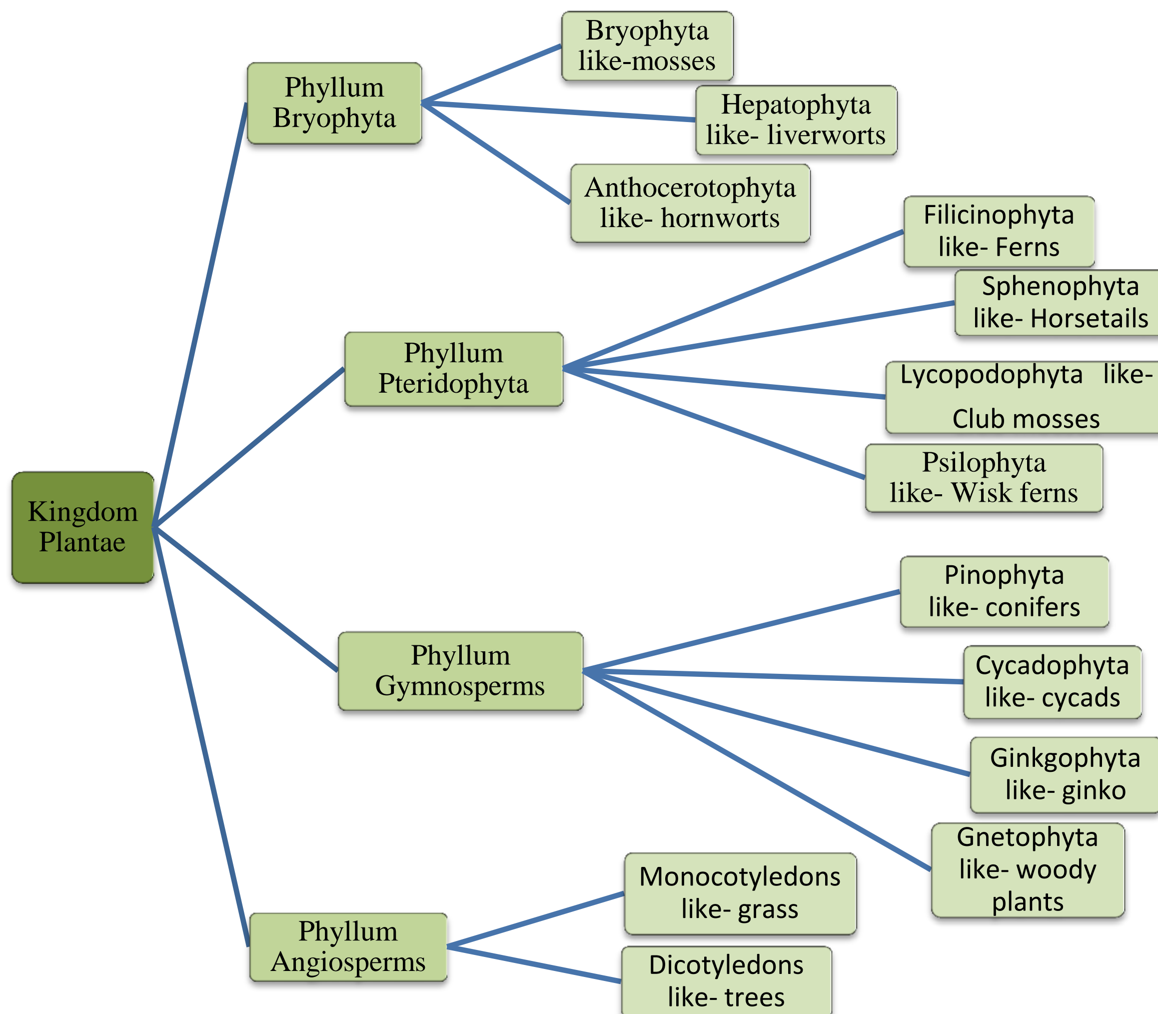


Table1.1: Divisions and subdivision of Kingdom Plantae

1.6 Medicinal plants or Herbs

Medicinal plant or herbs or tree, is a plant containing a wide verity of chemical constituent which help to elevated or treat illness. A medicinal plant and conventional pharmaceutical drugs has similar properties. From ancient period humans used medicinal plants either cure or lessen symptoms from an illness. Whereas, pharmaceutical drug is produced in a laboratory to

cure or help an illness which have also chance to create toxic or adverse effect. Typically, pharmaceutical drugs are modeled after compounds are found from medicinal plants. (Steven, James, 2014)

According to the WHO,

"A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." (Ghani, 1998)

According to the OPS, a medicinal plant is:

"Any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process"

Herbal remedies are the use of plants or plant extracts to medicate certain illnesses, minor or serious illnesses even cancer treatment. It has been used by our ancestors historically the Chinese, Arabs, Africans for centuries. Arjuna bark (*Terminalia arjuna*) contains Arjunolic acid. Arjuna bark mainly used to treat angina.

1.7 Herbal Medicine

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products, which contain as active ingredients parts of plants, or other plant materials, or combinations. Herbal medicine also called botanical medicine or phyto-medicine. Herbal medicine use a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Herbalism has a long tradition of use outside conventional medicine. The value of herbal medicine in treating and preventing disease need advances in clinical research, analysis and quality control to improvements in its activity. (Barnes, Anderson & Phillipson, 2007)

1.8 Plant Based Drugs and Medicines

Plants are an essential component of the universe. From the very beginning of time, human beings used plants as a medicine. Higher plants have been used as a source of drugs by mankind for several thousand years. After various observations and experiments medicinal

plants were identified as a source of important medicine for treatment of human civilization. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. However, plants are used as a basis of some of the most important drugs, even in the modern system of medicine. (Medicine Hunter, 2010)

1.8.1 Some Example of Plant Based Drugs and Medicines

The small fraction of flowering plants have been investigated and yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, digitoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine etc. In some cases, the crude extract of medicinal plants may be used as medicaments. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is currently available. (Medicine Hunter, 2010)

According to World Health Organization (WHO), about 80% of the world's population relies on traditional medicines. Some plant based drugs and medicines are given below-

- ✓ Turmeric possesses the blood clotting properties.
- ✓ Roots of the endive plant were used for treatment of gall bladder disorders.
- ✓ Paclitaxel from *Taxus brevifolia* used for the treatment of lung, ovarian and breast cancer.
- ✓ Mandrake was prescribed for pain relief.
- ✓ The alkaloid, forskolin from *Coleus forskohlii* and phytochemicals from *Stephania glabra*, are now being re-discovered as adenylate cyclase and nitric oxide activators, which may help in preventing conditions including obesity and atherosclerosis.
- ✓ Apomorphine is a semi synthetic compound derived from morphine (*Papaver somniferum*) used in Parkinson's disease.
- ✓ Cannabidiol obtained from cannabis plant (*Cannabis sativa*) and Capsaicin active compound from *Capsicum annuum* are used as pain relievers. (Veeresham, 2012)

1.9 Introduction to *Murraya* genus

Murraya is a genus of flowering plants in the Rutaceae family, which commonly known as the rue or citrus family. It is distributed in Asia, Australia, and the Pacific Islands. The center of diversity is in southern China and Southeast Asia. These plants are shrubs or trees whose leaves are pinnate, divided into several leaflets, and alternately arranged on the branches and also glandular, aromatic, and leathery to membranous in texture. This have small raceme of flowers growing at the ends of branches or in the leaf axils; some flowers are solitary. The fruit is a fleshy berry with pulp but without the juice. Usually these plant flowers have strong scents. It contains many types of coumarins, alkaloids, carbazole girinimbine etc. constituents. Plants various parts used to treat fever, pain, and dysentery. (Sayar, Paydar & Pingguan-Murphy, 2014)

1.10 Overview: *Murraya koenigii*

Curry leaves (*Murraya koenigii*) are popular leaf spice which used in very small quantities for their distinct aroma. For the presence of volatile oil, it gives distinct aroma and they have ability to improve digestion. Folk medicine was made by herbal and natural products which used for centuries in every culture of the world. Scientists and medical professionals have shown increased interest for this plant as they recognize its true health benefits. With their advantageous therapeutic uses for various ailments, they are also economical, effective and easy available.

So, for this curry leafs, we can say- “Food can be our medicine and medicine can be our food.” (Singh, More & Mohan, 2014)

Figure1.3: ***Murraya koenigii***

1.10.1 History of curry leaves

The curry leaf tree is native to India, Sri-Lanka, Bangladesh and the Andaman Islands. Later spread throughout Indian migrants and now grows in other areas of the world where Indian immigrants are settled. (Singh, More & Mohan, 2014)

1.10.2 Origin

Murraya koenigii Linn. is commonly known as *curry leaf*. The curry tree (*Murraya koenigii*) is a small, tropical to sub-tropical tree or shrub in the family Rutaceae. It is native to India and Sri Lanka. This deciduous shrub found mainly all around India and in other tropical parts of the Asian subcontinent as Bangladesh, Sri Lanka eastward to Myanmar, Indonesia, Southern China, and Hainan. In Bangladesh, it distributed at Forests of Chittagong, Chittagong Hill Tracts and Sal forests.

It is cultivated in India, Sri Lanka, and neighboring countries for its aromatic leaves and as an ornamental plant. Now, it is also cultivated in various other countries such as China, Australia, Nigeria and Ceylon. Height of the plant ranges from small to medium. The most useful parts of this plant are the leaves, root and the bark. (OrganicFacts, 2017)

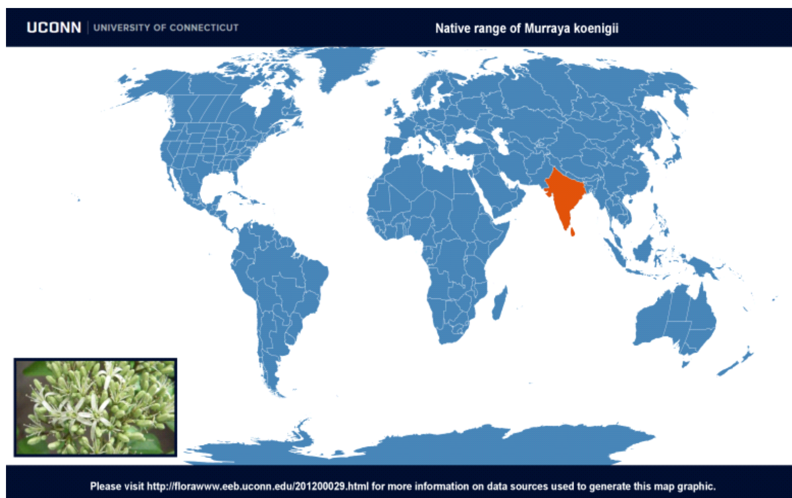


Figure1.4: Origin of *Murraya koenigii*

1.10.3 Others Common Names of *Murraya koenigii*

Murraya koenigii also known as 'sweet neem leaves', 'Karapincha', 'Karivepallai', 'Karivembu' or 'Kadipatta', etc.

It has several names in Bengali, they are- 'Nimbhut', 'Barsunga', 'Choto Kamini', 'Mitha Neem', 'Shurovi Neem', 'Girinim', 'Gandhal', 'Babsanga', 'Kariaphuli, 'Pahari Nim', 'Bhatraj' etc. It also have Tribal name like- 'Shatley (Khumi)'. (Parmar & Kaushal, 1982)

1.10.4 Plant Taxonomy (Scientific classification)

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Rosidae

Order: Sapindales

Family: Rutaceae – Rue family

Genus: Murraya J. Koenig ex. L – murraya

Species: ***Murraya koenigii (L.) Spreng***. –curry leaf tree (USDA, 2017)

1.10.5 Plant Morphology

Murraya koenigii is an easy to raise plant which needs full sunlight in moist forests to grow and needs minimum 55 degrees temperature, blooms in summer.

Figure1.5: ***Murraya koenigii*** **plant**

Plant grows 6 to 15 feet or 13–20 feet (4–6 m) tall and 4 to 12 feet wide.

Aromatic leaves are pinnate and evergreen. Each odd-pinnate leaf typically has 11 to 21 leaflets, each leaflet 2–4 cm (0.79–1.57 in) long and 1–2 cm (0.39–0.79 in) broad. These leaves are thin, ovate, shiny, dark green leaflets.

Figure1.6: ***Murraya koenigii*** **leaf**

This plant produces small but fragrant white flowers (each to 5 to 16 cm across) in many flowered panicles bloom irregularly throughout the year.

Figure1.7: ***Murraya koenigii*** **flower**

They can self-pollinate to produce small shiny bluish--black berries (each to 2/3" diameter) containing a single, large viable seed. These barriers are ovoid to oblong. Immature one is green, ripe one purplish black. Though the berry pulp is edible with a sweet but medicinal flavor, the pulp and seed are not used for cooking purposes. (Singh, More & Mohan, 2014)

Figure1.8: Fruits of *Murraya koenigii*

1.10.6 Cultivation and Collection

At the middle of April flowering starts and ends at the middle of May. The peak flowering season was observed at the last week of April. The fruiting season mainly observed from the middle of July to the end of August. The peak fruiting was season was observed at the last week of July to the first week of August.

Curry leaf tree is large shrub to small tree. Its pinnate leaves are mainly used. It needs full sun or light shade and fertilize (with palm or citrus) to advance leaf production. Ripe and fresh Seeds from plant are good for plant cultivation. If the pulp remove from fruit before planting in potting mix, it is best for plant cultivation. Stem cuttings can be also used for propagation. (Singh, More & Mohan, 2014)

1.10.7 Chemical Constituents

- ✓ The leaves of the plant contain various compounds such as essential oil, tannins, resin, glucoside girinimbin, isomahanimbin, koenine, koenigine, koenidine, koenimbine, koenigin, koenigicine, koemine, mahanimbicine, bi-cyclomahanimbicine, phebalosin, coumarine as murrayone imperatoxin, mahanine, and scopolin. (Brind , Misra & Srivastava, 2014)

- ✓ Triterpenoid alkaloids such as cyclomahanimbine, tetrahydromahanmbine and murrayastine, murrayaline, and pypayafoline alkaloids are the other chemical compounds in the leaves of *Murraya koenigii*.
- ✓ The leaves also are rich in carbazole alkaloids which are suggested to have stimulating effects on the central nervous system (CNS).

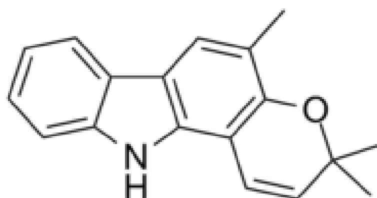


Figure1.9: Structure of Girinimbine

- ✓ Fruits and seeds contain the carbazole alkaloids, mahanimbine, murrayazolidine, girinimbine, koenimbine, koenigicine, mahanine, iso-mahanine and murrayanol.
- ✓ Stem-bark contains girinimbin, girinimbine, murrayanine, murrayacine and a carbazole carboxylic acid - mukoeic acid, curryangine and curryanine.
- ✓ Flowers contain a large number of mono- and sesquiterpenoids, the major ones being ß-caryophyllene, ß-ocimene and linalool.
- ✓ A new dimeric carbazole alkaloid, bismurrayafoline E, has been isolated from this plant. Plant also contains mukonine and mukonidine.
- ✓ Girinimbine, mahanimbine, isomahanimbine and murrayacine have also been isolated from roots. (Bangladesh Ethnobotany Online Database, 2012)

1.10.8 Activity of *Murraya koenigii*

It is used for several medicinal purposes like- astringent, aromatic, demulcent, depurative, antihelmintic, appetizing, carminative, anodyne, constipation, anti-inflammatory, antiseptic, tonic, etc. (Parmar & Kaushal, 1982)

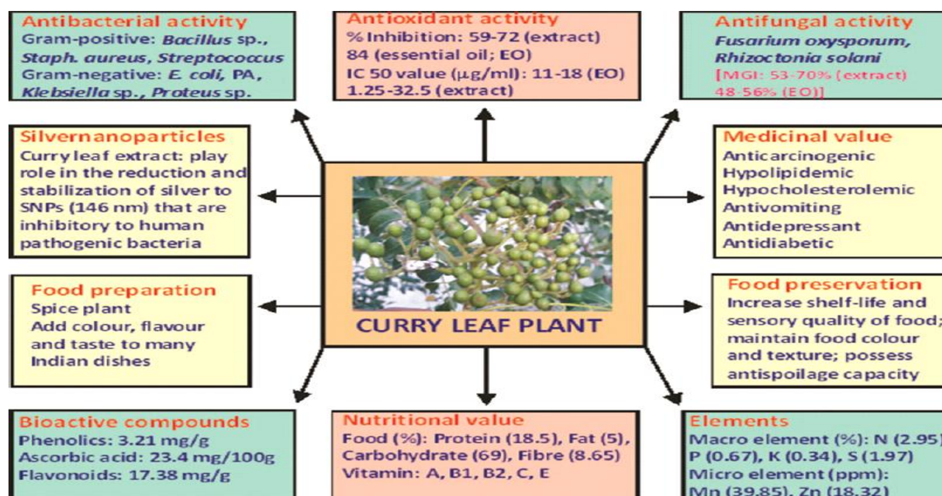


Figure1.10: Activities of *Murraya koenigii*

1.10.9 Nutrition Facts

Leaves:

The main nutrients found in curry leaves are proteins, carbohydrates, energy, fibers, calcium, phosphorous, iron, magnesium, copper and minerals, carotene, nicotinic acid, oxalic acid, crystalline glycosides, carbazole alkaloids, koenigin, and resin.

It also contains various vitamins like nicotinic acid and vitamin C, vitamin A, vitamin B, vitamin E, antioxidants, plant sterols, amino acids, glycosides and flavonoids. Also, nearly zero fat (0.1 g per 100 g) is found in them. Fresh leaves are containing yellow-colored volatile oil, and are rich sources of vitamin A and calcium. (Organic Facts, 2017)

Fruits:

The pulp of the fruit contains 64.9 percent moisture (water). The content of total soluble solids of the fruit juice is 16.8 percent. The pulp contains 9.76 per cent total sugars, 9.58 per cent reducing sugars, 0.17 percent non-reducing sugars and almost a negligible amount of tannins and acidity. (Parmar & Kaushal, 1982)

The vitamin C content of the fruit is 13.35 mg per 100 g of the pulp. The mineral content of the edible portion of the fruit is around 2.162 per cent.

Similarly, 100 g of the edible portion of the fruit contains protein, 1.97 g; phosphorus, 0.082 g, potassium, 0.811 g, calcium, 0.166 g; magnesium, 0.216 g; and iron, 0.007 g.

1.10.11 Traditional Uses

- ✓ For their unique pungent, aromatic flavor, they are used in Indian/Asian cuisine.
- ✓ In Ayurvedic medicine, curry leaves are used in believed, to have several medicinal properties such as anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic and hepato-protective properties. The roots are used for treating body aches and the bark is used for snake bite relief.
- ✓ In their fresh form, they have a short shelf life and do not keep well in the refrigerator. They are also available dried, though the aroma is largely inferior.
- ✓ For its characteristic aromatic properties, it can used in soap making, body lotions, diffusers, potpourri, air fresheners, body fragrances, perfumes, bath and massage oils, aromatherapy, towel scenting, spas and health clinics, incense, facial steams, and hair treatments etc.
- ✓ In the absence of basal (tulsi) leaves, curry leaves are used for Hindu rituals, such as pujas.
- ✓ It is traditionally used for medicinal and folk purposes like- astringent, aromatic, appetizing, constipation, anti-inflammatory etc.
- ✓ Timber is used as fuel and to prepare household materials. (Saini & Reddy, 2015)

1.10.12 Health Benefits of Curry Leaves

Most of the people think that curry leaves use only for add flavor to the food and throw leaves while eating their soup or curry. However, they have many important health benefits than people realize. *Murraya koenigii* (curry leaves) offers a wide range of health benefits without giving side effects of other medicines. (Always Ayurveda, 2017)

✓ Cure Diarrhea

Carbazole alkaloids present in curry leaves or *Murraya Koenigii* who have anti-diarrheal properties.

✓ Gives Gastrointestinal Protection

Use of curry leaves is recommended for cure of gastrointestinal problems in Ayurvedic methodology. It is considered to give mild laxative properties. So, mixture of curry leaves and lime juice is to be consumed for indigestion or paste from leaves can be added to buttermilk and taken every morning on an empty stomach.

✓ Contains Antioxidant Properties

Curry leaves or *Murraya Koenigii* is a good source of antioxidants. The presence of various vitamins like vitamin A, vitamin B, vitamin C and vitamin E help in reducing oxidative stress and free radical scavenging activity.

✓ Have Anti-diabetic Properties

Curry leaves is use to control diabetes. The anti-hyperglycemic properties of the leaves are beneficial in controlling blood glucose levels. The curry leaves extracts help in protecting the pancreatic beta cells and improving the functioning of pancreatic islets that are responsible to produce insulin.

✓ Fight against Cancer

The chemical constituents such as phenols (Carbazole alkaloids) are found in curry leaves, are helpful in fighting cancers such as leukemia, prostate cancer and colorectal cancers. It is known to improve cell immunity as well.

✓ Lower Cholesterol Levels

Curry leaves are also known to reduce bad LDL cholesterol level. It helps in preventing obesity. (The Times of India, 2017)

- **Good for Hair Growth & Prevents graying of hair**

Curry leaves are believed to help in strengthening hair roots. So, Dry curry leaf powder mixed in oil can be applied to hair with a quick massage. The curry leaves paste can also be applied in cases of gray hair. Doing these on a regular basis can improve hair growth as well.

- **Good for Eyesight**

Curry leaves contain high amounts of vitamin A. Vitamin A contains carotenoids which protect the cornea, which is the eye surface. Deficiency of vitamin A may cause night blindness, cloud formations in front of the eye and even the loss of vision loss in some cases.

- **Have Radio-protective and Chemo-protective activity**

Curry leaves have shown positive results in reducing the effects of chemotherapy and radiotherapy, it also offering protection against chromosomal damage, protection of bone marrow and prevention of free radicals becoming active in the body.

- **Protect Against Pathogen Attack**

Curry leaves are effective against fighting bacterial and fungal infections. The leaf extracts from the plant have been comparable to popular, mainstream antibiotic drugs.

- **Protect the Liver**

Curry tree have tannins and carbazole alkaloids present in the leaves, which exhibited good hepato-protective properties. The leaf extracts is reduce the levels of aspartae, bilirubin and alkaline phosphatase. They are also helpful in protecting the liver from various diseases such as hepatitis and cirrhosis and relieving hepatic complications. (Organic Facts, 2017)

- **Skin Care**

Curry leaves are also helpful in skin care. The juice or paste of leaves can be applied on burns, cuts, bruises, skin irritations and insect bites for quick recovery and clean healing.

1.10.13 Safety and Precaution

- The seeds of the curry leaf are poisonous and it should not be consumed.
- It can cause skin irritation or allergic reaction. (Organic Facts, 2017)

Chapter Two

Literature Review

2.1 Literature Review on *Murraya koenigii*

Murraya koenigii Linn (Rutaceae) have an ethnobotanical, economical and biological importance. It abundantly occurs in outer Himalayas, Assam, and Chittagong, upper and lower Burma, Andaman Islands, India. In traditional system of Medicine like ayurveda, bark, root, leaves, fruits and fruit pulp of *Murraya koenigii* are widely used as antiemetic, antidiarrhoeal, dysentery, diabetes, febrifuge, blood purifier, tonic, stomachic, obesity, vomiting, constipation, indigestion, piles, nausea, to relieve kidney pain etc. There have some paper reviews in which the data related to scientific works carried out with the plant and listed the bioactive compounds isolated from the plant till date. Based on the review made, present paper highlights the need of future research with *Murraya koenigii* so that more active principle for treating new ailments can be isolated and made available from the plant. (Saini & Reddy, 2015)

2.1.1 Antioxidative Activity of Carbazoles from Murraya koenigii Leaves

Murraya koenigii leaves extract contains antioxidative properties which were evaluated bases on oil stability index (OSI) and their radical scavenging ability against 1-1-diphenyl-2-picrylhydrazyl (DPPH). Methylene chloride (CH_2Cl_2) extract and the ethyl acetate (EtOAc) soluble fraction of the 70% acetone extract was drastically extended the OSI values compare to the α-tocopherol and BHT.

From the CH_2Cl_2 extract, five carbazole alkaloids structures were isolated and identified as euchrestine B (1), bismurrayafoline E (2), mahanine (3), mahanimbicine (4), and mahanimbine (5) ; was done based on ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) and mass (MS) spectral data. At 110 °C the OSI value of carbazoles was decreased in this order- 1 and 3 > α-tocopherol > BHT > 2 > 4, 5 and control. It was assumed that 1 and 3 no. compounds contributed to the high OSI value of the CH_2Cl_2 extract of Murraya koenigii. And the DPPH radical scavenging activity for these compounds was in this order- ascorbic acid > 2 > 1, 3 and α-tocopherol > BHT > 4 and 5.

(Tachibana *et al*., 2001)

2.1.2 Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (Murraya koenigii L.) extracts

By using various kinds of assays from curry leaves (Murraya koenigii L.) different extracts like- water, alcohol, alcohol:water, hexane or chloroform extracts , in vitro antioxidant properties were found. The highest antioxidant and free radical scavenging activity was showed by the alcohol:water (1:1) extract of curry leaves (AWEC) which not only inhibited the membrane lipid peroxidation by 76%, at 50 µg/ml, scavenged 93% of superoxides at 200 µg/3 ml but also scavenged approximately 90% of hydroxyl and 1,1-diphenyl-2-picrylhydrazayl radicals at 4–5-fold lower concentrations compared with the other tested extracts. Additionally, this alcohol:water extract reduced cytochrome c and ferric ion levels, chelated ferrous ions and inhibited ferrous sulfate:ascorbate-induced fragmentation and sugar oxidation of DNA. As a result, it established the antioxidant potential of AWEC, which could use as natural antioxidant source. (Ningappa, Dinesha & Srinivas, 2008)

2.1.3 Comparison of Antioxidative Properties of Carbazole Alkaloids from Murraya koenigii Leaves

From the CH_2Cl_2 extract of Murraya koenigii, a new dimeric carbazole alkaloid, 8,10‘-[3,3‘,11,11‘-tetrahydro-9,9‘-dihydroxy-3,3‘,5,8‘-tetramethyl-3,3‘-bis(4-methyl-3-pentenyl)]bipyrano[3,2-a]carbazole (12), was isolated together with six known carbazole alkaloids, koenimbine (6), O-methylmurrayamine A (7), O-methylmahanine (8), isomahanine (9), bismahanine (10), and bispyrayafoline (11).

Their structures were identified on the basis of ^{1}H and ^{13}C NMR spectroscopic and mass spectrometric (MS) data. The antioxidative properties of 12 carbazole alkaloids isolated from leaves of Murraya koenigii were evaluated on the basis of the OSI together with their radical scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. When the lag times reach a steady state, the 12 carbazoles were classified into three groups. It was suggested that an aryl hydroxyl substituent on the carbazole rings plays a vital role in stabilizing the thermal oxidation and rate of reaction against DPPH radical. (Tachibana *et al*., 2003)

2.1.4 Hypoglycemic action of Murraya koenigii (curry leaf) and Brassica juncea (mustard): mechanism of action.

Murraya koenigii and Brassica juncea showed effect on carbohydrate metabolism which was studied on rats as experimental animals. And both of them showed significant hypoglycemic action on rat.

For increased activity of glycogen synthetase, the increased concentration of hepatic glycogen and glycogenesis causes. For decreased activity of glycogen phosphorylase and gluconeogenic enzymes, the decrease concentration of glycogenolysis and gluconeogenesis causes.

(Khan, Abraham & Leelamma , 1995)

2.1.5 Immunomodulatory activity of methanolic extract of Murraya koenigii (L) Spreng. Leaves

Methanolic extract of Murraya koenigii leaves were used to evaluate Immunomodulatory activity on humoral and cell mediated immune response to ovalbumin, phagocytic activity by carbon clearance test, nitric oxide (NO) release from murine peritoneal macrophages and cyclophosphamide induced myelosuppression.

In culture of supernatants, significant increased of NO production occur in mouse peritoneal macrophages was detected. This indicated the increased phagocytic activity of macrophages. This extract showed not only significant increase in phagocytic index by rapid removal of carbon particles from blood stream but also increased the antibody titre against the ovalbumin and protection towards the cyclophosphamide induced myelosuppression. Still, the extract did not show any significant increase in delayed type hypersensitivity reaction which indicated the inability of the extract to stimulate T cells. Present study thus revealed that the extract holds promise as immunomodulatory agent, which acts by stimulating humoral immunity and phagocytic function.

(Shah, Wakade & Juvekar, 2008)

2.1.6 A review on *Murraya koenigii*: multipotential medicinal plant

Harish K Handral, Anup Pandith and Shruthi S D had done this present study which was aimed to review the ethanobotanical properties, pharmacognostic, phytochemical and pharmacological properties of *Murraya koenigii* plant. Medicinal plants mainly used in herbalism for medicinal properties which are easily available source for healthcare purposes in rural and tribal areas. The *Murraya koenigii* plant widely used as herb, spice, condiments and used to treat various types of ailments in Indian traditional system. World's about 80% populations including different tribal communities rely on herbal products by using various parts of plant for cure of numerous diseases and also for its safe, effective and economical side. Plant leaves used as tonic, stomachic, carminative, internally in dysentery, vomiting., antihelminthic, analgesic, cures piles, allays heat of the body, thirst, inflammation and itching.

Analysis of literature reveals some pharmacological activities of this plant such as activity on heart, anti diabetic and cholesterol reducing property, antimicrobial activity, antioxidative property, cytotoxic activity, anti diarrhea activity, phagocytic activity and many more medicinal values. (Handral, Pandith & Shruthi, 2012)

2.1.7 Phenolic antioxidants from herbs and spices

Nobuji Nakatani did his research on spices and herbs which are recognized as sources of natural antioxidants and plays an important role in the chemoprevention of diseases resulting from lipid peroxidation. Spices and herbs have over a hundred known and new compounds, having high antioxidant activity. From the Labiatae family, {it *Rosmarinus officinalis*, *Thymus vulgaris, Origanum vulgare*} and {it O. majorana} gave 26 active comopounds. Over 40 antioxidative compounds from {it *Zingiber officinale*}, 26 compounds from {it *Curcuma domestica* = C. *longa, C. xanthorrhiza*} and {it *Z. cassumunar*} were determined, these belonging to the family Zingiberaceae. From the family Myrtaceae, 25 compounds from the berries of {it *Pimenta dioica*} were determined and 3 carbazoles were isolated from {it *Murraya koenigii*}. Structure-activity relationships of some of the isolated compounds were also discussed. (Nakatani, 2000)

2.1.8 Antioxidant activity of plants methanolic extracts containing phenolic compounds

N Huda-Faujan, A Noriham, AS Norrakiah, and AS Babji published this paper which reported the antioxidative activities of some methanolic plant extracts namely 'ulam raja' (*Cosmos caudatus*), 'kesum' (*Polygonum minus*), 'selom' (*Oenanthe javanica*), 'pegaga' (*Centella asiatica*) and 'curry leaf' (*Murraya koenigii*). This analysis carried out total phenolic content, ferric reducing power, ferric thiocyanate (FTC) and thiobarbituric acid (TBA) tests. From the analyses, *M. koenigii* had the highest yield extraction (1.65%), highest total phenolic content (38.60 mg TAE/ 100 g fresh weight) and antioxidant activity (70.60%) using FTC method. Increasing the concentration of the extracts resulted in increased ferric reducing antioxidant power for all methanolic extracts tested. TBA analysis showed that C. caudatus extract had the highest antioxidant effect. Total phenolic content had positive correlation with antioxidant capacity which shows that the plants, especially *M. koeniigi*, can be potent source of natural antioxidants. (Huda-Faujan *et al.*, 2009)

2.1.9 Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic β-cells in experimental diabetes in rats

Oxidative stress and oxidative damage to tissues are common end points of chronic diseases such as atherosclerosis, diabetes, and rheumatoid arthritis. Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can further increase the deleterious effects of free radicals. The aim of the present study was to evaluate the possible protective effects of *Murraya koenigii* leaves extract against β-cell damage and antioxidant defense systems of plasma and pancreas in streptozotocin induced diabetes in rats. The levels of glucose and glycosylated hemoglobin in blood and insulin, Vitamin C, Vitamin E, ceruloplasmin, reduced glutathione and TBARS were estimated in plasma of control and experimental groups of rats. To assess the changes in the cellular antioxidant defense system such as the level of reduced glutathione and activities of superoxide dismutase, catalase and glutathione peroxidase were assayed in pancreatic tissue homogenate. The levels of glucose, glycosylated hemoglobin, insulin, TBARS, enzymatic and non-enzymatic antioxidants were

altered in diabetic rats. These alterations were reverted back to near control levels after the treatment of *M. koenigii* leaves extract. Transmission electron microscopic studies also revealed the protective nature of *M. koenigii* leaves on pancreatic β-cells. These findings suggest that *M. koenigii* treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress and pancreatic β-cell damage. The antioxidant effect of the *M. koenigii* extract was compared with glibenclamide, a well-known hypoglycemic drug. (Arulselvan & Subramanian, 2007)

2.1.10 Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats

Curry patta (*Murraya koenigii*) is traditionally consumed by diabetic's patients in southern part of India. So, Feeding of diet to normal rats containing various doses of curry leaves (5, 10 and 15%) for 7 days as well as mild diabetic (blood glucose levels >175 mg/dl induced by alloxan 35 mg/kg IP) and moderate diabetic rats (blood glucose levels >250 mg/dl induced by STZ 60 mg/kg IP) for 5 weeks showed varying hypoglycemic and anti-hyperglycemic effect. In normal rats, reduction in blood glucose was almost negligible (~ 4% with 10 and 15% diet). In mild and moderate diabetic rats, feeding of 5, 10 and 15% diet caused a maximal reduction in blood sugar by 13.1, 16.3 and 21.4% (NS, P<0.05 and 0.005) and 3.2, 5.58, 8.21% (NS), respectively.(Yadav *et al*., 2002)

2.1.11 Beneficial effect of the leaves of *Murraya koenigii* (Linn.) Spreng (Rutaceae) on diabetes-induced renal damage in vivo

The present study was designed to investigate the beneficial effect of the leaves of *Murraya koenigii* (Linn.) on diabetes-induced renal damage in vivo with regard to prove its efficacy by local traditional practitioners in the treatment of kidney frailties in diabetics. *Murraya koenigii* (Linn.) Spreng (curry leaf) is widely used as nephroprotective agent in kidney's infirmities among diabetics by the traditional practitioners in Malaysia. Aqueous (AQ) extract of the leaves of *Murraya koenigii* (Linn.) was administered to both normal and streptozotocin (STZ) induced diabetic male rats (Sprague–Dawley strain). Daily oral administration of variable dose levels of the AQ extract for 30 days, produced significant dose dependant decrease in

serum urea and creatinine levels ($p < 0.001$), and marked increase in the levels of plasma antioxidant capacity ($p < 0.01$) in diabetic treated rats, compared to the control (non-diabetic) subjects. However, the normal treated rats showed minimal variation in these parameters in comparison to normal controls. So the results of our study scientifically support the traditional belief for using the leaves of *Murraya koenigii* (Linn.) as adjuvant. (Yankuzo, *et al*., 2011)

2.1.12 Curry leaf (*Murraya koenigii*) or Cure leaf: Review of its curative properties

Murraya koenigii is not only important use in culinary but also used in the Ayurvedic system of medicine since many centuries. An analysis of literature reveals some notable pharmacological activities of the plant. Carbazole alkaloids which are abundantly present in the leaves, fruits, roots and bark of this plant. They have their antidiabetic, anticancer, antibacterial, and anti-nociceptive and antioxidant activities. Besides these activities, the plant is described to have a wide array of therapeutic activities. Phytochemistry and pharmacology of this plant necessitates a comprehensive review of its prospects as an important therapeutic agent for the management of numerous diseases commonly affecting humans. The current review provides a detailed report of the phytochemical, pharmacological, clinical and pre-clinical works carried out on this culinary plant and also throws light on its therapeutic prospects. (Bhandari, 2012)

2.1.13 An update on Murraya koenigii spreng: A multifunctional Ayurvedic herb

Murraya koenigii spreng (Rutaceae) is medicinally important herb which use as Ayurvedic system of medicine since many centuries. The plants leaves, fruits, roots and barks are abundant with Carbazole alkaloids which exarts antitumor, antidiabetic, anticancer, antibacterial, and anti-nociceptive and antioxidant activities. The current review provides a detailed report of the phytochemical, pharmacological, clinical and pre-clinical works are carried out on this culinary plant and also throws light on its therapeutic prospects. (Gupta, Nahata & Dixit, 2011)

2.1.14 Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of Murraya koenigii growing in Nigeria

The methanolic extract of *Murraya koenigii* leaf was screened for toxicological and biochemical effects on rats because of the folkloric uses as an anti-dysentery and anti-diabetes. The extract was moderately toxic (LD50=316.23 mg/kg body weight) for rats and had appreciable effect on the liver and kidney at higher doses leading to liver inflammation. It had little or no effect on haematology and relative organ weight of lungs, heart and spleen. Acute doses (⩾500 mg/kg) reduced significantly serum globulin, albumin, urea, glucose, total protein, aspartate transaminase (AST), and increased cholesterol and alanine transaminase (ALT) indicating hepatic injury. However, chronic administration for 14 days gave a significant ($p<0.05$) reduction in the serum cholesterol, glucose, urea, bilirubin, ALT and AST showing that the plant has hypoglycaemic and hepatoprotective effects after prolonged use. The activity demonstrated by some of the isolated carbazole alkaloids and their derivatives against Trichomonas gallinae confirmed that the anti-trichomonal activity of the leaf may be due to its carbazole alkaloids. The order of activity was C18>C23>C13. Girinimbine and girinimbilol with IC50 values of 1.08 and 1.20 μg/ml were the most active. Acetylation of girinimbilol and mahanimbilol improved their activities to 0.60 and 1.08 μg/ml. (Adebajo *et al*., 2006)

2.1.15 Hypoglycemic effects of Murraya koenigii on normal and alloxan-diabetic rabbits

In past there have been many medicinal plants, which have been used in traditional medicines for their antidiabetic properties without any scientific support and pharmacological evidence. The aqueous extract of *Murraya koenigii* leaves has been taken to evaluate the hypoglycemic activity in normal and alloxan induced diabetic rabbits. This plant is promising as it is widely and regularly used as a spice for food flavoring and as such it appears to be without any side effects and toxicity. Adequate characterization of hypoglycemic activity of aqueous extract has not been yet done, as no such reports are available in the literature though the activity is

reported. The scientific evaluation of its hypoglycemic activity was, therefore, explored and also compared with the effect of a standard hypoglycemic drug, tolbutamide. A single oral administration of variable dose levels (200, 300 and 400 mg/kg) of aqueous extract led to lowering of blood glucose level in normal as well as in diabetic rabbits. The maximum fall of 14.68% in normal and 27.96% in mild diabetic was observed after 4 h of oral administration of 300 mg/kg. The same dose also showed a marked improvement in glucose tolerance of 46.25% in sub-diabetic (AR) and 38.5% in mild diabetic rabbits in glucose tolerance test after 2 h. The findings from this study suggest that the aqueous extract of these leaves may be prescribed as adjunct to dietary therapy and drug treatment for controlling diabetes mellitus. (Kesari, Gupta & Watal, 2005)

2.1.16 Anti-diabetic effect of Murraya koenigii leaves on streptozotocin induced diabetic rats

The present study was aimed to evaluate the anti-hyperglycemic efficacy of *Murraya koenigii* in STZ-induced diabetic rats. Oral administration of ethanolic extract of *M. koenigii* at a dose of 200 mg/kg/b.w./day for a period of 30 days significantly decreased the levels of blood glucose, glycosylated hemoglobin, urea, uric acid and creatinine in diabetic treated group of animals. Determination of plasma insulin level revealed the insulin stimulatory effect of the extract. The results suggest that *M. koenigii* possesses statistically significant hypoglycemic potential in STZ-induced diabetic rats. The *M. koenigii* extract appeared to be more effective than glibenclamide, a known antidiabetic drug. (Arulselvan *et al*., 2006)

2.1.17 Effect of extracts of *Murraya koenigii* leaves on the levels of blood glucose and plasma insulin in alloxan-induced diabetic rats

The effect of daily oral administration of aqueous extract (600 Abstract : mg/kg b.wt.) and methanol extract (200 mg/kg b.wt.) of *Murraya koenigii* Spreng leaves for a period of eight weeks was studied on blood glucose and plasma insulin level in alloxan-induced diabetic rats. Blood glucose levels of diabetic rats treated with aqueous and methanol extracts of *Murraya koenigii* Spreng showed significant reduction ($P<0.05$) as compared to diabetic control groups. Plasma insulin showed significantly high on 43rd and 58th days of treatment in

aqueous and methanol extracts of *Murraya koenigii* treated groups. This suggests that the hypoglycemic effect may be mediated through stimulating insulin synthesis and/or secretion from the beta cells of pancreatic islets of Langerhans. (Vinuthan, Kumar & Ravindra, 2004)

2.1.18 Biosynthesis of silver nanoparticles using *Murraya koenigii* (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size

Biological synthesis of silver nanoparticles using *Murraya koenigii* leaf extract was investigated and the effect of broth concentration in reduction mechanism and particle size is reported. The rapid reduction of silver (Ag+) ions was monitored by using UV-visible spectrophotometry and showed formation of silver nanoparticles within 15 minutes. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis showed that the synthesized silver nanoparticle are varied from 10-25 nm and have the spherical shape. Further the XRD analysis confirms the nanocrystalline phase of silver with FCC crystal structure. From this study, it was found that the increasing broth concentration increases the rate of reduction and decreases the particle size. (Christensen *et al*., 2011)

2.1.19 Carbazole alkaloids from seeds of Murraya koenigii

Isomahanine and murrayanol were the carbazole alkaloids, which isolated from the *Murraya koenigii* fruits. This also contains another five carbazole alkaloids, such as- mahanimbine, murrayazolidine, girinimbine, koenimbine and mahanine. By methylation and cyclisation, structures of isomahanine and murrayanol were confirmed respectively by formatting 9-methoxymahanimbicine. (Reisch *et al*., 1992)

2.1.20 Protective effect of aqueous Curry leaf (Murraya koenigii) extract against cadmium-induced oxidative stress in rat heart

A low dose of cadmium chloride used in treatment of rat caused a serious damage in rat's cardiac tissue which was indicated by the increase level of serum glutamate oxaloacetate transaminase and lactate dehydrogenase1 activities. Also cause alterations in the activities of mitochondrial Kreb's cycle as well as respiratory chain enzymes. Histological studies showed

that cadmium-induced tissue damage was caused due to oxidative stress. Oxidative stress was clearly from changes in the levels of lipid per-oxidation and reduced glutathione, the protein carbonyl content and the altered the activities of cardiac antioxidant and pro-oxidant enzymes. These rats were pre-treated with an aqueous extract of Curry leaf *(Murraya koenigii)* and all these changes were improved. People consumed curry leaf in India and South-East Asian and some European countries; did not reported any side-effects and the results seem relevant for environmentally or occupationally exposed cadmium in humans. (Mitra. *et al*., 2012)

Chapter Three

Materials & Methods

3.1 Collection & preparation of plant material

Plant sample (Leaves) *Murraya koenigii* or curry leaf of was collected from national herbarium, Mirpur, Dhaka; in June 2017. Proper identification of *Murraya koenigii* plant sample (Leaves) was done by an expert taxonomist. After collecting leaves of the plant, they were sun dried for several days. Then, plant materials were oven dried for 24 hours at considerably low temperature for better grinding. After that the dried leaves was ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory of Department of Pharmacy at East West University.

3.2 Extraction of the plant material

About 478 gm of the powdered material was taken in separate clean, round bottomed flask (5 liters) and soaked in 3.5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by **Whatman No.1 filter paper** and the filtrate thus obtained was concentrated at 390°C with a Heidolph rotary evaporation.

Figure 3.1: Drying of extract using by rotary evaporator

The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 39.40 gm respectively.

3.3 Preparation of Mother Solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This is the mother solution.

3.4 Partition of Mother Solution

The mother solution was then partitioned off successively by two solvents of different polarity.

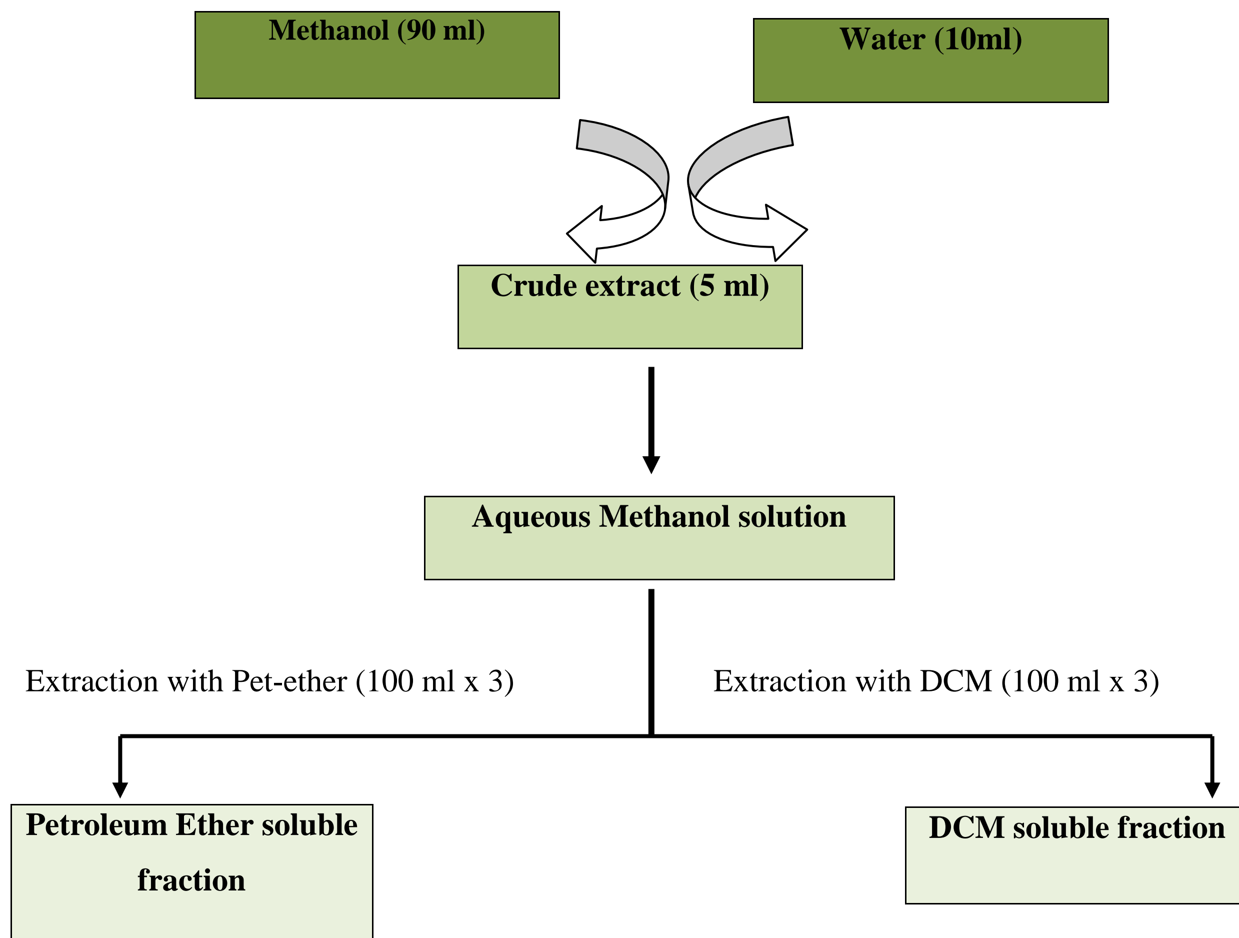


Figure 3.2: Schematic representation of the Partitioning of methanolic crude extract of *Murrya koeniggi* leaves.

3.4.1. Partition with Petroleum ether

The mother solution was taken in a separating funnel. 100 ml of the Petroleum ether (Pet-ether) was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml X 3). The Pet-ether fraction was then air dried for solid residue.

3.4.2 Partition with Dichloromethane

The mother solution was taken in a separating funnel. 100 ml of the Dichloromethane (DCM) was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml X 3). The Dichloromethane (DCM) fraction was then air dried for solid residue.

3.4.3 Collection of Pet-ether Fraction

After partitioning the mother solution with the two different solvents, the Petroleum ether fractions of them were collected and air dried. This Petroleum ether fraction was further investigated for different pharmacological properties such as Antioxidant, Cytotoxic and Antimicrobial. (Beckett & Stenlake, 1986)

3.5 Antioxidant Activity

3.5.1 Total Phenolic Content

The anti-oxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, it has been reported that there is an inverse relationship between the anti-oxidative status occurrences of human diseases. In addition, antioxidant compounds which are responsible for such antioxidants activity could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders. Therefore, research to identify anti-oxidative compounds is an important issue. Although it remains unclear which of the compounds, of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their

biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid per oxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their anti-oxidative effects have rarely been carried out. The purpose of this study was to evaluate extractives of Opuntiaelatior as new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity. 50 Cytotoxic and Antioxidant activity in aqueous fraction of Opuntiaelatior extract.

3.5.1.1 Principle

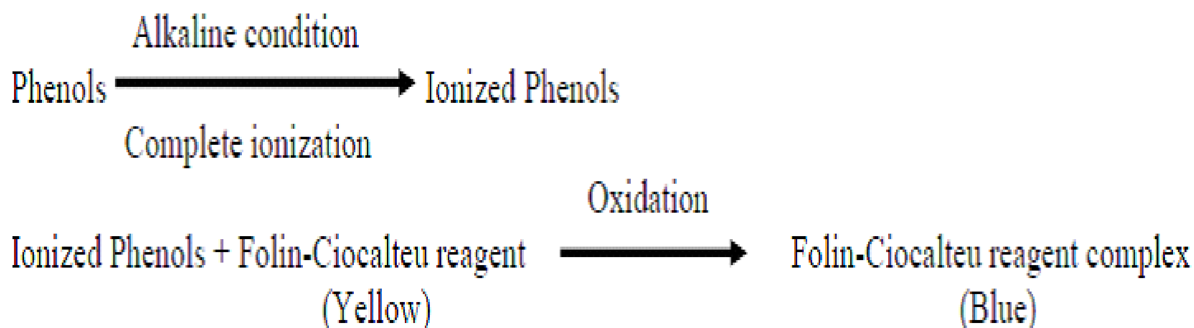
The content of total phenolic compounds in plant methanolic extracts was determined by Folin– Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. In the alkaline condition phenols ionize completely.

Table 3.1: Composition of 100 mg Folin-Ciocalteu Reagent

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

When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The exact chemical nature of the FC reagent is not known, but it is believed to contain hetero poly phosphotunstates - molybdates. Sequences of

reversible oneor two-electron reduction reactions lead to blue species, possibly (PMoW11O40) 4- . The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound. (Singleton *et al*., 1999).



3.5.1.2 Apparatus & Reagents

Table 3.2: Apparatus and reagents used for total phenolic content

Folin-Ciocalteu reagent (10 fold diluted)	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Na_2CO_3 solution (7.5%)	Test tube
Methanol	Micropipette (50-200 µl)
Distilled water	Cuvette

3.5.1.3 Procedure

3.5.1.3.1 Standard curve preparation

Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120 µg/ml to 80 µg/ml. 5 ml of FCR (diluted 10 times with water) and 4 ml of Na_2CO_3 (7.5% w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance

was measured at 765 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

3.5.1.3.2 Sample preparation

2 mg of the Opuntiaelatior aqueous fraction was taken and dissolved in 1 ml methanol to get a sample concentration of 2 mg/ml.

3.5.1.3.3 Determination of total phenol content

- ✓ 1.0 ml plant extract of different concentrations (120 µg/ml, 110 µg/ml, 100 µg/ml, 90µg/ml and 80 µg/ml) was taken in test tubes.
- ✓ 5 ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tube.
- ✓ 4 ml of Sodium carbonate solution was added into the test tube.
- ✓ The test tubes containing the samples were incubated for 1 hour at the room temperature to complete the reaction.
- ✓ Absorbance of solution was measured at 765 nm using a spectrophotometer against blank.
- ✓ A typical blank solution containing methanol was taken.

3.5.2 Total Flavonoid Content

3.5.2.1 Principle

Aluminium chloride ($AlCl_3$) colorimetric method is incorporated to determine the total flavonoid contents of the crude plant extract. The basic principle of the assay method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols of the crude extract. In addition aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A or B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorbance maximum at 510 nm. Therefore, the

amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510 nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard. (Chang *et al*., 2002)

Flavonoid (Extract) + $AlCl_3$ (reagent) = Formation of flavonoid- aluminium complex

(λ_{max} = 510 nm)

3.5.2.2 Apparatus & Reagents

Table 3.3: Apparatus and reagents used for total flavonoid content

Aluminium chloride	Spatula
Methanol	Analytical balance
Ascorbic acid	Pipette and pumper
Sodium hydroxide	Aqueous (sample) fraction
Sodium nitrite	Test tubes and beaker

3.5.2.3 Procedure

3.5.2.3.1 Preparation of 10% Aluminium Chloride ($AlCl_3$) Solution

10 mg of $AlCl_3$ was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

3.5.2.3.2 Preparation of 4% NaOH Solution

4 mg of NaOH was taken into a 100 ml volumetric flask and the volume was adjusted by distilled water.

3.5.2.3.3 Preparation of 5% (W/V) $NaNO_2$ Solution

5 mg of $NaNO_2$ was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

3.5.2.3.4 Preparation of Standard Solution

The stock solution was prepared by taking 10 mg of quercetin and dissolved into 50 ml of methanol. Concentration of this solution was 200µg/ml. The experimental concentrations were prepared from this stock solution.

Table 3.4: Preparation of standard solution

Concentration (µg/ml)	Solution taken from stock solution (ml)	Volume adjusted by methanol (ml)	Final volume (ml)
0	0.0	5	5
4	0.1	4.9	5
8	0.2	4.8	5
12	0.3	4.7	5
16	0.4	4.6	5

3.5.2.3.5 Preparation of Extract Solution

5 mg of each plant extracts were taken and dissolved into 5 ml of methanol. The concentration of the solution was 1 mg/ml of plant extracts. Then the following steps were carried out. 1.5 ml extract was taken in a test tube and then 6 ml of distilled water was added. Then 5% of $NaNO_2$was added and incubated for 6 minutes. 10% $AlCl_3$ was added and incubated for 6 minutes. 4% NaOH and 0.6 ml distilled water was added. Then it was incubated for 15 minutes. For blank solution 1.5 ml methanol was taken and same procedure was repeated.

Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank.

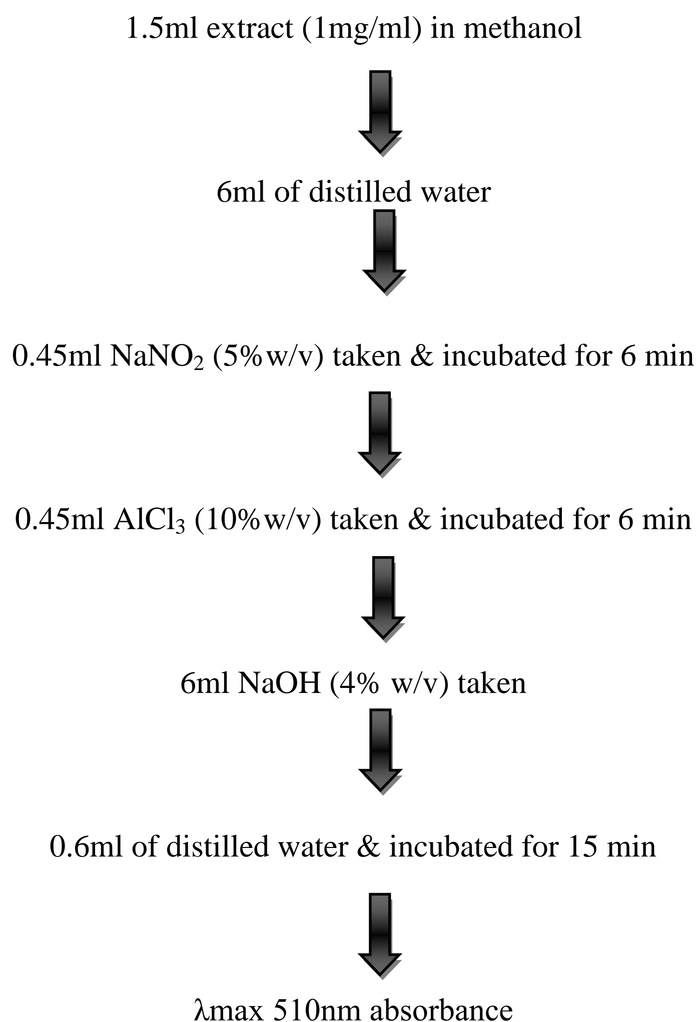
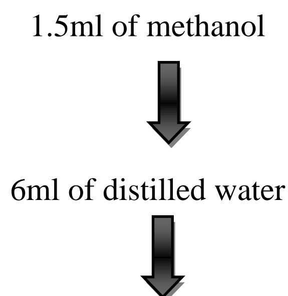


Figure 3.3: Schematic diagram of preparation of extract solution

3.5.2.3.6 Preparation of blank solution



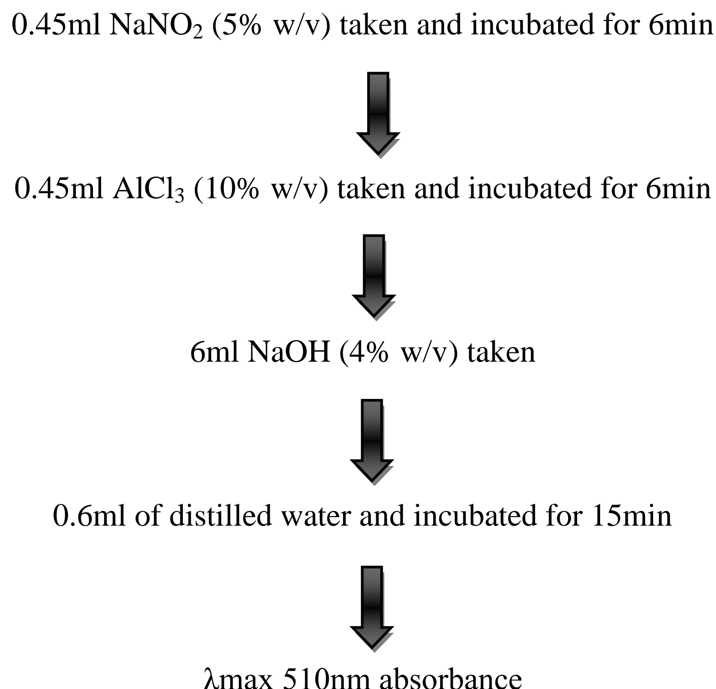


Figure 3.4: Schematic diagram of preparation of blank solution

3.6 Brine Shrimp Lethality Bioassay

3.6.1 Principle

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities such as- anticancer, antiviral and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (in-vivo) lethality, a simple zoological organism, (Brine shrimp napulii-*Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus Artemia of aquatic crustaceans. Artemia is the only genus in the family Artemiidae. (Olowa & Nuneza, 2013)

3.6.2 Apparatus & Reagents

Table 3.5: Apparatus and reagents for Brine shrimp lethality bioassay

Artemia salina leach (brine shrimp eggs)	Pipettes & Micropipette
Sea salt (NaCl)	Glass vials
Small tank with perforated dividing dam to hatch the shrimp	Magnifying glass
Lamp to attract shrimps	Test samples

3.6.3 Procedure

3.6.3.1 Preparation of Sea Water

To hatch the brine shrimp nauplii for the assay, sea water representing brine should be prepared at first. To prepare sea water 38gm of pure NaCl was dissolved in distilled water and then the volume made up to 1000ml by distilled water in a 1000ml beaker for *Artemia salina* hatching. 1-2 drops of1N NaOH solution was added with a dropper to obtain the pH 8.4 as sea water.

3.6.3.2 Hatching of Brine Shrimp

A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. Then a dry preserved egg of *Artemia salina* Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of *Artemia salina* were hatched at room temperature (25-30ºC) for 18-24hr. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay. (Niazi *et al*., 2009)

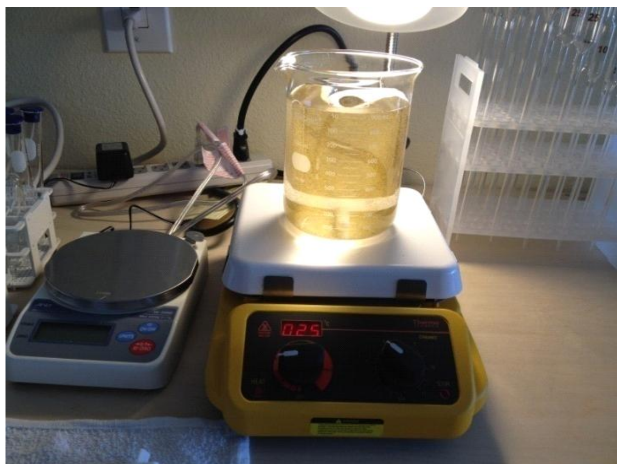


Figure 3.5: Hatching of brine shrimp

3.6.3.3 Preparation of Test Solutions

Clean test tubes were taken. These test tubes were used for ten different concentrations (one test tube for each concentration) of test samples and ten test tubes were taken for standard drug tamoxifen for ten concentrations of it and another one test tube for control test.

3.6.3.4 Preparation of the Test Samples of Experimental Plant

All the test samples of 4mg were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) in vials to get stock solutions. Then 100µl of solution was taken in test tube each containing 5ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100µl sample was added to test tube and fresh 100µl DMSO was added to vial. Thus the concentrations of the obtained solution in each test tube were 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml for 10 dilutions.

3.6.3.5 Preparation of the Positive Control Group

In the present study tamoxifen is used as the positive control. Measured amount of the tamoxifen is dissolved in DMSO to get an initial concentration of 20µg/ml. From that stock

solution serial dilutions are made using DMSO to get 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml. Then ten living brine shrimp nauplii in 5ml simulated seawater are added to the positive control solutions in the pre-marked test-tubes to get the positive control groups.

3.6.3.6 Preparation of the Negative Control Group

100µl of DMSO was added to the pre-marked test tube containing 5ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds. (Goldstein *et al*., 1974)

3.6.3.7 Counting of Nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

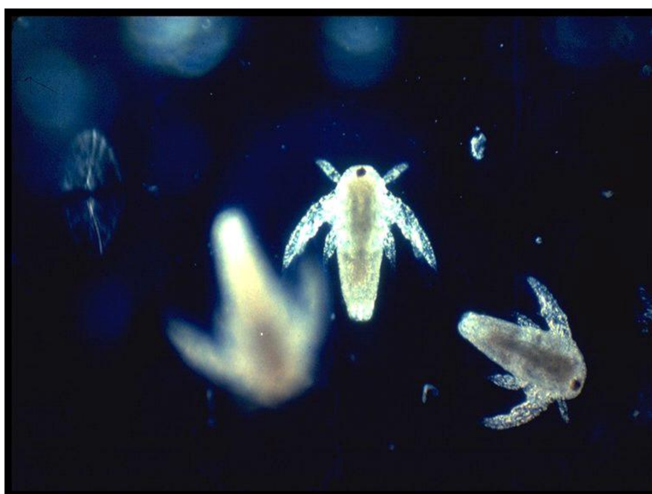


Figure 3.6: Alive Nauplii counting

3.7 Antibacterial Activity by Disc Diffusion Method

3.7.1 Principle

The disk diffusion susceptibility method is simple and well-standardized. Bacterial inoculums are applied to the surface of a large agar plate. Antibiotic discs and disc of test materials are placed on the inoculated agar surface. Plates are incubated for 16–24hr at 35°C prior to determination of results. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The zones of growth inhibition are measured to the nearest millimeter around each of the antibiotic disks. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. (Barry, 1976)

3.7.2 Apparatus &Reagents

Table 3.6: Apparatus and reagents for antibacterial test

Filter paper discs	Screw cap test tubes
Petri dishes	Nose mask and Hand gloves
Inoculating loop	Laminar air flow hood
Sterile cotton	Autoclave
Sterile forceps	Incubator
Spirit burner	Ethanol
Micropipette	Nutrient Agar Medium

3.7.3 Test Sample of *Murraya koinigii*

Petroleum ether fraction of methanolic extract of *Murraya koenigii* leaves were taken as test sample.

3.7.4 Test Organisms

The bacterial strains used for the experiment were collected as pure cultures from the East West University microbiology laboratory. Both gram positive and gram-negative organisms were taken for the test and they are listed in the following table.

Table 3.7: List of micro-organisms

Type of Bacteria	Name of Bacteria
Gram positive Bacteria	*Bacillus megaterium* *Bacillus subtilis* *Bacillus sereus* *Staphylococcus aureus*
Gram negative Bacteria	*Escherichia coli* *Salmonella paratyphi* *Salmonella typhi* *Vibrio parahaemolyticus* *Shigella dysenteriae* *Pseudomonas aureaus*

3.7.5 Procedure

3.7.5.1 Preparation of the Medium

To prepare required volume of this medium, 5.6gm of agar medium was taken in a bottle with a cap and distilled water was added to it to make 200ml volume. The contents were then autoclaved to make a clear solution.

Figure 3.7: Manual and Digital Autoclave

3.7.5.2 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Figure 3.8: Laminar Hood

3.7.5.3 Preparation of the Test Plate

The test organisms were transferred from the subculture to petri-dish containing about 10 ml of melted and sterilized agar medium. The bacterial and fungal suspension was taken by a loop mixed with normal saline with the help of vortex machine. Then a sterilized cotton bud was taken and dipped into the bacterial suspension. Then the bacterial sample is applied to the petri-dish with the help of this cotton bud.

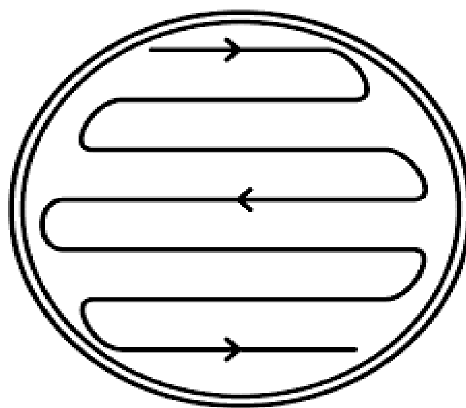


Figure 3.9: Preparation of test plate

3.7.5.4 Preparation of Discs

Three types of discs were used for antimicrobial screening.

- ✓ Standard Discs: These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, azithromycin (30µg/disc) disc was used as the reference.
- ✓ Blank Discs: These were used as negative controls which ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.
- ✓ Sample Discs: These discs were soaked with solutions of test samples of known concentration, dried and used to determine the anti-activity of the samples.

Figure 3.10: Disc preparation and placement

3.7.5.5 Preparation of Test Sample

Measured amount of test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical filter paper discs were taken in a blank petri-dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

3.7.5.6 Application of Test Samples

Standard azithromycin discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Methanol discs were used as negative controls which ensure that the residual solvents (left over the discs even after air drying) and the filter paper were not active themselves.

3.7.5.7 Diffusion & Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours.

Figure 3.11: Incubator

3.7.5.8 Determination of Antibacterial Activity by Measuring the Zone Of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

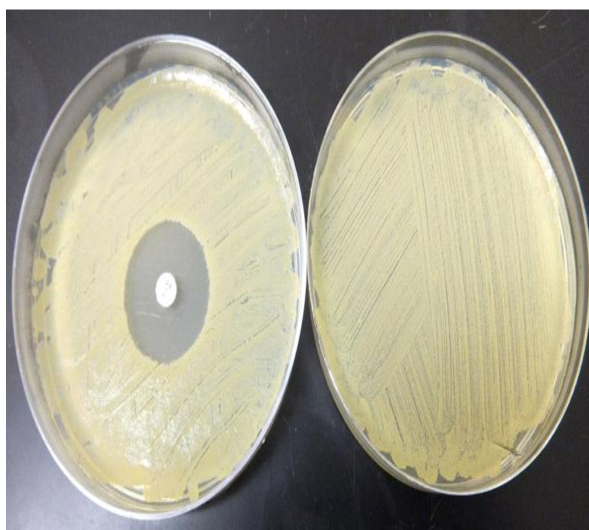


Figure 3.12: Zone Of Inhibition of antibiotic disc

Figure 3.13: Measuring the Zone Of Inhibition

Chapter Four

Results & Discussion

4.1 Antioxidant test results

Antioxidant tests are classified by various methods. Samples were subjected to various standard methods to determine various scavenging capacity and amount that is equivalent to the standard like ascorbic acids. Antioxidant property of Petroleum ether extract of *Murraya koenigii* (leaves) was determined by following methods-

- ✓ Determination of total phenolic content
- ✓ Determination of total flavonoid content

4.1.1 Result of total phenolic content

The Petroleum ether extract of leaves of *Murraya koenigii* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard.
(Singleton *et al*., 1999)

4.1.1.1 Preparation of Standard Curve

Table 4.1 Total Phenolic Content of Ascorbic Acid

Concentration (µg/ml)	Absorbance (at 765 nm)	Regression line	R^2 value
80	1.643	y = 0.009x + 0.931	0.753
90	1.848		
100	2.023		
110	2.089		
120	2.019		

A linear relationship was observed when the absorbance were plotted against concentrations, as shown in Figure 4.1. This linear curve was considered as a standard curve.

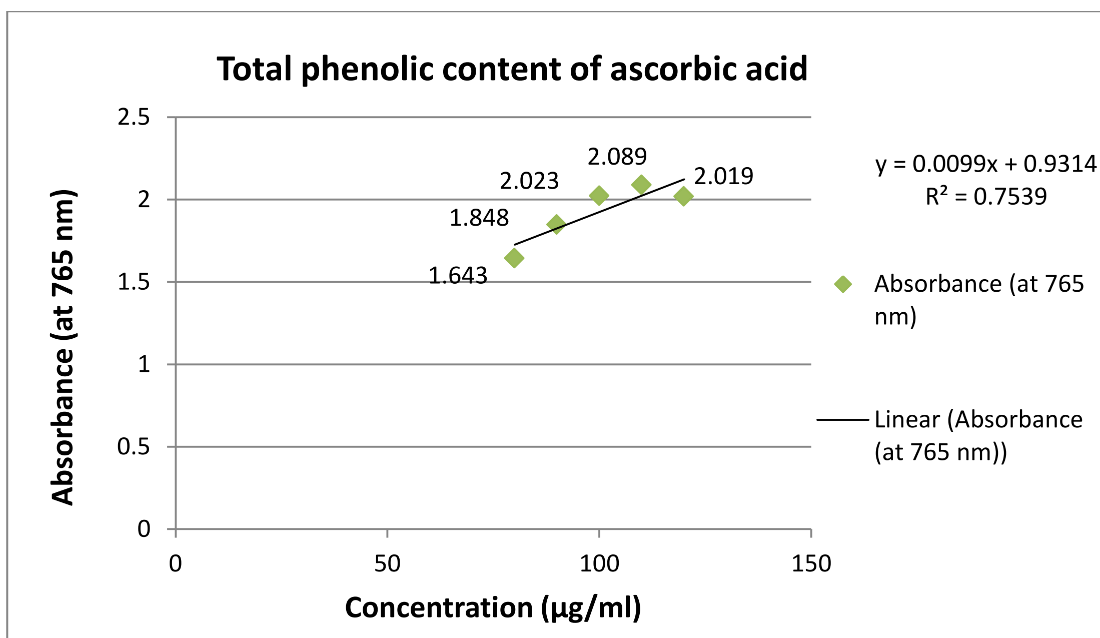


Figure 4.1: Graphical Representation of Assay of Phenolic Content of Ascorbic Acid

4.1.1.2 Total phenol content present in Petroleum ether extract of *Murraya koenigii*

Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of ascorbic acid equivalents (AAE), the total phenolic content present in the extract is calculated and given in the table below.

Table 4.2 Total Phenolic Content in Petroleum ether extract of *Murraya koenigii* (leaves)

Sample	Concentration (mg/ml)	Absorbance (Y value at 765 nm)	Total Phenolic (X) value (mg of AAE/gm of dried extract)
Petroleum ether extract of *Murraya koenigii*	**2**	**4**	**341**

4.1.1.3 Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the Petroleum ether fraction is higher than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 341 mg of AAE/gm of dried extract of phenol content was found in the Petroleum ether extract of *Murraya koenigii*.

4.1.2 Result of Total Flavonoid content

The Petroleum ether extract of *Murraya koenigii* (leaves) were subjected to determine total flavonoid content. Quercetin was used as reference standard.

4.1.2.1 Preparation of standard curve

Table 4.3: Total Flavonoid Content of Quercetin

Concentration (µg/ml)	Absorbance (at 510 nm)	Regression line	R^2 value
4	0.193	y = 0.053x - 0.013	0.999
8	0.422		
12	0.618		
16	0.834		

After absorbance's were taken of different solution of Quercetin of concentrations ranging from 4 µg/ml to 16µg/ml, a linear relationship was observed when the absorbance were plotted against concentrations, as shown in Figure 4.2. This linear curve was considered as a standard curve.

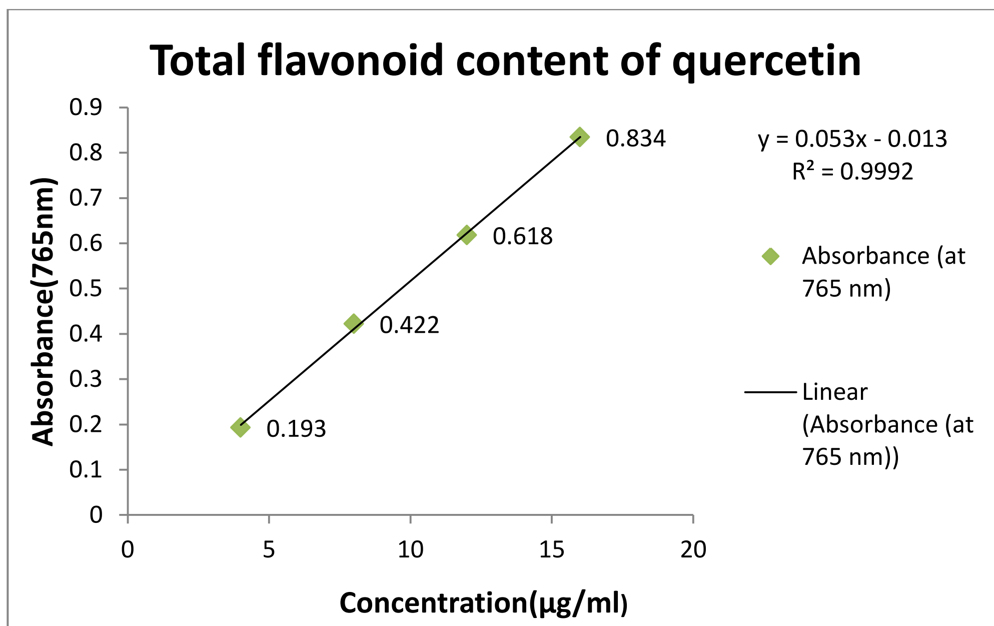


Figure 4.2: Graphical representation of Flavonoid Content of Quercetin

4.1.2.2 Total Flavonoid Content present in Petroleum ether extract of *Murraya koenigii* (leaves)

Based on the absorbance values of the extract solution and using the regression line equation of the standard curve, the total flavonoid content present in the extract is calculated and given in the table 4.4.

Table 4.4: Total Flavonoid Content in Petroleum ether extract of *Murraya koenigii* (leaves)

Sample	Concentration (mg/ml)	Absorbance (Y value at 510 nm)	Total Flavonoid (X) value (mg of quercetin/gm of dried extract)
Petroleum ether extract of *Murraya koenigii*	**1**	**4**	**75.717**

4.1.2.3 Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in flavonoid content. Absorbance of the Petroleum ether fraction is higher than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 75.717 mg of Quercetin/gm of dried extract of flavonoid content was found in the Petroleum ether extract of *Murraya koenigii* (leaves).

4.2 Result of Brine Shrimp Lethality Bio-Assay

The Petroleum ether fraction of the *Murraya koenigii* (leaves) extract was subjected to brine shrimp lethality bioassay. After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors counted. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a Median Lethal Concentration (LC50) value. LC50 represents the concentration of the standard and Petroleum ether extract that produces death in half of the test subjects after a certain period.

The percentage mortality at each concentration was determined using the following formula-

$$\% \textbf{ Mortality} = \frac{\textbf{Number of dead nauplii} \times \textbf{100}}{\textbf{Total number of nauplii}}$$

The LC50 of the test samples was obtained by a plot of percentage of the shrimps died (% Mortality) against the logarithm of the sample concentration (Log C) and the best-fit line was obtained from the curve data by means of regression analysis.

4.2.1 Preparation of Curve for Standard

Here, Tamoxifen was used as reference standard.

Table 4.5: Results of the bioassay of Tamoxifen (standard)

Concentration (C) (µg /*ml*)	Log C	Number of dead nauplii	% Mortality	LC50 (µg /*ml*)
20	1.301	10	100	0.315212
10	1	8	80	
5	0.699	9	90	
2.5	0.398	8	80	
1.25	0.097	7	70	
0.625	-0.204	4	40	
0.3125	-0.505	6	60	
0.156	-0.806	5	50	
0.078	-1.107	3	30	
0.039	-1.408	2	20	

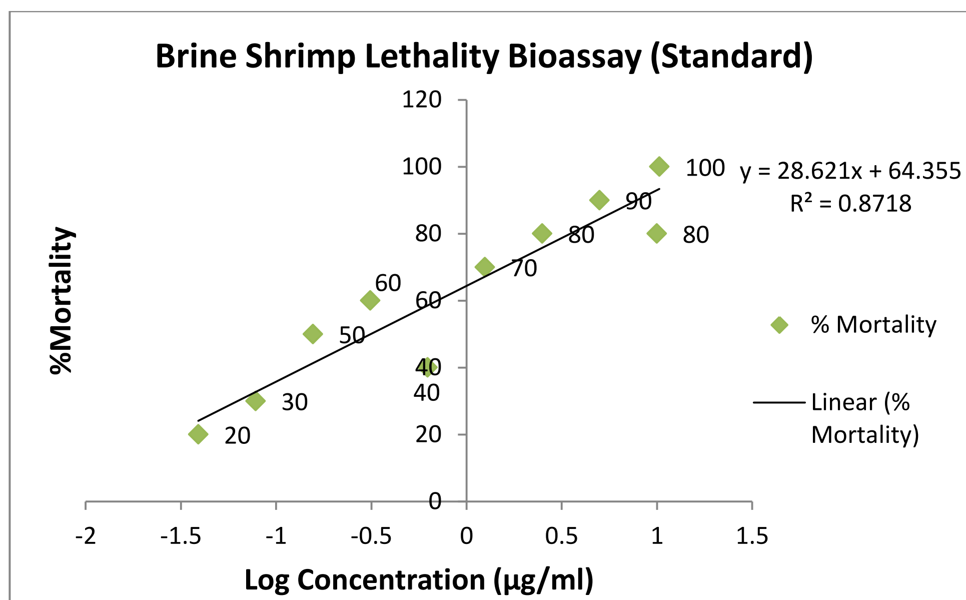


Figure 4.3: % Mortality and Predicted Regression Line of Tamoxifen (standard)

4.2.2. Preparation of Petroleum ether fraction Curve of *Murraya koenigii* (leaves)

Table 4.6: Results of the bioassay in Petroleum ether extract of *Murraya koenigii* (leaves)

Concentration (C) (µg */ml*)	Log C	Number of dead nauplii	% Mortality	LC50 (µg */ml*)
20	1.301	10	100	0.171947
10	1	9	90	
5	0.699	9	90	
2.5	0.398	6	60	
1.25	0.097	7	70	
0.625	-0.204	6	60	

0.3125	-0.505	6	60	
0.156	-0.806	5	50	
0.078	-1.107	5	50	
0.039	-1.408	3	30	

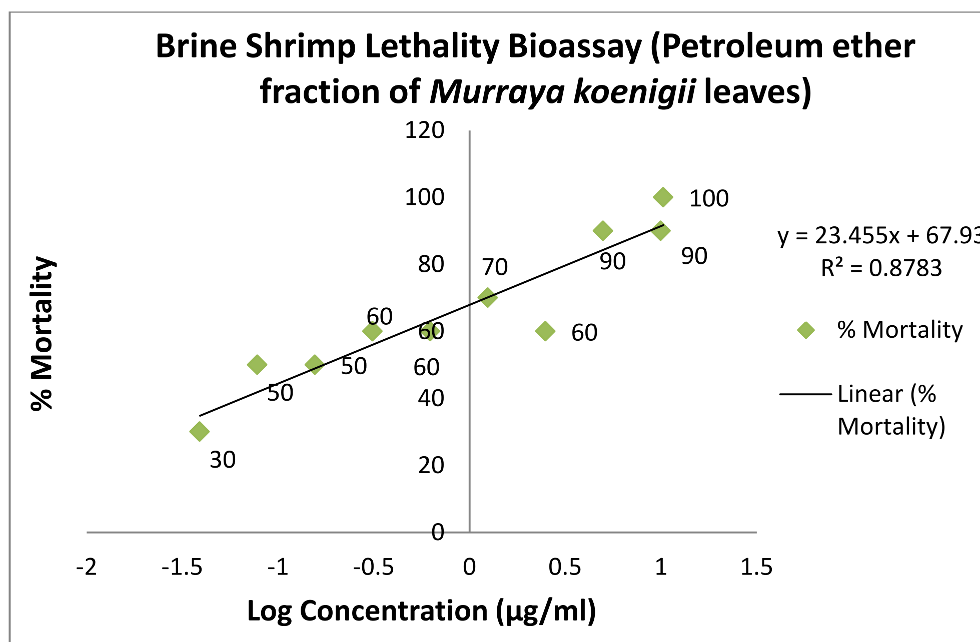


Figure 4.4:% Mortality and Predicted Regression Line in Petroleum ether extract of *Murraya koenigii* (leaves)

4.2.3 Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. The degree of lethality was found to be directly proportional to the concentration. Maximum mortalities took place at the concentration of 0.039 µg/ml, whereas the less mortalities at the concentration of 20µg/ml as shown in Table 4.6

Table 4.7: Cytotoxic activity of Tamoxifen and Petroleum ether extract of *Murraya koenigii* (leaves)

Sample	Linear regression equation	R^2 value	LC50 (µg/ml)
Standard (Tamoxifen)	y = 28.62x + 64.35	0.871	**0.315212**
Petroleum ether fraction	y = 23.45x + 67.93	0.878	**0.171947**

In this investigation, standard and Petroleum ether fraction exhibited cytotoxic activities with the LC50 values at 0.315212µg/ml and 0.171947µg/ml respectively as shown in Table 4.8. LC50 value of *Murraya koenigii* (leaves) in Petroleum ether fraction showed very less activity of it than Tamoxifen. Further investigation is needed to confirm the activity.

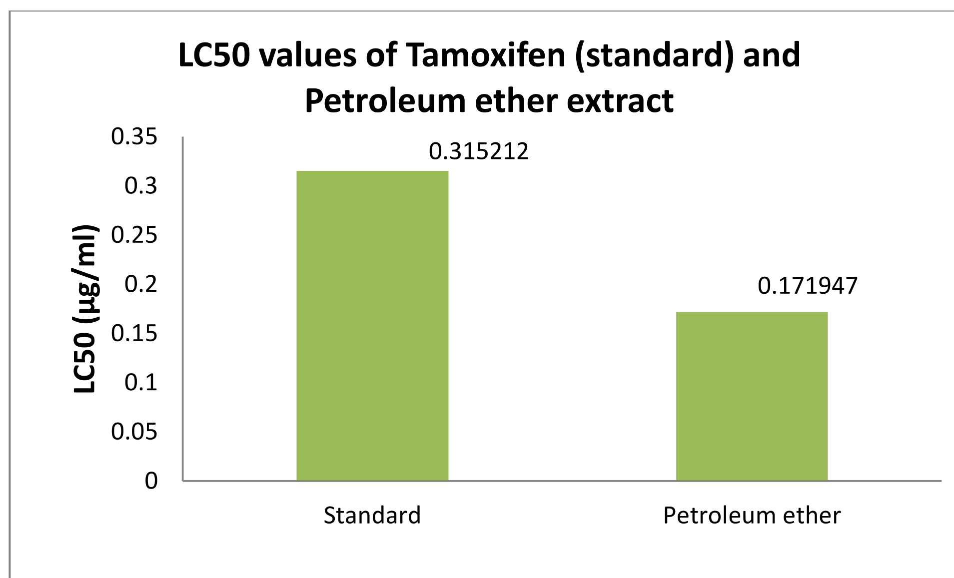


Figure 4.5: Comparison between LC50 values of standard and extract

4.3 Result of Antibacterial Test

The antibacterial activities of Petroleum ether extract of *Murraya koenigii* leaves extract were subjected in the study against various Gram positive bacteria and Gram negative bacteria. The Petroleum ether fraction was subjected to the various bacterial cultures and from that zones of inhibition were measured. Ciprofloxacin was used as standard reference.

4.3.1 Zone of Inhibition of Standard and Petroleum ether Fraction

Table 4.8: Antibacterial activity of standard (Ciprofloxacin) and Petroleum ether fraction

Type of microorganism		Zone of inhibition (mm)	
		Standard	Petroleum ether fraction
Gram positive bacteria	*Bacillus sereus*	22 mm	–
	Bacillus subtilis	13mm	–
	Bacillus megaterium	30mm	–
	Staphylococcus aureus	15mm	–
Gram negative bacteria	*Escherichia coli*	27mm	6mm
	Salmonella paratyphi	27mm	6mm
	Vibrio parahaemolyticus	27mm	5mm
		20mm	5mm

	Salmonella typhi		
	Shigella dysenteriae	27mm	6mm
	Pseudomonas aureaus	28mm	5mm

4.3.2 Discussion

Petroleum ether extract of *Murraya koenigii* (leaves) showed low to moderate antibacterial activity when compared to reference standard drug Ciprofloxacin. None of the zone of inhibition of Petroleum ether fraction is equal to Ciprofloxacin against any bacteria as shown in the figure 4.3. Among all the bacterial cultures, the fraction showed the best antibacterial activity against *Salmonella paratyphi*; *Shigella dysenteriae* and *Escherichia coli* (6mm) comparable to the standard (27mm).

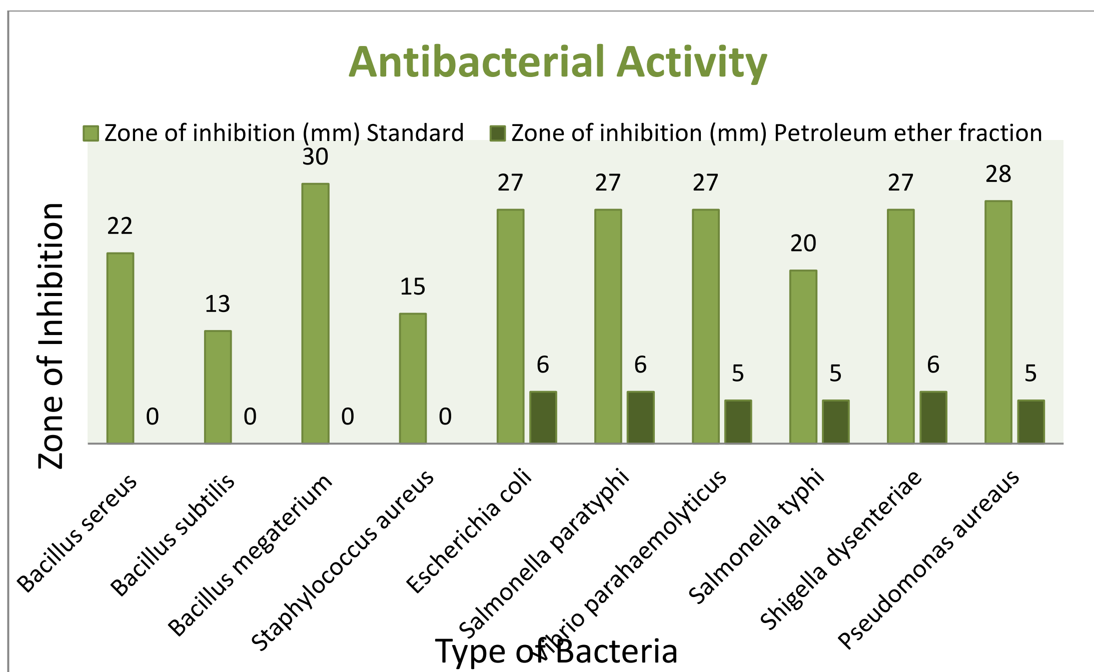


Figure 4.6: Comparison of antibacterial activity between standard (Ciprofloxacin) and Petroleum ether extract

Chapter Five

Conclusion

According to the literature reviews, presence of several phytochemical compounds in Petroleum ether fraction of *Murraya koenigii* (leaves) maked the plant pharmacologically active against several diseases.

After conducting some in-vitro tests with Petroleum ether fraction of *Murraya koenigii* (leaves), in the Total Phenolic Content assay, Phenolic content was found 341mg of AAE/gm and in Total Flavonoid Content assay; Flavonoid content was 75.717mg of quercetin/gm in Petroleum ether extract of *Murraya koenigii* (leaves). Thus, Petroleum ether extract of *Murraya koenigii* (leaves) showed significant antioxidant property.

The Brine Shrimp Lethality Bioassay gave LC50 value of *Murraya koenigii* (leaves) in Petroleum ether fraction which showed very low cytotoxic activity than Tamoxifen.

By using in vitro experiments in Petroleum ether extract of *Murraya koenigii* (leaves) which inhibited the bacterial growth low to moderately in compare to reference standard.

Mixture of compounds and impurities can lower antioxidant property in Petroleum ether fraction of *Murraya koenigii* (leaves) and also can reduce other pharmacological activity. So, further investigations can be carried out to isolate and identify the active compounds as novel and safe drugs for its respective pharmacological activities. This is only an introductory study but this plant can be further screened against a variety of diseases in order to explore its efficacy as potential source of biologically important components which can be significantly beneficial to mankind.

Chapter Six

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