"*In vitro* pharmacological investigations (cytotoxic, anti-oxidant activity) of Dichloromethane fraction of *Mentha arvensis*"

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EAST WEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF PHARMACY

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Declaration by the Research Candidate

I, Nusrat jahan, hereby declare that the dissertation entitled ***"In vitro* pharmacological investigations (cytotoxic, anti-oxidant activity) of Dichloromethane fraction of *Mentha arvensis*"** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2015-2016, under the supervision and guidance of Abdullah-Al-Faysal, Lecturer, Department of Pharmacy, East West University and the thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled **"*In vitro* pharmacological investigations (cytotoxic, anti-oxidant activity) of Dichloromethane fraction of *Mentha arvensis*"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by Nusrat Jahan, ID# 2012-1-70-053 in 2016, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Dedication

DEDICATED TO

MY PARENTS

Abstract

The plant *Mentha arvensis* has been used for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases like asthma, piles, diarrhea etc. The aim of the present study was to evaluate the cytotoxicity activity, and antioxidant activity of Dichloromethane extract of *Mentha arvensis*. The powdered leaves of *Mentha arvensis* were extracted with methanol and then partitioned with pet-ether, ethyl acetate and dichloromethane consecutively. The pet-ether fraction was used to evaluate cytotoxic, and antioxidant activities. The cytoxic activity was measured by brine shrimp lethality bioassay. Pet-ether fraction showed cytotoxic activity with LC_{50} value 17.79µg/ml in brine shrimp lethality test.The fraction contained 12.315mgAAE/g of total phenolic content and 56mg AAE/g total flavonoid content and 27.5mg AAE/g total reducing power content. The results of study clearly indicate the presence of cytotoxic, and antioxidant properties of pet-ether extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

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ABBREVIATION:

Meaning of abbreviated form	Abbreviated form
Ascorbic Acid Equivalent	AAE
Dimethyl Sulfoxide	DMSO
Gram	g or gm
Hour	hr
Lethal concentration required to kill 50% of the sample population	LC50
Microgram	µg
Micro liter	µl
Milligram	mg
Milliliter	ml
Mentha	*M.*
Ultraviolet	UV
World Health Organization	WHO

Chapter 1: Introduction

1.1 GENERAL INTRODUCTION

A medicinal plants 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 semisynthesis." When a plant is designated as medicinal it is implied that the said plant is used as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorousmammals.

In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants (such as myrrh and opium). In 1500 B.C., the Ancient Egyptians wrote the Ebers Papyrus, which contains information on over 850 plant medicines, including garlic, juniper, cannabis.

Ethnobotany, the scientific study of the relationships that exist between humans and plants, is a recognized way to discover new effective medicines for future and further use. In ancient Greece, plants were classified and descriptions of them were given by scholars. It aids in the identification process. Researchers identified in 2001, 122 compounds that were isolated and identified from "ethno medical" plant sources, are used in modern medicine. The current use of the active elements of the plants is 80% similar to those of ethno medical use (Fabricant & Farnsworth, 2001).

1.2 MEDICINAL PLANT

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. The therapeutic properties of medicinal plants are conditioned by the presence in their organs of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds, which physiologically affect the bodies of humans and animals or which are biologically active in relation to the causative agents of various diseases. A special group of medicinal plants are antibiotics. One of the aims of medicinal plant research is the isolation and identification of markers/ bioactive compounds. Isolation of the markers compounds and

bioactive plant constituents has always been a challenging task for the researchers. Separation of these components from the medicinal plants includes the use of

- column chromatography
- preparative thin layer chromatography
- preparative high performance liquid chromatography
- droplet counter current chromatography
- centrifugal thin layer chromatography, etc. (Sumai&Beham, 2016).

1.2.1 MEDICINAL PLANTS – HISTORY & CONTEXT

Archaeological evidence indicates that the use of medicinal plants dates at least to the Paleolithic, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years, to the Sumerians, who created lists of plants. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs. The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C,and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Seeds likely used for herbalism have been found in the archaeological sites of Bronze Age China dating from the Shang Dynasty. Over a hundred of the 224 drugs mentioned in the Huangdi Neijing, an early Chinese medical text, are herbs. Herbs were also common in the medicine of ancient India, where the principal treatment for diseases was diet. De MateriaMedica by Pedanius Dioscorides, a Roman physician, is a particularly important example of such writings. The documentation of herbs and their uses was a central part of both Western and Eastern medical scholarship through to the 1600s, and these works played an important role in the development of the science of botany (Nunn, 2002; Hong, 2004; Ackerknecht, 1982). Human beings have used plants for the treatment of diverse ailments for thousands of years. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements, since they cannot afford the products of Western pharmaceutical industries, together with their side effects and lack of healthcare facilities. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life.

These medicines are relatively safer and cheaper than synthetic or modern medicine. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Ernst, 2007). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

1.2.2 GLOBAL SCENARIO OF MEDICINAL PLANTS

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal medicine is a common element in Ayurvedic, homeopathic, naturopathic, traditional, and oriental, Native American & Indian medicine. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. The present global herbal market is worth about US$ 62 billion per annum. The annual growth of herbal market is about 15 percent and the global herbal market by 2050 is expected to be about US$ 5 trillion. Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs (Calixto, 2000) have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programs worldwide (Rates, 2001).

1.2.3 MEDICINAL PLANTS AS DRUGS

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal and human body are generally designated as medicinal plants (Ghani, 1998).

1.2.4 FUTURE OF MEDICINAL PLANTS

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

1.2.5 CHARACTERISTICS OF MEDICINAL PLANT

- Synergic Medicine: It has been verified that in many cases the application of an isolated component has not had the wished effect, because it does not have the same curative power that it has when it has taken altogether with the rest of components or because it has turned out to be toxic. The components of the plants have the synergistic effect, that is to say they all interact simultaneously, so that their uses can complement or damage others or neutralize their possible negative effects.
- Support of Official Medicine: The treatment of very complex diseases can require in some cases the support of the medicinal properties of the plants or the derivatives that they provide.
- Preventive Medicine: Finally we do not have to forget the preventive character that the plants have regarding the appearance of disease. In this sense the plants are better than the chemical remedies that are applied essentially when the disease has already appeared.

1.2.6 IMPORTANCE OF MEDICINAL PLANTS

- The importance of medicinal plants becomes more patent at the present time in developing countries. In Pakistan it is estimated that a 80% of its population depend on plants to cure themselves, a 40% in China. In technologically advanced countries as the United States, it is estimated that 60% of its population use medicinal plants habitually to fight certain ailments. In Japan there is more demand of medicinal plants than of official medicines.

- A future medicine bank to discover. There are approximately half a millon plants with flowers, most of which have not been investigated and which principles could be decisive in the treatment of present or future diseases.

- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.

- Some food crops have medicinal effects, for example garlic.

1.2.7 SIGNIFICANCES OF MEDICINAL PLANTS TO MANKIND

Even if we only consider the impact of the discovery of the penicillin, obtained from microorganisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-ZhongShu, 1998). The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger & Hostettmann, 1991).

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

a) Synergic medicine- The plants ingredients all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

b) Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

c) Preventive medicine- It has been proven that the component of the plants are also characterized by their ability to prevent the appearance of some diseases. This will help to

reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Ghani, 1998).

Plants are valuable for modern medicine in four basic ways:

i. They are used as sources of direct therapeutic agents.

ii. They serve as raw materials base for elaboration of more complex semi synthetic chemical compounds. iii.The chemical structures derived from plant sources can be used as models for new synthetic compounds. iv.Finally plants can be used as taxonomic markers for the discovery of new compounds (Reddy, *et al.*2010).

1.2.8 CLASSIFICATION OF MEDICINAL PLANTS

Of the 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification (Joy *et.al.,* 1998).

Table-1.1: Based on part used (Joy.*et.al.,*1998).

Part	**Name of plant**
Whole plant	*Phyllanthusneruri*, *Saracaindica*
Root	*Panax ginseng*, *Polemoniumreptans*
Stem	*Tinosporacordifolia*, *Acoruscalamus*
Bark	*Saracaasoca*, *Cinchona rubra*
Leaf	*Medicago sativa*, *Indigoferatinctoria*, *Lawsoniainermis*, *Aloe vera*
Flower	*Biophytumsensitivum*, Mimusopselenji, *Bellisperennis, Calendula officinalis*
Fruit	*Solanum*species, *Arctiumlappa*
Seed	*Daturastramonium*, *Apiumgraveolens*

Table-1.2: Based on habit (Joy *et.al.,* 1998).

Part	**Name of the plant**
Grasses	*Cynodon dactylon*
Sedges	*Cyperus rotundus*
Herbs	*Rosemarinus officinalis*
Shrubs	*Solanum species*
Climbers	*Asparagus racemosus*

1.3 ADVANTAGES OF DRUG DISCOVERY FROM NATURAL RESOURCES

Usage of botanical sources as starting point in the drug development program is associated with few specific advantages:

a) Mostly, the selection of a candidate species for investigations can be done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active com-pounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use. At certain time point afterward, one may attempt upon synthesis of active molecule and reduce pressure on the resource. Drug development from *Rauwolfia serpentine*, *Digitalis purpurea*, etc. in the past fall under this category of approach.

b) Sometimes, such approaches lead to development of novel molecules derived from the source due to inherent limitations of the original molecule. For instance, podophyllin derived from *Podophyllum hexandrum* was faced with dose-limiting toxicities. Such limitations could be overcome to a great extent by semi-synthesis of etoposide, which continues to be used in cancer therapy today. Similar was the case with camptothecin (originally isolated from *Camptotheca sp*. and subsequently from *Mappia sp*.), which led to development of novel anticancer molecules like topotecan and irinotecan.

c) Natural resources as starting point has a bilateral promise of delivering the original isolate as a candidate or a semi-synthetic molecule development to overcome any inherent limitations of original molecule.

1.3.1 TRIBAL MEDICINES

In different localities of Rangamati and Bandarban Districts of Bangladesh a survey was carried out between 2001 and 2002 to document medicinal plants. Kaukhali proper is about 10 km west to Rangamati town. Betbunia is a Union under Kaukhali P.S. situated about 9 km south of Kaukhali proper and about 18 km south-west to Rangamati town. Being a hilly area they are rich in floral diversity. Inhabitants of those areas are mostly tribal, dominated by Chakma and Marma. Many of them still depend on local medicinal plants for the treatment of different diseases. A good number of Bengali families are also living there. They also use quite a good number of medicinal plants for the treatment of different diseases. In recent years due to development of good communication, modern doctors and medicines have reached there, resulting decline in the use of traditional medicine. Therefore the knowledge of traditional use of medicinal plants by the local people is likely to be lost in near future, and for this it is necessary to document as much as possible the existing available information. Keeping this in mind this survey was done to document those valuable ethno-medico-botanical knowledge. The survey was carried out for about a year. During this work 34 species representing 23 genera and 17 families were documented which are used for the treatment of 31 diseases. Local names of those plants, locality and method of use are mentioned (Yusuf *et.al.,*2006).

1.3.2 APPROACHES FOR ISOLATION OF ACTIVE COMPUNDS FROM NATURAL ORIGIN

1.3.2.1 RANDOM APPROACH

Two approaches have been followed for screening of the plants selected randomly for the purpose of new drug discovery (Katiyar et al., 2012):

a) Screening for selected class of compounds like alkaloids, flavonoids, etc**:** While this route is simple to perform, however, it is flawed in the sense.

b) provides no idea of the biological efficacy. However, chances of getting novel structures cannot be denied following this approach.

c) Screening of randomly selected plants for selected bioassays**:** Central Drug Research Institute, a premier R and D organization of Council of Scientific and Industrial Research of India, followed this approach about three decades ago. They screened almost 2000 plants for biological efficacy. However, the screening did not yield any new drug. National Cancer Institute (NCI) of National Institute of Health, USA, studied about 35,000 plant species for anticancer activity, spending over two decades from 1960 to 1980.

1.3.2.2 ETHNOPHARMACOLOGY APPROACH

The approach of ethnopharmacology essentially depends on empirical experiences related to the use of botanical drugs for the discovery of biologically active New Chemical Entities (NCEs). This process involves the observation, description, and experimental investigation of indigenous drugs, and is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines like anthropology, archaeology, history, and linguistics. This approach based on ethnomedicinal usage history has seen some success, e.g. *Andrographis paniculata* was used for dysentery in ethnomedicine and the compounds responsible for the activity were isolated as andrographolide. Morphine from *Papaver somniferum*, Berberine from *Berberis aristata* are some examples of this approach. Some of the plants which are not selected on the basis of ethnomedical use also had some success stories (Katiyar et al., 2012).

1.3.2.3TRADITIONAL SYSTEM OF MEDICINE APPROACH

Countries like India and China have a rich heritage of well-documented traditional system of medicine in vogue. Though these codified systems of medicine use largely botanical sources asmedicines, however, these stand apart from ethnomedicine specifically on three accounts (Katiyar et al., 2012):

a) The ethnomedicinal practice is based on empirical experiences. On the other hand, these codified systems built up the empirical practices on strong conceptual foundations of human physiology as well as of pharmacology (though the tools of their investigations in those times were far different from the existing ones).

b) The pharmaceutical processes have been more advanced as against the use of crudely extracted juices and decoctions in ethnomedicinal practices. Due to this phenomenon, the concept of standardization was known to the system.

c) They are well documented and widely institutionalized. On the other hand, the ethnomedicinal practices are localized and may be largely controlled by few families in each of the community. (Katiyar et al., 2012).

1.4 PHYTOCHEMISTRY

Phytochemistry can be defined as the biochemical study of plants which is concerned with the identification, biosynthesis, and metabolism of chemical constituents of plants, especially used in regard to natural products. Phytochemistry is considered as one of the early subdivisions of organic chemistry. It has been of great importance in the identification of plant substances of medicinal importance. Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants. Effect of extracted plant phytochemicals depends on:

- ✓ The nature of the plant materials
- ✓ Its origin
- ✓ Degree of processing
- ✓ Moisture content (Tiwari & Kumar, 2011)

1.4.1 PROCEDURE FOR DEVELOPMENT

Since drug development is an expensive practice, careful phytochemical analysis and pharmacological screening and if promising clinical tests are required. The way of developing drugs from plants involves several stages (Ghani, 1998), which include:

a) Selection and correct identification of the proper medicinal plant.
b) Extraction with suitable solvent(s).
c) Detection of biological activity of crude extract and establishment of a bioassay system to permit the identification of the active fractions and rejection of the inactive ones.

d) Fractionations of crude extract using the most appropriate chromatographic procedures, biological evaluation of all fractions and separation of the active fractions.

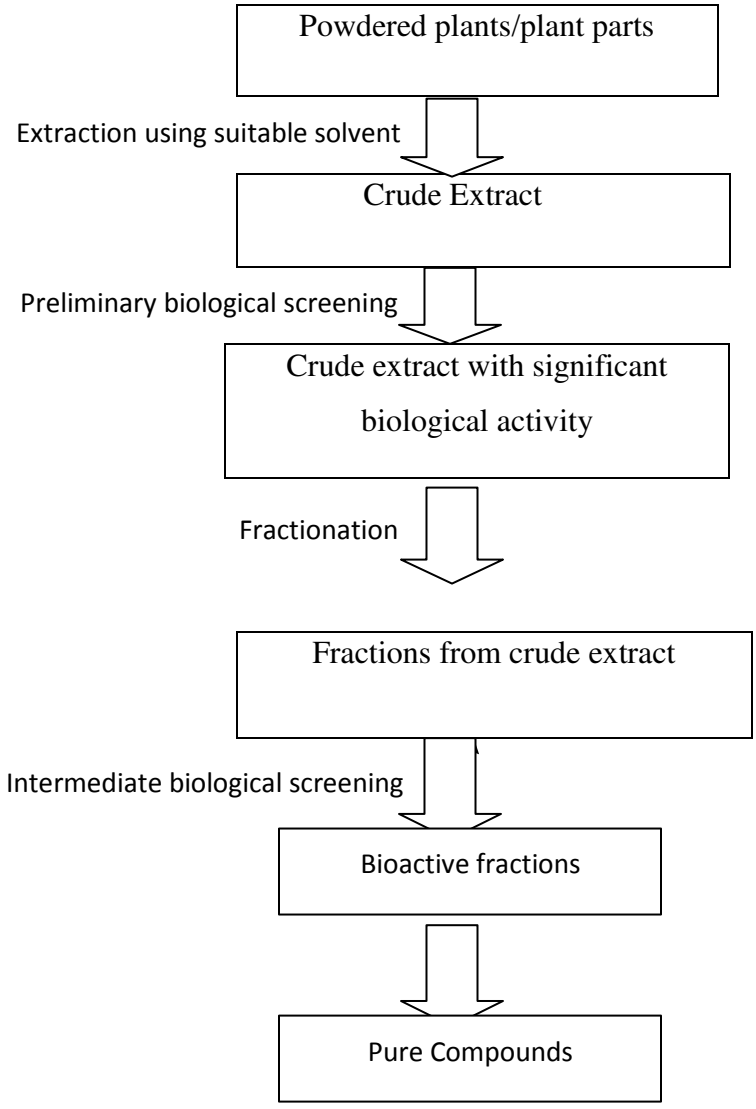
e) Repeated fractionation of active fractions to isolate pure compound(s).

f) Elucidation of chemical structure of pure compound(s) using spectroscopic methods.

g) Evaluation of biological activity of pure compound(s)

h) Toxicological tests with pure compound(s).

1.4.2 SCHEMATIC DIAGRAM OF BIOACTIVITY GUIDED RESEARCH OF MEDICINAL PLANTS



1.4.2.1 BIOACTIVE COMPOUNDS IN MEDICINAL PLANTS

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (Bernhoft, 2010):

a) Primary metabolites such as sugars and fats, which are found in all plants; and
b) Secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination.

It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development (Bernhoft, 2010). Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs (Bernhoft, 2010):

- Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine.
- Polyphenols (also known as phenolics) are compounds contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics.
- Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis.

- Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpenesqualene. When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

The goals of using plants as sources of therapeutic agents are (Bernhoft, 2010):

- ✓ to isolate bioactive compounds for direct use as drugs, e.g. digoxin, digitoxin, morphine , reserpine, taxol, vinblastine, vincristine etc.
- ✓ to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics), taxotere which are based respectively on galegine, Δ9- tetrahydrocannabinol, morphine, taxol
- ✓ to use agents as pharmacologic tools, e.g., lysergic acid diethylamide, mescaline, and ✓to use the whole plant or part of it as a herbal remedy, e.g., cranberry, garlic etc.

1.5 REVIEW ON PLANT (*MENTHA ARVENSIS*):

Figure 1.1: *Mentha arvensis*

1.5.1 VERNACULAR NAMES OF PLANT

Table 1.3: Showing the vernacular names of *Mentha arvensis* in different regions(Kirtikar and Basu, 1999).

Region/Tribal name	Vernacular names
Arabic	Naana al hind.
Bengali	Podina
Burmese	Bhudina
Chinese	Po-ho
Danish	Agermynte
Dutch	Akkermunt
Finnish	Peltominttu, Pelto-minttu, Rantaminttu.
French	Baume des champs, Menthe des champs, Menthe du japon.
German	Ackerminze, Feldminze, Kornminze, Minze
Gujarati	Phudno, Pudina.
Hindi	Pudinah.
Italian	Mentaselvatica, Minta.
Japanese	Mensa arubenshisu, Mentaarubenshisu, Youshuhakka
Kannada	Chetnimaraga, Chetnimaragu.
Malayalam	Putiyina.
Marathi	Pudina
Persian	Pudinah

Country	Name
Polish	Miętapolna
Portuguses	Hortelã-comum, Vique (Brazil)
Tamil	Puthina.
Telegu	Pudina
Sanskrit	Pudina, Putiha
Spanish	Mentajaponesa, Mentasilvestre, HierbaBuena
Swedish	Åkermynta
Urdu	Pudinchkohi

1.5.2 TAXONOMY OF *MENTHA ARVENSIS* (Robinson, 1994)

Kingdom	Plantae
Superdivision	Embryophyta
Division	Tracheophyta
Class	Magnoliopsida
Order	Lamiale
Family	Lamiaceae
Genus	Mentha L.
species	*M. arvensis L.*

1.5.3 HABITATS

Woodland Garden Sunny Edge; Shady Edge; Cultivated Beds.

1.5.4 EDIBLE USE

- Edible Parts: Leaves
- Edible Uses: Condiment; Tea

1.6 LAMIACEAE FAMILY

Lamiaceae, formerly called Labiatae, the mint family of flowering plants, with 236 genera and more than 7,000 species, the largest family of the order Lamiales. Lamiaceae is distributed nearly worldwide, and many species are cultivated for their fragrant leaves and attractive flowers. The family is particularly important to humans for herb plants useful for flavour, fragrance, or medicinal properties.

Most members of the family are perennial or annual herbs with square stems, though some species are woody shrubs or subshrubs. The leaves are typically simple and oppositely arranged; most are fragrant and contain volatile oils.

1.6.1 PHYSICAL DRESCRIPTION OF LAMIACEAE FAMILY

The species of Lamiaceae are mainly herbs or shrubs of various sizes, rarely trees. Life cycles of the herbaceous members may be annular or perennial. The stems are usually square, especially when young, erect or procumbent. The leaves are opposite or whorled, decussate (in opposite pairs, with each pair at right angles to the one above and below it), and gland-dotted. The leaves are often strongly aromatic due to ethereal oils located in the glandular hairs. The leaf blades are simple, rarely pinnately lobed (*Teucrium*) or digitately compound with entire or toothed margins. The flowers are arranged in compact axillary cymes (verticillasters), e.g. in the white *Leonotis,* or are sometimes single in the axils of the leaves. Sometimes the inflorescences are congested as in *Pycnostachys*. The calyces are usually persistent with 5 teeth or lobes. Zygomorphic (irregular) flowers are very characteristic of the family.

1.6.2 DISTRIBUTION

Worldwide the Lamiaceae, a member of the seed-forming, dicotyledonous plants, comprises over 240 genera and 6 500 species, following the family circumscription. The main centre of diversity

is the Mediterranean region to central Asia. Members are found in tropical and temperate regions. About 60 genera with ± 980 species occur in the Sub-Saharan African region (Klopper*et al.* 2006).

In South Africa, there are ± 255 species in 35 genera. The species occur predominantly in the summer rainfall areas, but are also found in the winter rainfall areas. The habitats vary to a great extent.

1.6.3 CONVERSATION STATUS

Many species of Lamiaceae are currently not threatened. However, habitats in which they occur are threatened by human impact and thus may change their status in future. Many species are endemic to a restricted area, and are regarded as rare because of their narrow distribution but are not considered as threatened at this stage, for example, *Syncolostemonmacranthus,* known from the Natal Drakensberg between Cathedral Peak and Van Reenen's Pass and just extending into the eastern Free State.

1.6.4 ECONOMIC & CULTURAL VALUE

Lamiaceae is characterized by aromatic plants which have been widely used since ancient times. The family is known for culinary herbs such as basil, mint, oregano, rosemary, sage and thyme. Members are a source of essential oils for the flavouring and perfume industry, for example, the widely cultivated lavender, *Lava,* meaning the beer of birds, referring to the copious nectar produced by the plant which is relished by sunbirds.

1.6.5 GROWING PLANTS OF THE LAMIACEAE FAMILY

Many species of the Lamiaceae are attractive to grow in the garden, for example, the genus *Plectranthus*. *Leonotisleonurus*is grown as an ornamental in various parts of the world. Plants are easily propagated from stem cuttings. *Ocimum,* an important economic and medicinal herb, requires warmth for growth and should be protected from frost.

1.6.6 DRESCRIPTION OF *MENTHA ARVENSIS*

Podina is an herbaceous perennial herb that grows to a height of 10 to 60 cm. This downy herb has running rootstocks and a rigid branching stem. The lanceolate oblong leaves are simple and sharply toothed, paired in opposites with minute hair. They are 2 to 6.5 cm in length and 1 to 2 cm in width. The pale purple flowers (sometimes white or pink) are found in clusters at the stem where each flower measures 2 to 4 mm in length. The plant is native to the temperate regions of Europe, western and central Asia (eastern Siberia and east of the Himalayas) and North America.

It is commonly known as pudina in Hindi.

1.6.6.1 CONSTITUENTS

- Plant yields a volatile oil (0.22%) containing pulegone, menthol, menthene, menthenone.
- Study showed the shoot leaf gave the highest yield of oil, 0.62%; while the stems had negligible yield. Menthol was the major component of all the oils. Other oils identified were: B-caryophyllene oxide, a-phellandrene, terpinolene, limonene, menthone and pulegone.
- Phytochemical screening of powdered plant samples (root, stem, and leaves) yielded alkaloids, polyphenols, flavonoids, tannins, saponins, cardiac glycosides, and diterpenes.
- Study of aerial parts for essential oil yielded major components of (Z,Z,Z)-9,12,15octadecatrien-1-ol (50.06%), 2-hydroxy-4-methoxyacetophenone (7.50%), and 3,4dihydro-8-hydroxy-3-methyl-1H-2-ben- zopyran-1-one (6.60%).

1.6.6. TRADITIONAL USE OF *MENTHA ARVENSIS*:

If the leaves are rubbed on a new beehive, it will attract bees. Pennyroyal is a lesser known species of mint often used in the past. The oil of Pennyroyal is applied to the skin to ward off mosquitoes and other biting insects or strewn in cupboards and beds to deter ants and fleas. Peppermint is used to increase the flow of digestive juices and bile while relaxing the muscles of the digestive tract. It reduces colic, cramps, and gas, and helps to soothe the lining and muscles of the colon, relieving spastic episodes. Peppermint is also helpful for diarrhea and constipation.

Applied to the skin, peppermint relieves pain and reduces sensitivity. Since it reduces nausea, mint is helpful for travel sickness. Mint can be used to promote sweating in fevers and influenza.

- In northern Spain, mint tea (called poleo) is offered as an after-dinner beverage. Other cultures also have long known that it is a digestive tonic plant. The oils in mint help to break down fats making them easier to digest. In Latin America, yerba buena (the good herb) or mint is used as a tonic for lingering illneses.

- Today, peppermint is preferred in the West, while the Chinese prefer to use field mint and have done so for at least a thousand years to treat fevers, flu, colds, nosebleeds, diseases of the nose and throat, snake and insect bites, and nervous disorders in children.

  In China, the leaves are often added to salads and vegetable dishes.

- In Djakarta, mint grows wild and used for headaches and colds. The leaves are ground with a bit of lime and put on the temples as a poultice to relieve throbbing headaches or are brewed for serious coughs.
- In New Zealand, mint is used as a tonic and to treat colds, flu, headaches, colic, gas, and nausea. Oil of peppermint is put on burns to relieve the pain and, when taken on a regular basis, dissolves gallstones.
- In India, mint is used to tone the stomach, stimulate the mind and body, rid the intestines of gas, and relieve muscle spasms. There, chutney is not just a condiment, but also a way of taking medicinal herbs. Mint is added to fruit chutneys to be taken as a tonic with every meal. The western practice of using mustard and ketchup came from the Indian chutneys, but these certainly do not possess any of the healthful qualities that the Indian chutneys contain.
- The Arabs use mint for many ailments, including skin diseases and as a general tonic. They are also one of the few nations who believed that mint was a tonic for the mind as well as the stomach.

1.6.7 USES

1.6.7.1 Nutritional

- Cultivated as a spice for cooking.
- Leaves used for tea.
- Used in salads to provide flavor.
- Used as a flavoring in confections and dentrifices.

1.6.7.2 Folkloric

- One of the oldest household remedies known.
- In the Philippines, tops and leaves are considered carminative; when bruised used as antidote to stings of poisonous insects.
- Mint is used in neuralgic affections, renal and vesical calculus.
- Used for stomach weakness and diarrhea.
- Decoction and infusion of leaves and stems used for fever, stomach aches, dysmenorrhea, and diuresis.
- Pounded leaves for insect bites, fevers, toothaches, headaches.
- Crushed fresh plants or leaves are sniffed for dizziness.
- Powdered dried plant as dentifrices.
- Crushed leaves are applied on the forehead and temples for headaches.
- For toothaches:
    - Wet a small piece of cotton with juice expressed from crushed leaves; apply this impregnated cotton bud to the tooth.
    - Boil 6 tbsp. of leaves in two glasses of water for 15 minutes; strain and cool. Divide the decoction into 2 parts and take every 3 to 4 hours.
- For flatulence: Boil 4 tbsp of chopped leaves in 1 cup water for five minutes; strain. Drink the decoction while lukewarm. Facilitates expulsion of flatus.
- Alcohol or ether extract used as local anesthetic for affections of the nose, pharynx, and larynx.

- Used for obstinate vomiting of pregnancy.
- An alcoholic solution of menthol has been used as inhalation for asthma. Menthol is also used as local anesthesia for headache and facial neuralgia.
- Decoction or vapor from menthol used with lemon grass as febrifuge. Also used in hiccups.
- Plant used as emmenagogue; also used in jaundice.
- Dried plant used as dentifrice.
- Leaves and stems used as carminative, antispasmodic, and sudorific.
- Infusion of leaves used for indigestion, rheumatic pans, arthritis and inflamed joints.
- For coughs, boil 6 tbsp of chopped leaves in 2 glasses of water for 15 mins; cool and strain. Divide the decoction into three parts; take 1 part 3 times a day.
- Diluted essential oil used as wash for skin irritations, burns, pruritus, scabies, ringworm and as mosquito repellent.
- For arthritis, warm fresh leaves over low flame; then pound. Apply pounded leaves while warm on the painful joints or muscles.
- As mouthwash, soak 2 tbsp chopped leaves in 1 glass of hot water for 30 minutes; strain. Use the infusion as mouthwash.
- Peppermint oil is often used in pharmaceutical preparations to subdue unpleasant medicinal smells.
- Menthol derived from the essential oil is used in pharmaceutical, perfumery and food industries.

1.6.7.3 Reversible Male Contraceptive Effect: Study of aqueous extract solution in male mice caused inhibition of fertility while maintaining normal sexual behavior. All induced effects returned to normalcy within 30 days of withdrawal of 60-day treatment.

1.6.7.4 Anti-candida: A study of essential oils and ethanolic extracts of leaves/roots of 35 medicinal plants in Brazil screened for anti-Candida activity. *M. arvensis* was one of 13 essential oils that showed anti-candidal activity.

Figure 1.2: *Mentha arvensis*

1.6.7.6 Anti-fertility / Male Contraceptive: A study of the ether extract of MA on male mice showed reduction of number of offspring, with decrease in testes weight, sperm count and motility, among others. Results suggest that the ether extract of MA possess reversible antifertility properties.

1.6.7.7 Potentiatiing Effect with Chlorpromazine Against Bacterial Resistance: Study showed extracts of M arvensis could be used as a source of plant-derived natural products with resistance-modifying activity, such as in the case of aminoglycosides - a new weapon against bacterial resistance to antibiotics, as with chlorpromazine.

1.6.7.8 Anti-Gastric Ulcer: Study of various extracts of *Mentha arvensis* showed a protective effect against acid secretion and gastric ulcers in ibuprofen plus pyloric ligation-induced and 90% ethanol-induced ulcer models.

1.6.7.9 Herbal Liniment/Analgesic: *M. arvensis* provides potent analgesic action and is used externally in rheumatism, neuralgia and headaches. In an herbal liniment where it was combined with four other medicinal plants, the liniment was found effective in ligament or muscle injury pain (sprains, strains, spasms, tennis elbow, etc), less so in osteoarthritis of the joint and periarthritis of the shoulder. No adverse reactions were reported. Efficacy was noted better in synergism with oral or parenteral analgesics.

1.6.7.10 Volatile Constituents/Menthol: Study showed the shoot leaf gave the highest yield of oil, 0.62%; while the stems had negligible yield. Menthol was the major component of all the oils. Other oils identified were: β-caryophyllene oxide, α-phellandrene, terpinolene, limonene, menthone and pulegone.

Chapter 2: Literature Review

2.1 ANTIFERTILITY INVESTIGATION AND TOXICOLOGICALSCREENING OF THE PETROLEUM ETHER EXTRACT OF THELEAVES *MENTHA ARVENSIS* L. IN MALE ALBINO MICE

The petroleum ether extract of the leaves of *M. arvensis* L., at the doses 10 and 20 mg/mouse per day for 20, 40 and 60 days, when administered orally, showed a dose and duration dependent reduction in the number of offspring of the treated male mated with normal females. Negative fertility was observed in both dose regimens after 60 days of treatment The levels of serum protein, bilirubin, GOT, GPT and acid phosphatase, blood urea and haematological indices were unaltered throughout the course of investigation. All the altered parameters were reversible following withdrawal of treatment. The results suggest that the petroleum ether extract of the leaves of *M. arvensis* possess reversible antifertility property without adverse toxicities in male mice.

2.2 INHIBITION OF IMMUNOLOGIC AND NONIMMMUNOLOGICSTIMULATION MEDIATED ANAPHYLACTIC REACTIONS BY THE AQUEOUS EXTRACT OF *MENTHA ARVENSIS*

The effect of aqueous extract of *M. arvensis* on immunologic and nonimmunologic stimulationmediated anaphylactic reactions was studied. Nonimmunologic anaphylactic reaction was induced by compound 48/80 injection immunologic anaphylactic reaction was generated by sensitizing the skin with anti-dinitrophenyl (DNP) IgE followed 48 h later with an injection of antigen..MAAE (0.001 to 1 mg/ml) dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-DNP IgE. Moreover, MAAE

(0.1 mg/ml) had a significant inhibitory effect on anti-DNP IgE-mediated tumor necrosis factor-α (TNF-α) production. These results indicate that MAAE inhibits immunologic and nonimmunologic stimulation-mediated anaphylactic reactions and TNF-α production from RPMC.

2.3 ANTIBACTERIAL ACTIVITY OF LEAVES AND INTER-NODALCALLUS EXTRACTS OF *MENTHA ARVENSIS*

- To determine the anti-bacterial efficacy of chloroform, ethanol, ethyl acetate and water extracts of inter-nodal and leaves derived calli extracts from *M. arvensis (M. arvensis)* against *Salmonella typhi(S. typhi), Streptococcus pyogenes.*
- The inter-nodal and leaves segments of *M. arvensis* were cut into 0.5-0.7 cm in length and cultured on Murashige and Skoog solid medium supplemented with 3% sucrose, gelled with 0.7% agar and different concentration of 2, 4-Dichlorophenoxyacetie acid (2,4-D) either alone or in combinations. The preliminary phytochemical screening was performed by Brindha*et al* method. Antibacterial efficacy was performed by disc diffusion method and incubated for 24 h at 37 °C.
- Maximum percentage of callus formation was obtained on Murashige and Skoog's basal medium supplemented with 3% sucrose and 1.5 mg/L of 2, 4-D. The ethanol extracts of leaves derived calli showed the maximum bio-efficacy than other solvents. The leaves and stem derived calli extracts on *Proteus* sp. showed that the plants can be used in the treatment of urinary tract infection.

2.4 ANTIMICROBIAL ACTIVITY OF *MENTHA ARVENSIS* AGAINSTCLINICAL ISOLATES OF HUMAN CARIOGENIC PATHOGENS-AN IN-VITRO STUDY

- The objective of this study is to evaluate the efficacy of herbal crude extract of *M. arvensis* in human Cariogenic pathogens.Crud extract of *M. arvensis* in different solvent 50% and 10% methanol, ethyl acetate, chloroform and was tested against human Cariogenic pathogens *Streptococcus mutans, Streptococcus sangunis, Staphylococcus aurues, Lactobacillus casei* were isolated from patients having dental disease.
- The crude extracts activity were studied by disc diffusion and both dilution methods in different concentration. Studies were also undertaken to assess the phytochemical composition of the *M. arvensis* extract.
- 50% methanolic extract at 2.5mg/ml and 5mg/ml concentration shows slightly bigher zone of inhibition (ranging from 26 to 30 mm and 28 to 32 mm), and 10% methanolic

2.5mg/ml and 5mg/ml extract shows slightly small zone (ranging from 20 to 24 mm and 22 to 27 mm) and comparison with ethyl acetate and chloroform shows small zone at 5mg/ml ranging from 15 to 18 mm and 13 to 17 mm and in 2.5gm/ml ranging from 14 to 15mm and 09 to 16 mm or to be moderately sensitive. MIC results exhibit the profound and promising activity of *Mentha arvensis*.

- The secondary metabolites commonly present in the test leaves are Alkaloids, Tannins, Flavonols, Steroids, Xantones and glycosides. These data suggest that extracts of *M. arvensis* contain significant amounts of phytochemicals with antioxidative properties which could serve antimicrobial property of the *Mentha arvensis* and it is exploited as a potential source for plant-based pharmaceutical products. These results could form a sound basis for further investigation in the potential discovery of new natural bioactive compound.

2.5 STUDIES ON ACTIVITY OF VARIOUS EXTRACTS OF *MENTHAARVENSIS* LINN AGAINST DRUG INDUCED GASTRIC ULCER INMAMMALS

To examine the antiulcerogenic effects of various extracts of *Mentha arvensis* Linn on acid, ethanol and pylorus ligated ulcer models in rats and mice. Various crude extracts of petroleum ether, chloroform, or aqueous at a dose of 2 g/kg po did not produce any signs or symptoms of toxicity in treated animals. In the pyloric ligation model oral administration of different extracts such as petroleum ether, chloroform and aqueous at 375 mg/kg *po*, standard drug ranitidine 60 mg/kg *po* and control group 1% Tween 80, 5 mL/kg *po* to separate. There was a decrease in gastric secretion and ulcer index among the treated groups (Londonkar, 2009).

2.6 PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF*GYMNEMA SYLVESTRE, MENTHA ARVENSIS, SOLANUMSURRATENSE*,EXTRACTS IN DENTAL CARIES

This is the first report testing the antibiotic resistance-modifying activity of *M. arvensis* against MRSA (methicillin - resistant *Staphylococcus aureus*).In this study an ethanol extract of *M. arvensis* L. and chlorpromazine were tested for their antimicrobial activity alone or in combination with conventional antibiotics against MRSA strains. A potentiating effect of this

extract on gentamicin, kanamycin and neomycin was demonstrated. Similarly, a potentiating effect of chlorpromazine on the same aminoglycosides was observed, indicating the involvement of an efflux system in the resistance to these antibiotics. It is therefore suggested that extracts from *M. arvensis* could be used as a source of plant-derived natural products with resistancemodifying activity.

2.7 ANTIOXIDANT PROPERTIES AND COMPOSITION OF AQUEOUSEXTRACTS FROM *MENTHA* SPECIES

Water-soluble extracts from the *Mentha* species *M. aquatica* L. and *M. haplocalyx* Briq, the hybrids and *M*. "Native Wilmet" cultivars were screened for potential antioxidative properties. These properties included iron(III) reduction, iron(II) chelation, 1,1-diphenyl-2-picrylhydrazyl radical scavenging, and the ability to inhibit iron(III)−ascorbate-catalyzed hydroxyl radicalmediated brain phospholipid peroxidation. Total phenol content and qualitative and quantitative compositional analyses of each extract were also made. The extracts demonstrated varying degrees of efficacy in each assay, with the *M*. x *piperita* "Frantsila" extract being better than the other extracts, except for ferrous iron chelation. With the exception of iron chelation, it appeared that the level of activity identified was strongly associated with the phenolic content.(Pubs.acs.org, 2016

2.8 EFFECTS OF DAYLENGTH ON THE MONOTERPENE COMPOSITION OF LEAVES OF *MENTHA X PIPERITA*

The monoterpene composition of leaves of *Mentha x piperita* has been studied by direct analyses of samples (Desorption-Concentration-Introduction Technique). Kinetic studies on individual pairs of leaves from plants subjected to different photoperiodic treatments (long or short photoperiod followed or not by an inversion of the initial photoperiod) show that the photoperiod received by the young leaves directly influenced the oil composition. Therefore the photoperiod is such as responsible for the variations of the monoterpene metabolic pathway. This conclusion is based on the likelihood that the reducing pathway (menthone-menthol) was developed in young leaves on condition that they are subjected to a long photoperiodic treatment and that the

metabolic pathway originally induced by the daylength in the oldest leaves was not changed by the inversion of the initial photoperiodic treatment.

2.9 ANTI – INFLAMMATORY AND SEDATIVE – HYPNOTIC ACTIVITYOF THE METHANOLIC EXTRACT OF THE LEAVES OF *MENTHA ARVENSIS*

Mentha arvensis Linn, a plant used as traditional medicine and in perfumery, has now been explored for its pharmacological activities as an anti-inflammatory and also as sedative hypnotic plant drug. The methanolic extract of the leaves after being processed, was taken for the pharmacological study. Anti-inflammatory activity was carried out on albino rats. Further, the activity was compared to that of a standard anti-inflammatory drug – nimesulide and the percent inhibition of oedema determined. The sedative hypnotic activity, when carried out on mice, showed the potentiation of pentobarbitone induced sleeping time. The data of average recovery time was analyzed to show the standard deviation from the mean.

2.10 ORGANOGENESIS AND TERPENOID SYNTHESIS IN *MENTHA ARVENSIS*

Leaf discs obtained from field grown plants of *Mentha arvensis* were used to initiate multiple shoots on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine 5 mg and naphthaleneacetic acid 0.5 mg. Profuse rooting was achieved when the well-grown shoots were cultured on half strength MS medium supplemented with indole-3-acetic acid (2 mg l^{-1}). The regenerated plantlets were hardened and successfully transferred to soil and grown to maturity. Tissues at different stages of differentiation were analyzed for their essential oil content and characteristic monoterpene pattern. Tissue culture raised plants show the same essential oil profile as that of the parent plant. However, tissues at early stages of growth show distinct changes in oil composition, such as high levels of pulegone in shoot cultures. (Sciencedirect.com,2016)

2.11 QUALITY EVALUATION OF THE ESSENTIAL OILS OF THEPREVALENT CULTIVARS OF COMMERCIAL MINT SPECIES *MENTHA ARVENSIS,SPICATA,PIPERITA,CARDIACA*

- *Mentha arvensis, Mentha piperita, Mentha spicata,* were planted in autumn and winter rabi seasons in India. The autumn crops were harvested after 135 and 170 days from planting. The winter crops were harvested at 70, 100 and 175 days from planting. The 100 days crop were ratooned and harvested again at 75 and 115 days from the time of first harvesting.
- The fresh herb from each harvest was distilled in Clevenger apparatus to obtain essential oil which were characterized by GC and GC/MS analyses for their profile to determine the contents of the major terpenoids. The oil contents in the *Mentha arvensis* herb was about twice than the cultivars of other species.
- The most economic crops were the winter planted and harvested at 70 or 100 days from the time of planting and also ratoon crops harvested at 75 days. All the cultivars of *Mentha spicata* yielded oil rich in carvone. The carvone content was highest in the oil of cultivars Arka and MSS-5. The Neer-Kalka cultivar of *Mentha spicata* had menthone and neo-menthol in its oil. The essential oil of *Mentha piperita* cv. Kukrail crops when harvested at 70 and 100 days. The implications of the difference in the oil profile in terms of developmental stage of the crop are discussed.

2.12 YIELD AND COMPOSITION OF THE ESSENTIAL OIL OFMENTHA PIPERITA L.GROWN WITH BIOSOLID

- This research evaluated the effects of biosolid levels on yield and chemical composition of *Mentha piperita* L. essential oil. Mint plants were grown in a greenhouse containing the equivalent to 0, 28, 56, and 112 $t.ha^{-1}$ biosolid. Three evaluations were made at 90, 110, and 120 days after planting (DAP).
- The oil was extracted from the dry matter of shoots by hydrodistillation, and composition was determined by GC/MS. Oil production was slightly affected by the biosolid, increasing when plants were grown with a condition which did not result in quality

improvement. Menthyl acetate was the component obtained at the highest percentage in all treatments.

- Since the production of biosolid is on the rise, a suitable destination must be given to it, and restrictions exist for its use in relation to the environment and plants. Thus, although cultivation with 28 $t.ha^{-1}$ is within the limits allowed by law, such a rate, which increased oil yield, did not improve oil quality.

2.13 PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF*GYMNEMA SYLVESTRE*, *MENTHA ARVENSIS* EXTRACTS IN DENTAL CARIES

Hydro alcoholic dry extracts of *Gymnema sylvestre*, *Mentha arvensis*, for treatment of a dental caries were screened for antimicrobial activity by Agar well diffusion method against *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus mitis*, and *Candida albicans*. Among them the extracts of *Gymnema sylvestre* dry extract showed strong antimicrobial activity against the bacteria and fungi with the zone of inhibition ranges from 16-20 mm at 25 mg/ml.
The other extracts such as *Solanum surratense*, *Mentha arvensis* showed concentrationdependent activity against all the tested micro-organisms with the zone of inhibition ranges from 12-24 mm at various concentrations

2.14 INFLUENCE OF WATER STRESS ON JAPANESE MINT

Changes in plant growth and development, micronutrient accumulation, and essential oil yield and composition were studied in Japanese mint (*Mentha arvensis*) growing under selected water stresses from deficiency to sufficiency. Water stress resulted in significant reductions in CO_2 exchange rate, total assimilatory area, fresh and dry matter, chlorophyll, carotenoids, Fe, Mn, Zn, and essential oil yield. Some changes in essential oil composition were observed, but these changes generally were not correlated with water stress.

2.15 CORN MINT (*MENTHA ARVENSIS*) EXTRACT DIMINISHESACUTE *CHLAMYDIA PNEUMONIAE* INFECTION IN VITRO AND INVIVO

Corn mint (*Mentha arvensis*) provides a good source of natural phenols such as flavone glycosides and caffeic acid derivatives, which may have prophylactic properties against inflammations. This study investigated whether corn mint extract would be beneficial against a universal respiratory tract pathogen, *Chlamydia pneumoniae* infection. The extract inhibited the growth of *C. pneumoniae* CWL-029 in vitro in a dose-dependent manner. The inhibition was confirmed against a clinical isolate K7. The phenolic composition of the extract was analyzed by UPLC-ESI/Q-TOF/MS, the main components being linarin and rosmarinic acid. These compounds were active in vitro against *C. pneumoniae*. Linarin completely inhibited the growth at 100 μM. Inbred C57BL/6J mice were inoculated with *C. pneumoniae* K7. *M. arvensis* extract was given intraperitoneally once daily for 3 days prior to inoculation and continued for 10 days postinfection. The extract was able to diminish the inflammatory parameters related to *C. pneumoniae* infection and significantly (p = 0.019) lowered the number of *C. pneumoniae* genome equivalents detected by PCR at biologically relevant amounts.

Chapter 3 : Methods & Materials

3.1 COLLECTION & PREPARATION OF PLANT

Plant sample (Leaves) of *Mentha arvensis* was collected from Narsingdi in January 2016. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24hours at considerably low temperature for better grinding. The dried leaves was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 EXTRACTION OF THE PLANT MATERIAL

About 650gm 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 39^{o}C with a Heidolph rotary evaporation.

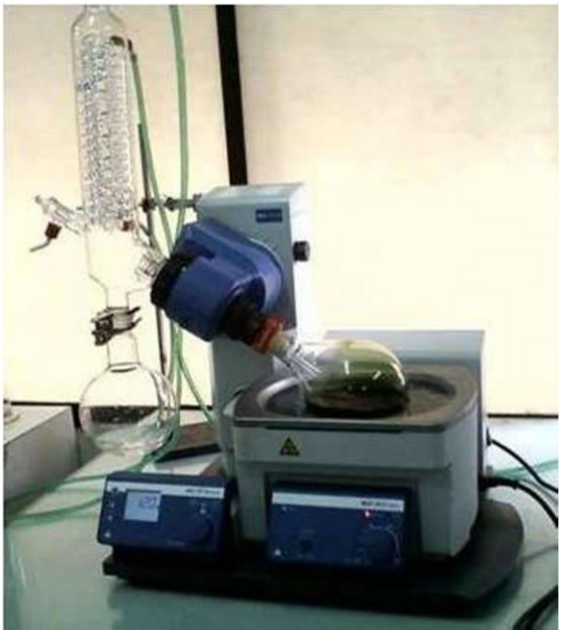


Figure 3.1: Drying of extract using rotary evaporator.

The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 25.18 gm respectively.

3.3 PREPARATION OF MOTHER SOLUTION

5gm of methanol extract was triturated with 90ml of methanol containing 10ml 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 three solvents of different polarity.

3.4.1 PARTITION WITH N-HEXANE

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

3.4.2 PARTITION WITH DICHLOROMETHANE

To the mother solution left after partitioning with n-hexane, 12.5ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with dichloromethane (DCM). The process was repeated thrice (100ml X 3). The DCM fraction was then air dried for solid residue.

3.4.3 PARTITION WITH ETHYLE ACETATE

To the mother solution that left after washing with n-Hexane and DCM was then taken in a separating funnel and extracted with Ethyl acetate (100ml X 3). The Ethyl acetate soluble fractions were collected together and air dried.

3.4.4 COLLECTION OF ETHYL ACETATE FRACTION

After partitioning the mother solution with the three different solvents the ethyl acetate fraction was collected and air dried. This ethyl acetate was further investigated for different pharmacological properties (antioxidant, cytotoxic and antimicrobial)

3.5 BRINE SHRIMP LETHALITY BIOASSAY

3.5.1 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 e.g. 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- *Artemiasalina*) 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 andNuneza, 2013; Rishikesh*et.al,*. 2013).

3.5.2 APPARATUS & REAGENTS

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

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

3.5.3PROCEDURE

3.5.3.1PREPARATION 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 thevolume made up to 1000ml by distilled water in a 1000ml beaker for *Artemiasalina* hatching. 1-2 drops of NaOH solution of 1N was added with a dropper to obtain the pH 8.4 as sea water.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 *Artemiasalina* 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 *Artemiasalina*were hatched at room temperature (25-30ºC) for 18-24hr. The larvae (nauplii) wereattracted 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 Hatching of Brine Shrimp.

3.5.3.2 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.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.

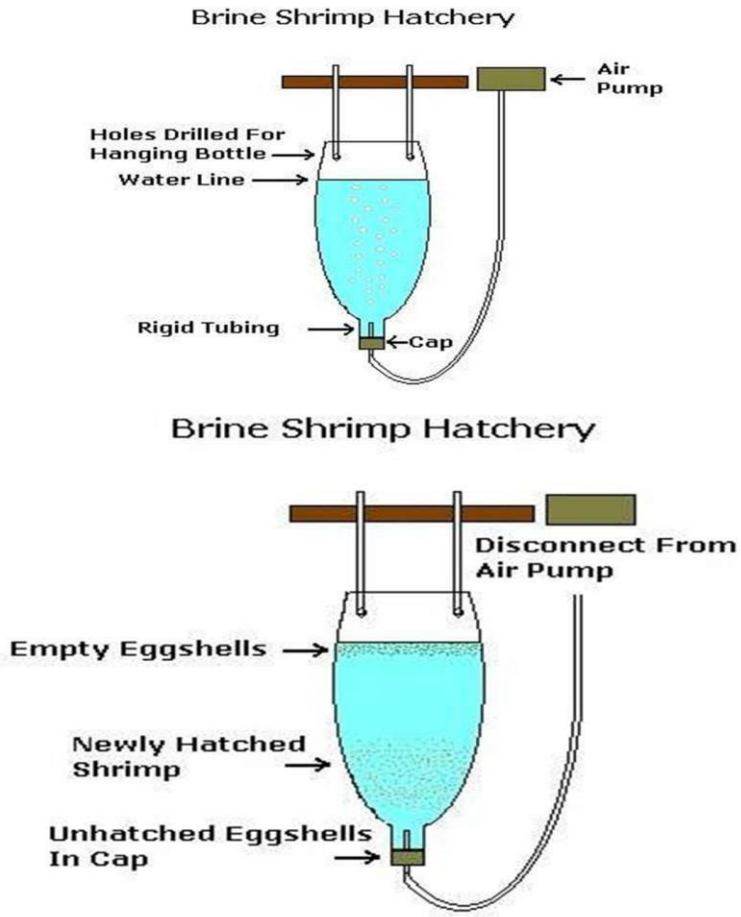


Figure 3.2: Brine shrimp Hatchery

3.5.3.3PREPARATION OF THE TEST SAMPLES OF EXPERIMENTALPLANT

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.

3.5.3.4 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 testtubes to get the positive control groups.

3.5.3.5 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.

3.5.3.6 COUNTING OF NAUPILI

After 24 hours,the vials were inspected using a magnifying glasst 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.

3.6ANTIOXIDANT ACTIVITY

3.6.1 TOTAL PHENOLIC CONTENT

3.6.1.1 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. 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 heteropolyphosphotunstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{-4}$.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; Vinson *et.al,.*, 2005).

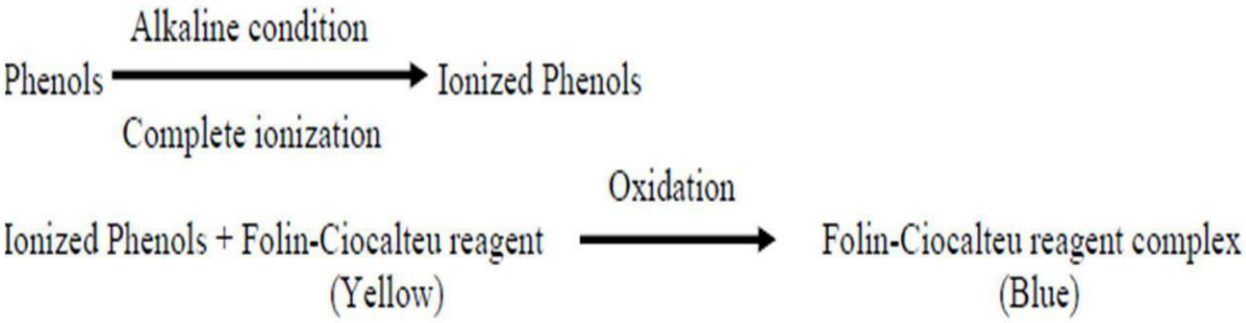


Table 3.4: Composition of 100mg Folin-Ciocalteu Reagent

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

3.6.1.2 APPARATUS & REAGENTS

Table 3.5: Apparatus and reagents used for total phenolic content

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

3.6.1.3 PROCEDURE

STANDARD CURVE PREPARATION

Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120µg/ml to 80µg/ml. 5ml of FCR (diluted 10 times with water) and 4ml of Na2CO3 (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 765nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

SAMPLE PRPARATION

2mg of the *Mentha arvensis* ethyl acetate fraction was taken and dissolved in 1ml of distilled water to get a sample concentration of 2mg/ml.

DETERMINATION OF TOTAL PHENOL CONTENT

- 1.0ml of plant extract (200µg/ml) of different concentrations (120µg/ml, 110µg/ml, 100µg/ml, 90µg/ml and 80µg/ml) was taken in test tubes.

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

- 4ml of Sodium carbonate solution was added into the test tube.

- The test tubes containing the samples were incubated for 1hr at the room temperature complete the reaction.

- Then the absorbance of the solution was measured at 765nm using a spectrophotometer against blank.
- A typical blank solution containing methanol was taken.

3.6.2 TOTAL FLAVONOID CONTENT

3.6.2.1 PRINCIPLE

Aluminium chloride (AlCl3) 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 absorptivity maximum at 510nm (Chang *et.al,.*, 2002). Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510nm 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) + AlCl3 (reagent) = Formation of flavonoid-aluminium complex (λ max 510nm)

3.6.2.2 APPARATUS & REAGENTS

Table 3.6: Apparatus and reagents used for total flavonoid content

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

3.6.2.3 PROCEDURE

PREPARATION OF 10% ALUMINIUM CHLORIDE (ACL3) SOLUTION

10mg of AlCl3 was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

PREPARATION OF 4% NAOH SOLUTION

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

PREPARATION OF 5% (W/V) NANO2 SOLUTION

5mg of NaNO2was taken into a 100ml of a volumetric flask and the volume was adjusted by distilled water.

PREPARATION OF STANDARD SOLUTION

The stock solution was prepared by taking 0.025gm of ascorbic acid and dissolved into 5ml of ethanol. The concentration of this solution was 5µg/µl of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Table 3.7: Preparation of standard solution

Concentration (µg/ml)	Solution takenfrom stock solution (µl)	Volume adjustedby ethanol (ml)	Final volume (ml)
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

PREPARATION OF EXTRACT SOLUTION

5ml of each plant extracts were taken and dissolved into 5ml of methanol. The concentration of the solution was 1mg/ml of plant extracts. Then the following steps were carried out. 1.5ml extract was taken in a test tube and then 6ml of distilled water was added. Then 5% of NaNO2 was added and incubated for 6 minutes. 10% AlCl3 was added and incubated for 6 minutes. 4% NaOH and 0.6ml distilled water was added. Then it was incubated for 15 minutes. For blank solution 1.5ml methanol was taken and the same procedure repeated. Then the absorbance of the solution was measured at 510nm using a spectrophotometer against blank.

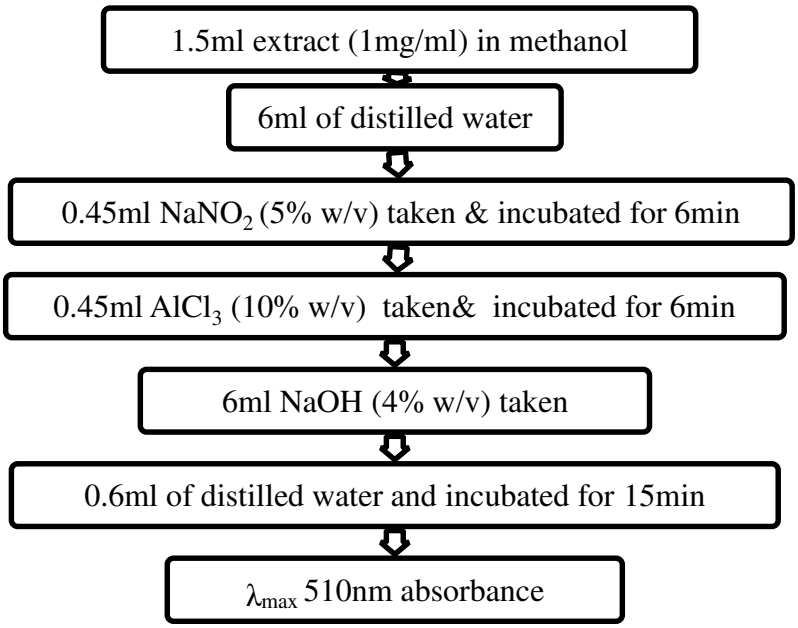


Figure 3.8: Schematic diagram of preparation of extract solution

PREPARATION OF BLANK SOLUTION

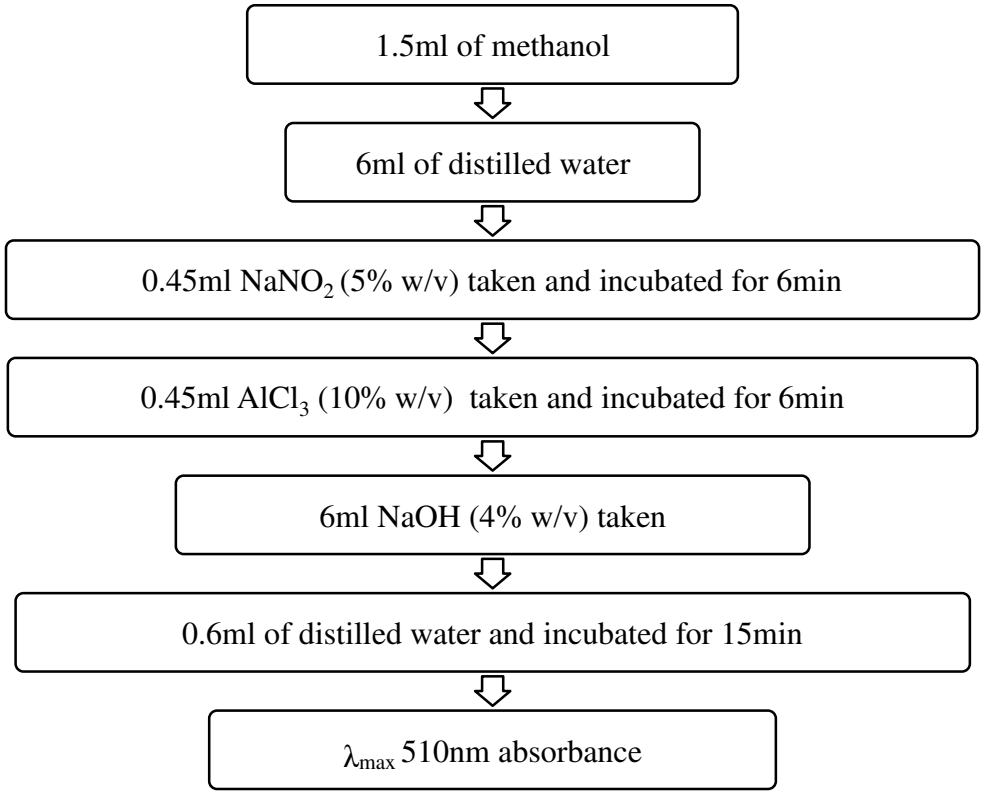


Figure 3.9: Schematic diagram of preparation of blank solution

3.6.3 REDUCING POWER ASSAY:

3.6.3.1PRINCIPLE: The reducing power of Dichloromethane of extract *Mentha arvensis* was determined by the method osf Oyaizu,substances which have reduction potential determined by the method of react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride

Antioxidant ↓

Potassium ferrocyanide + ferrous chloride

3.6.3.2 APPARATUS & REAGENTS

Table 3.2: Apparatus and reagents for antimicrobial test

Spatula	Potassium ferricyanide
Analytical balance	Methanol
Pipette and pumper	Ascorbic acid
Aqueous fraction	Trichloro acetic acid
Test tubes	Phosphate buffer
Beaker	Ferric chloride

3.6.3.3 PROCEDURE:

PHOSPHATE BUFFER (0.2 M, BUFFER ,pH 6.6) PREPARATION

Dibasic sodium phosphate (18.75 ml of 0.2M) is mixed with 31.25 ml monobasic sodium phosphate and diluted to 100 ml with water.

POTASSIUM FERRICYANIDE (1% W/V) PREPARATION

1 mg of potassium ferricyanide(K_3 [Fe (CN) $_6$]) was taken into a 100 ml of volumetric flask and the volume was adjusted by distilled water.

TRICHLORO ACETIC ACID (10%)PREPARATION

10 mg of trichloro acetic acid(CCl_3COOH) was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

FERRIC CHLORIDE (0.1%)PREPARATION (0.1%)

0.1mg of ferric chloride ($FeCl_3$]) was taken into a 100 ml of a volumetric flask 0.2and the volume was adjusted by distilled water.

STANDARD SOLUTION PREPARATION

The stock solution was prepared by taking 0.025 gm of ascorbic acid and dissolved into 5 ml of methanol. The concentration of this solution was 5000 µg/ml of ascorbic acid.

Table 3.7: Different concentrations of ascorbic acid solution preparation

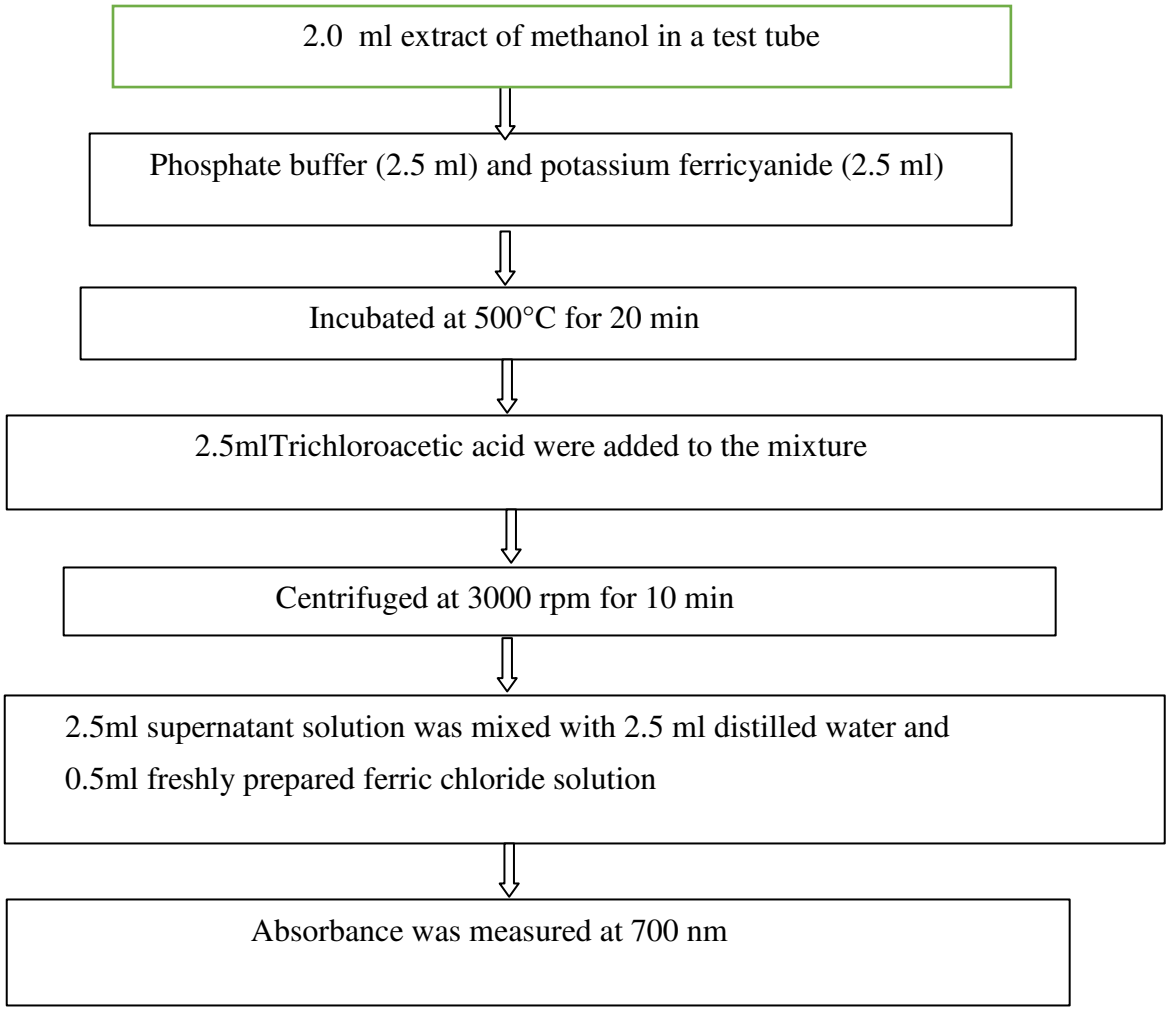
Concentration (µg/ml)	**Solution taken from stock solution (µl)**	**Volume adjusted by methanol (ml)**	**Final volume (ml)**
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

EXTRACT SOLUTION PREPARATION

5 mg of plant extract was taken and dissolved into 5 ml of methanol. The concentration of thesolution was 1 mg/ml of plant extract.

DETERMINATION OF REDUCING POWER

2.0 ml plant extract solution and ascorbic acid in different concentrations were taken in test tubes and mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 500°C for 20 min. 2.5 ml Trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. 2.5 ml upper layer (supernatant solution) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract.



$$\% \text{ increase in Reducing Power} = \left(\frac{A_{test}}{A_{blank}} - 1\right) 100\%$$

Where A_{test} is absorbance of test solution; A_{blank} is absorbance of blank. Increased absorbance of the reaction mixture indicates increase in reducing power (Oyaizu M, 1986).

Chapter 4 : Results & Discussion

4.1 RESULT OF BRINE SHRIMP LETHALITY BIO-ASSAY

The dichloromethane fraction of the *menthe arvensis* extract were subjected to brine shrimp lethality bioassay following the procedure Meyer *et.al,.*, (1982). After 24hrs, 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 (LC_{50}) value. This represents the concentration of the standard or ethyl acetate 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:

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

The LC_{50} 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.

4.1.1 PREPARATION OF CURVE FOR STANDARD

Here, Tamoxifen was used as reference standard

Table 4.1: Results of the bioassay of Tamoxifen (standard)

Test tube no.	Concentration (C) (µg/ml)	LogC	Number of Naupliialive	Number of Naupliidead	% Mortality	LC_{50} (µg/ml)
1	400	2.602	0	10	100	
2	200	2.301	1	9	90	
3	100	2.000	2	8	80	
4	50	1.699	3	7	70	
5	25	1.398	4	6	60	12.5
6	12.5	1.097	5	5	50	
7	6.25	0.796	6	4	40	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	.078125	-0.107	9	1	10	

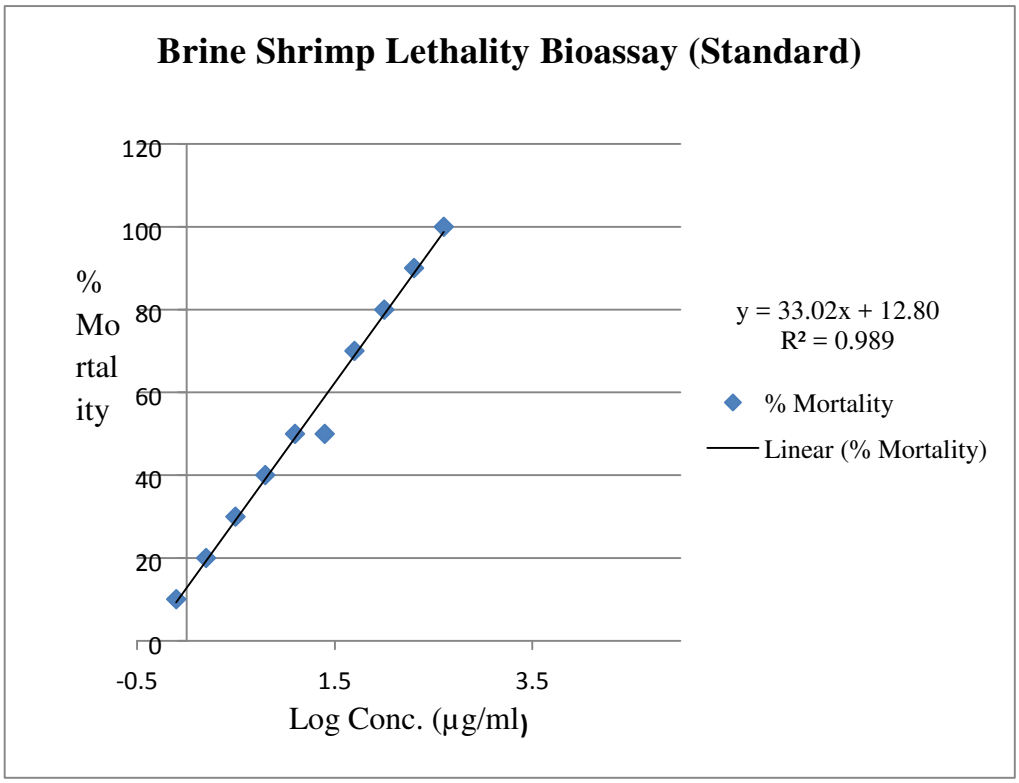


Figure 4.1: % mortality and predicted regression line of Tamoxifen (standard)

4.1.2 PREPARATION OF DICHLOROMETHANE FRACTION CURVE

Table 4.2: Results of the bioassay of dichloromethane fraction (extract)

Test tube no.	Concentration (C) (µg/ml)	LogC	Number of nauplii alive	Number of naupliidead	% Mortality	LC_{50} (µg/ml)
1	400	2.602	00	00	100	
2	200	2.301	03	07	100	
3	100	2.000	04	06	90	
4	50	1.699	08	02	70	
5	25	1.398	02	08	60	
6	12.5	1.097	05	05	40	17.68
7	6.25	0.796	03	07	40	
8	3.125	0.495	07	03	30	

9	1.5625	0.194	08	02	20	
10	.078125	-0.107	07	03	20	

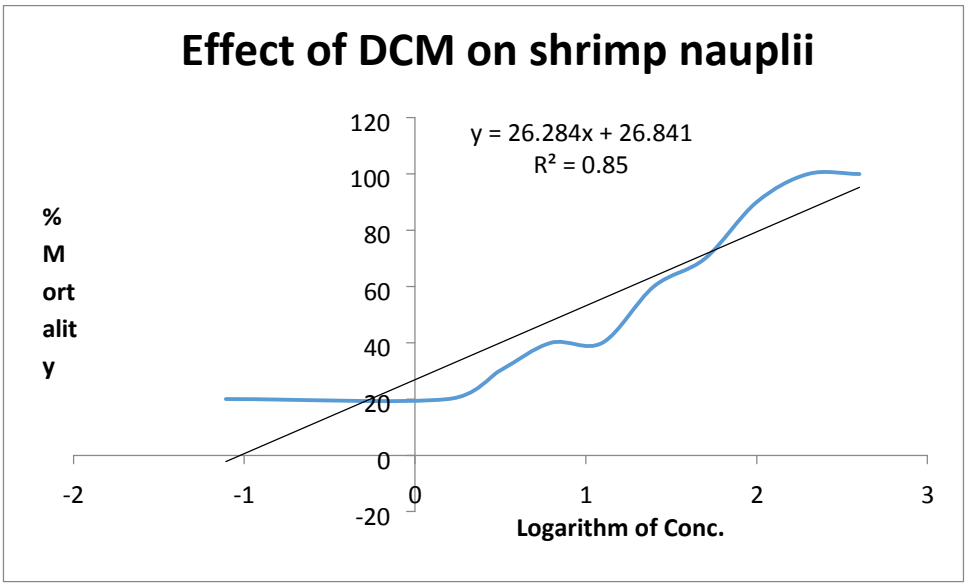


Figure 4.2: % mortality and predicted regression line of dichloromethane fraction (extract).

4.1.3 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 ranging from the lowest concentration to the highest concentration in both standard and dichloromethane fraction samples. Mortality increased gradually with an increase in concentration of the test samples. Maximum mortalities took place at the highest concentration of 400µg/ml, whereas the least mortalities at lowest concentration 0.78125µg/ml as shown in Table 4.1 and Table 4.2.

Table 4.3: Cytotoxic activity of Tamoxifen and dichloromethane fraction of *Mentha arvensis*

Sample	Linear regression equation	R^2 value	LC_{50} (µg/ml, 24hr)
Standard (Tamoxifen)	y = 33.021x + 12.806	0.989	13.38
Extract (DCM fraction)	y = 0.0282x - 0.3616	0.9606	17.68

In this investigation, standard and dichloromethane fraction exhibited cytotoxic activities with the LC_{50} values 12.5 µg/ml and 17.68µg/ml respectively as shown in Table 4.3. For dichloromethane fraction value LC_{50} is more than the standard which indicates that the extract has less potent activity than standard against brine shrimp nauplii.

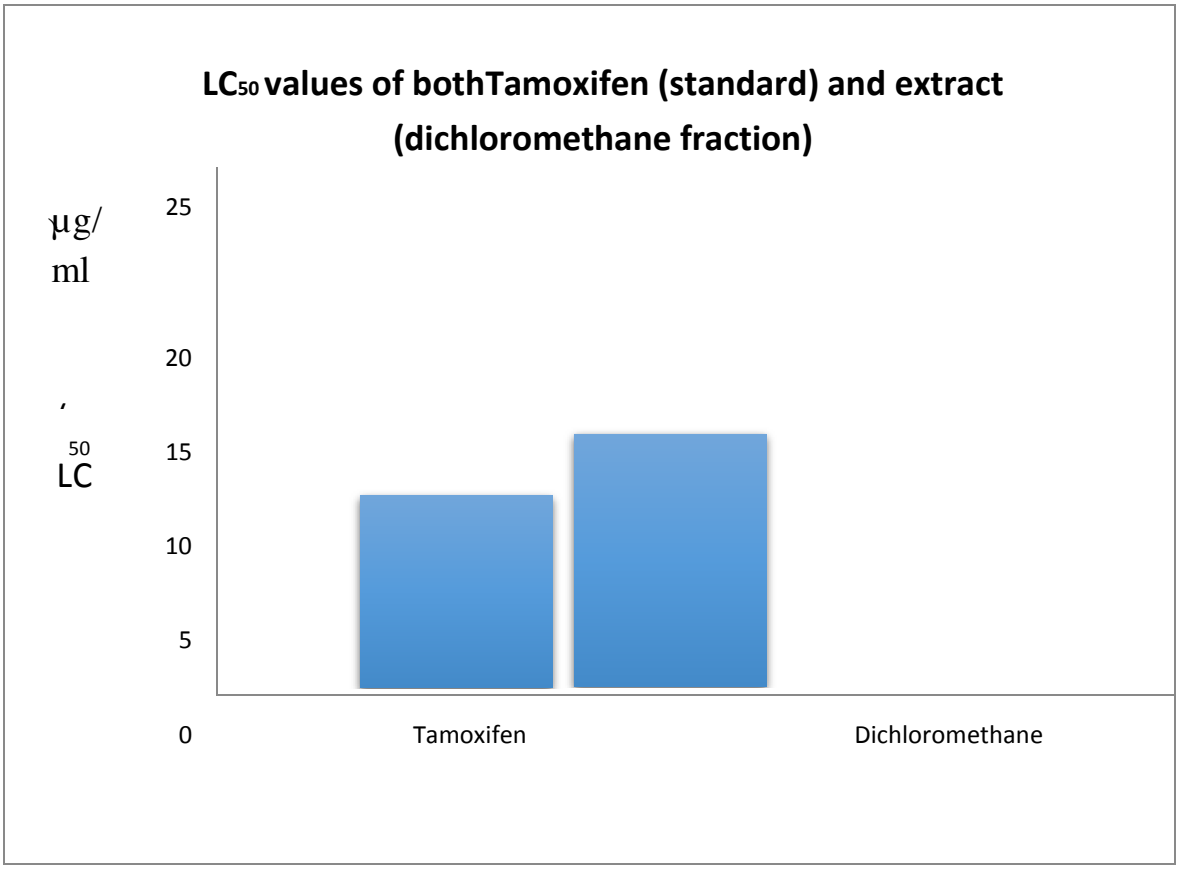


Figure 4.3: Comparison between LC_{50} values of standard and extract

From the above figure it can be concluded that for dichloromethane fraction the lethal concentration required to kill 50% of the sample population is higher than the standard. So the extract is less potent than Tamoxifen (Standard) at lower concentration.

4.2ANTIOXIDANT 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 the aqueous fraction of *mentha arvensis* leaves extract was determined by following methods-

- Determination of total phenolic content
- Determination of total flavonoids content
- Total reducing power assay

4.2.1RESULT OF TOTAL PHENOLIC CONTENT

The dichloromethane extract of leaves of *Mentha arvensis* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard.

4.2.1.1 PREPARATION OF STANDARD CURVE

Table 4.5: Total phenol content of ascorbic acid

Concentration (µg/ml)	Absorbance (at 765 nm)	Regression line	R^2value
80	2.406		
90	2.473		
100	2.767	y = 0.019x+0.824	0.937
110	3.057		
120	3.080		

A linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.4. This linear curve was considered as a standard curve.

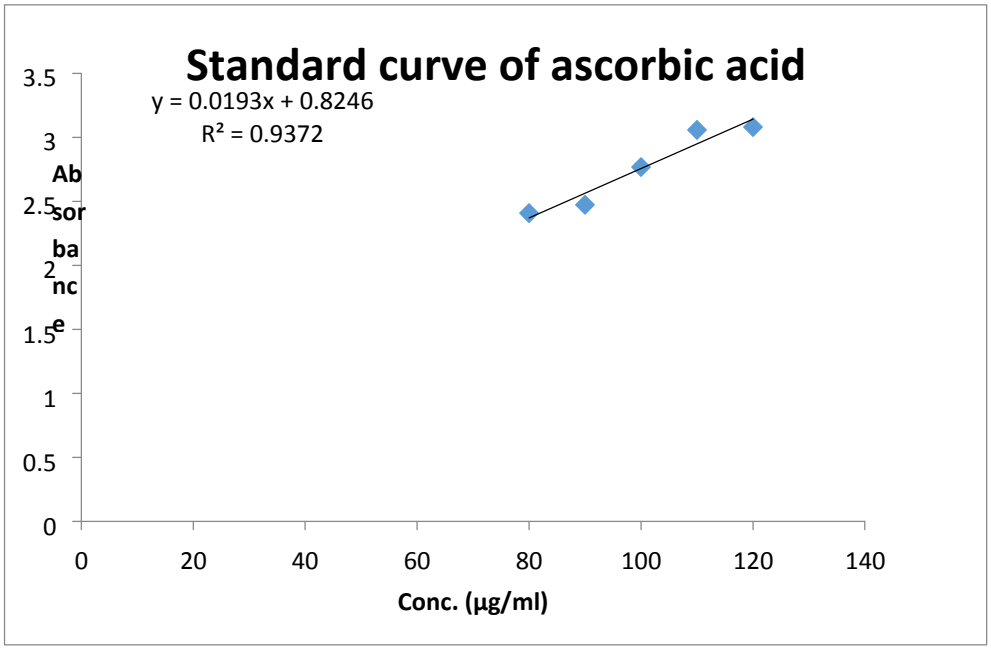


Figure 4.4:Graphical representation of assay of phenolic content of ascorbic acid

4.2.1.2 TOTAL PHENOL CONTENT PRESENT IN EXTRACT

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 4.6: Total phenolic content of dichloromethane fraction of leaves of *mentha arvensis*

Concentration (mg/ml)	Absorbance	mg AAE/g
2	**1.728**	**31.546**

4.2.1.3 DISCUSSION

The plant extract contain phenolic content. The total phenolic content of crude extract, dichloromethane fraction 31.546 mg AAE/g. Further research is needed on the determination of the correlation between the antioxidant capacity and the chemical components of the plant.

4.3.2 RESULT OF TOTAL FLAVONOID CONTENT

The dicholoromethane fractions of *Mentha arvensis* leaves were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard.

4.3.2.1 PREPARATION OF STANDARD CURVE

Table 4.7: Total flavonoid content of ascorbic acid

Concentration (µg/µl)	**Absorbance (At 510 nm)**	**Regression line**	**R^2 value**
50	0.05	y = 0.0017x-0.042	0.991
100	0.13		
150	0.19		
200	0.29		
250	0.39		

After absorbances were taken of different solution of ascorbic acid of concentrations ranging from 50µg/µl to 250µg/µl, a linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.10 This linear curve was considered as a standard curve.

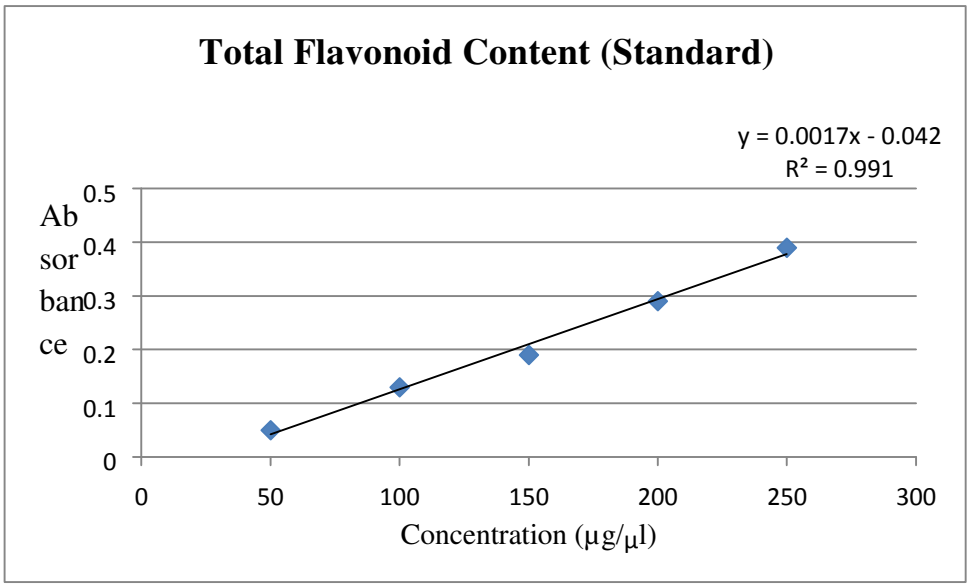


Figure 4.5: Graphical representation of assay of flavonoid content of ascorbic acid

4.3.2.2TOTAL FLAVONOID CONTENT PRESENT IN EXRACT

Based on the absorbance value of extract solution and using the regression line equation of the standard curve, the total flavonoid present in the extract is calculated and is given in Table 4.8.

Table 4.8: Total flavonoid content of dichloromethane fraction of *mentha arvensis* extract

Sample	**Concentration (mg/ml)**	**Absorbance**	**Total flavonoid content (mg of AAE/g of dried extract)**
Dichloromethane fraction of *Mentha arvensis*	1	0.673	715

4.3.2.3DISCUSSION

To determine the total flavonoid content of the test samples the standard curve was used. For 1mg/ml concentration of dichloromethane fraction of *Mentha arvensis* (leaves), 715 mg of AAE/gm of dried extract of flavonoid content was found. So it can be said that, the extract contains antioxidative compounds.

4.2.3 RESULT OF TOTAL REDUCING POWER ASSAY:

The Dichloromethane extract of *Mentha arvensis*were subjected to determine total reducing power. Ascorbic acid was used asreference standard (Oyaizu M, 1986).

4.2.3.1 PREPARATION OF STANDARD CURVE:

Table 4.8: Total Reducing power of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 700 nm)**	**Regression line**	**R^2 value**
250	2.657		
200	2.126		

150	2.284	y = 0.010x + 0.266	R² = 0.821
100	1.603		
50	0.355		

A linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.6. This linear curve was considered as a standard curve.

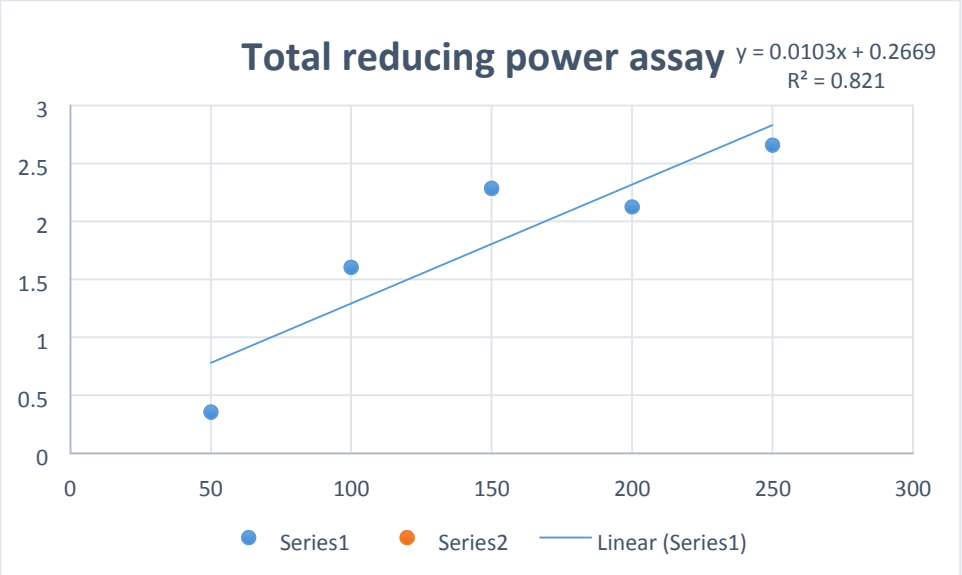


Figure 4.6: Graphical representation of Reducing power of ascorbic acid

4.2.3.2 TOTAL REDUCI NG POWER ASSAY IN DICHLOROMETHANE OF*MENTHA ARVENSIS*

Based on the absorbance values of the extract solution, reacted with potassium ferricyanide reagent and compared with the standard solutions of ascorbic acid equivalents (AAE), the total reducing power present in the extract is calculated and given in the table below.

Table 4.9: Total reducing power in dichloromethane fraction of *Mentha arvensis*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 700 nm)**	**Total reducing power (X) value (mg of AAE/gm of dried extract)**
Dichloromethane fraction of *Mentha arvensis*	1	0.571	30.5

4.2.3.3 DISCUSSION:

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in reducing power content. Absorbance of the Dichloromethane is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 30.5 mg of AAE/gm of dried extract of reducing power content was found in the dichloromethane fraction of *Mentha arvensis*.

Chapter 5 : Conclusion

5.1 CONCLUSION:

As the literature review suggests, the presence of several phytochemical compounds in *Mentha arvensis* makes the plant pharmacologically active. The present study showed that it has very good antioxidant activity that could make it a potent drug against free radical mediated diseases. The dichloromethane extract possesses cytotoxic activity that could be a better treatment in tumor as well as cancer. However, studies are required on higher animal model and subsequently on human subjects to prove efficacy as an antioxidant, cytotoxic.

The medicinal values of the leaves of this plant may be related to their phytochemical constituent. So, further investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be tested. It will help in the development of new novel and safe drugs for the treatment of various diseases.

Chapter 6: Reference

Anderson, D., Phillips, B., Tian, WY., Edward, A &Ayesh, R. (2000) 'Effects of vitamin C supplementation in human volunteers with a range of cholesterol levels on biomarkers of oxygen radical generated damage', *Pure Applied Chemistry*, 72:973-983.

Barbera G., Carimi F. and Inglese P., 1992. Past and present role of the Indian-fig prickly-pear Miller, Cactaceae) in agriculture in Sicily. *Economic Botany* 46, 10-20.

Beckett, A.H. and Stenlake, J.B. (1986) *Practical Pharmaceutical Chemistry.* 2: 7576. 3rd edition. London: Athlone P.

Datta, S.C., 1988. ―A handbook of systematic botany‖ , fourth edition, New Age Publishers, New Delhi, page no. 229-231.

Ennouri, M., Fetoui, H., Bourret, E., Zeghal, N., Guermazi, F., Attia, H., (2006). Evaluation of some biological parameters of *Mentha arvensis* 2. Influence of seed supplemented diet on rats. *Bioresource Technology* 97, 2136–2140.

Ernst, E. (2007). "Herbal medicines: balancing benefits and risks". *Novartis Foundation Symposia*:8-282.

Finkel, T. and Holbrook, N. J. (2000).Oxidants: oxidative stress and biology of ageing. *Nature*, 408, pp.239–247.

Galati, E.M., Pergolizzi, S., Miceli, N., Monforte, M.T., Tripodo, M.M., 2002a. Study on the increment of the production of gastric mucus in rats treated with *Mentha arvensis* (L.) Mill. cladodes. *Journal of Ethnopharmacology* 83, 229-233.

Ghani, A. (1998). *Medicinal Plants of Bangladesh*. Dhaka: Asiatic Society of Bangladesh, 14460.

Grabley, S. and Thiericke, R. (1999) 'Bioactive agents and natural sources: trends in discovery and application'. *Adv Biochem Eng Biotechnol*. 64: 101–154.

Halliwell, B &Gutteridge, J. (1999)'The definition and measurement of antioxidant in biological systems, Free Finkel, T & Holbrook, NJ. (2000) 'Oxidants: oxidative stress and biology of ageing', *Nature*, 408:239–247.

Hanna, B. (1998). *Mentha arvensis - field mint*. [online] PDX. Available at: http://web.pdx.edu/~maserj/ESR410/Menthaarvensis13.html [Accessed 14 May 2016].

Hasan, B.A.R. (2012) Medicinal Plants (Importance and Uses), *Pharmaceutica AnalyticaActa*, 3(10).

Jahan, A.I., Nahar, N., Mosihuzzaman, M., Rokeya, B., Ali, L., Khan, A.K.A., Makhmur, T &Choudhury, I.M.(2009) 'Hypoglycaemic and antioxidant activities of *Mentha arvensis* Linn.Fruits',*Natural Product Research*, 23(4):399-408.

Jebashree, H., Kingsley, S., Sathish, E. and Devapriya, D. (2011). Antimicrobial Activity of Few Medicinal Plants against Clinically Isolated Human Cariogenic Pathogens—*An In Vitro StudyISRN Dentistry*, 2011, 4(4), pp.1-6.

Joy, PP., Thomas, J., Mathew, S., Skaria, BP. (1998) 'Medicinal Plants', *Aromatic and Medicinal Plants Research Station*, 1:10-11.

Li, Y &Trush, M. (1994) 'Reactive oxygen dependent DNA damage resulting from the oxidation of phenolic compounds by a copper redox cycle', *Cancer Res*, 54:1895–1898.

Londonkar, R. (2009). Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals. *World Journal of Gastrointestinal Oncology*, 1(1), p.82.

Meyer, B. (1982) Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Med.* 45: 31-34.

Mtambo M. M. (1999) Evaluation of the efficacy of the crude extracts of Capsicum frutescens, Citrus limon against Newcastle disease in domestic fowl in Tanzania. *Ethnopharmacol.* 68: 55-61.

Ojika, L. F. and Nuneza, O. M. (2013) Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. *InternationalResearch Journal of Biological Sciences.* 2: 74-77.

Organogenesis and terpenoidsynthesis in Mentha arvensis. [online] Available at: http://www.sciencedirect.com/science/article/pii/S0367326X01003471 [Accessed 14 May 2016].

Phillipson, J. D. (2001) Phytochemistry and medicinal plants. *Phytochemistry.* 56: 237−243.

Piga, A., 2004. Cactus Pear: A fruit of Nutraceutical and Functional Importance. Journal of the Professional Association for Cactus Development, 9 – 22.

Ponce M, 1989. Distribucion de cactaceas en Venezuela y su ambito mundial (Distribution of cacti in Venezuela and worldwide). *Venezuala, South America*: pp. 21-25.

Pubs.acs.org, 2016)Pubs.acs.org. (2016). *Corn Mint (Menthaarvensis) Extract Diminishes Acute Chlamydia pneumoniae Infection in Vitro and in Vivo - Journal of Agricultural and Food Chemistry (ACS Publications)*. [online] Available at: http://pubs.acs.org/doi/abs/10.1021/jf2032473 [Accessed 14 May 2016].

Raja, R. and Devi, B. (2010). Phytochemical and antimicrobial screening of *Gymnemasylvestre, Mentha arvensis, Solanumsurratense*, extracts in dental caries. *Journal of Pharmacy Research*, 3(1), pp.21-23. Available at: http://www.cabdirect.org/abstracts/20103155668.html [Accessed 14 May 2016].

Rates, S. (2001). ―Plants as source of drugs.*Toxicon.*39(5):603-613.

Robinson, H. 1974. Scanning electron microscope studies of the spines and glochids of the Opuntioideae (Cactaceae). *American Journal of Botany* 61, 278-283.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., Latha, L. Y. (2011) 'Extraction, Isolation an Characterization of Bioactive Compounds from Plants Extract', *African Journal ofTraditional, Complementary and Alternative medicines,* 8:1-10.

Scavroni, J., Boaro, C., Marques, M. and Ferreira, L. (2005). <![CDATA[Yield and composition of the essential oil of *Menthapiperita L.* (Lamiaceae) grown with biosolid]]>. *Brazilian Journal of Plant Physiology*, 17(4).

Shu, Y.Z. (1998). ―Recent natural products based drug development: a pharmaceutical industry perspective.‖ *Journal of Natural Products*. 61:1053–1071.

Singh, S., Sharma, S. and Singh, L. (2013). A review on medicinal plants having antioxidant potential. *Indian Journal of Research in Pharmacy and Biotechnology*, 1(3), pp.404-409.

Singleton, V. L., Rudolf, O. & Rosa, M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent', *Methods in Enzymology*, pp.152-178. .

Sreekanth, D., Arunasree, M.K.,. Roy, K.R., Reddy, T.C., Reddy, G.V., Reddanna, P., 2007. Betanin a betacyanin pigment purified from fruits of *Mentha arvensis* induces apoptosis in human chronic myeloid leukemia Cell line-K562. *Phytomedicine* 14, 739-746.

Tilburt, J. (2008) Herbal Medicine Research And Global Health: An Ethical Analysis. *Bulletin ofthe World Health Organization.* 86: 594-599.

Tiwari, P., Kumar, B. (2011) _Phytochemical screening and Extraction: A Review', *Journal of Internationale Pharmaceutica Sciencia*, 1:98-106.

Veeresham, C. (2012) Natural Products Derived From Plants As A Source Of Drugs. *J AdvPharm Tech Res.* 3: 200-201.

Voirin, B., Brun, N. and Bayet, C. (1990). Effects of daylength on the monoterpene composition of leaves of *Mentha x piperita*. *Phytochemistry*, 29(3), pp.749-755.

Yang, N. Anti-Diabetic Effects Of Polysaccharides From Normal And Streptozotocin-Induced Diabetic Rats. *Innovative Food Science & Emerging Technologies.* 9: 570-574.

Zhishen J., Mengcheng T. and Jianming W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64: 555-559.