BIOLOGICAL INVESTIGATION OF ETHYL ACETATE EXTRACT OF *Mentha arvensis*

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

Submitted by:

Suvra Sarker

ID No : 2011 - 3 - 70 - 029

Department of Pharmacy

East West University

DECLARATION BY THE CANDIDATE

I, Suvra Sarker , hereby declare that this dissertation, entitled ***"Biological investigation of ethyl acetate extract 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 (honors) is a genuine & authentic research work carried out by me under the guidance of Abdullah-Al-Faysal, Lecturer, Department of Pharmacy, East West University, Dhaka. The contents of this dissertation, in full or in parts, have not been submitted to any other institute or university for the award of any degree or diploma of fellowship.

Suvra Sarker
ID: 2011 - 3 - 70 - 029
Department of Pharmacy
East West University
Dhaka.

CERTIFICATION BY THE SUPERVISOR

This is to certify that the dissertation , entitled ***" Biological investigation of ethyl acetate extract of Mentha arvensis"*** is a bonafide research work done, under my guidance and supervision by Suvra Sarker (ID: 2011 - 3 - 70 - 029), in partial fulfillment of the requirement for the degree of bachelor of pharmacy.

Abdullah-Al-Faysal

Lecturer

Department of Pharmacy

East West University

Dhaka

ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled ***"Biological investigations of ethyl acetate extract of Mentha arvensis"*** is a bonafide research work done by Suvra Sarker (ID: 2011 - 3 - 70 - 029), in partial fulfillment of the requirements for the degree of bachelor of pharmacy.

Dr. Shamsun Nahar Khan

Chairperson & Associate Professor

Department of Pharmacy

East West University

Aftabnagar, Dhaka

ACKNOWLEDGEMENT

All praise is for almighty for all the bounties granted to me and only with his guidance and help this achievement has become possible.

It is my pleasure and proud privilege to express my heartiest regards and gratitude to my respected teacher and supervisor Abdullah-Al-Faysal, Lecturer, Department of Pharmacy, East West University, for his expert supervision, constructive criticism, valuable advice, optimistic counseling, constant support and continuous backup and encouragement throughout the very phase of the project as well as to prepare this dissertation.

I would also like to put forward my most sincere regards and profound gratitude to Dr. Shamsun Nahar Khan, Associate professor and Chairperson, Department of Pharmacy, East West University, for giving me the opportunity to conduct such an interesting project and for facilitating a smooth conduction of my study.

I would also like to extend my thanks to all the research students in the lab, lab officers and other staffs of the department of pharmacy for their help and assistance, friendly behavior and earnest co-operation which enabled me to work in a very congenial and comfortable ambience. I owe special thanks to my fellow research group members for their immense support and contribution in my research work.

DEDICATION

This research paper is dedicated

To my beloved parents,

Who are my biggest inspiration...

ABSTRACT

The study was designed for biological investigations of ethyl acetate fraction of *Mentha arvensis*. The powder of *Mentha arvensis* were extracted with methanol and then partitioned with ethyl acetate, petroleum ether and DCM consecutively & the ethyl acetate fraction remaining at the end. The ethyl acetate fraction was remaining at the beaker was investigated for the antioxidant & cytotoxic test. The fraction contained 106 mg AAE/g of total flavaniod content,153 mg AAE/g of total phenolic content & 14.9 mg AAE/g of total reducing power content. Screening for cytotoxic properties using brine shrimp lethality bioassay with Tamoxifen (LC_{50} value of 12.5 µg/ml) as positive control showed that the fraction have considerable more cytotoxic potency exhibiting LC_{50} value 6.25 µg/ml. The ethyl acetate fraction showed more cytotoxic activity & less antioxidant activity. Further investigations are needed for the proper identification and isolation of these bioactive compounds to produce safer drugs for treatment of harmful diseases.

CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

CHAPTER TWO

LITERATURE REVIEW

CHAPTER THREE

METHOD

CHAPTER FOUR

RESULTS AND DISCUSSION

CHAPTER FIVE

CONCLUSION

CHAPTER SIX

REFERENCE

1.1 MEDICINAL PLANT

Since the dawn of history, man has relied so much on medicinal plants for health and food needs. The traditional use of medicinal plants for curing and preventing illnesses, including the promotion of both physical and spiritual well-being among human beings. Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies.

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans. These plants are either “wild plant species” those growing spontaneously in self maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or the contrasting “Domesticated plants species” those that have arisen through human actions such as selection or breeding and depend on management for their existence (Nwachukwu et. al,. 2010).

These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste (Hasan, 2013).

All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care. From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1 and 10% are used as food by humans and other animals. Brazil has the world’s highest biodiversity, accounting for over 20% of the total number of known species. This country presents the most diverse flora, with more than 55 thousand described species, which corresponds to 22% of the global total. Such biodiversity is followed by a wide acceptance of the medicinal plant use. Most of the Brazilian population (80%) consumes only 37% of the commercially available drugs and depend almost exclusively on medicines of natural origin. Thus, phytotherapics entered the market promising a shorter and cheaper production, since basic requirements to use medicinal plants do not involve strict quality control regarding safety and efficacy compared to the other types of drugs.

Recently, the World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care. In Germany, about 600-700 plant-based medicines are available and are prescribed by some 70% of German physicians. In the last 20 years in the United states, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use. During the past three decades, the demand and utilization of medicinal plants has increased globally. There is now a consensus regarding the importance of medicinal plants and traditional health systems in solving the health care problems, efficacy and safety of medicinal plants in curing various diseases. Because of this growing awareness, the international trade in plants of medicinal importance is growing phenomenally, often to the detriment of natural habitats and mother populations in the countries of origin (Motaleb, 2011).

1.2 THE NATURE OF MEDICINAL PLANT

The role of food crops on which most human nutrition is based depends on the primary product of photosynthesis, the carbohydrate, protein, triglycerides (fats and oil). In the case of most drugs, herbs, ethnomedicines, essential oils and cosmetics are derived from the secondary products of plant metabolism such as the alkaloids, terpenoids and flavonoids (Alaribe, 2008). These substances have evolved as responses of plants to stress, predation and competition constituting to what is regarded as the vast chemical library of biological systems. Thus, it is usually “extracts” not the plants themselves or their parts such as fruits, seeds leaves etc; that are used for medicinal effects. However, medicinal plants possess what is referred to as pathological niche and they assume pathogenomic structure. This means that medicinal herbs can be used for different ailments with respect to its on human physiology (Nwachukwu et. al,. 2010).

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

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

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

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

1.3 HISTORY OF MEDICINAL PLANT

The history of medicinal plants is essentially the history of mankind itself, every culture and civilization has used plants extensively since prehistory. Ancient Chinese scriptures and Egyptian Papyrus hieroglyphics describe medicinal uses for plants. 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. 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.

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (WHO and IARC, 2002).

Human beings have used plants for the treatment of diverse ailments for thousands of years. According to the World Health Organization (WHO), 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 effect 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. 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.

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Van Wyk & Wink, 2004).

1.4 SIGNIFICANCES OF MEDICINAL PLANT

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.
- Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.

- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.

- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants (North carolina state university, 2016).

1.5 ALTERNATIVE MEDICINE

These days the term “Alternative Medicine” became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti- malaria medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively (Hasan, 2013).

1.6 SCENARIO OF MEDICINAL PLANT IN BANGLAESH

It has been recorded that about 450 to 500 plants growing or available in Bangladesh have therapeutic values. In Bangladesh, people living in the remote hilly areas, such as, ethnic communities are relying mostly on herbal medicines. In addition, realizing the huge potential of herbal medicine, twenty pharmaceutical companies applied for manufacturing herbal medicine in Bangladesh. About six thousand metric tons of medicinal plants are required annually by the relevant industries in Bangladesh for producing traditional medicines. In an estimate, the international market of medicinal plants related to trade stood at 60 billion US Dollar per year. The demand for medicinal plants based raw materials are growing at an approximate rate of 10-15% per year internationally. Medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of Bangladesh. In recent years, the growing demand for herbal product has led to a quantum jumping in volume of plants materials trade within and across the country. Bangladesh there is no systematic cultivation process or conservation strategies about medicinal plants. The local

people conserve traditional knowledge through their experience and practice, which is handed down orally without any documentation. This knowledge is now under threat to extinction. This is a very alarming situation with regard to natural growth of medicinal plants in the wilderness in this country. In this scenario, the survey on ―Traditional and industrial use and market Scenario of Medicinal plants in Bangladesh. It has been conducted by the DEBTEC researchers at Chakbazar, Dhaka, Bangladesh. We have found that there is worth of 11 million US dollars medicinal plant market in Bangladesh, which have been imported but not in the name of medicinal plants rather in the name of spices and other products. This research aimed at documenting the Present Status and Market Scenario of Medicinal Plants in Bangladesh. Our research finding shows that 84.1% of the respondent use medicinal plants in health care. 18.3% of the villagers use Kabirazi in the disease in medium category.55.0% of our respondent‘s source of knowledge of using medicinal plant is family where 34.7% gained knowledge from neighbor. Only 14.3% of the respondents are involved with trading of medicinal plant. About 10.4% of the villagers are involved in cultivation, collection or business of medicinal plant. From the survey report it has been found that 46.6% industries are using above 60% of imported medicinal plants as their raw materials and 53.3% of the industries are using below 40%. The study revealed that 86.7% industries are importing Indian raw materials, 53.3% are importing the Pakistani one and very few of them are importing the raw materials from Nepal, Iran and Korea. According to the response of shop owners, the local raw materials of their products are mostly coming from 5 different areas of the country. Among those 90% are coming from Chittagong and again 76.6% from Tangail, 30% from Gazipur and another 30% from Khulna. In this scenario, appropriate steps must therefore be taken immediately in order to save this situation with regard to growth, conservation and supply of medicinal plants in the country. The best possible way of doing this is to bringing this more and more of these plants under planned cultivation. The cultivation of medicinal plants in Bangladesh will lead to the conservation and also protect the biodiversity. Ecological and biotic factors are suitable in Bangladesh for the cultivation of medicinal plants. We have been successful to sensitize the policy makers. In Bangladesh there is no facilities and skilled manpower for the processing of MPs. Our research is now aiming to develop processing unit and to train the garden owner for skilled manpower to value addition of MP, which will create the income generating women in rural areas (Bregum, 2010; Motaleb, 2011) .

1.7 PLANT INFORMATION *(Mentha arvensis)*

Mentha arvensis is a perennial plant belonging to the Mentha genus and Lamiaceae family. This mint plant is found in various parts of Europe, Asia and North America. Its common names include Field Mint, Wild Mint or Corn Mint; however, it is better known by its scientific name *Mentha arvensis*. The leaves of this herbal plant have a fresh minty flavor and are used for culinary and medicinal purposes. The essential oil extracted from the leaves also has many uses.

Figure 1.1: *Mentha arvensis* plant

1.7.1 History of *Mentha arvensis*

These herbal plants have been used by humans for approximately 2000 years. It is believed that these plants were first cultivated in Europe during ancient times. However, Japan started cultivating these mint plants for commercial purposes in the late nineteenth century. Countries

like China and Japan used them for various medicinal purposes. Later they were introduced to India where they are still known as Pudina (Exportindia, 2016).

1.7.2 Taxonomy of *Mentha arvensis*

Domain : Eukarya

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatohyta

Phylum : Angiospermophyta

Class : Magnoliopsida

Order : Lamiales

Family : Lamiaceae

Genus : Mentha

Species : Mentha arvensis (Johnson, 2009)

1.7.3 Botanical Name

Mentha arvensis

1.7.4 Synonym of *Mentha arvensis*

Mentha arvensis var. canadensis, Mentha arvensis var. glabrata, Mentha Canadensis (Evergreen, 2015).

1.7.5 *Mentha arvensis* Description

The *Mentha arvensis* is generally described as a perennial herb. Wild mint is found primarily on moist soils in full sun. It is Suitable for : light (sandy), medium (loamy) and heavy (clay) soils

and can grow in heavy clay soil. Suitable pH : acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. It prefers dry or moist soil. This is native to the U.S. (United States) has its most active growth period in the spring .These herbaceous plants have a strong minty fragrance.

- Height: The height of different sub species of this species varies from 10 cm to 60 cm.
- Leaves: The green, simple leaves of *Mentha arvensis* have toothed edges and grow somewhere between 2 cm and 6.5 cm in length and 1 cm and 2 cm in breadth. They grow in opposite pairs. The leaves of each pair grow in opposite directions from each other.
- Flowers: The flowers are pale purple; however, white or pink flowers can also be seen in some subspecies. They grow in clusters with each flower being an average 3-4 mm in size.
- Fruits: 4 small, egg shaped nutlets. (Montana plant life, 2016).
- Stem: The color of the hairy stem varies from brownish- green to green.

Figure 1.2: Leaves of *Mentha arvensis*

1.7.6 Location

These plants grow well in moist places, especially along streams and shores and edges of marshes. These plants are native to the temperate regions of western and central Asia and Europe. They are also found in the eastern regions of Himalaya, various regions of North America and Eastern Siberia.

1.7.7 Cultivation

The preference is full sun to light shade, and moist conditions with rich organic soil. This plant likes low-lying open grassy areas. It is little bothered by disease, and can be very aggressive.

1.7.8 Growing Condition

Soil : Moist and wet soils are ideal for them

Water : Use of water is medium

Sun light : Sun to part shade

Temperature : These plants can survive in cold weathers up to -15^0 C

$CaCO_3$ tolerence: Medium (Lady Bird Johnson Wildflowers center, 2016)

1.7.9 Planting Time

Plant seedlings indoors in late winter or early spring. Once plants reach out 10cm tall, they can be moved outside.

1.7.10 Harvesting

Mentha arvensis leaves can be harvested as soon as they begin growing in the springtime. The new leaves have the best flavor. Make sure to leave the smaller leaves to grow, and pinch back the new stem ends off of the new stem branch growth. This will keep your mint compact and manageable. When harvesting, leave at least 1/3 of the plant intact. The volatile oils of mint are

most potent when harvested in the morning after the dew has dried off (Global Healing Center, 2016)

1.7.11 Habitat

It is a perennial, the root-stock, as in all the Mints, creeping freely, so that when the plant has once taken hold of the ground it becomes very difficult to eradicate it, as its long creeping roots bind the soil together and ultimately overrun a considerable area. It is generally an indication that the drainage of the land has been neglected. It is abundantly distributed throughout Britain, though less common in the northern counties and flourishes in fields and moist ground, and Peppermint growers must be ever watchful for its appearance.

The Corn Mint (*Mentha arvensis*) is the type species of the Japanese Menthol plant, but is not endowed with useful medicinal properties, great care indeed, as has been mentioned, having to be taken to eradicate it from Peppermint plantations, for if mingled with that valuable herb in distilling its strong odor affects the quality of the oil (Grieve, 1971).

1.7.12 Chemical Constituent

The *Mentha arvensis* leaf and oil contain acetaldehyde, amyl alcohol, methyl esters, limonene, -pinene, -phellandrene, cadinene, dimethyl sulphide , and traces of -pinene, sabinene, terpinoline, g-terpinene, fenchene, citronellol and luteolin-7-O-rutinoside.

M. arvensis consists of menthol (35-70%), menthone (15-30%), (-)-menthyl acetate (4-14%) and pulegone (1-4%). And more recently, linarin (acacetin -7-O--rutinoside) was extracted from the flower of *Mentha arvensis* (Globinmed, 2015).

1.7.13 Properties

- antispasmodic, sudorific, emmenagogue.
- Oil is rubefacient and - Carminative, stimulant, stomachic, aromatic, antiseptic, stimulant.
- Tops and leaves are carminative (Godofredo, 2015).

1.7.14 Plant Part Used

Entire plant, aerial parts, leaf and oil.

Figure 1.3: Flower and stem of *Mentha arvensis*

1.7.14.1 Edible uses

- ✓ The mint flavored leaves are used as herb in various cuisines.
- ✓ Sometimes raw leaves are added to salads and other preparations to add flavor to the food.
- ✓ Fresh or dried leaves are used to make herbal tea.
- ✓ The oil extracted from these plants is used as a flavoring agent for beverages and sweets.

1.7.14.2 Medicinal uses

- ✓ The Mentha arvensis leaves are used as a herbal remedy for stomach disorders like indigestion and gas.
- ✓ The leaves are used as anti inflammatory. It is used in the treatments of fever, headache, cold and asthma.
- ✓ Leaves are used to make cough syrups.
- ✓ A decoction made from the Field Mint plants are used to treat stomach pain, diarrhea, vomiting and influenza.
- ✓ Sometimes, dried Field Mint leaves are eaten to reduce chest pains and other heart ailments.
- ✓ It can also be used to reduce toothaches and swellings of gum.
- ✓ The leaves are used to alleviate arthritis.
- ✓ The oil extraction of these leaves is used in the treatments of insomnia and nervous tension.

1.7.14.3 Other uses-

- ✓ *Mentha arvensis* oil is used in soaps, perfumes and other cosmetic products.
- ✓ It is also used as a fragrance element in detergents.
- ✓ This oil is used in aromatherapy.
- ✓ The leaves as well as the oil of these plants are used in toothpastes and mouthwashes.
- ✓ These leaves are also used as insect repellant (Exportersindia, 2016).

1.7.15 Health Benefit Of *Mentha arvensis*

- ✓ Many health benefits can be derived from the Field Mint plants and their essential oil.
- ✓ The Mint leaves can work as blood cleanser with their antibacterial and antiseptic properties.
- ✓ It can alleviate mouth ulcers, toothache and swollen gum.
- ✓ Fresh leaves can cure headache and dizziness.
- ✓ Mint leaves can relief arthritis and joint pains.
- ✓ The leaf infusion helps in curing health disorders like dysmenorrheal, stomachache and diuresis.
- ✓ These plants have antispasmodic and anesthetic properties.
- ✓ Having expectorant properties makes it beneficial for cough, cold, sore throat and fever.
- ✓ The oil extraction of these plants cures various skin problems like acne, ulcer and boils.
- ✓ The oil is beneficial for the nervous system (Exportersindia, 2016).

2.1 ANTIBACTERIAL ACTIVITY OF LEAVES AND INTER-NODAL CALLUS 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 *Mentha arvensis* against *Salmonella typhi(S. typhi) , Streptococcus pyogenes(S. pyogenes) , Proteus vulgaris(P. vulgaris) and Bacillus subtilis(B. subtilis).*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 associated with Proteus sp. Through the bacterial efficacy studies, it is confirmed that the in vitro raised calli tissue was more effective compared to in vivo tissue. The bio-efficacy study confirmed that the calli mediated tissues showed the maximum zone of inhibition. The present study paved a protocol to establish high potential cell lines by in vitro culture (Johnson et. al,. 2011).

2.2 POTENTIATING EFFECT OF *Mentha arvensis* AND CHLORPROMAZINE IN THE RESISTANCE TO AMINOGLYCOSIDE OF METHICILLIN – RESISTANT *Staphylococcus aureus*

This is the first report testing the antibiotic resistance-modifying activity of *Mentha arvensis* against MRSA (methicillin - resistant *Staphylococcus aureus*). In this study an ethanol extract of *Mentha arvensis*. 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 resistance-

modifying activity, such as in the case of aminoglycosides, constituting a new weapon against bacterial resistance to antibiotics, as with chlorpromazine (Coutinho et. al,. 2009).

2.3 ANTIFERTILITY INVESTIGATION AND TOXICOLOGICAL SCREENING OF THE PETROLEUM ETHER EXTRACT OF THE LEAVES OF *Mentha arvensis* IN MALE ALBINO MICE

In male albino mice, the petroleum ether extract of the leaves of *Mentha 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 body weight and libido of the treated ammals remain unaffected. However, a significant decrease in the weight of the testis, epididymis, cauda epididymal sperm count, motility, viability and normal morphology of the spermatozoa was observed. 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 toxicity in male mice (Sharma & Jacob, 2001).

2.4 ENHANCEMENT OF THE ANTIBIOTIC ACTIVITY AGAINST A MULTIRESISTANT *Escherichia coli* BY *Mentha arvensis* AND CHLORPROMAZINE

This is the first report testing the antibiotic resistance-modifying activity of *Mentha arvensis.* 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 strains of Escherichia coli. The growth of two E. coli strains tested was not inhibited by the extract. The minimum inhibitory concentration and minimal bactericide concentration values were ≧1 mg/ml for both strains of E. coli used. A potentiating effect of this extract on gentamicin was

demonstrated. Similarly, there was a potentiating effect of chlorpromazine on kanamycin, amikacin and tobramycin, indicating the involvement of an efflux system in the resistance to these aminoglycosides. It is therefore suggested that extracts from *M. arvensis* could be used as a source of plant-derived natural products with resistance-modifying activity, such as in the case of gentamicin, constituting a new weapon against bacterial resistance to antibiotics, as with chlorpromazine (Countinho et. al,. 2008).

2.5 STUDIES ON ACTIVITY OF VARIOUS EXTRACTS OF *Mentha arvensis* AGAINST DRUG INDUCED GASTRIC ULCER IN MAMMALS

To examine the antiulcerogenic effects of various extracts of *Mentha arvensis* on acid, ethanol and pylorus ligated ulcer models in rats and mice. There was a decrease in gastric secretion and ulcer index among the treated groups i.e. petroleum ether (53.4%), chloroform (59.2%), aqueous (67.0%) and in standard drug (68.7%) when compared to the negative control. In the 0.6 mol/L HCl induced ulcer model in rats (n = 6) there was a reduction in ulcerative score in animals receiving petroleum ether (50.5%), chloroform (57.4%), aqueous (67.5%) and standard. drug (71.2%) when compared to the negative control. In the case of the 90% ethanol-induced ulceration model (n = 6) in mice, there was a decrease in ulcer score in test groups of petroleum ether (53.11%), chloroform (62.9%), aqueous (65.4%) and standard drug ranitidine (69.7%) when compared to the negative control. It was found that pre-treatment with various extracts of *Mentha arvensis* Linn in three rat/mice ulcer models i.e; ibuprofen plus pyloric ligation, 0.6 mol/L HCl and 90% ethanol produced significant action against acid secretion (49.3 ± 0.49 vs 12.0 ± 0.57, $P < 0.001$). Pre-treatment with various extracts of *Mentha arvensis* Linn showed highly -significant activity against gastric ulcers (37.1 ± 0.87 vs 12.0 ± 0.57, $P < 0.001$). Various extracts of *Mentha arvensis*. 375 mg / kg body weight clearly shows a protective effect against acid secretion and gastric ulcers in ibuprofen plus pyloric ligation, 0.6 mol/L HCl induced and 90% ethanol-induced ulcer models (Londonkar & Poddar, 2009).

2.6 INHIBITION OF IMMUNOLOGIC AND NONIMMUNOLOGIC STIMULATION- MEDIATED ANAPHYLACTIC REACTIONS BY THE AQUEOUS EXTRACT OF *Mentha arvensis*

The effect of aqueous extract of *Mentha arvensis* (MAAE) on immunologic and nonimmunologic stimulation-mediated anaphylactic reactions was studied. Nonimmunologic anaphylactic reaction was induced by compound 48/80 injection. MAAE (0.005 to 0.5 g/kg) inhibited systemic anaphylactic reaction induced by compound 48/80. 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 g/kg) dose-dependently inhibited passive cutaneous anaphylaxis (PCA) when intraperitoneally, intraveneously and orally administered. 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 (Shin, 2003).

2.7 ANTIOXIDANT POTENTIAL OF METHANOL ROOT EXTRACT OF *Mentha arvensis*

The study was carried out to evaluate the phytochemical constituents and antioxidant potential of methanolic root extract of *Mentha arvensis*. Methanolic root extract of *Mentha arvensis* posses string antioxidant activity, evidenced by the free radical scavenging property, iron chelating, reducing power property, Nitrous oxide scavenging and H_2O_2 scavenging, which may be due to the presence of phenolic and flavonid components in the extra ct. Overall, the methanolic root extract of *Mentha arvensis* is a source of natural antioxidant that can be important in disease prevention, health preservation and promotion of longevity promoter (Dar et. al,. 2014).

2.8 IN VITRO THROMBOLYTIC AND CYTOTOXIC EVALUATION OF *Mentha arvensis*

Atherothrombotic diseases or coronary artery thrombosis are common disorders which are treated by streptokinase (SK), urokinase (UK) or tissue plasminogen activators (t - PA). Because of the high risk of bleeding, severe anaphylactic shock, intracranial hemorrhage and lacks of specificity of these drugs, they are restricted to those patients who have undergone surgery or those with a history of gastrointestinal bleeding or hypertension. Therefore, plant based drugs are used because they are cheap, safe, low side effects and effective against many diseases. The study was carried out to check the clot lysis effect and cytotoxic effects of *Mentha arvensis* using streptokinase as a positive control and water as a negative control. In this experiment, the *M. arvensis* showed 32.56% clot lysis activity in case of methanol extract , 32.04% clot lysis activity in case of ethanol extract , 31.87% clot lysis activity in case of chloroform extract and 30.29% clot lysis activity in case of acetone extract respectively . In brine shrimp cytotoxic assay the methanol extracts *M. arvensis* showed LC_{50} value s of 2.088 which was referred to Vincristine sulfate. From this study, it can be said that the *M. arvensis* has clot lysis activity and low cytotoxic activity. So, these plant s could be incorporated as a thrombolytic agent with I n vivo effects to improve the atherothrombotic patients (Shahik et. al,. 2014).

2.9 PHYTO-CHEMICAL ANALYSIS, ANTI-ALLERGIC AND ANI-INFLAMMATORY ACTIVITY OF *Mentha Arvensis* IN ANIMALS

Allergic diseases are fairly common in all parts of the world and involve all ethnic groups with bronchial asthma, allergic rhinitis, conjunctivitis and eczema being the commonest manifestations. The qualitative and quantitative determination of phyto - chemicals and assessment of the anti - allergic and anti - inflammatory activities using aqueous and organic extracts of different plant parts (root, stem and leaves) of *Mentha arvensis* in animals showed that all parts of *M . arvensis* (specifically leaves) are rich source of secondary phytoconstituents, which impart their therapeutic effects against allergic and inflammatory diseases. These results support the claim about the use of this herb in folk medicines (Malik et. al,. 2012).

2.10 ANTIMICROBIAL ACTIVITY OF *Mentha arvensis* AND *Zingiber officinale* ESSENTIAL OILS

The aim of the study was to evaluate the antimicrobial effects of essential oils in vitro for a possible application to reduce the content of microorganisms in the air of animal farms. The essential oils *Mentha arvensis* and *Zingiber officinale* were screened against bacteria *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani and Chalmers, *Proteus mirabilis* Hauser and yeast *Candida albicans* Berkhout. The minimal inhibitory concentrations of the active essential oils were tested using broth dilution assay at concentrations ranging from 0.1–50.0%. The oils showed a wide spectrum of antibacterial activity: concentrations of 0.1–0.8% of *Mentha arvensis* reduced the total bacterial counts of *Proteus mirabilis* Hauser and *Candida albicans* Berkhout . The dilution method revealed that essential oil *Zingiber officinale* only at high bacteriocidal concentrations was able to stop the bacterial growth. *Zingiber officinale* R . at 50.0% completely inhibited the growth of *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani and Chalmers, *Proteus mirabilis* Hauser and yeast *Candida albicans* Berkhout (Mickiene, Ragazinskiene & Bakutis, 2011).

2.11 HEPATOPROTECTIVE ACTIVITY OF *Mentha arvensis* LEAVES AGAINST CCl_4 INDUCED LIVER DAMAGE IN RATS

To study the Hepatoprotective activity of ethanol, chloroform and aqueous extracts of *Mentha arvensis* leaves against CCl_4 induced liver damage in rats. Hepatotoxicity was induced by CCl_4 and the biochemical parameters such as serum glutamate pyruvate transminase (sGPT), serum glutamate oxaloacetate transaminase (sGOT), alkaline phosphatase (sALP), serum bilirubin (sB) and histopathological changes in liver were studied along with silymarin as standard Hepatoprotective agents. The Phytochemical investigation of the extracts showed presence of flavonoids, steroids, triterpenoids, alkaloids, glycosides, carbohydrates, tannins, phenolic compounds. Treatment of the rats with chloroform, ethanol and aqueous extract with CCl_4 administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB ($P <$

0.01) almost comparable to the silymarin. The Hepatoprotective was confirmed by histopathological examination of the liver tissue of control and treated animals. From the results it can be concluded that *Mentha arvensis* possesses Hepatoprotective effect against CCl_4 induced liver damage in rats (Patil & Mall, 2012).

2.12 PHARMACOGNOSTIC STANDARDIZATION, PHYSICO AND PHYTOCHEMICAL EVALUATION OF AERIAL PARTS OF *Mentha arvensis*

The present study deals with the macroscopical and microscopical studies of aerial parts of M*entha arvensis*. Microscopically, aerial parts showed glandular trichomes, helical to spiral xylem, palisade tissues with columnar cells, diacytic stomata. Powder microscopical examination showed the presence of glandular and uni to multi celled trichomes, helical to spiral xylem vessel, stomatal epidermal cells, abundant xylem vessels with pitted thickenings, abundant thin walled parenchymatius cells, epidermis with cuticle and collenchymatous cells, parenchymatous cells with reddish tannin contents. Physicochemical parameters and preliminary phytochemical studies of the powdered aerial parts were also carried out. Total ash was approximately sixteen and four times more than acid insoluble and water soluble ash, respectively. Water soluble extractive was slightly higher than ethanol soluble extractive. T.L.C. of petroleum-ether, chloroform and ethanol extract showed eight spots, nine spots and six spots, respectively. Phytochemically, it exhibited alkaloids, glycosides, steroids and sugars. These findings might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs (Sandeep et. al,. 2010).

2.13 MENTHOL TOLERANT CLONES OF M*entha arvensis*: APPROACH FOR IN VITRO SELECTION OF MENTHOL RICH GENOTYPES

In vitro raised shoots of *Mentha arvensis* L. were screened for menthol tolerance level by growing them in media containing 0–100 μg ml^{-1} menthol. A total of 2850 regenerated shoots

were step wise screened for menthol tolerance at the concentrations of 50 μg ml^{-1} followed by 60 and 70 μg ml^{-1}. In this screening, only 30 individual regenerated shoots were able to survive. The clones from the primary screen were inoculated into rooting medium and, after rooting, transferred to pots in the greenhouse. Ultimately, these 30 menthol tolerant clones were multiplied and grown in the field in replicated plots of 2.5×2.5 m sizes. Twigs of 30 clones from the replicated trials were rechecked for tolerant phenotypes at a concentration of 70 μg ml^{-1} menthol wherein, these survived even after 7 days (secondary screening). These clones were checked for oil and menthol content and were found to be better than the control plants. Out of these 30 plants, five tolerated 80 μg ml^{-1} menthol (tertiary level screening) and were found to contain the highest amount of menthol per g leaf biomass. Molecular analysis through RAPD showed distinct variation in the profiles of these five plants, in comparison to the control. Using this method the relationship between the primer OPT 04, menthol tolerance and high menthol content character of the genotype was established. Further, a cultivar `Saksham' was released from the selections by CIMAP for superior performance (Dhawan et. al,. 2003).

2.14 ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF M*entha arvensis* AGAINST PROTEUS MIRABILIS FROM URINARY TRACT INFECTED PATIENTS

In the present study 63 urine samples were collected from Urinary tract infected patients from various hospitals in Salem District, Tamilnadu, India. The collected samples were subjected to microscopic observation and biochemical characterization to identify the presence of bacteria The Proteus mirabilis were isolated on specific medium using XLD (xylose lysine deoxycholates agar deficient) , Macconkey agar , Mullen hinton agar, CLED and UTI agar. The purified Proteus mirabilis were performed in biochemical tests such as IMVIC tests etc. The positive isolate was used. Leaves of *Mentha arvensis* were extracted by using acetone, isopropyl alcohol and petroleum ether, the compound was purified and preliminary phytochemical studies was done. A comparative study on the total antibiotic activity of plant extract were found to be effective against the tested isolated organism Proteus mirabilis and MTCC 442 strain. MIC and MBC was performed by agar dilution method and the range was found to be 0.97mg/ml to 250

mg/ml. from the result that particular plant extract (*Mentha arvensis*) showed high antibacterial activity against tested organism (Srinivas & Arun, 2012).

2.15 ANTIBACTERIAL ACTIVITY AND MODE OF ACTION OF MENTHA ARVENSIS ETHANOL EXTRACT AGAINST MULTI-DRUG RESISTANT *Acinetobacter baumannii*

To evaluate the antibacterial effect of ethanol extract of *Mentha arvensis* against multi-drug resistant *Acinetobacter baumannii* using liquid chromatography – mass spectrometry (LC - ESI - MS). Disc diffusion and microdilution assays were used t o evaluate the antibacterial effect of the extract by measuring the zone of inhibition, minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of the extract against the test bacteria. Scanning electron micros copy (SEM) was employed to evaluate the morphological changes induced by the extract in cellular membrane of the bacteria. Reactive oxygen species (ROS) generation and protein leakage from the bacterial cells induced by the extract were also evaluated. The ex tract showed dose - dependent growth inhibitory effects against *A. baumannii* with MIC and MBC of 23.5 and 72.1 μg/mL, respectively. The extract also induced potent ROS generation and protein leakage in *A. baumannii* bacterial cells. SEM findings revealed that the extract induced potential cellular damage which increased with increasing extract concentration. Conclusion: The ethanol extract of *Mentha arvensis* is a potent antibacterial agent against *A. baumannii* and acts by inducing lethal cellular damage to the bacterium (Zhang et. al,. 2015).

2.16 IDENTIFICATION OF FLAVONE AGLYCONES AND FLAVONOL GLUCOSIDES IN ETHYL ACETATE EXTRACT OF *Mentha arvensis*

Four flavonoids (two flavonol glycosides and two flavones) were isolated from ethyl ace- tate extract of *Mentha arvensis* leaves. The structures were determined by usual spectroscopic methods (UV and 1 H NMR). This study confirmed that *Mentha arvnsis* can be a good source of antioxidant polyphenols. In recent study kaempferol and apigenin and their derivatives, have

been found to possess preventive and therapeutic potential against several kinds of cancer (Boureghda, Tebboub & Benayache, 2013).

2.17 CHEMICAL COMPOSITION OF THE ESSENTIAL OIL AND NITROGEN METABOLISM OF MENTHOL MINT (*Mentha arvensis*) UNDER DIFFERENT PHOSPHORUS LEVELS

The purpose of this work was to evaluate the effects of different phosphorus levels (0.05, 0.5, 1 and 2 mm) under nitrogen metabolism and the essential oil profile of menthol mint (*Mentha arvensis*). The relationship between the leaf maturity and the essential oil profile was also explored. The experiment was conducted in a hydroponic system located in a grow chamber during 41 days and after the harvest, nitrate reductase activity, and the NO_3^--N, amino -N, and soluble sugars levels of each plant part were evaluated. Also the essential oil from young leaves (6th to 8th node) and adult leaves (3rd to the 5th node) was analyzed. An uptake mechanism related to the increase in fresh weight of the roots was promoted with the use of low P levels (0.05 and 0.50 mmol·L^{-1}). With 1 mmol·L^{-1} P plants showed in all parts an increased nitrate reductase activity and high levels of nitrate and amino -N in leaves. Plants submitted to the lowest P level (0.05 mmol·L^{-1}) presented high levels of menthol. In leaves from 6th to 8th node (mature leaves) men-thol level was approximately 87% of the essential oil however leaves from the 3rd to the 5th node (young leaves) showed high levels of pulegone, that can be toxic for humans. The results indicate that the essential oil quality in menthol mint is influenced by the leaf maturity and the P levels. Also it was showed that the oil extracted from mature leaves of plants under low P levels has the best commercial profile (Souza et. al,. 2014).

3.1 COLLECTION & PREPARATION OF PLANT MATERIAL

Plant sample (Leaves) of *Mentha arvensis* was collected from Gopalgong in February 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 24 hours 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 39^0C 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 Parttion with Ethyl 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 fraction was further investigated for different pharmacological properties (antioxidant and cytotoxic).

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- Artemia salina) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus Artemia of aquatic crustaceans. Artemia is the only genus in the family Artemiidae (Olowa and Nuneza, 2013; Rishikesh et.al,. 2013).

3.5.2 Apparatus and reagents

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

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

3.5.3 Procedure

3.5.3.1 Preparation of sea water

To hatch the brine shrimp nauplii for the assay, sea water representing brine should be prepared at first. To prepare sea water 38gm of pure NaCl was dissolved in distilled water and then the

volume made up to 1000ml by distilled water in a 1000ml beaker for Artemia salina hatching. 1-2 drops of NaOH solution of 1N was added with a dropper to obtain the P^H 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 Artemia salina Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of Artemia salina were hatched at room temperature (25-30ºC) for 18-24hr. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay Hatching of Brine Shrimp.

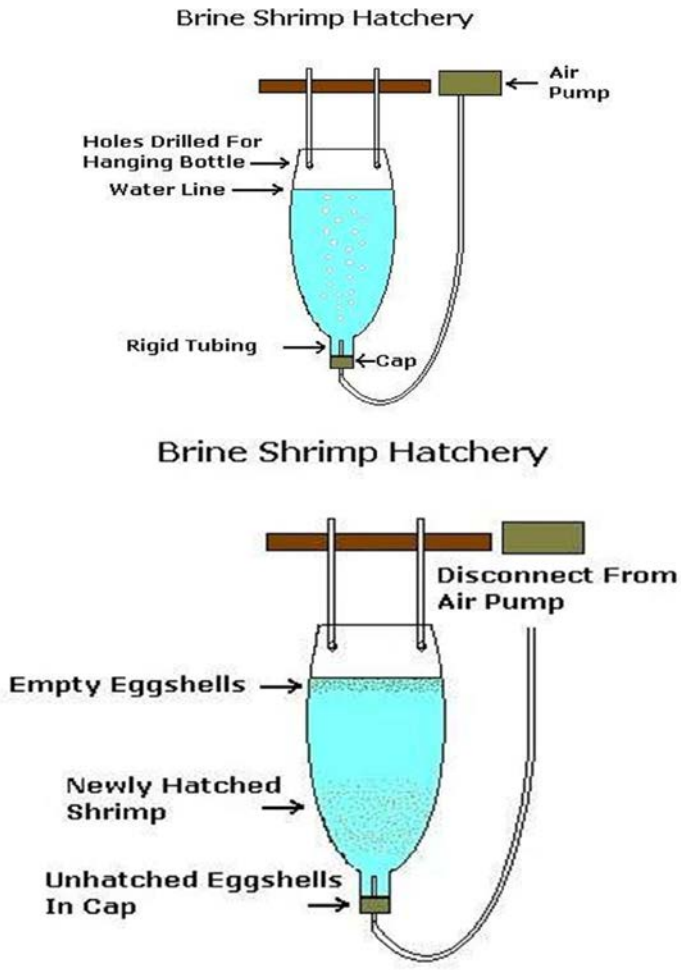


Figure 3.2: Brine shrimp Hatchery

3.5.3.2 Preparations 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.

3.5.3.3 Preparation of the test sample of experimental plant

All the test samples of 4mg were taken and dissolved in 200µl of pure dimethylsulfoxide (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 test-tubes to get the positive control groups.

3.5.3.5 Preparation of 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 nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. 3.6 Antimicrobial Activity by Disc Diffusion Method.

3.6 ANTIOXIDANT ACTIVITY

3.6.1 Total Phenolic Content

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, it has been reported that there is an inverse relationship between the antioxidative status occurrences of human diseases. In addition, antioxidant compounds which are responsible For Such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders. Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the compounds, of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid per oxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidative effects have rarely been carried out. The purpose of this study

was to evaluate extractives of *Mentha arvensis* as new potential sources of natural antioxidants and phenolic compounds. This study also demonstrates a possible relationship between phenolic content and antioxidant activity.

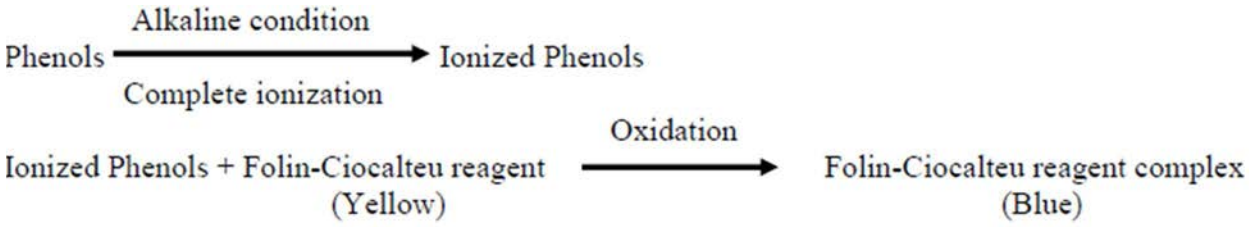
3.6.1.1 Principle

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.

Table 3.2: Composition of 100 mg Folin-Ciocalteu Reagent

Ingredient	Amount
i. Water	57.5 ml
ii. Lithium Sulfate	15.0mg
iii. Sodium Tungstate Dihydrate	10.0mg
iv. Hydrochloric Acid (25%)	10.0mg
v. Phosphoric Acid 85% solution in water	5.0mg
vi. Molybdic Acid Sodium Dihydrate	2.5mg

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 oneor two-electron reduction reactions lead to blue species, possibly (PMoW11O40)-4.The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound (Singleton et.al,., 1999; Vinson et.al,., 2005).



3.6.1.2 Apparatus & reagents

Table 3.3 : Apparatus & 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 (50-200 µl)
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 Na_2CO_3 (7.5%w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 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 preparation:

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

3.6.2.2 Apparatus & reagents

Table 3.4: Apparatus & reagents used for total flavonoid content

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

3.6.2.3 Procedure

Preparation of 10% Aluminium Chloride ($AlCl_3$) Solution: 10mg of $AlCl_3$ 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) $NaNO_2$ Solution: 5mg of $NaNO_2$ was 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 3.5: Preparation of standard solution

Concentration (µg/ml)	Solution taken from stock solution (µl)	Volume adjusted by 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 $NaNO_2$ was added and incubated for 6 minutes. 10% $AlCl_3$ 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 was repeated. Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank.

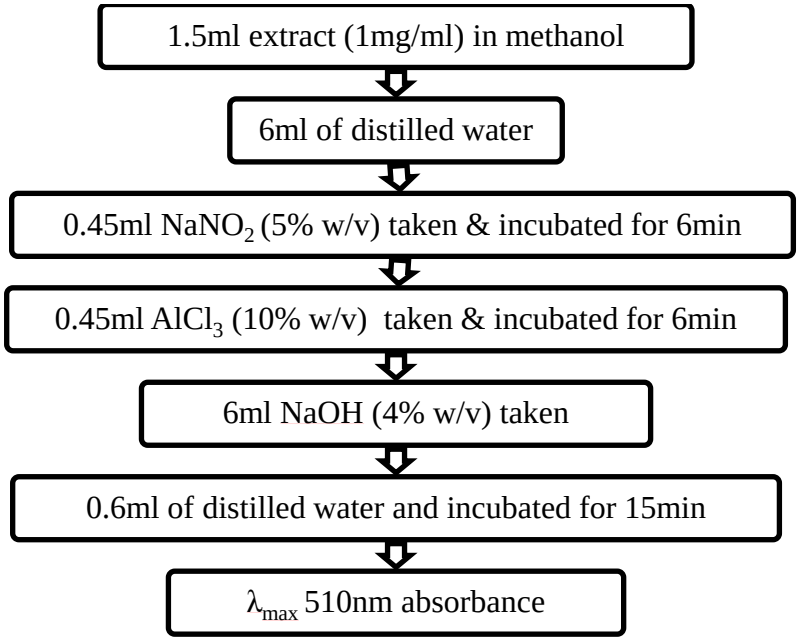


Figure 3.3: Schematic diagram of preparation of extract solution

Preparation of blank solution:

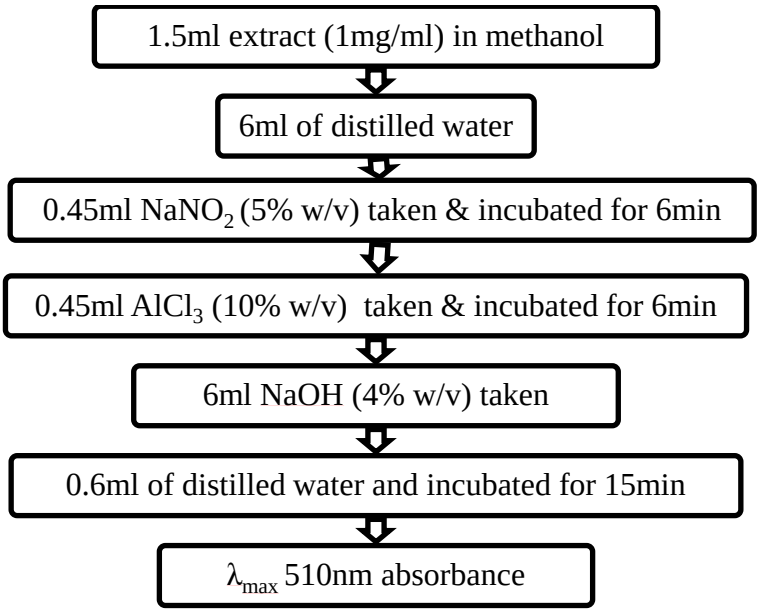


Figure 3.4: Schematic diagram of preparation of blank solution

3.6.3 Reducing Power Assay:

3.6.3.1 Principle:

The reducing power of ethyl acetate extract of *Mentha arvensis* was determined by the method of Oyaizu. Substances, which have reduction potential, 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 $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + ferrous chloride

3.6.3.2 Apparatus and reagents:

Table 3.6: Apparatus and reagents used for reducing power assay

Spatula	Potasium farricyanide
Analytical balance	Methanol

Pipette and pumper	Ascorbic acid
Aqueous fraction	Trichloro acetic acid
Test tubes	Phosphate buffer
Beaker	Farric chloride

3.6.3.3 Procedure:

Phosphate buffer (0.2 M, P^H 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.

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

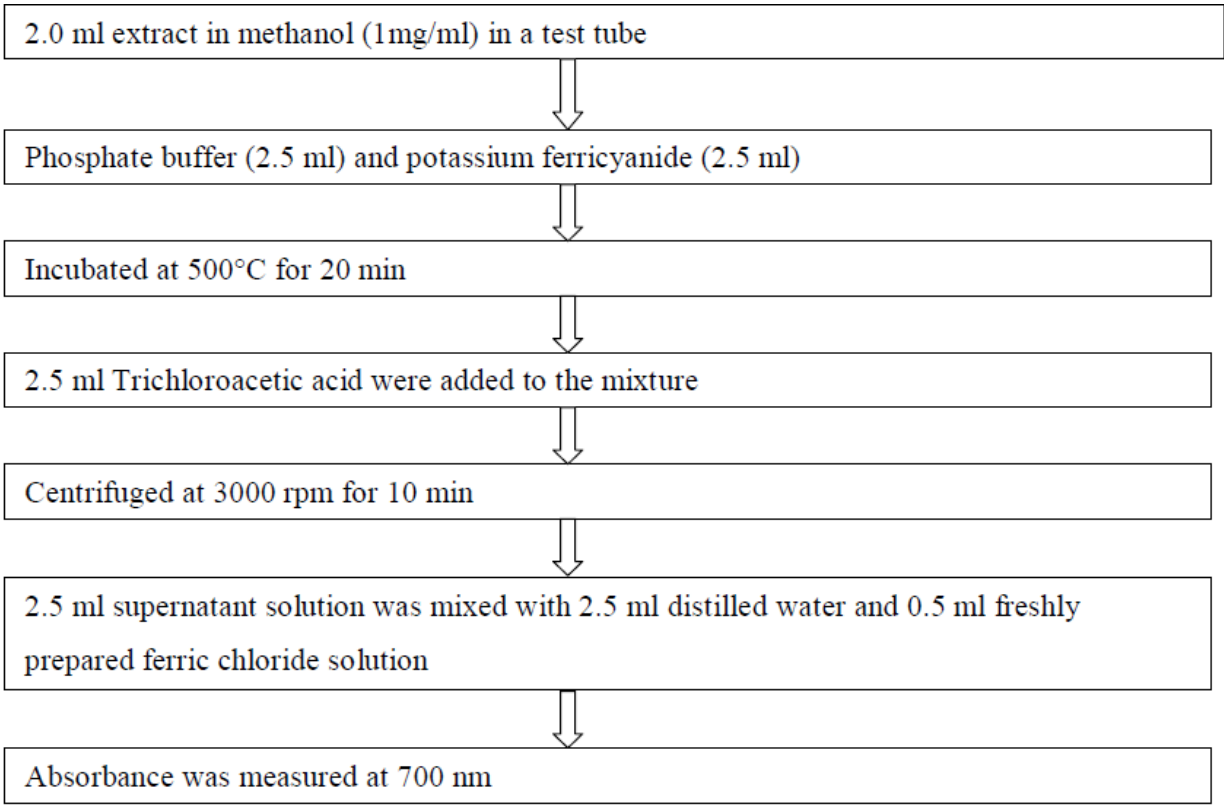
Standard solution preparation: The stock solution was prepared by taking 0.025 gm of ascorbic acid and dissolved into 5 ml of methanol. The concentration of this solution was 5000 µg/ml of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

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 the solution 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.



$$\% \text{ increase in reducing} = \frac{A_{test}}{A_{blank}} - 1 \times 100\%$$

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).

4.1 RESULT OF BRINE SHRIMP LETHALITY BIO-ASSAY:

The ethyl acetate fraction of the *Mentha arvensis* extract was subjected to brine shrimp lethality bioassay. After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors counted. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a median lethal concentration (LC_{50}) value. LC_{50} represents the concentration of the standard and 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:

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

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 (LogC) 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 nauplii alive	Number of nauplii dead	% 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	

6	12.5	1.097	5	5	50	12.5
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	0.78125	-0.107	9	1	10	

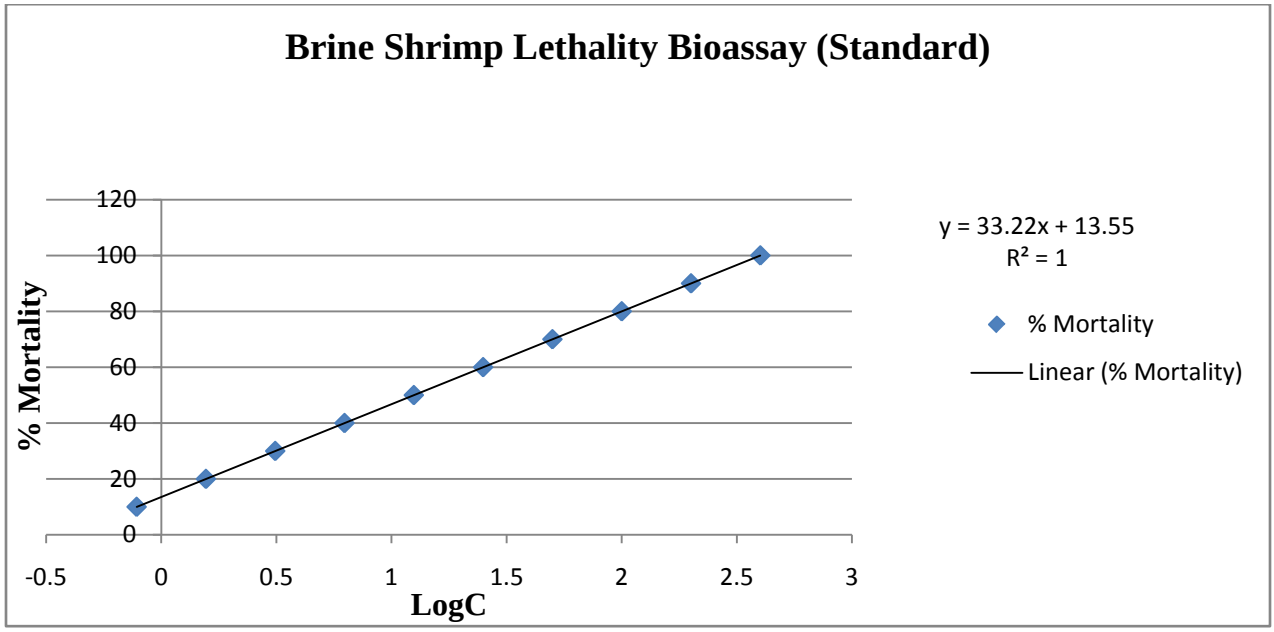


Figure 4.1: % Mortality and predicted regression line of tamoxifen (standard)

4.1.2 Preparation of Ethyl Acetate Fraction Curve:

Table 4.2: Results of the bioassay of ethyl acetate fraction (extract)

Test tube no.	Concentration (c) (µg/ml)	LogC	Number of nauplii alive	Number of nauplii dead	% 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	
6	12.5	1.097	4	6	60	
7	6.25	0.796	5	5	50	6.25
8	3.125	0.495	5	5	40	
9	1.5625	0.194	7	3	30	
10	0.78125	-0.107	8	2	20	

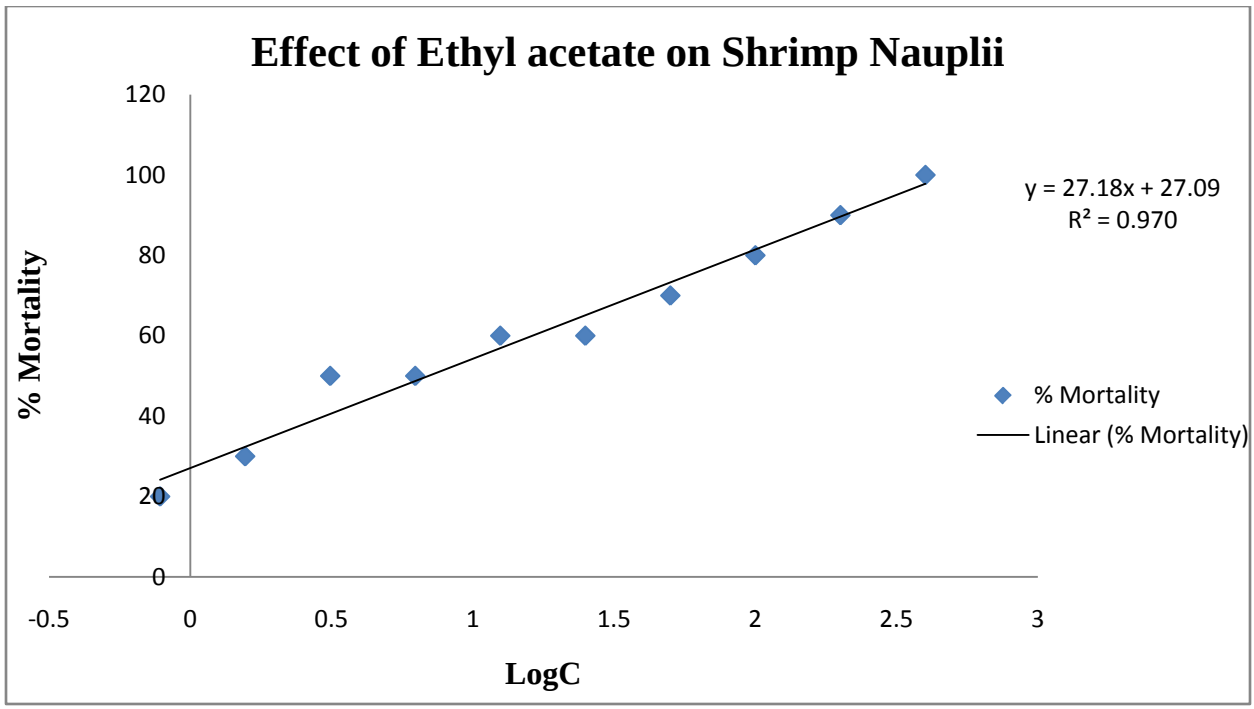


Figure 4.2: % Mortality and predicted regression line of ethyl acetate fraction (extract).

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 ethyl acetate 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 ethyl acetate fraction of *Mentha arvensis*

Sample	Linear regression equation	R^2 Value	LC_{50} (µg/ml)
Standard (Tamoxifen)	y = 33.22 x + 13.55	1	12.5
Extract (Ethyl acetate fraction)	y = 27.18 x + 27.09	0.970	6.25

In this investigation, standard and ethyl acetate fraction exhibited cytotoxic activities with the LC_{50} values 12.5µg/ml and 6.25µg/ml respectively as shown in table 4.3. For ethyl acetate fraction LC_{50} value is less than the standard which indicates that the extract has more potent activity than standard against brine shrimp nauplii.

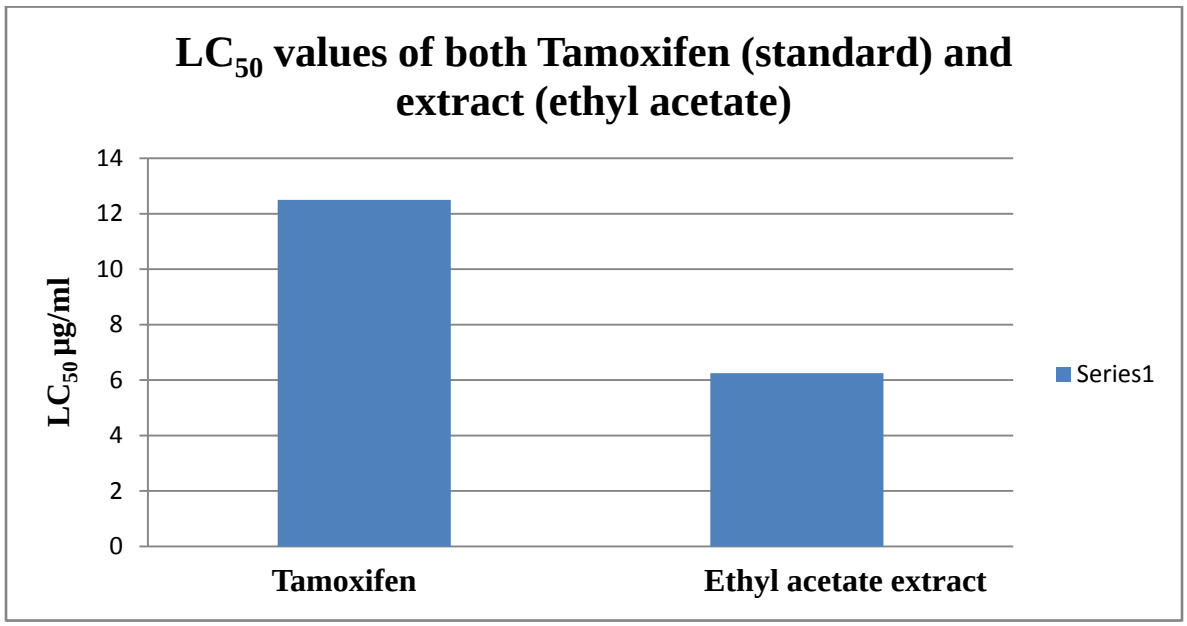


Figure 4.3: Comparison between LC_{50} values of standard and extract

From the above figure it can be concluded that for ethyl acetate fraction the lethal concentration required to kill 50% of the sample population is lower than the standard. So the extract is more potent than tamoxifen (standard) at lower concentration.

4.2 RESULT OF ANTIOXIDANT TESTS:

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 ethyl acetate fraction of *Mentha arvensis* extract was determined by following methods:

- ✓ Determination of total phenolic content.
- ✓ Determination of total flavonoids content.
- ✓ Determination of total reducing power content.

4.2.1 Result of Total Phenolic Content:

The ethyl acetate extract of *Mentha arvensis* were subjected to determine total phenolic content. Ascorbic acid was used as reference standard (Singleton et al., 1999).

4.2.1.1 Preparation of standard curve:

Table 4.4: Total phenolic content of ascorbic acid

Concentration (µg/ml)	Absorbance (at 765 nm)	Regression line	R^2 value
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.

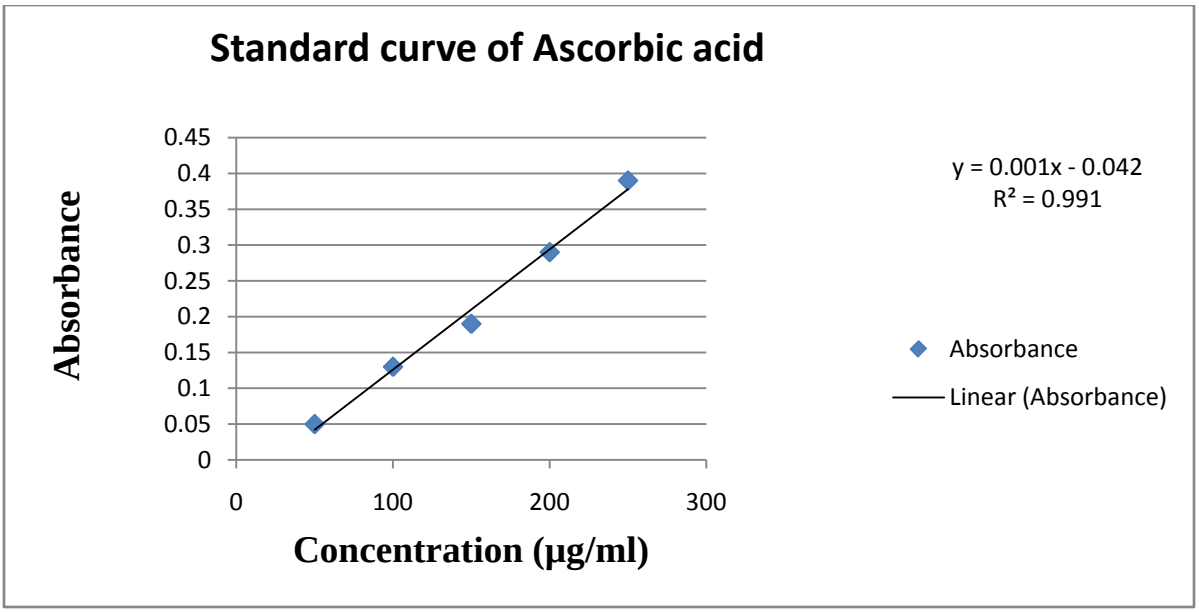


Figure 4.4: Graphical representation of assay of phenolic content of ascorbic acid

4.2.1.2 Total phenolic content present in ethyl acetate extract of *Mentha arvensis*:

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 4.5: Total phenolic content of ethyl acetate fraction of *Mentha arvensis*

Concentration (mg/ml)	Absorbance	Total phenolic value (mg of AAE/g of dried extract)
2	0.111	153

4.2.1.3 Discussion:

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the

ethyl acetate fraction 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, 153 mg of AAE/gm of dried extract of phenol content was found in the ethyl acetate fraction of *Mentha arvensis.*

4.2.2 Result of Total Flavonoid Content:

The ethyl acetate fractions of *Mentha arvensis* were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard (Chang C et al., 2002).

4.2.2.1 Preparation of standard curve:

Table 4.6: Total flavonoid content of ascorbic acid

Concentration (µg/ml)	Absorbance	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 4.5. This linear curve was considered as a standard curve.

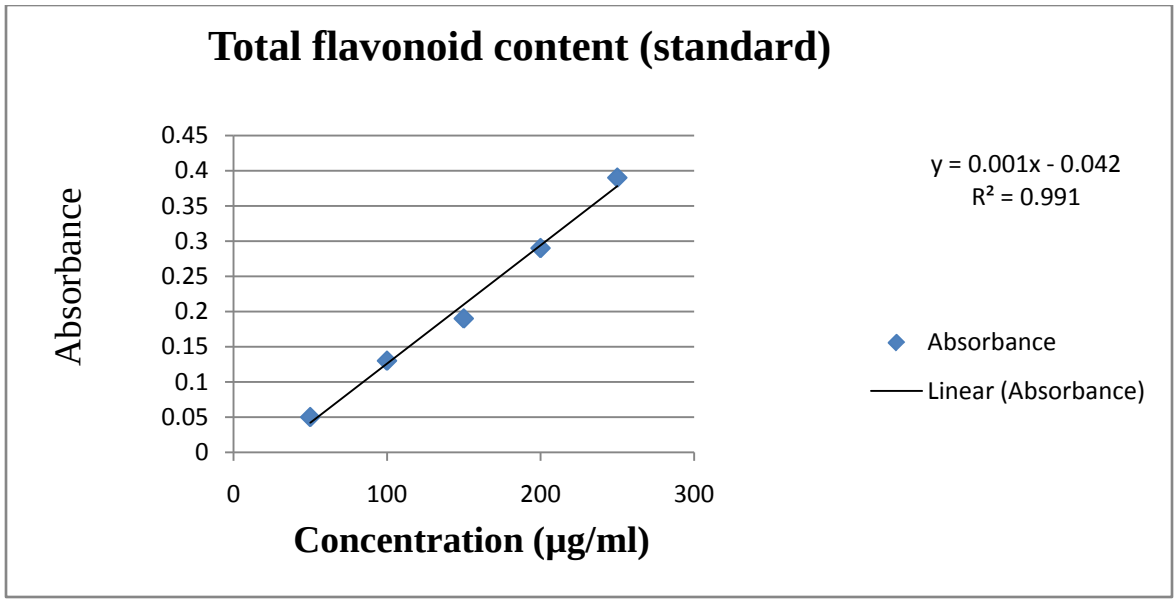


Figure 4.5: Graphical representation of assay of flavonoid content of ascorbic acid

4.2.2.2 Total flavonoid content present in ethyl acetate fraction of *Mentha arvensis*:

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 4.7.

Table 4.7: Total flavonoid content of ethyl acetate fraction of *Mentha arvensis*

Concentration (mg/ml)	Absorbance	Total flavonoid content (mg of AAE/g of dried extract)
1	0.064	106

4.2.2.3 Discussion:

To determine the total flavonoid content of the test samples the standard curve was used. For 1 mg/ml concentration of ethyl acetate fraction of *Mentha arvensis* 106 mg of AAE/gm of dried extract of flavonoid content was found.

4.2.3 Result of Total Reducing Power Assay:

The ethyl acetate extract of *Mentha arvensis* were subjected to determine total reducing power. Ascorbic acid was used as reference 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	Regression line	R^2 value
250	2.657	y = 0.010x + 0.266	0.821
200	2.126		
150	2.284		
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.

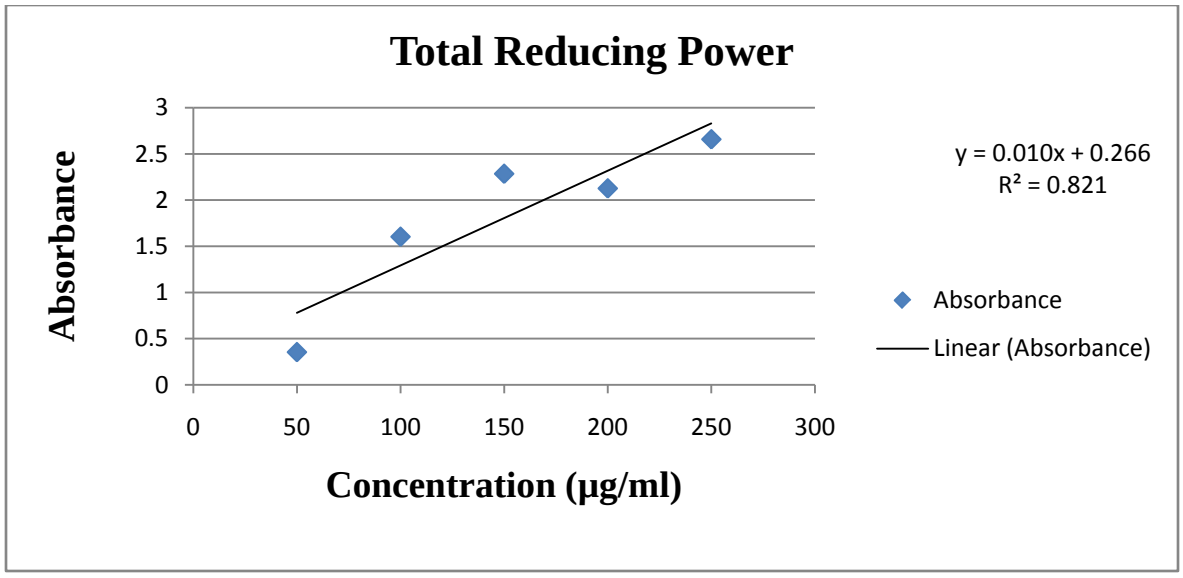


Figure 4.6: Graphical representation of reducing power of ascorbic acid

4.2.3.2 Total reducing power assay in ethyl acetate extract 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 ethyl acetate fraction of *Mentha arvensis*

Concentration (mg/ml)	Absorbance	Total reducing power (mg of AAE/g of dried extract)
1	0.415	14.9

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 ethyl acetate fraction 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, 14.9 mg of

AAE/gm of dried extract of reducing power content was found in the ethyl acetate fraction of *Mentha arvensis*.

5.1 CONCLUSION:

As the literature review suggests, the presence of several phytochemical compounds in ethyl acetate fraction of *Mentha arvensis*, makes the plant pharmacologically active.

LC_{50} value of *Mentha arvensis* in ethyl acetate fraction showed more cytotoxic activity than tamoxifen. Since ethyl acetate fraction of *Mentha arvensis* exhibited potent cytotoxic activity, so it can be investigated for anticancer, pesticidal and antitumor properties in future.

Antioxidant property in ethyl acetate extract of *Mentha arvensis* was determined by phenolic content assay, flavonoid content assay and reducing power assay. Phenolic content was 153 mg/gm, flavonoid content was 106 mg/gm and reducing power was 14.9 mg/gm in ethyl acetate extract of *Mentha arvensis*. So ethyl acetate extract of *Mentha arvensis* have poor antioxidant property. Mixture of compounds can lower antioxidant property in ethyl acetate fraction of *Mentha arvensis*, if any counteracting compounds were present in mixture. So pure compound isolation should be done in future to confirm antioxidant property of ethyl acetate fraction of *Mentha arvensis*.

Further investigations can be carried out to isolate and identify the active compounds present in the plant that are responsible for pharmacological activity in the development of novel and safe drugs. Other tests can be performed to evaluate some other pharmacological activities.

Boureghda, A., Tebboub, O. and Benayache, S. (2013). Identification of flavone aglycones and flavonol glucosides in ethyl acetate extract of *Mentha arvensis*. *International Journal of Pharma and Bio Sciences*. 4 (3). p.69-73.

Bregum, F. (2010). The present status of Medicinal Plants in Bangladesh. *Iranian Journal of Pharmaceutical Research*. p.34-34.

Chang, C., Yang, M., Wen, H. and Chern, J. (2002). Estimations of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*. 10.p.178-182.

Coutinho, H.D., Costa, J.G., Lima, E.O., Falcao-Silva, V.S. and Siqueira-Junior, J.P. (2009). Potentiating effect of *Mentha arvensis* and chlorpromazine in the resistance to aminoglycosides of methicillin-resistant *Staphylococcus aureus*. *In vivo*. 23 (2). p.287-289.

Coutinho, H.D., Costa, J.G., Lima, E.O., Falcao-Silva, V.S. and Siqueira-Junior, J.P. (2008). Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. *Chemotherapy*. 54 (4). p.328-330.

Dar, M.A., Masoodi, M.H., Wali, A.F., Mir, M.A. and Shapoo, N.S. (2014). Antioxidant potential of methanol Root extract of *Mentha arvensis* L. from Kashmir Region. *Journal of Applied Pharmaceutical Science*. 4 (3). p.50.

Dhawan, S., Shasany, A.K., Naqvi, A.A., Kumar, S. and Khanuja, S.P. (2003). Menthol tolerant clones of *Mentha arvensis*: approach for in vitro selection of menthol rich genotypes. *Plant cell, tissue and organ culture*. 75 (1). p.87-94.

Evergreen. (2015). Native plant database [Online]. Canada. Available at: http://nativeplants.evergreen.ca/search/view-plant.php?ID=00429 [Accessed 17th April 2016]

Exportersindia. Full details of menthol products [Online]. India. Available at: http://img1.exportersindia.com/product_images/bc full/dir_20/579706/tbl_spec_41388_p_1268399.pdf [Accessed 15th April 2016]

Global Healing Center. Tips for growing peppermint [Online]. Houston,Texas. Available at: http://www.globalhealingcenter.com/organic-herbs/growing-peppermint [Accessed 20th April 2016]

Globinmed. (2015). *Mentha arvensis* Linn. [online]. Malaysia. Available at: http://www.globinmed.com/index.php?option=com_content&view=article&id=79226:mentha-arvensis-linn&catid=199&Itemid=139#2 [Accessed 25th April 2016]

Godofredo U. Stuart Jr., M.D. (2015). Yerba buena [Online]. Phillipine. Available at: http://www.stuartxchange.com/Yerba.html [Accessed 25th April 2016]

Grieve, M. (1971). A Modern Herbal | Mints. [online] Botanical.com. Available at: https://www.botanical.com/botanical/mgmh/m/mints-39.html [Accessed 18 June 2016].

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, World Health Organization and International Agency for Research on Cancer. (2002). *Some traditional herbal medicines, some mycotoxins, naphthalene and styrene*. 82.

Hassan B. A. R. (2012). Medicinal Plants (Importance and Uses). *Pharmaceut Anal Act.* 3 (139). p. 2153-2435.

Johnson, M., Wesely, E.G., Kavitha, M.S. and Uma, V. (2011). Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L. *Asian Pacific journal of tropical medicine*. 4 (3). p. 196-200.

Lady Bird Johnson Wildflowers center. (2016). NPIN: Native Plant Database [Online]. Texas: The University of Texas at Austin. Available at: http://www.wildflower.org/plants/result.php?id_plant=MEAR4 [Accessed 20th April 2016]

Londonkar, R.L. and Poddar, P.V. (2009). Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals. *World J Gastrointest Oncol*. 1 (1). p. 82-88.

Malik, F., Hussain, S., Sadiq, A., Parveen, G., Wajid, A., Shafat, S., Channa, R.A., Mahmood, R., Riaz, H., Ismail, M. and Raja, F.Y. (2012). Phyto-chemical analysis, anti-allergic and anti-

inflammatory activity of *Mentha arvensis* in animals. *African Journal of Pharmacy and Pharmacology*. 6 (9). p. 613-619.

Mickienė, R., Ragažinskienė, O. and Bakutis, B. (2011). Antimicrobial activity of *Mentha arvensis* L. and *Zingiber officinale* R. essential oils. *Biologija*. 57 (2). p. 92-97.

Montana plant life. Wild Mint *Mentha arvensis* L. [Online]. Montana, USA. available at: http://montana.plant-life.org/species/mentha_arven.htm [Accessed 18th April 2016]

Motaleb, M.A. (2011). *Selected Medicinal plants of Chittagong Hill Tracts. IUCN Bangladesh*. p. xii+116

Nicole Johnson. (2009). Peppermint Menthol X Piperita [Online]. Wisconsin: University of Wisconsin-La Crosse. Available at: http://bioweb.uwlax.edu/bio203/s2009/johnson_nic4/Classification.htm [Accessed 17th April 2016]

North carolina state university. Medicinal plants Introduction [Online]. Available at: https://www.cals.ncsu.edu/plantbiology/Faculty/dxie/Chapter1-1.pdf [Accessed 7th March 2016]

Nwachukwu, C.U., Umeh, C.N., Kalu, I.G., Okere, S. and Nwoko, M. (2010). Identification and traditional uses of some common medicinal plants in Ezinihitte Mbaise L.G.A., of Imo State, Nigeria. *Report and Opinion*. 2 (6). p. 1-2.

Olowa, L. F. &Nuneza, O. M. (2013). Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. *International Research Journal of Biological Sciences*. 2 (11). p. 74-77.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*. 44. p. 307-315.

Patil, K. and Mall, A. (2012). Hepatoprotective activity of *Mentha arvensis* Linn. leaves against CCl_4 induced liver damage in rats. *Asian Pacific Journal of Tropical Disease*. 2. p. S223-S226.

Sandeep, G., Dheeraj, A., Deenanath, J., Kumar, S.N. and Bharti, A. (2010). Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial Parts of *Mentha arvensis* Linn. *IJPSD R*. 2 (4). p. 261-264.

Shahik, S.M., Sikder, M.O.F., Patwary, N.I.A., Sohel, M., Islam, M.S., Nishi, T.F., Sultana, T. and Barua, R. (2014). In vitro thrombolytic and cytotoxic evaluation of *Mentha arvensis* L., *Mentha spicata* L. and *Mentha viridis* L. *IOSR Journal of Pharmacy and Biological Sciences (IOSR - JPBS)*. 9 (5). p. 97-102.

Sharma, N. and Jocob, D. (2001). Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of *Mentha arvensis* L. in male albino mice. *Journal of Ethnopharmacology*. 75 (1). p. 5-12.

Shin, T.Y. (2003). Inhibition of immunologic and nonimmunologic stimulation-mediated anaphylactic reactions by the aqueous extract of *Mentha arvensis*. *Immunopharmacology and immunotoxicology*. 25 (2). p. 273-283.

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*. p. 152-178.

Souza, M.A.A., Araujo, O.J.L., Brito, D.M.C., Fernandes, M.S., Castro, R.N. and Souza, S.R. (2014). Chemical Composition of the Essential Oil and Nitrogen Metabolism of Menthol Mint under Different Phosphorus Levels. *American Journal of Plant Sciences*. 5. p. 2312-2322.

Srinivas pidugu and Arun T. (2012). Antibacterial Activity and Phytochemical Screening of *Mentha arvensis* Linn. against *Proteus mirabilis* from Urinary Tract Infected Patients. *International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN : 0974 – 4304*. 4 (4) . p. 1735 – 1744.

Van Wyk, B.E. and Wink, M. (2004). Medicinal plants of the world. *Australian journal of medical herbalism*. *16* (1). p. 36-36.

Vinson, J., Zubik, L., Bose, P., Samman, N. &Proch, J. (2005). Dried fruits: excellent in vitro and in vivo antioxidants. *Journal of the American College of Nutrition*. 24 (1). p. 44–50.

Zhang, L., Xu, S.G., Liang, W., Mei, J., Di, Y.Y., Lan, H.H., Yang, Y., Wang, W.W., Luo, Y.Y. and Wang, H.Z. (2015). Antibacterial Activity and Mode of Action of *Mentha arvensis* Ethanol Extract against Multidrug-Resistant *Acinetobacter baumannii*. *Tropical Journal of Pharmaceutical Research*. 14 (11). p. 2099-2106.