Studying The Effect of *Mentha Longifolia* Plant Extract In Inhibition Growth of Some Bacteria and Inhibiting the Emergence Fourth Stage Larvae of Mosquitoes Aedes Aegypti

Alaa Adnan Al-janabi[1], Atyaf Ali Sahib[1], Farah Jabbar Ali[1]

[1]AlSafwa University College, Iraq

Abstract

This study make as research about activity of secondary compounds in Mentha longifolia plant extracts as crude , the effect of crude extract of Mentha longifolia leaves make as inhibitory growth factor for two types of bacteria Staphylococcus aureus and Escherichia coli , and inhibit emerge four stage larvae of mosquitoes . watery and alcohol plant extracts of Mentha longifolia leaves were prepared , were appear variation effect in bacteria , the water plant extract show simple effect in Staph. aureus , while alcohol extract show clear effect in E. coli . in state of emerge four stage larva of mosquitoes , there are clear effect for alcohol extract in larvae comparing with water extract .

Keywords: *Mentha longifolia, plant extract, inhibition growth, bacteria.*

Introduction

Plant extracts of various types are an important and effective tool for the development and progress of studies and scientific research at present. The studies took the plant extracts of today a great place and space [1]. The man turned to seek a way to get rid of the pain and disease with compounds with a few side effects and began to treat some herbs and plants that are believed to be connected to healing [2]. One of the important tests in which plant extracts and their inhibitory effects are used are bacteria, which are small organisms and very important to humans because of their damage and benefits in many aspects [3]. Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 μm and characterised by individual cocci , which divide in more than one plane to form grape-like clusters. To date, there are 32 species and eight sub-species in the genus Staphylococcus, many of which preferentially colonise the human body [10,11]

The staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however, in general they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine, and B vitamins, including thiamine and nicotinamide [17].

Staph. aureus is an opportunistic pathogen and a hospital pathogen. It is often acute and fatal. If untreated, the infection may spread to the surrounding tissue or through bacteremia and blood clots to other sites to include other organs [5] . The other intestinal bacteria that cause diarrhea is E. coli , a Gram negative bacilli , non-sporing , motile , some of which have the ability to form capsules and are a member of the intestinal family enterobacteriaceae. Their colonies appear to be small, slightly convex, that kill at 55 ° C for an hour, but some of them can resist the temperature of pasteurization (2-8) m for 30 minutes. Although the therapeutic effects of many herbs have not been proven in practice, research is continuing to learn more about how these plants work and to identify the effective ingredients given by the therapeutic properties. Scientists hope that these research will reveal new, effective plant ingredients that can someday form the basis of cancer-fighting drugs and AIDS. [4] The treatment of medicinal plants in today's place and place in the great science of medicine preferred herbs and medicinal plants and no pharmacy in the world devoid of herbal medicines, so we find in Europe pharmacies especially to describe medicinal herbs, knowing that more medicines and chemical drugs are of plant origin, For patients who use herbs and

medicinal plants to treat their diseases, thus avoiding the side effects caused by the chemical drugs, as well as that the active substances in plants reduced and easy and the objects can interact with them gently in its natural form. [20] As a result of the ongoing increase in scientific research on the importance of the plant kingdom as the inexhaustible source of organic compounds of medicinal importance against microorganisms, many studies have been conducted in the use of plants as anti-bacterial agents that cause wounds and assist in the healing and healing of wounds. In many tropical and subtropical countries diseases transmitted by arthropods are among the major causes of illness and deaths (WHO, 1992). Mosquitoes serve as vector for various tropical and subtropical

diseases which cause destructive effects to human health (WHO, 1998). The most common disease associated with mosquitoes are Japanese encephalitis, chikungunya, filariasis, malaria, dengue and yellow fever. Chemical pesticides are still one of the most important means in the control of insect pests . However, the intensive use of different pesticides in plant protection programs has led to environmental malfunction and the emergence of pests and resistant strains and their high toxicity and toxicity in the environment and its harmful effects on humans and animals. Not to distinguish between harmful insects and beneficial, resulting in an imbalance in the ecosystem and the cumulative nature of chemical pesticides in the food chain and the high economic costs in the manufacture and its effects on the genetic side of the plant cell and contamination of surface water to river and sea and air pollution.

Therefore, specialists are trying to find alternative methods such as the use of biological control methods, genetic control and the use of pesticides of plant origin [7]

Plant extracts are natural plant compounds that affect several methods, either as direct substances, toxic or infertile, or organized for the growth of the insect or modified their behavior and found many chemical groups have an anti-feeding effect or to lay eggs in many insects [14]

Material & Methods

Preparation of raw extracts of mentha longifolia leaves

Preparation of hot water extract for Mentha longifolia leaves for the purpose of obtaining the hot water extract of the leaves of the Mentha longifolia plant, a method of [1] was followed with some modifications and as follows :

- Place 50 gm. of the Mentha longifolia leaves in a volumetric flask of 1 liter
- Add 500 ml containers of distilled water and close the hole by the cellophane.
- Place the beaker in the vibrator and let it blend well, at room temperature, and quickly (60 cycles, min) .
- After 24 hours, the solution was filtered using medical gauze first and then with filter paper Wattman No. 1.
- Distribute the leachate in the centrifugal discharge tubes at a speed of 3000 cycles / minute for 10 minutes.
- Ignore the precipitate and take the leak to the rotary evaporator device for the purpose of concentration and then the weight of the extracted raw extract.

Preparation of the crude extract of ethanol for the leaves of the Mentha longifolia

Follow the steps of preparing the raw water extract to obtain a dry plant extract. Ethyl alcohol was used with 80% distillation of distilled water. (Anessiny and Perez , 1993) except for the difference in time period where the solution was placed in the vibrator for 72 hours rather than 24 hours.

Preparation of a medium to activate the bacteria

Dissolve 0.13 g of nutrient broth in 10 ml of distilled water in a vial of volume. The mixture should be placed in the autoclave after closing the bottle with medical cotton for the purpose of sterilization and then left to cool a sample of Staph. aureus is activated by a needle isolated to the center and placed in the bottle after closing tightly to the incubator where incubated 37 ° C for 24 hours and the same method was followed to prepare the activation medium E. coli bacteria and applied this paragraph in the room sterile hood

Preparation of concentrations of extracts

* Concentration of 200 mg / ml

2 g of the extract and add to 10 ml of distilled water in the baker where the mixture is then blended after being placed in a bottle of volume and using the method of dilution obtained the following concentrations:

100 mg / ml , 50 mg / ml and 25 mg / ml

Preparation of the agricultural medium used for the growth of bacteria

Mueller-Hinton agar was found to dissolve 12.16 g of sugar in 320 distilled water in a conical flask and sealed the flask with cotton and sterilized with a sterilizer.

Left for 10 minutes to cool down. The medium was then poured into the Petri dishes near the flame to ensure that the germs did not enter and contaminate the dishes and left until the medium solidified. The diffusion method was followed by well diffusion in the medium of mm6 by means of the thorny fellini , the dishes were fertilize by a swab immersed in a bacterial suspension with a mass equivalent to the standard McFarland tube in tightly sealed tubes that passed on the surface of the center homogeneously except for the inside and sides of the well

Effect of extracts used to inhibit the onset of mosquitoes

A concentration of 1% of concentrations used in the treatment of bacteria was prepared

Treatment of fourth stage larvae

The fourth phase larvae were treated after isolating them from the sample by ten larvae per replicate (and repeaters for each concentration) in 100 mL plastic dishes. After the desired concentrations were prepared in other dishes, they were transferred using a boring cloth and the dishes were monitored for one week to calculate the percentage of inhibition that could result effect of extracts .

Result and Discussion

The inhibitory effect of the types of Mentha longifolia extracts

The effect of plant extracts used in this study showed at the two types of gram positive bacteria Staph. aureus and the negative E. coli by using the diffusion method of drilling has different effect on the two types of bacteria

Effect of plant extracts on bacteria Staphylococcus aureus :

The results in Table (1) show that there is a slight inhibitory effect of the hot water and alcohol extracts of Mentha longifolia leaves in bacteria. We note that treatment with concentration (25-50-100-200 mg / ml) of two types of extracts have no effect in inhibiting the growth of bacteria , compared with 18.2 + 0.5 when using Gentamicin

Table (1): shows the effect of hot water and alcohol extracts of Mentha longifolia leaves in bacteria Staph. aureus :

Concentrate mg / cm 3	rate of inhibition diameter	
	Hot water extract	Alcohol extract
200	-	-
100	-	-
50	-	-
25	-	-
Gentamicin)10mg/ ml)	18.3+0.5	

The reason for the low and ineffective inhibition of the studied plant extracts may be due to plant extracts may contain compounds that inhibit the growth of some microorganisms, but some may lose its ability .

In addition to the reduction of this activity originally in some plant extracts or during certain reactions. The method of preparation and concentration of plant extracts is an important and influential factor in the inhibitory action of each extract .

The study were agreed with Majid & Al- Shatti (2002), who found that there was no effect of water extract in Staph. aureus bacteria at a concentration of 10% .

Effect of plant extracts in bacteria Escherichia coli :

The hot water extract of the Mentha longifolia leaves has no effect in bacteria . With the alcohol extract, the rate of inhibition increased with increasing concentration. Inhibition with 25 mg / ml concentration was 3.95 and increased with 50 mg / ml concentration to 4.75, 6.45 and 6.35 with concentrations of 100 and 200 mg / ml, respectively , This was not consistent with Akroum et. al. (2009) [2] and there was no effect of alcoholic extract in the inhibition of bacterial growth . Gentamycin 10 mg / ml, which reached 17.3

Table (2) : Shows the effect of alcohol extract on bacteria E. coli :

Concentrate mg / cm 3	Rate of inhibition diameter
200	6.35+0.48
100	6.45+3.4
50	4.75+0.24
25	3.95+1.1
(ml/10mg)Gentamicin	17.3+0.6

The high effect of the ethanol extract may be due to the high content of flavonoids , which are known to have a strong effect against bacteria (Cushnie et al , 2003 ; Martini et al , 2004)

The results obtained have confirmed the benefits offered by medicinal plants. In fact, some of the flavonoids found in them have shown a significant anti-bacterial activity and the effect of acute toxicity is very low [2]

Ethanol extract has a significantly greater effect than the water extract the degree of polarity of the solvents used as the coefficient of extinction , For water 9 and ethanol 5.2 polarity index [7]

The polarity of the solvent by choosing another solvent may increase its effectiveness in dissolving active compounds.

Effect of the extracts on inhibiting the emergence of fourth stage larvae of mosquito Aedes aegypti

The first three concentrations of the alcohol extract were 100% inhibition, while the concentration of 25 was 81.6 .

When using the water extract, When the concentrations were used 25 mg / ml, the percentage of inhibition was 13.6 % and increased with concentration increased to 57.1% when using concentration 200 mg / ml. As shown in table (3) . we will notice a positive relationship between the concentrations used for two types of extracts .

Table (3) : Shows the effect of treatment of fourth stage larvae of mosquitoes with Mentha longifolia extracts in percentage of inhibition of onset :

Concentration mg/ml	Water extract	Alcohol extract
200	57.1	100
100	51.2	100
50	44.6	100
25	13.6	81.6

Accidental loss can be attributed to the fact that active compounds can be concentrated in the channel , These substances may interfere with the functioning of the endocrine system, leading to malfunction , The process of growth and increase in insect loss [19]

Conclusions

1. The hot water extract of the leaves of the Mentha longifolia crushed has a inhibitory effect on the positive bacteria of the gram stain .

2. Alcoholic extract showed a disincentive effect on the bacteria.

3. The alcohol extract has a clear effect in inhibiting the larvae of the fourth phase mosquitoes compared to the water extract.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the AlSafwa University College , Iraq and all experiments were carried out in accordance with approved guidelines.

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