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Antimicrobial Effects of *Mentha Pulegium* Extract against *Staphyloccocus aureus* Bacteria

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Abstract
The effects of aqueous and alcoholic extracts of *pulegium* leaves were studied on the growth of *Staphylococcus aureus* burn isolate. The Phytochemical analysis of the aqueous and alcoholic extracts of *Mentha pulegium* leaves show the presence of bioactive constituents like comarins, steroids, tannins, glycosides, saponins, alkaloids, flavonoids, phenols and essential oil. The ethanolic extract shows an inhibition zone larger than aqueous extract. Results show that the ethanolic extract inhibits bacterial growth with inhibition zone(30.33mm) but the aqueous extract give an inhibition zone(8mm). In this study, minimum inhibitory concentration (MIC) also detected and the result varies according to the type of extract.

Keywords: *Mentha pulegium*, Ethanolic extract, Aqueous extract , *Staphylococcus aureus.*

الخلاصة
تم دراسة تأثير المستخلص الكحولي الحار والمستخلص المائي الحار لنبات النعناع *Mentha pulegium* ضد بكتريا *Staphylococcus aureus* المعزولة من الجروح باستخدام طريقة الانتشار في الحفر, تم الكشف عن مكوناته الكيميائيه للمستخلصات المائية والكحولية لاوراق نبات النعناع ووجد بأنها تحتوي على كل من الكومارينات ,التانينات, الكلايكوسيدات, الصابونينات, القلويدات, فلافونات, فينولات والزيوت الطيارة. وكان للمستخلص الكحولي فعاليه تثبيطيه اعلى من المستخلص المائي في نمو العزلات البكتيرية. بينت النتائج أن المستخلص الكحولي قد ثبط نمو البكتريا بقطر تثبيط (30.33 ملم) أما المستخلص المائي اعطى قطر تثبيط (8 ملم). تم في هذه الدراسة تحديد قيمه التركيز المثبط الادنى MIC للمستخلصات النباتيه وقد تباينت النتائج تبعاً لاختلاف نوع المستخلص.

Introduction

A staphylococcus aureus bacterium is a spherical, gram-positive bacteria belonging to the Micrococaceae family [1]. *S. aureus* bacteria can causes urinary tract infection and many diseases including pneumonia, mastitis, meningitis, osteomyelitis, endocarditis, superficial skin lesions, furunculosis [2], and the cause of its ferocity is due to the proliferation of many enzymes, for example Protease, the enzyme Hyaluronidase in addition to the enzyme leukocidin causes lyses of leukocytes cells.

They also have the ability to produce the hemolysin hemolytic enzyme, causing Leukotoxin poisoning,as it leads to the decomposition of the eukaryotic cell membrane in addition to the production of catalase enzyme which gives it the ability to survive inside the cell gland [3,2] *S. aureus* could secretion of enterotoxin toxins causing food poisoning and Toxic shock syndrome [4]. For treatment of diseases of many antibiotics, have been used increased use of these antibiotics, which are often random and for long periods led to the emergence of side effects that harm the health of the individual on the one hand and the emergence of resistant strains on the other hand, I see that these interests have been increased for two decades to detect for effective alternatives and medicinal plants contains more than one active substance [5].

Mentha pulegium plant:

The genus mint belongs to the Labiata family and the genus mint contains more than 25 species of plants [6]. Peppermint is a very important plant for its nutritional and therapeutic benefits and has been used as a food conditioner and as a treatment for diseases [7].

It is have medium growth and limited to the existing branch, up to (50) cm, cardio or rectangular leaves, the length is between (3-5) cm and width (1 - 2.5) cm. [7].

Peppermint is a very important plant for its nutritional and therapeutic benefits and has been used as a food conditioner and as a treatment for diseases [8]. Different peppermint species grow under similar climatic conditions of temperature ranging from (20-35)

The plant contains a number of chemical compounds, including: Cineol, Menthol, Limoneno, bitter metilacelate, pulegona, Jasmone, piperitone, vitamins C and D, Cetonas, Tanins, flavonoids, Cumaric acid, fernlic acid, Cafeic acid [6].

It is used in the treatment of nausea and heart palpitations and activation. It also helps in stimulating liver and yellow secretions, relieving the sensitivity of the gastric mucosa with the expulsion of intestinal gases and removing convulsions.

The important compounds of mint oil, the most important of which are menthol and methionate, which are used in the manufacture of medicines for the alleviation of nerve crises, can be separated, and each is used in the manufacture of cigarettes for the characteristic flavor of mint to reduce its harm [8].

Peppermint is also used as a peppermint as it is added to many kinds of sweets to absorb the distinctive flavor.

Mint oil has some disinfectant properties, as well as toothpaste and soap making. Other compounds containing mint oil include lemon in pineapple, and tannic acid. [9]

(Peppermint Acute Aromatic aroma prevents nausea and stomach aches and colic and large winds and go diets, gout, sciatica, itching and scabies coating and drinking, and benefit from leprosy and joint pain and spleen drink, and sugar drink break the types of headaches and brain weakness and purify Chest of all diseases)). [9]

Materials and Methods

Plant collection:

Leaf leaves were collected from Baghdad during the month of October dried in shade at room temperature.

Preparation of the extract:

Alcoholic extract: Prepare the alcohol extract of the plant using method [10] by continuous extraction using the device Soxhlet Continuous extraction, using ethanol as a solvent at 80% concentration for 7 hours, then concentrate the extract using rotary evaporator Rotary Evaporator and then dry the extract electric oven at 40 ° C and kept in sterilized bottles in the refrigerator until use.

Water extract: The hot water extract was prepared using [11], weighing 10 g of dry matter leaves of the plant and placed in a 500 mL flask. Add 100 mL of distilled water and leave the flask in a 35 ° C incubator for 3 hours after Concentrate the extract using rotary evaporator, then dry the extract with electric oven at 40 ° C and store in sterilized bottles in the refrigerator until use.

Sensitivity testing of extracts in the growth of bacteria:

The inhibitory effect of the water extract and the ethanolic extract was studied against the isolated *Staphylococcus aureus* bacteria isolated in the laboratories of Educational laboratories in the city of medicine where the agar well diffusion method was used based on [12]. When was the bacterial suspension present, comparative with the McFarland solution 0.5, then 0.1ml was seeded on the Mueller hinton agar medium and worked holes in the culture medium by using the cork hole with the addition of 100µl from each concentration of extracts used in the study with the control tube.

Chemical detection of active compounds:

The presence of active compounds has been investigated using chemical detection methods as follow:

1. Detection of Tannins Test: Add 1 ml of water lead acetate (1%) to 1 ml of extract when a white deposit is found, the result is positive indicating the Tannins [13].
2. Carbohydrate detection using a molash detector, mix 1 ml of the model with 5 drops of Alpha Naphthol Alcoholic in a tube and good well. Add 2.5 ml of sulfuric acid and a blue ring to indicate the presence of carbohydrates [14].
3. Detection of glycosides Test: Detection of the glycosides by the fehling reagent, and the emergence of red deposit indicate the presence of the glycosides [13].
4. Phenols test: Dissolve (0.1 g) of the model in (1) ml of distilled water and add (1-2) a drop of FeCl3 solution. When the blue or green color appears, the result is positive and indicates on the presence of phenols [15].
5. Resins test: Add 1 ml lead acetate (1%) to 1 ml of the model. When white precipitation is found, the result is positive and indicates the presence of resins [13].
6. Detection of Flavonoids test: Detection of Flavonoids by addition of (1) ml of the Alcoholic potassium hydroxide reagent (5) N to (1) ml of the model, and when a yellow deposit appears, the result is positive and indicates the presence of flavonoids [16].
7. Detection of Saponin test: Add 1 ml of Mercury Water Chloride Reagent (5%) to (1) ml of the model. When white precipitation is found, the result is positive and indicates the presence of soap [17].
8. Alkaloid test: Detection of alkaloids using the wagners reagent by adding several drops of reagent to (1) ml of the model and When an acorn appears, the result is positive and indicates the presence of alkaloids [18].
9. Protein detection: Detection of proteins using a papurite detection consisting of (80%) copper sulphate dissolved in distilled water and (1) ml of (10%) of the reagent. When the violet color indicates the presence of proteins [19] .
10. Coumarins test: Detect coumarins by placing a quantity of the sample in a test tube, then cover the tube with a filter paper moistened with diluted sodium hydroxide solution, then heat the tube on boiling water bath for a few minutes and then expose the filter paper to the source of ultraviolet radiation, The coloring of the paper in bright green yellow indicates the presence of coumarin [20].
11. Terpens test and steroids Test: Dissolve (1) gm from the model to a little chloroforms and adds a drop of acetic anhydride and a drop of concentrated sulfuric acid. When the brown color appears to indicate the containment of the soil model, the dark blue Fidel color contains the steroids [20].

Determination of the minimum inhibitory concentration of plant extracts MIC:

Biological activity included the calculation of the MIC inhibitor based on [21] agencies:

1. Add 0.8 mL of brain heart infusion broth to small test tubes, sterilized by autoclave.
2. Add 0.1 ml of the prepared concentrations (20, 40, 60, and 80) mg/ml of the Mentha plant extract.
3. Add 0.1 ml of the comparative bacterial strain broth with McFarland's number 0.5.

The tubes were well fed and incubated at 37 ° C for 24 h. The results were then recorded on the basis of the turbidity observation, as shown in Table (2).

Results and Discussion

Chemical detection of the essential components of mint leaves:

Table (1) shows the results of the chemical detection by using the mentioned reagents that the hot water extract of the mint plant contains the tannins, the Glycosides, the soap and the volatile oils, and as for the terpenes, alkaloids, phenols, flavones and resins did not notice. While the hot alcoholic extract contains all the soap, Tannins, alkaloids, steroids, flavones, phenols, volatile oils, turbines, coumarins and Glycosides. Resins were not found. pH variance of the hot water and hot alcoholic

extract of mint leaves was 5.9 and 5.5 respectively.

Table (1) Chemical components of the extract of alcohol and water for mint leaves.

Active chemical compounds	Mentha plant leaves extract	
	Water	Alcoholic
Temperature	24	25
Saponins	-	+
Flavonoids	-	-
Turbines	-	-
Steroids	-	-
Coumarins	-	-
Volatile oils	-	+
Tannins	+	+
Resins	_	_
Alkaloids	_	-
Glycosides	+	+
Phenols	_	-
Protiens	-	-
Carbohydrate	+	+
pH	6.4	6.6

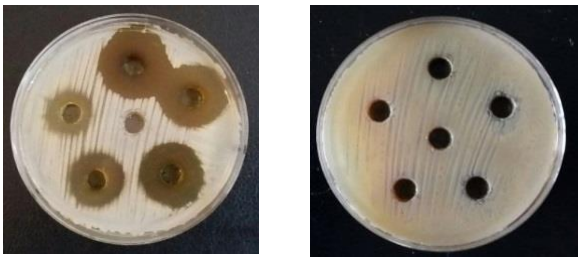
(+)=The substance is present in the plant.
(-)=the substance is not present in the plant

Effectiveness of peppermint leaves: Peppermint leaves differed in their effect on the growth of bacteria and yeast under study. Some plant extracts affected the growth of bacteria and did not affect others. The results, shown in Table 2 and in Figure 1a and 1b show that mint leaves have varying inhibitory efficacy in the studied bacteria. The concentration of the inhibitory effect of the plant at the highest concentration (stock) was determined because it gave the highest efficacy compared to the other shaking. The highest inhibitory of the ethanolic extract was against Staphylococcus aureus in the inhibition zone of (30.33) mm, while the water extract gave a diameter of inhibition (8 mm).

Table (2) The inhibitory effect of the extract of alcoholic and water for the leaves of mint.

Bacteria	S.aureus	
Ethanolic extract(mm)	St.	30.33 ±1.10
	0.8	27.66 ±1.93
	0.6	26.66 ± 1.01
	0.4	23.33 ±0.45
	0.2	18.33 ±0.88
Water extract(mm)	St.	8 ± 0.25
	0.8	- ±0.0
	0.6	- ±0.0
	0.4	- ±0.0
	0.2	- ±0.0

*(-)= No inhibition
*P<0.01
*(±)=the standard error value
*%=percentage *100=stock mg / ml



(a) (b)

Figure 1: The inhibitory effect of the ethanolic (a) and water. (b) extracts of Mentha pulegium leaves in the growth of S.aureus bacteria

Determination of the minimum inhibitory concentration of plant extracts MIC: -

The minimum inhibitory concentration MIC was determined by mixing the different concentrations with the liquid and macrophage medium with the reduced bacterial ligation (broth) as mentioned in the articles and methods of work Paragraph (5). The results show that the antimicrobial activity of the

extract is increased by increasing the concentration of the extract as shown in Table (3).

Bacteria	Ethanolic extract	Water extract
S.aureus	%20	-

Table (3): The minimum inhibitory concentration of mint leaf extracts in microbial growth.

Discussion:

The results of active compounds detection showed that the leaves of peppermint plant contain steroids, tannins, Glycosides, soaps, and volatile oils, which are attributed to inhibitory activity. Sapnins have the ability to analyze the plasma membrane of bacterial cells [22]. Tannins possess high inhibition ability because of their ability to inhibit protein synthesis In the bacterial cells or their ability to stimulate phagocytic cells [23]. The effect of volatile oils extracted from some Iraqi plants, including mint, was studied on microorganisms and found that these volatile oils had great effectiveness in preventing the growth of microorganisms [24].

In addition to the presence of phenolic compounds, which have an important role in inhibiting bacterial growth because of its effect on the inhibition of enzymes responsible for the basic metabolic reactions through its non-specialized interaction with proteins leading to the protein mutant and thus the inability of bacteria to continue [25], and some studies indicated the effectiveness Volatile oils on the bacteria due to the presence of phenolic compounds thymol & carvacrol in their oils [23]. There was a marked difference in the inhibitory effect between positive and negative bacteria for gram stain due to the different composition and components of the plasma membrane of the bacteria. For MIC, it has been observed that the MIC of the ethanolic extract has been shown to be effective, while the water extract has not been effective. Several factors affect the value of MIC, such as bacterial production of some enzymes, bacterial concentration and the nature of the cell wall structure. Based on the results presented in this study and previous studies, the mint extract has proven to be effective against *staphylococcus aureus.*

Conclusion

Antimicrobial activity of leaves parts of Mentha pulegium L. essential oil against (S.aureus) was examined and it showed that the oil of alcoholic extract of Mentha pulegium has a potent antimicrobial activity and belongs to piperitone/piperitenone type. Further research is required to evaluate the practical values of therapeutic applications. Mentha pulegium can be considered as asource of gallocatechin

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