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Effect of Flavones Extracted from Lavender Flowers Against Some Bacteria that Cause Food Poisoning

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Abstract

The lavender plant is a shrub-shaped plant. It contains many active substances such as volatile oils, terpenes, and flavonides are considered antioxidants, antibacterial and antifungal substances. Take up the research extract and detect for flavonoids in the flowers of the lavender plant, especially the anthocyanin pigment, due to its important demand. The dried alcoholic extract (flavonoids) was prepared at different concentrations, estimated in milligrams per milliliter (mg/ml). And to study its effect on the bacteria Staphylococcus aureus and the bacteria Bacillus cereus that cause food poisoning, the active ingredient (flavonoids) was detected using ethyl alcohol and the base potassium hydroxide (KOH) at a concentration of 50%. The reagent was added to the aqueous extract of lavender flowers, and a yellow-brown color appeared indicating a positive detection. Then the extraction process was carried out using ethyl alcohol at a concentration of 80% to obtain the pure extract containing the anthocyanin dye. The anthocyanin pigment was identified using HPLC device compared with the standard solution. The active substance appeared at a retention time of 2 minutes. Then prepare a series of half-dilutions of the dried extract from the original concentration of 800mg/ml. For the purpose of testing the sensitivity of staphylococcus and bacillus bacteria to the plant extract using the wells method, and measuring the diameter of the inhibition zone mm. The results indicate that it is in the inhibition zone for Staphylococcus bacteria, it is 17 mm at a concentration of 800 mg/ml which is minimum bactericidal concentration (MBC) value, and the lowest inhibition zone is 12 mm at a concentration of 400 mg/ml, which is the value of the minimum inhibitory concentration (MIC). As for Bacillus bacteria, its highest zone of inhibition was 22 mm at a concentration of 800 mg/ml which is minimum bactericidal concentration (MBC) value, and its lowest zone of inhibition was 15 mm at a concentration of 200 mg/ml. which is its MIC value. . This result encourages the use of anthocyanin pigment as a preservative in food due to its being an inhibitor of the bacteria that cause food poisoning.

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1. Introduction

The lavender plant, (Lavendula), is an attractive plant with its beautiful violet flowers and a strong aromatic scent. It belongs to the Lamiaceae family, of which there are 39 species, and it is sometimes known as Ficus. One of its important species is Lavendula dentate. It is found in the highest mountains and rocky areas and is spread in North Africa, Asia, and Europe. It is a perennial herbaceous plant that reaches a height of about one meter [1]. The most important active components of this plant are volatile oils, monoterpenes, coumarins and flavonoids [2]. This plant is important from a medical standpoint and has a long history in medicine. It is used to facilitate digestion, relieve the pain of irritable bowel syndrome and asthma, and treat Depression, insomnia, migraines, and insect bites. It is considered a skin disinfectant, anti-inflammatory, antioxidant, antibacterial, and antifungal. It is also used in the manufacture of perfumes, spices, soap, and shampoo [3]. One of the important studies on this plant is that when volatile oils are mixed with nanoparticles, they show good effectiveness against microbes, especially those resistant to antibiotics such as Staphylococcus aureus, and E.coli bacteria [4]. Flavonoids have received a lot of attention in scientific research, and they are natural compounds of polyphenols. It is easily absorbed in the intestines and has many benefits, such as antioxidants, anti-inflammatory, anti-allergic and anti-bacterial, and protects the heart, blood vessels and cancer. It is considered crystalline compounds that dissolve in water, organic acids, bases and alcohol and give a pale green or brown color with ferric chloride [5]. The anthocyanin pigment is considered one of the very important compounds that protect plants. From ultraviolet rays and infection with parasites and fungi, this dye dissolves in water and gives different colors to plants, such as red, orange, and violet [3]. This research aim to extract flavonoids, which are the important active substances, diagnose the anthocyanin pigment as a type of flavonoid, and effect it against Staphylococcus aureus and Bacillus cereus bacteria which cause food poisoning.

2. Materials and Methods

Plant: lavender was obtained in the form of purple dried flowers from local markets. It was diagnosed based on the Encyclopedia of Life, 2013, and was washed and dried at room temperature (35°C) and stored in nylon bags until use.

Chemicals: Chemicals were obtained, such as the base potassium hydroxide KOH (Analar Company), with a purity of 99%, and ethyl alcohol (Hayman Company), with a purity of 99%. From the laboratories of Al-Adda Medical Center - Department of Materials Research.

Laboratory equipment: I used devices such as the rotary evaporator (Heidolf Company) to get rid of the solvent, the Oven oven (Gsc Company) to get rid of liquids and moisture, and dry the extract at a temperature of 40°C, and the High Performance Liquid Chromatography (HPLC) device from Shimazo Company) for the purpose of diagnosis. Active ingredient (anthocyanin).

Agricultural media: Nutrient agar and Nutrient broth produced by LAP were used for the purpose of growing and preserving bacterial isolates and also testing sensitivity to the plant extract.

Bacterial isolates: They were obtained from the Diroctorate of Environment and Water laboratories - and they were diagnosed in the Central Health Laboratory / Ministry of Health and include:

Staphylococcus aureus bacteria, which causes food poisoning, is of the Gram-positive type.

Bacillus cereus, bacteria that causes food poisoning, is of the Gram positive type.

Detection of flavones (specific detection): Flavones were detected using KOH base and ethyl alcohol at a concentration of 50%. The reagent was added to the plant extract in equal proportions, and a brown color appeared, indicating a positive test [6]

Extraction of the active ingredient (flavones): The extraction was done using ethyl alcohol at a concentration of 80%, added to the plant powder (for flowers) and left for 3 days. Then it is filtered using filter papers. After filtration, it is concentrated with a rotary evaporator and dried in an oven to obtain the dried extract, and its weight is calculated in grams or milligrams [6].

Quantitative detection: The active substance in the flavonoid extract, which is anthocyanin (violet pigment), was identified using a Japanese Shimazo high-pressure liquid chromatography device. The comparison was made with the standard solution at a retention time of 2 minutes and a wavelength of 520 Nm and the liquid volume of the extract (20 μL). The column ODs c18 (250 x 4.6 1d mm) was used. Then the concentration of the active substance was calculated and its value was 5 ppm. According to the following equation:

$$Concentration\ of\ Sample = \frac{Concentration\ of\ standard\ solution\ \times area\ of\ sample}{area\ of\ standard\ solution} \quad ...(1)$$

Biological tests: The method of spreading in the culture medium (well method Agar) was followed using a sterilized cook borer with a diameter of (6 mm) and the liquid extract was distributed in each hole using a fine pipette with a size of (0.3 ml) and at concentrations (50, 100, 200, 400, 800) mg/ ml, while the control pit contains only distilled water. The plates were then placed in the incubator at a temperature of 37 °C for 24 hours, then the diameter of the inhibition zone around each hole was measured with a ruler, expressed in millimeters (mm) [7].

Statistical analysis method: The results derived from the experiments were analyzed to demonstrate the effect of the prepared extract on the growth of bacterial isolates using the ANOVA test SPSS. Method at the probability level of 0.05 in order to evaluate the results in terms of whether they were significant differences or not.

3. Results and Discussion

Figure 1 shows the qualitative detection of flavones and the appearance of a yellow (brown) color indicating a positive (+) test when the reagent is added to the aqueous extract of lavender flowers. As for Figure 2, the appearance of the active substance (anthocyanin) at a retention time of 2 minutes compared to the standard solution using an HPLC device at a wavelength of (520 Nm) and the concentration of the active substance was5 ppm. Table (1) represents the diameter of the inhibition zone in millimeters against two types of bacteria, Bacillus cereus and Staph aureus, which cause food poisoning, treated with several concentrations of plant extract mg/ml (50, 100, 200, 400, 800). The number 6 indicates the absence of an inhibition zone around the hole and the control is distilled water. Similar letters mean there are no significant differences, and different letters mean there are significant differences at the 0.05 probability level.

It is clear from Table (1) that the highest zone of inhibition for Staphylococcus aureus (17mm) is at a concentration of 800 mg/ml, whicht is Minimum Bacteriocidal Concentration(MBC) value and the lowest zone of inhibition (12mm) is at a concentration of 400 mg/ml, which is the minimum inhibitory concentration (MIC) value, and the highest zone of inhibition for Bacillus cereus (22mm) is at The concentration was 800 mg/ml which is (MBC) , the minimum inhibition zone was 15 mm, and the MIC value was 200 mg/ml, as shown in Figure (3) and (4). The study, which dealt for the first time with the extraction of the active ingredient (anthocyanin pigment) from the lavender flower, showed that it has an anti-bacterial effect against the bacteria Bacillus and Staphylococcus aureus, which cause food poisoning, after drying the flavonoid alcoholic extract.

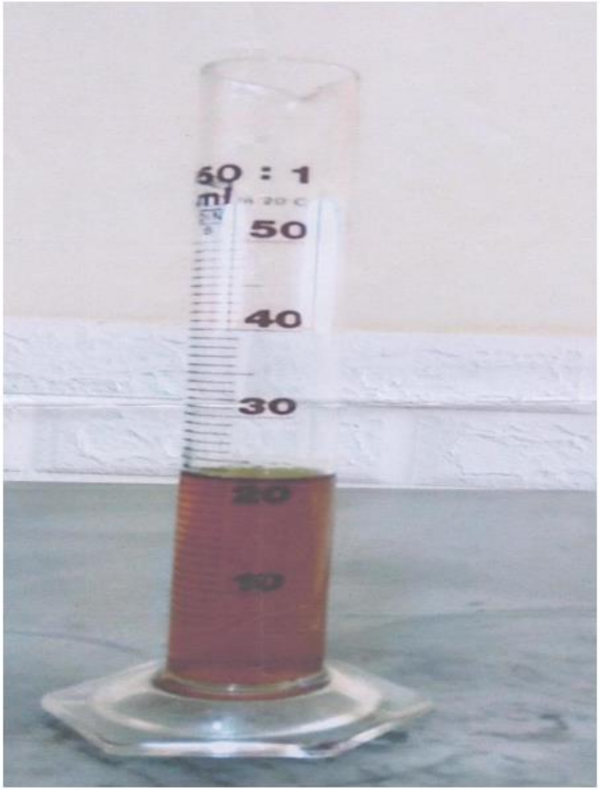
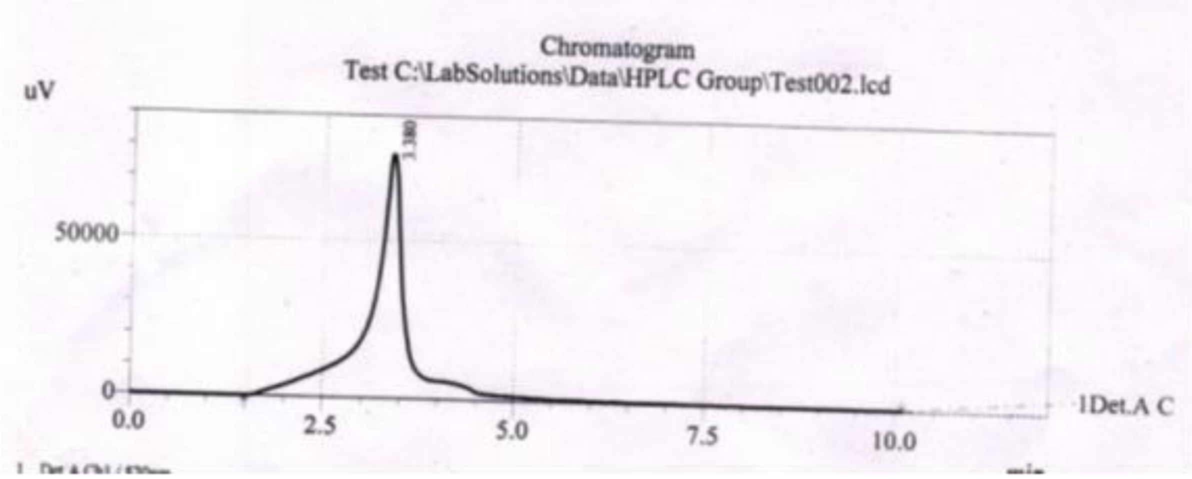
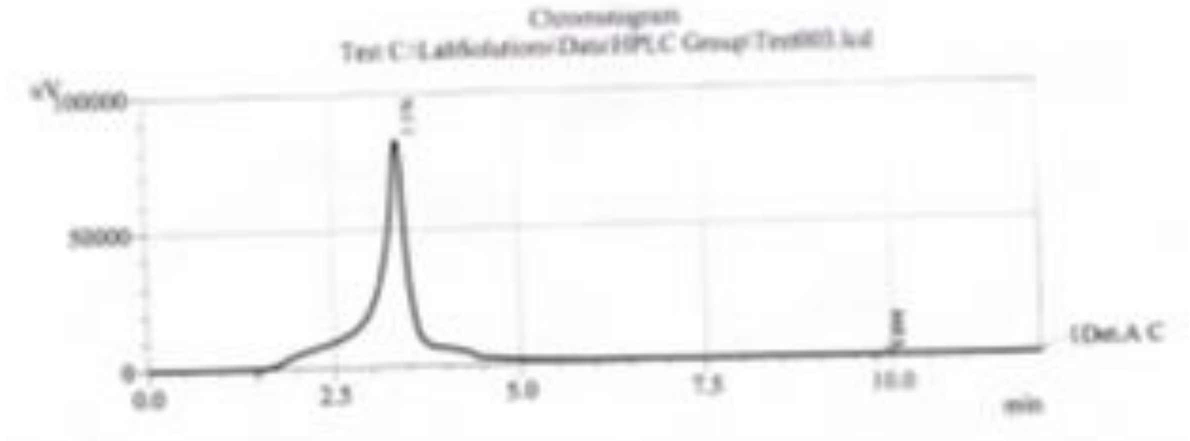


Figure 1. Appearance of a yellow-brown color when adding the reagent to the aqueous extract of lavender flowers, indicating a positive detection of the presence of flavones.



Standard



Sample

Figure 2. Appearance of the active ingredient (anthocyanin) at retention time = 2 compared to the standard solution using an HPLC device

Table 1. Diameter of the inhibition zone in millimeters for two types of bacterial isolates treated with several concentrations of plant extract of lavender flowers.

Bacterial Isolates	Plant extract concentrations are estimated in mg/ml					Control
	800	400	200	100	50	Distilled water
Staph aureus	17a	12b	6c	6c	6c	6c
Bacillus cereus	22a	20a	15b	6c	6c	6c

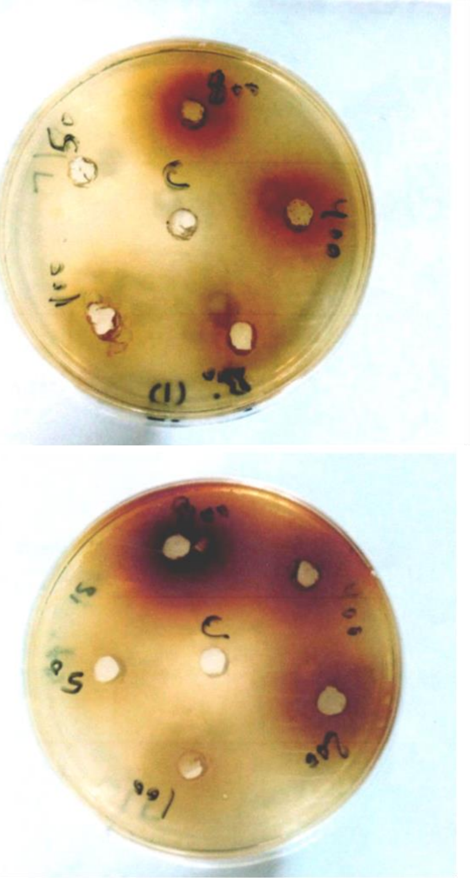


Figure 3. The diameter of the inhibition zone in millimeters for the bacterial isolates Staph aureus and Bacillus cereus at different concentrations of the plant extract of lavender flowers. C -control (distilled water), A - bacteria Bacillus cereus, B - Staph aureus bacteria

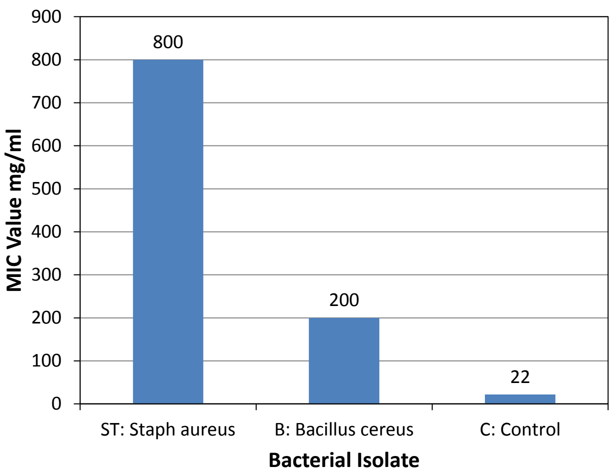


Figure 4. Minimum inhibitory concentration (MIC) values estimated in mg/ml (well difussion method)for the plant extract (lavender flowers) against two types of bacterial isolates.

This result is consistent with the result of researcher Ciocarlan, who studied the microbial activity of the lavender plant. Aqueous and alcoholic extracts have given high effectiveness against Bacillus bacteria and some fungi such as Pencillium, and. SAspergillus [8]. Researcher Panel [9] and others demonstrated the possibility of mixing the volatile oils of lavender flower at concentrations of 2%, 4%, and 6% with starch, phenolsilaran, or gelatin in the form of coatings. This will enhance the antimicrobial property of these three coatings and the possibility of using them in food packaging in industry. While the researcher Marin[7] studied the chemical components, especially the volatile oils of the Spanish lavender plant, he determined the total phenolic content and studied the antioxidant and antibacterial activity. He found that the phenolic compounds were very high, as they gave the highest percentage of inhibition of free radicals (64%), and that the extracted volatile oils gave The highest percentage of inhibition was against the bacteria Listeria with a diameter of the inhibition zone of 31 mm and a size of µL (20-40) per hole, while the bacteria Pseudomonous aeruginosa was resistant to oil. Another previous studies researcher in Palestine, Mohammad, conducted research on one of the types of lavender, Lavendula pubescence. He found that the oils extracted from this plant have been used since ancient times as a local treatment for many diseases, such as depression, anti-inflammatory, and antiseptic substances. The volatile oils were separated by water distillation and were tested as antioxidants, antibacterial substances, and enzymes. For fats. Chemical analysis was done by GC_mass to obtain different types of oils, such as 65% carvacrol. These oils have powerful antioxidants at a concentration of µL/ml (0.16-0.18), and anti-enzymatic at a concentration of 1.08. They also gave these oils effectiveness against bacteria. Such as Staphylococcus aureus with an inhibition rate of 95%, and against the yeast Candida albicans the MIC value was in the range of µL/ml 0.47, and against skin fungi such as Microsporum canis, Trichophyton rubrum, and Epidermatophyton floccosum at a concentration of µL/ml 0.06- 0.05 [10] There is another study on water lavender, Lavendula angustifolia. It gave little activity against microbes, about 0.05%, while its effectiveness against antioxidants was high at a concentration of 266 µmol, especially the oxygen radical [11]. Researcher Quedrini studied mixing thyme oil, lavender oil, and marjoram oil, which is a way to preserve foods from microbial spoilage. He noticed that mixing thyme oil with marjoram oil gave an inhibitory effect on Staph aureus and E.coli bacteria, and when mixing marjoram oil with lavender oil. It also gave an inhibitory effect on both types of bacteria, and this condition is called (synergism). These oils are considered preservatives that do not affect the properties of the food, but rather inhibit the growth of bacteria [12]. The researchers, Adaszynka & Szezerbinska, noted that lavender oil, when added to chicken water at a concentration of (0.4 ml/L), gave good effectiveness against intestinal bacteria E.coli [13]. Also in the same field, researcher Valkova three types of oils, such as peppermint oil, lavender oil, and rosemary oil, and found that lavender oil, such as (cineol and urinol), had a high inhibition against Pseudomonous aeruginosa bacteria and the yeast Candida albicans, and peppermint oil gave a high inhibition against salmonella bacteria. It also had high effectiveness. In reducing free radicals, rosemary oil gave inhibition against Staphylococcus aureus bacteria [14]. The biological study was also applied in the bread industry, considering that the lavender plant is a food preservative because it contains volatile oils, terpenes, and phenols as antioxidants and antimicrobials. When 5% lavender is added, the shelf life of bread increases by more than 96 hours compared to the control. There is no fungal or bacterial rot at a temperature of (22, 30, 37°C) for four days.[15]. The best combination of lavender or lavender with the antibiotic ciprofloxacin has been studied against pathogenic bacteria in food, especially Salmonella, the MIC value of the volatile oils mixed with the antibiotic was 0.2-0.3 mg/ml due to the occurrence of synergy, and this is useful in the food industries [16]. There is a study indicating that cinnamon oil is effective against Gram-positive and Gram-negative bacteria. And some fungi that cause rot, such as Aspergillus. As for lavender oil, it inhibited 4 types or strains of Staphylococcus aureus Listeria, Aspergillus, and Candida, with an MIC value of 1 microliter/ml [17] Researcher Chao [18] also proved that lavender oil is effective against staphylococcus bacteria, P. aeruginosa bacteria, and fungi like Candida. These oils gave a clear synergy with antibiotics against methicillin-resistant Staphylococcus aureus (MRSA) [19] and are added to food packaging to protect food from microbial spoilage [20].

4. Conclusions

The experiments showed that the flavonoid extract of lavender flowers has good inhibitory activity against Staphylococcus aureus and Bacillus cereus, which causes food poisoning. The value of the minimum inhibitory concentration (MIC) for Staphylococcus bacteria was (400 mg/ml) and for Bacillus cereus (200 mg/ml), (MBC) value for two isolates were (800 mg/ml) therefore, the favonoid extract of Lavender flower plant can be used as a preservative in food.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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