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Study of the antibacterial properties of *Spirulina platensis* and ethanolic extracts of *dill (Anethum graveolens) and mint (Mentha spp.)*

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ABSTRACT

The importance of this researcher is to understand the significance of medicinal extracts mint plants (*Mentha* spp.) And dill (*Anethum graveolens*) and algae *Spirulina platensis* at different concentrations (control,10,20,30 and 40 mg/g) on three different bacterial species: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, The algae and mint ,dill extracts were extracted using the Soxhlet method .The Vitek device was used to diagnose every bacterial isolate under investigation, indicated the results significant differences among all concentrations, with 40 mg/g algae *Spirulina platensis* showing the highest antibacterial activity at the inhibition area, the rate 14.19,14.65,13.19 mm for *E. coli,P. aeruginosa,S. aureus* with 40 mg/g for mint extract at the inhibition area, the rate (13.12,14.18,12.68mm)for *E. coli,P. aeruginosa,S. aureus* with 40 mg/g for dill extract At the inhibition area, the rate (15.49,15.49,15.49mm) for *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively .

Key words : Green algae, *S. platensis*, *Mentha* spp, *A.graveolens*.

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Introduction

Antibiotic-resistant pathogenic bacteria are now one of the biggest threats to world health, as it has led to a decrease in the effectiveness of many traditional treatments and an increase in the rates of infection and mortality associated with bacterial infections [1]. This challenge has prompted researchers to explore alternative natural sources with antimicrobial activity, especially medicinal plants and micro-algae, due to their effective bio-active compounds that possess multiple mechanisms in inhibiting bacterial growth [2]. The blue-green algae Spirulina (*S. platensis*) has attracted a lot of interest in the medical and nutritional domains because of its high protein, unsaturated fatty acid, and bioactive pigment content, including phycocyanin, as well as phenolic compounds that have demonstrated antioxidant and antibacterial properties. [3,4]. Numerous investigations have demonstrated that spirulina can stop both Gram-positive and Gram-negative bacteria from growing by interfering with the bacteria's metabolic activity and cell membrane integrity [5].On the other hand, mint (*Mentha* spp.) Among the commonly used aromatic medicinal plants, it is characterized by containing volatile oils, the most important of which are menthol and menthone, which possess strong antibacterial and anti-fungal properties. It has traditionally been used to treat various inflammations [6]. Dill (*A.graveolens*) is also considered one of the well-known medicinal plants, containing flavonoids, terpenes, and essential oils that contribute to inhibiting the growth of microorganisms by disrupting cellular enzymes and affecting the cell wall [7].This study focuses on evaluating the antibacterial effect of spirulina algae and the extracts of mint and dill on three clinically significant pathogenic bacteria: *Gram-positive bacteria like S. aureus and Gram-negative bacteria like E. coli and P. aeruginosa*, in order to investigate their potential as substitute or supplementary natural sources for conventional antibiotics.

Material and Method

The American company Amazon provided the ready-made algae powder.On January 12, 2024, early in the morning, the mint and dill plants were gathered from nearby markets. To get a pure extract, the plants were cleaned of dust and debris using distilled water. After that, the sample was allowed to dry outdoors to produce dry leaves. An electric blender was used to grind the plants, and the powder was refrigerated in plastic containers until extraction [8].

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Extract method :The active ingredients of the algae *S. platensis* , mint plants (*Mentha* spp. And dill (*A. graveolens*) were extracted using 4 grams of the powdered algae, which were placed in a thimble and positioned in the designated place of the Soxhlet apparatus. 80 mL of 70% methanol was added, and it was left for 7 minutes without extraction to allow the powder to saturate with the solvent. The extraction process was carried out for about 20 hours until a green filtrate was obtained. The filtrate was dried using an electric oven at a temperature of 40 degrees[9,10].

Revealing the active compounds

The active compounds Phenol, Flavonoid, Tannins, Glycoside and Alkaloid were revealed thru chemical assays.

Alkaloids

Iodine and potassium iodide were combined to create Wagner reagent, which gave the collected filtrate a reddish-brown hue. .

Phenols

Lead acetate test: 2.5 mg of volatile oil extract was mixed with 0.2 mL of a 1% lead acetate solution; a precipitate was produced.

Glycoside

Following the dissolution of 0.5 mg of volatile oil extract from basil in 1 mL of distilled water (D.W.), an aqueous sodium hydroxide (NaOH) solution was added, causing the mixture to turn yellow.

Tannins

The mixture was heated to 80 to 100 degrees Celsius for 10 minutes in a water bath apparatus after 5 mL of (D.W.) was added to 5 mL of basil volatile oil extract. The mixture was then filtered, and five drops of 1% ferric chloride were added to create the dark green color.

Flavonoids

Four mL of each sample of basil volatile oil extracts were combined with 1.5 milliliters of 50% methanol. After combining, magnesium metal was used to heat it and acidified with five drops of

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concentrated hydrogen chloride (HCl) until a crimson hue developed. The presence of flavonoids is indicated by the color red [11,12].

Diagnosis of bacterial isolates using the Vitek device.

The Vitek gadget, which uses diagnostic kits for both Gram-positive and Gram-negative bacteria, was utilized to diagnose all of the bacterial isolates under investigation [13 and 14]. Study of the antibacterial activity of *S. platensis*, mint plants (*Mentha* spp.) and dill (*A. graveolens*) In order to assess the efficacy of algae and the extracts of mint and dill against the bacterial strains E. Coli, P. aeruginosa, and S. aureu used in this study, the agar diffusion method was utilized using wells in accordance with the method [15].

Statistical analysis

The data were statistically using SPSS v.27. The results were represented by the mean and standard deviation. They were tested using multiple analyses of variance according to the least significant difference test for comparing the means[16] .

Results

At table (1), phytochemical screen study was to determine the active compounds at extract of *S. platensis* ,*M.* spp and *A. graveolen* shows Phenol ,Flavonoid, Tannins, Glycoside and Alkaloid positive results.

Table 1. phytochemical screen of *S.platensis* ,*M.* spp and *A. graveolens*.

phytochemical	*S.platensis*	*M. spp*	*A.graveolens*
Phenol	+	+	+
Flavonoid	+	+	+
Tannins	+	+	+
Glycoside	+	+	+
Alkaloid	+	+	+

The results presented in Table (2) show that the green algae *S. platensis* have a clear and concentration-dependent effect in enhancing antibacterial activity. No inhibitory activity was recorded in the control treatment, confirming that the observed effect in the other treatments is exclusively attributed to the *spirulina* extract. With the increase in the concentration of *S.*

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platensis from 10 to 40 mg/g, a gradual rise in the average diameters of the inhibition zones (Multivariate) was observed, with values of 11.46 , 12.27 , 13.12 , and 14.76 mm, respectively. This increasing trend indicates a strong correlation between the extract's concentration and its level of antibacterial activity, indicating the effectiveness of the active bio active compounds in spirulina at high concentrations. The low values of the standard deviation also show a relatively uniform bacterial response, which enhances the reliability of the results and reduces the likelihood of experimental variation. The fact that the inhibitory effect was comparable across the tested bacterial species suggests that *S. platensis* possesses broad antibacterial action against both Gram-positive and Gram-negative microorganisms.

Table 2. Effect of the green algae *S.platensis on the antibiotic* .

Con. bacterial strains	***E. Coli(mm)***	***P.aeruginosa (mm)***	***S. Aureus (mm)***	**Multivarite**
Mean ± SD				
Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00
10 mg/g	11.35 ± 0.80^{d}	10.35 ± 0.78^{d}	11.65 ± 0.99^{d}	11.46
20 mg/g	12.35 ± 0.6^{c}	11.37 ± 0.64^{c}	12.65 ± 0.61^{c}	12.27
30 mg/g	13.36 ± 0.45^{b}	13.64 ± 0.45^{b}	12.75 ± 0.45^{b}	13.12
40 mg/g	14.19 ± 0.58^{a}	14.65 ± 0.58^{a}	13.19 ± 0.58^{a}	14.76

LSD test indicated significant differences among all concentrations, with 40 mg/g showing the highest antibacterial activity.

Based on the table (3) that the mint plant extract possesses clear antibacterial activity, and this activity increases directly with the concentration. The control treatments showed a complete absence of any inhibition of bacterial growth, confirming that the observed effect in the other treatments is due to the active compounds in mint. With concentrations of 10, 20, 30, and 40 mg/g, a gradual increase in the diameters of the inhibition zones was observed for *E. coli*, *P. aeruginosa*, and *S. aureus*. For example, the Multivariate values (mean response for all bacteria) were 11.04, 11.95, 12.65, and 13.33 (mm), respectively. This upward trend reflects a clear direct relationship between the concentration of mint extract and the intensity of its antibacterial effect,

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which is evidence of the effectiveness of the active bio-compounds in mint, especially at higher concentrations. It also appears that the standard deviation was relatively low in most treatments, indicating the homogeneity of the bacterial response and the reliability of the measurements. It is also noted that the effect of mint was greater on *P. aeruginosa* compared to other bacteria at all concentrations, indicating a different relative sensitivity among bacterial species toward the mint extract.

Table 3. Effect of the mint plant on the antibiotic .

Con. Bacterial	***E.coli* (mm)**	***P.aeruginosa* (mm)**	***S.aureus* (mm)**	**Multivarite**
Mean ± SD				
Control	0.00 ± 0.00[e]	0.00 ± 0.00[e]	0.00 ± 0.00[e]	0.00
10 mg/g	10.50 ± 0.06[d]	11.79 ± 0.21[d]	10.84 ± 0.71[d]	11.04
20 mg/g	11.61 ± 0.06[c]	12.50 ± 0.45[c]	11.75 ± 0.99[c]	11.95
30 mg/g	12.39 ± 0.54[b]	13.22 ± 0.54[b]	12.35 ± 0.61[b]	12.65
40 mg/g	13.12 ± 0.57[a]	14.18 ± 0.51[a]	12.68 ± 0.89[a]	13.33

LSD test indicated significant differences among all concentrations, with 40 mg/g showing the highest antibacterial activity.

Based on the table (4) that dill plant extract possesses a clear antibacterial activity, which increases with higher concentrations. In the control treatment, no effect on bacterial growth was observed, confirming that the antibacterial activity is due to the effective compounds in dill. With the increase in extract concentration from 10 to 40 mg/g, a gradual rise in the average diameters of the inhibition zones for all three bacteria combined (Multivariate) was observed, with values reaching 11.78,13.89,14.27 and 15.66 mm, respectively. The efficacy of the bioactive compounds in dill at high concentrations is demonstrated by this increase, which shows a direct correlation between the extract concentration and the strength of the antibacterial effect.

The standard deviation was also relatively low, indicating the homogeneity of the bacterial response and the reliability of the results. It is also noteworthy that the effect of dill was similar between Gram-negative bacteria (*E. coli* and *P.aeruginosa*) and Gram-positive bacteria (*S. aureus*), indicating that dill has a broad spectrum of activity against different types of bacteria.

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Table 4. Effect of the dill plant on the antibiotic .

Con.	*E.coli*	*P.aeruginosa*	*S.aureus*	Multivarite
Mean ± SD				
Control	0.00 ± 0.00[e]	0.00 ± 0.00[e]	0.00 ± 0.00[e]	0.00
10 mg/g	12.35 ± 0.45[d]	11.35 ± 0.45[d]	11.65 ± 0.45[d]	11.78
20 mg/g	13.45 ± 0.47[c]	13.76 ± 0.47[c]	14.45 ± 0.47[c]	13.89
30 mg/g	14.83 ± 0.07[b]	13.46 ± 0.50[b]	14.53 ± 0.48[b]	14.27
40 mg/g	15.49 ± 0.45[a]	15.76 ± 0.45[a]	15.74 ± 0.49[a]	15.66

LSD test indicated significant differences among all concentrations, with 40 mg/g showing the highest antibacterial activity.

Discussion

The study's findings demonstrated that the various natural extracts had differing inhibitory effects on the tested bacteria's growth. This variation is explained by the characteristics of the active compounds found in each extract as well as the structural distinctions between Gram-positive and Gram-negative bacteria. Gram-negative bacteria, such as *E. coli* and *P. aeruginosa*, possess an outer membrane rich in lipopolysaccharides, which reduces the permeability of many antibacterial compounds compared to Gram-positive bacteria like *S. aureus* [17 and 18]. Due to the presence of substances like phycocyanin and unsaturated fatty acids, which damage the integrity of the bacterial cell membrane and obstruct vital metabolic processes, spirulina algae demonstrated strong antibacterial activity against specific bacterial strains. This resulted in the inhibition of bacterial growth or cell death [4]. These findings align with earlier research demonstrating spirulina's efficacy against a range of harmful bacteria[19,20]. When compared to Gram-negative bacteria, the mint extract was more efficient against Staphylococcus aureus. This is explained by the fact that volatile oils, particularly menthol, can more readily pass through Gram-positive bacteria's cell walls., leading to increased membrane permeability and leakage of cellular contents [21]. In contrast, *P. aeruginosa* exhibited higher resistance, which aligns with its known nature of possessing multiple resistance mechanisms, such as efflux pumps and enzymes that degrade antimicrobial compounds. Because the dill extract contains flavonoid and

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terpenoid components that prevent bacterial enzyme function and disrupt cell development, it also demonstrated an inhibitory effect on bacteria [22]. However, its effectiveness was relatively lower compared to some other extracts, which may be related to the concentration of active compounds or the extraction method used [23]. In general, the results of this study confirm that plant extracts and micro-algae represent promising sources of natural compounds with antibacterial activity, and they can be used as alternatives or supplements to antibiotics, especially in light of the increasing problem of bacterial resistance [24]. However, there is still a need for further studies to determine the precise mechanisms of action, optimal concentrations, and safety of these extracts before their clinical application [25].

Conclusion

The results showed that 40% ethanol extracts of *Spirulina platensis* and *Mentha* spp. Additionally, the antibacterial activity of *Anethum graveolens* varies according to the type of extract and the strain of bacteria.

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