The effect of Pimpinella anisum extracts on the activity of a protease enzyme isolated from clinical samples of bacteria

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Abstract
Background This study included the use of medicinal herbs in the treatment of many infections, especially urinary tract infections, due to the presence of great resistance to antibiotics. Anise plant extract was used in this study to inhibit the virulence factors of some types of bacteria isolated from pathological cases. Methodology The Ramadi Teaching Hospital collected 30 scientific specimens from a variety of sources, including burns, wounds, and UTI infections. These samples were collected using sterile cotton swabs. A sterile container was used to collect samples from patients with urinary tract infections, protease activity Casein substrate solution and bacterial growth supernatants were mixed together and incubated at 37°C for 20 minutes to measure the activity of the enzyme protease. A 3 ml injection of 5 percent Trichloroacetic acid (TCA) stopped the reaction, and the sample was centrifuged at 2500 rpm for 20 minutes. The experiment was set up using the same protocols except for the addition of TCA reagent before the supernatants, and the absorbance at 275 nm was then measured. Result the effect of anise extract on inhibiting the activity of some types of gram-positive bacteria such as staphylococcus and gram-negative bacteria such as Pseudomonas aeruginosa. In the activity of the protease enzyme produced by staphylococcus bacteria 16%, while the same concentration of the same extract gave the highest inhibition in the activity of the enzyme in Pseudomonas aeruginosa, reaching 25%, the analysis of variance for the effect of anise plant extract on gram-positive and gram-negative bacteria. There were significant differences regarding the effect of this extract on gram-negative and gram-positive bacteria. There were significant differences for the concentrations used for this extract in inhibiting the activity of this enzyme

1. Introduction

Anise is one of the aromatic plants that was used in the past in alternative medicine for the manufacture of medicines, and anise extracts were used in the preparation of some cosmetics, as well as the nutritional value of anise and the desired taste of everyone.(1) Anise is one of the most important herbs that improve digestion, and contribute to reducing digestive problems such as, indigestion, vomiting, diarrhea, and constipation. (2)The content of anise from various nutrients and antioxidants contributes to fighting the causes of bronchial infections, and drinking anise helps to get rid of phlegm from the throat and lungs and reduce dry coughing attacks, and this makes aniseed an excellent choice for colds, asthma, sinusitis, and pneumonia.(3) One of the most important benefits of anise on an empty stomach is that anise is one of the anti-epileptic and hysterical herbs, as it has a narcotic and sedative effect, so it relieves these seizures by reducing the excessive response.(4.5) The content of anise contributed to controlling cholesterol and fat levels in the body and preventing the accumulation of both cholesterol and fat in the blood vessels, as well as lowering blood pressure levels and preventing episodes of sudden high blood pressure.(6.7) Drinking anise on an empty stomach helps stimulate the metabolism process by stimulating hormones inside the body, which improves the metabolism and burns body fat in a healthy way. Excessive use of anise may cause a sharp drop in blood sugar level, especially for diabetic patients.(8.9) Estrogen-like effects The use of anise by people with hormonal problems may cause a range of hormonal disorders.

2. Material and Methods

Collection of clinical specimens

The Ramadi Teaching Hospital collected 30 scientific specimens from a variety of sources, including burns, wounds, and UTI infections. These samples were collected using sterile cotton swabs. A sterile container was used to collect samples from patients with urinary tract infections.

Bacterial isolation and identification

Various biochemical and cultural methods have been used to identify these bacterial isolates.[10.11]

Extraction of Pimpinella anisum

In order to extract the active organic cloth crude from plant components, the Soxhlet device was utilized. used two plants and ground into a fine powder, 50 grams of the plant powder placed in Thumble, 250 mL of ethanol then placed 95 percent after extraction; the extraction method was completed within 24 hours and then focused by way the Rotary

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Evaporator. Then put in frozen under 20 degrees Celsius until ready to be used. (12)

Protease production

Casein substrate solution and bacterial growth supernatants were mixed together and incubated at 37°C for 20 minutes to measure the activity of the enzyme protease. A 3 ml injection of 5 percent Trichloroacetic acid (TCA) stopped the reaction, and the sample was centrifuged at 2500 rpm for 20 minutes. The experiment was set up using the same protocols except for the addition of TCA reagent before the supernatants, and the absorbance at 275 nm was then measured. (13)

3. Result and Discussion

Table 1 The nutritional value of 100 grams of anise seeds is shown in the following	
nutritional element	Nutritional value
water	9.54 g
energy	337 calories
protein	17.6 g
Fates	15.9 g
carbohydrate	50 g
fiber	9.54 g

Table 2 effect of Pimpinella anisum extracts on the activity of a protease enzyme isolated from clinical samples of bacteria

Dependent Variable: protease activity			
gram positive bacteria	concentration of plant extract	Mean	Std. Deviation
staphylococcus aureus	20%	34.67	1.528
	40%	26.00	1.000
	80%	16.00	1.000
	Total	25.56	8.156
pseudomonas aerginosa	20%	40.67	5.774
	40%	34.00	1.000
	80%	25.00	2.000
	Total	33.22	7.480
Total	20%	37.67	5.007
	40%	30.00	4.472
	80%	20.50	5.128
	Total	29.39	8.555

Table 2 shows the effect of anise extract on inhibiting the activity of some types of gram-positive bacteria such as staphylococcus and gram-negative bacteria such as Pseudomonas aeruginosa. In the activity of the protease enzyme produced by staphylococcus bacteria 16%, while the same concentration of the same extract gave the highest inhibition in the activity of the enzyme in Pseudomonas aeruginosa, reaching 25%. Anise contains many nutrients necessary for health, which may contribute to enhancing the benefits of anise for the health of the body and hair. for oxidation. (14) That anise oil capsules may contribute to improving other symptoms of Irritable Bowel Syndrome, including: gastroesophageal reflux disease, headache, fatigue, general satisfaction and quality of life, and accordingly; This study concluded that anise may have a remarkable efficacy in improving symptoms of Irritable Bowel Syndrome.(11)

Table 3 ANOVA table of effect of Pimpinella anisum extracts on the activity of a protease enzyme isolated from clinical samples of bacteria

Dependent Variable: protease activity					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1158.944[a]	5	231.789	32.595	.000
Intercept	15546.722	1	15546.722	2186.258	.000
bacteria	264.500	1	264.500	37.195	.000
concentration	887.444	2	443.722	62.398	.000
bacteria * concentration	7.000	2	3.500	.492	.623
Error	85.333	12	7.111		
Total	16791.000	18			
Corrected Total	1244.278	17			
a. R Squared = .931 (Adjusted R Squared = .903)					

Table 3 shows the analysis of variance for the effect of anise plant extract on gram-positive and gram-negative bacteria. There were significant differences regarding the effect of this extract on gram-negative and gram-positive bacteria. There were significant differences for the concentrations used for this extract in inhibiting the activity of this enzyme. Bacteria, as shown in Figure 1. Anise contains powerful compounds with anti-bacterial and anti-fungal properties, the most important of which is anethole, which contributes to enhancing the benefits of anise in preventing infection with yeasts and dermatophytes that cause skin diseases. It also prevents the growth of several strains of bacteria,

such as the bacteria that cause cholera. (3) Antioxidant Thanks to its antioxidant effect, anise slows down the aging of cells. They are very rich in nutrients as they contain vitamins B and C, iron, zinc, calcium, magnesium and potassium. Thus, anise has many benefits for the immune system. This aromatic plant also helps purify the blood. (6)

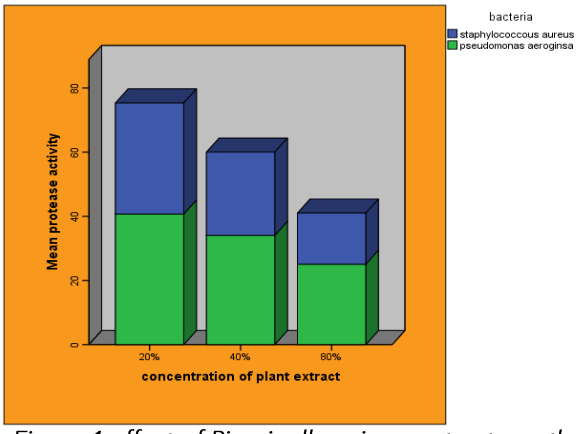

Figure 1 effect of Pimpinella anisum extracts on the activity of a protease enzyme isolated from clinical samples of bacteria

References

Al-Wendawi, S. A., & Gharb, L. A. (2021). ANTIOXIDANT, ANTIBACTERIAL AND ANTIBIOFILM POTENTIALS OF ANISE (Pimpinella anisum) SEEDS EXTRACTED ESSENTIAL OILS. IRAQI JOURNAL OF AGRICULTURAL SCIENCES, 52(2), 348-358.

Ashry, A. M., Habiba, M. M., El-Zayat, A. M., Hassan, A. M., Moonmanee, T., Van Doan, H., ... & Dawood, M. A. (2022). Dietary anise (Pimpinella anisum L.) enhances growth performance and serum immunity of European sea bass (Dicentrarchus labrax). Aquaculture Reports, 23, 101083.

Bharti, S. K., Pathak, V., Arya, A., Alam, T., Singh, V. K., Verma, A. K., & Rajkumar, V. Characterization of composite active edible film functionalized through reinforced Pimpinella anisum essential oil. Journal of Food Processing and Preservation, e16766.

Bontzolis, C., Plioni, I., Dimitrellou, D., Boura, K., Kanellaki, M., Nigam, P. S., & Koutinas, A. (2022). Isolation of antimicrobial compounds from aniseed and techno-economic feasibility report for industrial-scale application. International Journal of Food Science & Technology, 57(8), 5155-5163.

Das, S., Singh, V. K., Dwivedy, A. K., Chaudhari, A. K., & Dubey, N. K. (2021). Nanostructured Pimpinella anisum essential oil as novel green food preservative against fungal infestation, aflatoxin B1 contamination and deterioration of nutritional qualities. Food Chemistry, 344, 128574.

Fathi-Achachlouei, B., Babolanimogadam, N., & Zahedi, Y. (2021). Influence of anise (Pimpinella anisum L.) essential oil on the microbial, chemical, and sensory properties of chicken fillets wrapped with gelatin film. Food Science and Technology International, 27(2), 123-134.

Fradi, A. J. (2022). Evaluation the Effectiveness of Different Concentrations Phenols, Alkaloids and Terpenes Extracted from Pimpinella anisum against Phytophthora Fungi. Ibn AL-Haitham Journal for Pure and Applied Sciences, 35(2), 1-6.

Hasan, N., Chakraborty, T., & Das, T. (2021). PHARMACOLOGICAL PROPERTIES OF PIMPINELLA ANISUM: A.

Lavaee, F., Moghaddas, A., Modarresi, F., & Nowrouzi, M. (2022). The Effect of Pimpinella Anisum and Origanum Vulgare Extracts Against Streptococcus Sanguinis, Streptococcus Mutans, and Streptococcus Salivarius. Journal of Dentistry, 23(2), 113-120.

Nasır, A., & Yabalak, E. (2021). Investigation of antioxidant, antibacterial, antiviral, chemical composition, and traditional medicinal properties of the extracts and essential oils of the Pimpinella species from a broad perspective: a review. Journal of Essential Oil Research, 33(5), 411-426.

Sayadi, M., Mojaddar Langroodi, A., & Jafarpour, D. (2021). Impact of zein coating impregnated with ginger extract and Pimpinella anisum essential oil on the shelf life of bovine meat packaged in modified atmosphere. Journal of Food Measurement and Characterization, 15(6), 5231-5244.

Noori, N., Khanjari, A., Rezaeigolestani, M., Karabagias, I. K., & Mokhtari, S. (2021). Development of antibacterial biocomposites based on poly (lactic acid) with spice essential oil (Pimpinella anisum) for food applications. Polymers, 13(21), 3791.

Jafari, R., Zandi, M., & Ganjloo, A. (2022). Effect of ultrasound and microwave pretreatments on extraction of anise (Pimpinella anisum L.) seed essential oil by ohmic-assisted hydrodistillation. Journal of Applied Research on Medicinal and Aromatic Plants, 100418.

Mehrabanjoubani, P., Ghorbani Nohooji, M., Karimi, E., & Abdolzadeh, A. (2021). The differences between Froriepia subpinnata (Ledeb.) Baill. and Pimpinella anisum L. commonly named as anarijeh based on major components of the essential oil, a marker for resolve ambiguities. Journal of Medicinal Plants, 20(79), 59-71.