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Introduction

The significance of any natural drug lies in its ability to treat various diseases and infections medicinally. Proteins are considered among the multi-chemical compounds with therapeutic efficacy, especially those derived from fungi and algae. Chemically, proteins contain the active chemical group represented by the peptide bond present in the structure of the polypeptide chain. (1,2) The active proteins were successfully extracted from various medicinal plants, and these biological molecules demonstrated their high capacity to treat multiple infections and inflammatory conditions caused by diverse pathogenic microorganisms. Also, natural proteins isolated and characterized from some fungi and algae have been used as effective remedies in medicine. Therefore, many pre-

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Evaluation of Medicinal Efficacy of Proteins Isolated from Various Fungi and Algae against Some Pathogenic Microorganisms

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Abstract

Proteins are polymeric polypeptidic compounds that consist of many various amino acids, which are bound by peptidic bonds with each other. They are essential components of humans, animals, and plants because they participate in the biochemical processes that sustain life. Some proteins are active chemical compounds used to treat various diseases. In this study, active proteins were extracted and isolated from diverse fungi and algae. The results of the medicinal efficacy of these proteins showed various inhibition zone diameters against the pathogenic microorganisms, including *Alternaria alternata*, *Candida tropicalis*, *Candida krusei*, *Candida albicans*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In conclusion, this study approved the possibility of extraction and using of fungi and algae medical proteins as natural therapies to treat diverse diseases caused by pathogens (fungi and bacteria), rather than synthetic drugs, which have various side effects.

Keywords: Active proteins, Fungi, Alga, Medical efficacy, *Candida albicans*.



studies demonstrated the biochemical and medicinal roles of proteins naturally extracted from microorganisms, such as algae and fungi. (3,4). Various vital organisms, such as fungi and algae, can serve as biological sources for the production of active proteins, making them beneficial for the production of natural active proteins. Some pre-research demonstrated and ensured the medicinal efficacy of active proteins isolated from diverse algae and fungi. Also, these proteins have been used as effective drugs for the treatment of multiple diseases caused by different pathogens. (5,6). Pathogenic microorganisms such as *Candida tropicalis*, *Candida krusei*, *Candida albicans*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are highly hazardous to humans, as they cause serious infections and inflammation, leading to diverse diseases. (7–9). Review of literature revealed scarce publications regarding the extraction medicinal proteins from various Fungi and Algae in Iraq. Consequently, the present research was conducted to evaluate the medicinal and biochemical efficacy of chemically extracted proteins from some algae and fungi against the growth of various pathogenic fungi and bacteria.

Materials and Methods

Biological sources of proteins

Some local living microorganisms were used in this study, namely *Penicillium sp.* and *Spirulina sp.*, for the biochemical production of active proteins. A specialist microbiologist characterized these microorganisms.

Chemical extraction of proteins

The potent proteins were extracted from fungi and algae by growing *Penicillium spp.* on a solid medium for 5 days. Next, the media was divided and placed in other media containing the modified toxin for 11 days. Then the mycelium was collected by filtration through a layer of Miracloth. After this process, the contents were ground carefully in a cold chamber. Concentrations of 1000 mg/mL of each isolate of fungus were prepared in ethanol (50% v/v). After that, the fungal mixtures were centrifuged, then filtered through 0.2 µm paper. The extracts obtained from filtration were used to evaluate antifungal efficacy. The protein extract was treated with 3 mL of ethanol (50 % v/v), the volume was adjusted to 15 mL with a pipette, and the mixture was placed in focal tubes. The protein extract was precipitated using protein buffer three times. The buffer and extract were mixed well by inversion 20 times, and the mixture was incubated for 2 hours at -20 °C. The protein and buffer mixture was centrifuged. The supernatant was collected and incubated at -22 °C, and the buffer was precipitated overnight. The protein pellets were washed gently three times with cold acetone containing DDT after centrifugation. Afterward, the pellets were air-dried, and proteins were solubilized at another time using labeling buffer, and the pH was adjusted to 8. Then, 30 ml of the sample was removed for total protein quantification, and the remaining supernatant was kept at -25 °C until protein electrophoresis (10,11)

Biological sources of proteins

The alga (*Spirulina sp.*) and the fungus (*Penicillium sp.*) were used as natural biological sources for the extraction of total active proteins.



Pathogenic fungi and bacteria

Diverse types of fungi represented by *Alternaria alternate*, *Candida tropicalis*, *Candida krusei*, *Candida albicans*, and different kinds of bacteria represented by *Bacillus subtilis*, *Pseudomonas areginosa*, and *Staphylococcus aureus* were used in the current study.

Estimation of total protein concentration

The concentration of total protein was determined using bovine serum albumin (0.2 mg), and the absorbance at 595 nm was recorded for each concentration. Linearity was measured across all concentrations, and a standard calibration graph was generated for these concentrations and absorbance. (12,13). As shown in Figure 1.

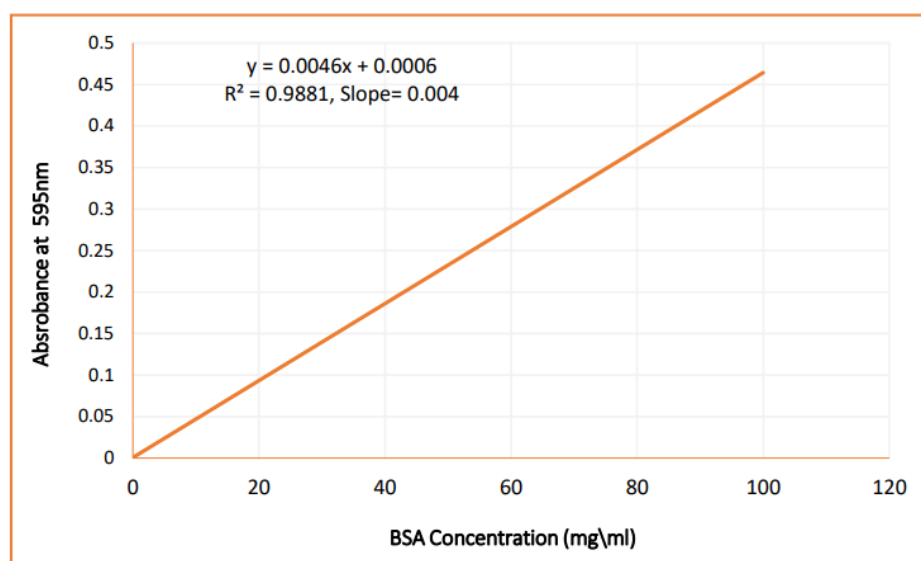


Figure. 1: Standard calibration graph of the bovine standard protein.

Assay of medicinal efficacy of active proteins

Various protein concentrations (0.2ml) extracted from algae and fungi were used to estimate antibacterial and antifungal efficacy. The well agar diffusion method was carried out. The petri dishes were cultured with a bacterial and fungal suspension containing 1×10^8 cells/ml using a glass spreader, and then wells were made in the culture medium. Each algal or fungal sample was placed in a well containing 100 mL. After that, all weights were incubated at 37 °C for 24 hours. Finally, inhibition zone diameters (cm) were measured for all protein concentrations. (14,15).

Results

Qualitative detection of proteins extracted from fungi and algae showed formation of a violet color when the proteins were treated with the biuret reagent. This result indicates the presence of active proteins in the extracts isolated from algae and fungi.

Extraction percentage of proteins.

The weights of proteins extracted from *Spirulina sp.* and *Penicillium spp.* were 2 and 2 g, respectively; therefore, the extraction percentages were 0.5% and 10.42%, as shown in the following equation.

$$\text{Extraction percentage} = \frac{\text{Weight of protein extract (gm/ml)}}{\text{Weight of alga or fungus (gm/ml)}} \times 100$$

The extraction percentage was perfect, ensuring the presence of active proteins in high quantities in both *Spirulina sp.* and *Penicillium spp.* However, the rate was higher in fungi than in algae, as shown in Table. 1.

Table. 1: Extraction percentages of proteins extracted from *Penicillium spp.* and *Spirulina sp.*

Microorganism type	Weight of living organism (gm)	Weight of protein extract	Extraction percentage(%)
<i>Spirulina sp.</i>	2	0.011	0.5 gm/ml
<i>Penicillium spp.</i>	2	0.2085	10.42 gm/ml

Medicinal Efficacy of Protein Extracts

Concerning *Spirulina sp.* 0.2 g recorded inhibition zone diameter represented by 0.4, 0.4, and 0.3mm against pathogenic bacteria (*Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). At the same time, the same weight belongs to the protein extract of *Penicillium spp.* Showed different inhibition zone diameters equal to 0.2,0.1, and 0.2 mm, against the growth of the same pathogenic bacteria as referred to in Table. 2.

Table. 2: Medicinal efficacy of proteins extracted from algae and fungi against pathogenic bacteria.

Type of living organism	Weight (mg)	Inhibition zone diameters (mm)		
		<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
<i>Penicillium spp.</i>	0.2	0.2	0.1	0.2
		0.2	0.1	0.2
<i>Spirulina sp.</i>	0.2	0.4	0.4	0.3

Concerning *Spirulina sp.* 0.2 gm recorded inhibition zone diameters represented by 0.7, 3.1, 3.2, and 3.1mm against pathogenic fungi (*Alternaria alternata*, *Candida tropicalis*, *Candida krusei*, and *Candida albicans*). At the same time, the same weight belongs to the protein extract of *Penicillium spp.* Showed different inhibition zone diameters equal to 0.1, 1.3, 1.2, and 2.1mm against the growth of the same pathogenic fungi as illustrated in Table. 3.

Table. 3: Shows the medicinal efficacy of proteins extracted from algae and fungi against pathogenic fungi.

Type of living organism	Weight (mg)	Inhibition zone diameters (mm)			
		<i>Alternaria alternata</i>	<i>Candida tropicalis</i>	<i>Candida krusei</i>	<i>Candida albicans</i>
<i>Penicillium spp.</i>	0.2	0.1	1.3	1.2	2.1
		0.7	3.1	3.2	3.1
<i>Spirulina sp.</i>	0.2	0.7	3.1	3.2	3.1

The results of the biological activity values in the case of combining the protein extracted from mushrooms with the protein extracted from algae in a ratio of 0.1 mg of each type, with the total weight being 0.2 mg for both types together, were as follows: 0.7, 0.9 and 0.9 mm against pathogenic bacteria (*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) . were as follows: 0.9, 4.1, 3.5 and 3.7 mm against pathogenic fungi (*Alternaria alternata*, *Candida tropicalis*, *Candida krusei* and *Candida albicans*) as referred in table. 4.

Table. 4: Shows the medicinal efficacy of proteins extracted from *Penicillium spp.* and *Spirulina sp.* against pathogenic microorganisms.

Pathogenic microorganisms	Inhibition zone diameters (mm)
<i>Alternaria alternate</i>	0.9
<i>Candida tropicalis</i>	4.1
<i>Candida krusei</i>	3.5
<i>Candida albicans</i>	3.7
<i>Bacillus subtilis</i>	0.7
<i>Pseudomonas aeruginosa</i>	0.9
<i>Staphylococcus aureus</i>	0.9

Discussion

The biological importance of natural proteins lies in their biochemical and medicinal functions, especially those derived from organisms such as algae and fungi. Therefore, these proteins play therapeutic roles in treating various diseases caused by pathogenic bacteria, fungi, and parasites (16). The results of the current study, revealed that potent proteins were successfully extracted from *Spirulina sp.* and *Penicillium spp.* in high yields. These observation give these microorganisms a valuable biological source for obtaining various proteins via a simple, inexpensive process. The extracted proteins were confirmed using the Biuret reagent, as the formation of a violet color indicated the presence of natural proteins.

Pre-diverse studies were conducted on the extraction and isolation of active proteins from various classes of algae and fungi, confirming their biological and medicinal effectiveness as active chemical compounds. Additionally, these compounds were used as agents to inhibit the growth of pathogenic fungi, parasites, and bacteria (17–19). Crude protein was

chemically extracted, purified, and isolated from medicinal mushrooms, and its biological and medical activity was evaluated against *Candida albicans*. Simultaneously, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined, and the crude protein demonstrated superior potential. It is known that *Candida albicans* is a pathogenic fungus responsible for serious inflammatory diseases; therefore, algal protein extract is a highly effective agent for inhibiting the biological and chemical processes of various fungi (20,21). Based on the current study's results, it was concluded that protein extracts from both *Spirulina sp.* and *Penicillium spp.* Exhibit excellent antimicrobial activity against the tested microorganisms. The activity of the fungal crude protein was higher than that of the algal protein against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, which is attributed to the presence of distinct active compounds with strong ability to inhibit or kill pathogenic bacteria (22,23). In contrast, the algal protein extract was more effective than the fungal extract against *Alternaria alternata*, *Candida tropicalis*, *Candida krusei*, and *Candida albicans*, likely because the algal protein contains more potent proteins that disrupt the chemical systems of pathogenic fungi, including carbohydrates, lipids, nucleic acids, proteins, and enzymes (24,25). The combination of algal and fungal protein extracts showed greater efficacy against *Alternaria alternata*, *Candida tropicalis*, *Candida krusei*, and *Candida albicans* than either extract alone, although this mixture exhibited less biological activity against bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This suggests that bacteria are more resistant to the protein extract mixture than fungi (26).

Conclusion

In conclusion, active protein extracts were isolated from *Spirulina sp.* and *Penicillium spp.*. The results of the current study demonstrated the medicinal efficacy against certain pathogenic fungi and bacteria, significantly inhibiting their growth. Also, the mixture of proteins extracted from both algae and fungi showed the highest biological activity, especially against pathogenic fungi and bacteria, leading to the destruction of their chemical and biological systems. Therefore, proteins extracted from algae and fungi, as well as protein mixtures, can be used as natural therapies to treat diverse diseases caused by these pathogenic fungi and bacteria.

Declarations

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Competing Interests

The authors declare that they have no competing interests, financial or non-financial, relevant to the content of this article.



Author Contributions

SQB: Conceptualization, Methodology, Investigation, DNA: Writing – Review and Editing, ADMA: Supervision, Editing, and Writing. All authors read and approved the final manuscript.

Compliance with Ethical Standards

All experiments were conducted in accordance with institutional guidelines and applicable national regulations. This study did not involve human participants, human tissues, or animals.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Generative AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used Grammarly for language polishing. They also consulted ChatGPT and Papers Pal to clarify scientific concepts. After using these tools, the authors thoroughly reviewed and edited the content, and take full responsibility for the final version of the manuscript.

Consent to Publish / Consent to Participate

Not applicable. This study did not involve human participants

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