



Alginin: A Natural Alternative to Antibiotics Used for *In vitro* Inhibition of Clinically *Propionibacterium acnes* Growth from Adolescents in Mosul City/Iraq.

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Abstract:

Context: Acne is an inflammatory disease of the sebaceous glands that results in the formation of papules, pustules, and more advanced complicated scars by opportunistic micro-organisms that are known as *Propionibacterium acnes* which feed mainly on fat. The need to reduce the use of antibiotics and the discovery of medicinal plant treatments, due to the resistance of Bacteria over time. *Propionibacterium acnes* is a gram-positive bacterium that plays the main role in the development of some diseases. **Aims:** This study aimed to investigate the effect of Alginin which was extracted from *Pleurotus ostreatus* fungus on *Propionibacterium acnes*. **Method:** The study involved (100) samples, which were collected from 75 patients suffering from acne, 65 samples of comedones, and 35 pustules, ages ranging between (14-20) years. All samples were collected from adolescent patients in Mosul City/Iraq. All samples indicated the presence of *P. acnes*.

Results: A marked effect was observed through the inhibition of the growth of *Propionibacterium acnes* as the diameter of inhibition of the extract of Alginin ranged between 9.5 mm to 11.5 mm. So, we suggest the possibility of using Alginin as an alternative to antibiotics in the treatment of skin affected by acne.

Keywords: Alginin, Bacteria, *acne*, damaged cells, *Pleurotus ostreatus*.

1. Introduction

Acne is a common skin disorder marked by the formation of blackheads, whiteheads, pimples, and scars. Acne is often treated with antibiotics, but there are increasing concerns about antibiotic resistance and side effects, so it was necessary to use natural alternatives, so the fungi were the first choice because they contained many effective compounds

[1,2]. Basidiomycetes produce antibiotics through secondary metabolism. These compounds are produced in response to environmental stresses, Such as the presence of harmful bacteria [3]. Basidiomycota is one of the finest fungal groups, they include saprophytic types that absorb simple organic matter and analyze complex organic matter by the decomposer enzymes that they secrete in the

growth medium and types that establish a symbiotic relationship as mycorrhiza with the roots of some plants [4,5]. Among the most prevalent and well-researched species is *Pleurotus ostreatus*. It is a member of the family Pleurotaceas, order Agaricales, phylum Basidiomycota. For *P. ostreatus* to develop fruiting bodies, solid-state culture is frequently employed, with lignocellulosic side-streams from agro-industrial food production serving as the substrate [6,7] *P. ostreatus* fruiting body production can take up to twenty to twenty-five days. Due to its potential to produce mycelia with consistent, repeatable properties and useful metabolites in a significantly shorter amount of time, submerged culture has drawn more interest recently [8]. Humans have been consuming *P. ostreatus* fruiting bodies for thousands of years because they have outstanding organoleptic qualities and high nutritional content. The mycelia and basidiocarp metabolites have excellent medicinal qualities [9]. Numerous bioactive substances, including polysaccharides, soluble and insoluble glucans, dietary fibers, proteins, polyphenols, and macro- and microelements, are found in large amounts in these mushroom species. Alginin is a natural compound found in Basidiomycetes. It treats acne in several ways. Anti-inflammatory: Alginin reduces inflammation, which is one of the main factors of acne, and kills acne-causing bacteria, such as *Propionibacterium acnes*, which protects skin cells from damage caused by free radicals, which may contribute to acne. helps remove dead skin cells from skin pores, preventing them from becoming clogged and reducing the appearance of blackheads and whiteheads. Several studies indicate that Alginin is effective in treating acne. For example, one study found that alginate cream was as effective as clindamycin cream, a common antibiotic for acne. Another study found that alginate gel significantly improved acne severity in people with moderate to severe acne [10]. Alginin is a natural substance that has been used for centuries in traditional medicine. They are generally safe to use and have little risk of

side effects they have shown activity against antibiotic-resistant strains of *Propionibacterium acnes*. It can help reduce inflammation, which is one of the main factors of acne, and help improve overall skin health by moisturizing and nourishing it. Disadvantages of using alginate. May not be effective for everyone. Alginin may not be effective for all people with acne. May cause some mild side effects, such as dryness and itching. One of the most extensively investigated Alginin is lignin, which is a complex organic compound that forms a significant part of the cell wall in fungi. As it resists biodegradation, lignin is important as a component in numerous natural materials. Properties of *Pleurotus ostreatus* fungus extracted lignins [11]. Antioxidant: According to studies, Alginin sourced from *Pleurotus ostreatus* mushroom has potent antioxidant properties that keep cells safe from free radicals that damage them. Anti-inflammatory: Alginine obtained from fungal species *Pleurotus ostreatus* is believed by some researchers to have anti-inflammatory characteristics meaning they could be used to manage conditions such as rheumatism and other inflammatory diseases. Immune booster: Some studies suggest that lignin taken out from the *Pleurotus ostreatus* mushroom species can support up or lift immune systems which implies it might safeguard against infections. Bactericidal: Lignins isolated from *Pleurotus ostreatus* mushrooms are also effective antibacterial agents in vitro thereby it may be useful when dealing with bacterial infections. Anti-cancer: In several experiments conducted on *Pleurotus ostriates* extracts there was indication that they possess antitumor attributes meaning they can be used both for prophylactic and therapeutic purposes [12].

2. Materials and Methods:

Extraction of the active component (Alginin)

Extraction by Soxhlet is a chemical process used to extract substances from liquids or solids. It is a more complicated method than simple hydrolysis but can extract specific compounds such as active ingredients in a sample. The following are the steps

of Soxhlet extracting from the fruiting body parts of the fungus, a dry sample was prepared and prevented contamination during the sample process. Twenty-five (25) g of powder of the fruiting body for extraction were used. The sample was put in the filtered funnel, and it was inserted into the Soxhlet device and the water was added. The cup was heated until the solvent began to boil. The steam was condensed on the condenser and dripped back into the cup. Repeat all the above steps many times to obtain the maximum amount of active extraction.

HPLC Technique

Extraction Procedure: Two different extraction methods were employed, including petroleum ether extraction and water extraction. For the first one (25) of dried and powdered *Pleurotus ostreatus* were mixed with (400) ml of petroleum ether for (24) hours. The extract was then filtered through filter paper and concentrated using a rotary evaporator. The second method of extraction was carried out by boiling (25 gm) dried and powdered *Pleurotus streams* with (100) ml of distilled water for (24) hours. The extract was then filtered and Lyphliyer to obtain it as powder. **Preparation of HPLC Samples:** The extracts obtained from the two extraction methods were dissolved in water and (10) mg to prepare the HPLC samples. The samples were then filtered through a (0.22) μm filter before being injected into the HPLC system.

HPLC Analysis

HPLC analysis was carried out using Japanese Shimadzu. The column used was C18. The mobile phase consisted of (80%) methanol and (20 %) water, with a wavelength of (280) nm (Detector) and a flow rate was (1 ml/minute) for ten minutes. **Identification of Active Ingredient:** The active ingredient in the extracts was identified by comparing the retention time and UV spectra with those of the standard (Alginin) compound. **Determination of Extract Yield:** The extract yield was determined by dividing the weight of the extract obtained by the weight of the plant material used for the extraction. **Determination of Purity:** The purity of the active

ingredient in the extracts was determined by calculating the peak area of the active ingredient and comparing it with the total peak area of all the compounds in the extract. Purity was expressed as a percentage (Alginin).

Microorganism suspensions:

Bacterial suspension: in this report, 100 samples from 75 patients suffering from acne, for both genders, ranged in age from 14-25 years. The samples were collected from Mosul Hospitals: (dermatology outpatient clinics) while the laboratory steps were done at Northern Technical University / main lab. Samples included comedones and pustules were taken after wiping the skin 3-5 times with ethyl alcohol at a concentration of 70%, comedones samples were collected by a comedy extractor, whereas pustules samples were collected by sterile lancet (StenleLancet), the Samples had placed separately in the tube containing thioglycolate broth and transported to the laboratory [13]. The samples were then taken out by mixer tubes to homogenize the bacteria in the medium, and incubated anaerobically for 5-7 days at 37 ° C, After incubation, the anaerobic samples were incubated in a jar containing anaerobic gas Pak and demonstrate the cultural characteristic of colonies growing on the medium in an anaerobic medium according to the shape and color, then preparing a bacterial gram-stained smear, and were examined under a microscope to see cell shapes and their positivity for gram stain [8], and also the biochemical reactions that important in the diagnosis were made such as Oxidase, Catalase, indole, urease, gelatin printing, and blood hemolysis test [12].

Bacterial Suspension:

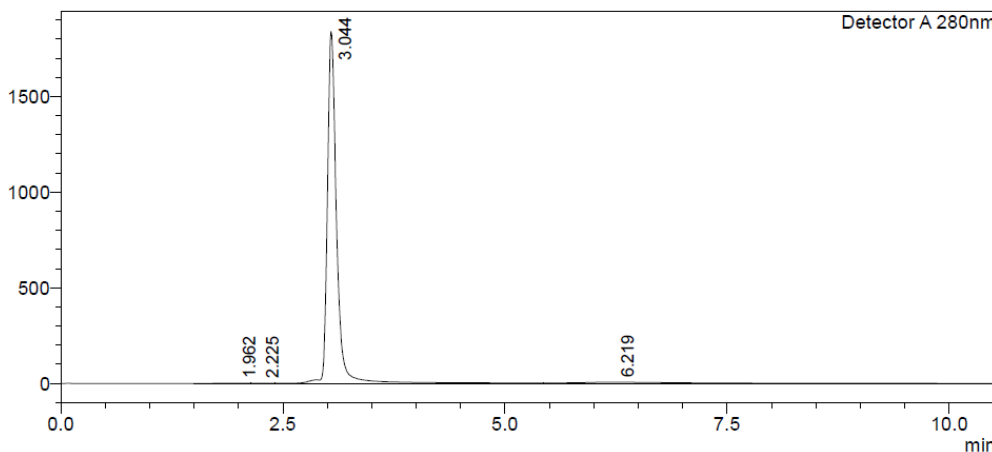
Prepared by taking pure colonies and culturing them in test tubes containing thioglycolate broth and mixing well to ensure spreading the bacteria in the nutritive medium and incubated anaerobically at 37° for 3 days. The culture after incubation was compared to McFarland standards by using several concentrations until reaching 1.5×10^8 CFU /ml and incubated at 37 °C for 3 days [14].

3. Results:

The chromatography analysis using the HPLC technique revealed the availability of a number of active compounds based on standards, as shown in Figure 1. When the active compound Alginine was extracted from the basidiomycetes *Pleurotus Ostreatus* from several areas of the city of Mosul, the results revealed that Alginine was a higher concentration in the Alasania region (19.7 g/ml), followed by Alsalam ' farms, Mosul Forest and Rashidia, (0.58, 0.099, 0.084 g/ml) respectively. While the lower levels were Alginin (0.044 g/ml) in Aldhabi's farms. *Pleurotus ostreatus*, also known as oyster mushroom, shows a marked difference in the concentration of the active ingredient asinine from

one region to another. From the Factors Affecting Alginine Concentration Environmental factors such as climate, temperature, humidity, amount of sunlight, and soil type play an important role in determining the concentration of Alginine in mushrooms. See Figure 1.

Table 1 shows the results of Alginin concentration among study regions. The results revealed that higher concentration in Alasania (19.72996 g/ml) followed by Amsalem farms, Mosul Forest, and Rashidia regions (0.581078, 0.099868, 0.084377 g/ml) respectively. While the lower concentration of Alginin was 0.044495 in Alhadba's farms' region (table 1).



<Peak Table>

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.962	16730	1147	0.000			
2	2.225	10312	705	0.000		V	
3	3.044	13497663	1839380	0.000		V	
4	6.219	613313	6137	0.000		V	
Total		14138018	1847368				

Figure 1: Concentrations of Alginin in an extraction by HPLC Technique

Table 1: Alginin concentration among Mosul regions under study.

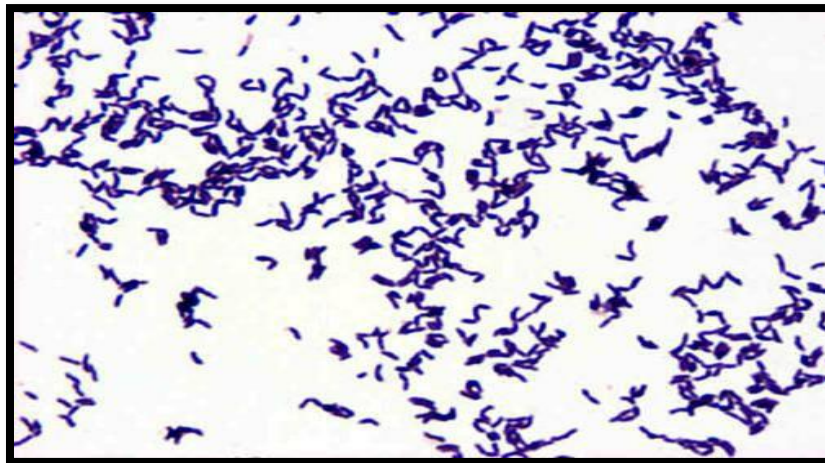
Site	Ret. Time(min)	Area	Concentration (1g/ml)
Standard (Alginin)	3.044	1839380	(1g/ml)
Alginin			
Alasania	59861	3034015	19.72996
Rashidia	256	3034015	0.084377
Alhadba' farms	135	3034015	0.044495
Alsalam farms	1763	3034015	0.581078
Mosul forest	303	3034015	0.099868

In the present study, we diagnosed *P. acne* depending on the colonies that appeared greyish white as shown in Figure 2, and that the colonies grow in size over time, see Figure 2.



Figure 2 " Colonies of Propionibacterium acnes on Blood Agar.

The bacteria are gram-positive and take on different shapes, including Cocci or Bacilli, and often arranged in a Y or V shape, as shown in Figure 3:



"Figuer3 Propionibacterium acnes under Microscope,100x

Biological efficacy of Alginin against *p. acne*:

The results showed the capacity of Alginin compound supernatants against the growth of *p. acne* bacteria used in the study, the table 2 indicates that the bacteria *p. acne* was the most sensitive to the Alginin compound, reaching OD 0.387 at 600 nm at a concentration of 200 µg / ml which is the highest concentration used (Table 2).

Table 2: The effect of Alginin compound against *p. acne*.

Treatment Alginin bacteria <i>P. acne</i>	200	100	50	25	12.5	6.25	+ve	-ve
	µg / ml							
<i>p. acne</i>	0.387	0.387	0.387	0.445	0.445	0.591	0.637	0.336

4. Discussion:

Alginin, a polysaccharide extracted from the oyster mushroom (*Pleurotus ostreatus*), emerges as a promising candidate for acne therapy. Its antibacterial properties, particularly against *Propionibacterium acnes*, a key bacterium implicated in acne development, suggest its potential to disrupt the inflammatory process underlying the condition [15]. The antibacterial attributes of Alginin and its anti-inflammatory potential make it valuable in the therapy of acne. Alginin has been found, through clinical and preclinical studies, to be effective against *P. acne* which is crucial for acne formation. These anti-inflammatory features can enhance skin health by reducing any redness or irritation associated with acne. Studies have shown that alginin can be used in both preclinical and clinical contexts to treat acne [11]. Nonetheless, more research is needed to determine whether it works well enough and if it's safe when used on different groups of patients suffering from acne. Further investigations should look at the right dosage, appropriate treatment duration as well as likely drug interactions [16]. The concentration of Alginin in mushrooms increases as they age, then decreases again when they are fully mature. The harvesting method, like with sharp tools, may affect the concentration of Alginine in the mushrooms. There are studies on the variation of Alginine concentration. Study in Finland: A study conducted in Finland showed that the concentration of Alginine in the fungus *Pleurotus austriates* decreases significantly from north to south, according to [17]. Study in China: A study conducted in China showed that the concentration of alginine in *Pleurotus austriates* varies greatly between different regions of the same province, according to [18]. Study in the USA: A study across various forests in the USA reported that there was a high variation in alginine concentration in *Pleurotus austriates* [19]. The difference in alginin concentration is important because the following are medical uses of *Pleurotus ostreatus* mushroom, a source of traditional medicine

against such conditions as cancer, heart diseases, infections, and so on. Thus, the differences in its alginine concentration would impact the effect of the mushroom on such conditions. Nutritional value: The *Pleurotus ostreatus* mushrooms are rich in proteins, fiber content, vitamins, and essential minerals. Differences in alginin concentration may affect the nutritional value of mushrooms. Differences in alginin concentration may affect the taste of mushrooms [20]. For biochemical tests of *p. acnes* bacteria are Catalase positive, gelatin liquefy [21]. 50 samples of *p. acnes* bacteria were identified from 65 samples, 35 samples of which were collected from patients suffering from comedones, and 15 samples were isolated from patients suffering from pustules, the dominance of bacterial type in the comedones samples is virulent due to the increase in oxygen pressure in comedones, which creates favorable conditions for bacterial growth and its reproduction supernatant of Alginin compound effect in inhibition the growth of *p. acnes* bacteria [22].

The differences in concentrations of compounds and their quality can be attributed to geographical and climatic conditions, which are represented in temperatures and the amount of rainfall, as well as the age of fruiting bodies, which have a major role in the yield of secondary metabolic results, as well as the nature of developing organic matter. On it mushrooms. The variation in the inhibitory effect on bacterial growth may be due to yeast genera and their ability to produce toxins and also due to the process of purification of the yeast to obtain the Alginin compound in pure form and free from bacterial products, it may be reaching to 41% and lead to impairment its ability for production toxins [23,24] discovered that the dietary mushroom *P. ostreatus* contains an active compound called alginin, which may have anti-bacterial properties. These properties are characterized by mechanisms of action that include cell membrane lysis, proteolytic enzymes, microbial adhesion, and inhibition of protein

synthesis. Additionally, organic extracts of the mushroom have demonstrated effectiveness against Gram-positive bacteria, suggesting that they may be a potential source of anti-bacterial agents.

Conclusion:

Alginin compound has an inhibitory effect on the growth of *P. Acne* which was revealed by the study. Due to the increasing resistance of *P. acne* against various antibiotics in addition to the vigorous use of antibiotics against the disease, it will be a good opportunity to conduct further studies to include the biological treatment for such diseases with bacterial origin by using Alginin compound or other biological agents.

Ethical statement

This study was conducted according to the standards accepted in the medical manuscripts, where official approvals were obtained from the Mosul Medical Technical Institute / Northern Technical University, where the study was conducted on a sample of students after obtaining their approvals, we the authors are committed to protecting the privacy of students participating in the study.

Conflict of interest

There is no conflict of interest.

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