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Effect of *Cladosporium herbarum* Extracts on Some Bacterial Species

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Abstract

Background: The results of the chemical detection showed that the active groups in the *Cladosporium herbarum* extracts are Sumiki's acid and Acetyl Sumiki's acid, and knowing its biological effectiveness against bacteria

Aim: To evaluate the effect of *Cladosporium herbarum* extracts on some bacterial species (*E. coli & Staph. aureus)*

Materials and methods: The fungal extract of *Cladosporium herbarum* was prepared and tested for its inhibitory effect on *Escherichia coli* and S*taphylococcus aureus*. Three concentrations were tested (50, 100, 250 mg/ml). The active compounds of the extract were then identified.

Results: The fungus producing the fungal extract was isolated from various soil samples and was found to be *C. herbarum*. After growing on the production medium for seven days at 30°C and chemically identifying the active components in the extracts, the presence of sumiki's acid and acetyl sumiki's acid35 in the *Cladosporium herbarum* fungal extract was found at concentrations of 0.32 and 0.97 mg per 100 ml, respectively. The inhibitory capacity was tested at concentrations of 50, 100, and 250 mg/ml against Staph. aureus and E. coli bacteria. The results showed that the fungal extract varied in its inhibitory effect on the isolated bacteria at a concentration of 50 mg/ml, with an average diameter of inhibition of 13 mm. The average diameter of inhibition at a concentration of 100 mg/ml was 17 and 20 mm, respectively.

Keywords: *Cladosporium herbarum, E. coli, Staph. aureus*, secondary metabolites.

Introduction

Fungi are eukaryotic organisms that are widespread in nature and capable of growing and reproducing [1]. They are found wherever organic matter is present, due to their broad ability to exploit and utilize organic and inorganic materials of various compositions, as well as their tolerance to harsh environmental conditions, including low pH and temperatures [2]. Some fungi can live symbiotically, or as parasites, on other organisms. However, some of them have therapeutic and nutritional benefits for humans, animals, and plants alike. Some of them are pathogenic to other organisms, especially humans, causing a number of diseases called mycobacterial diseases, i.e., poisoning by fungal metabolites. These belong to four groups: Zygomycetes, Ascomycetes, Deuteromycetes, and Basidiomycetes [3, 4].

Microorganisms, especially fungi, are the most important source for discovering new drugs and agricultural chemicals. Fungi have played a major role in this field since the discovery of penicillin as an effective antibiotic against many types of pathogenic bacteria [5]. It is worth noting that the compounds with biological activity discovered from fungi do not match their huge numbers, although there are many secondary metabolite compounds that have been discovered and modified. For example, more than 145 compounds have had their chemical structures identified from the Aspergillus niger species [6]. Secondary metabolites are not produced by fungi, as is the case with the production of primary metabolites. Their production is affected by changes in external factors, such as changes in nutritional components, competition, and environmental changes, such as temperature, humidity, drought, and the type of culture media in which fungi grow [7].

Fungi can produce a wide range of chemical compounds during primary or secondary metabolism. Unlike primary metabolism, the compounds produced by secondary metabolism are generally not essential for normal fungal growth, as they are produced after the fungus has completed its primary growth stages, and often have bio- or physiological activities [8]. These secondary metabolite compounds are of great importance and have wide and varied uses. Some of them are used in the field of manufacturing treatments, while others are used in the field of nutrition and biotechnology. In addition, some of them have a severe toxic effect, as in the mycotoxins they produce [9].

Materials and methods:

Preparation of culture media

The culture media used in the study were prepared by dissolving a certain amount of them in distilled water, as recommended by the manufacturer of each medium. They were dissolved by boiling, then sterilized at a temperature of 121°C for 15 minutes, then cooled to a temperature of 50°C, poured into dishes, and left until they solidified. It was confirmed that they were not contaminated by leaving them for 24 hours in the incubator at a temperature of 30°C, as in [10], except for the media in which special processes were indicated in their preparation.

Preparation of inoculum:

On days (7) and (5) of the research, the fungus was harvested in slanted cultures, and then 5 ml of sterile distilled water with Tween 80 was added at a concentration of 1%. To get a spore suspension with a concentration of (1. 8 x 106 spores/ml, the fungal spores (conidia) were thoroughly separated in the tubes and collected in sterile glass flasks with a volume of (250) ml. [11].

Cultivation conditions:

The liquid yeast extract media was split at a rate of (100) ml/flask into (250) ml conical glass flasks after it had been prepared. Using an Autoclave, the flasks were sterilized for 20 minutes at a temperature of 121°C and a pressure of 1 pound per cubic inch after being securely sealed with cotton plugs. The spore suspension was inoculated into the culture media at a rate of 2% and then incubated for 72 hours in a shaking incubator at 25°C and 200 cycles per minute.

Preparation of fungal extract:

Following the incubation time, the work was done in a sterile atmosphere, and the created mycelium was then separated by filtering the filtrate through a 0. 45 mm Agene filter unit before centrifuging it for 15 minutes at 1000 rpm. The filtrate was then gathered and filtered once more via a 0. 45 mm Nalgene filter system. The fungal extract was put into glass tubes after it was obtained, and the tubes were sealed with a wax adhesive tape (Parafilm). [12].

The principle of the Lyophilizer

The fungal extract was then frozen using a Lypholizer equipment. The test was conducted for a certain amount of time at a temperature of 70°C, until a dry powder was produced [13]. Istanbul University's central laboratory was used to carry out the experiment under aseptic circumstances.

Estimation of fungal metabolites:

The metabolites of fungi were determined after extraction in the laboratories of the Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University (Turkey), using an LCMS-IT-TOF device, as in [14].After the separation column was washed with methanol for 15 minutes, followed by a 10-minute wash with acetonitrile, the molecular weights of each of the compounds produced were estimated from the extracted filtrate to determine their concentrations. Concentrations were determined using the JOEL Mass Center program.

Testing the inhibitory activity of fungal extract against bacteria:

The drilling method was followed [15] as follows:

(1). Put 25 milliliters of nutrient agar into each dish. (2). Using a sterile spreader, spread 0.1 ml of the bacterial culture onto the nutrient agar, then allow the plates to dry at room temperature. (3). Using a sterile cork borer, three holes with a diameter of 5 mm were drilled into the culture medium. (4). A sterile micropipette was used to add a quantity of (0. 2) ml of the produced graded concentrations of the fungal extract (50, 100, 250 mg/ml), and an antibiotic was added to create a positive control well. (5). Following the preparation of three replicates for each plate, the plates were incubated in the incubator for 24 hours at 37 degrees Celsius. Measuring the diameter of the inhibition zone allowed us to assess the efficacy of each concentration of the fungal extract.

Statistical analysis:

The study results were statistically analyzed using Duncan's multiple range test at a 5% probability level. This was performed using Excel software on an electronic calculator.

Result and Discussion:-

Biological activity of (*C. herbarum*) fungal extract against bacterial species: -

The extract produced by growing the fungus *C. herbarum* showed high inhibitory activity compared to the inhibition by the antibiotic ciprofloxacin. The average diameter of inhibition of the fungal extract at concentrations of 50, 100 and 250 mg/ml against *E. coli* bacteria reached 13, 20, and 28 mm, respectively (Table 1). The ability of the same extract

against Staph. aureus bacteria reached 13, 17, and 28 mm, respectively, at the same concentrations.

The results were consistent with previous studies that indicated that secondary metabolites from *C. herbarum* had antibiotic activity against *B. subtilis*, *Staph. aureus*, and *E. coli* after extraction using ethyl acetate and n-butanol. These metabolites were polar metabolites that were later identified as sumiki acid, a furonic acid derivative [16]. The ability of the metabolic extract produced from the fungus *C. Herbarum*, which was identified after diagnosis to contain a concentration of the compound Acetyl Sumix acid, may be due to its ability to penetrate the cytoplasmic membrane and affect both Gram-positive and Gram-negative bacteria cells [17, 18]. In addition to its saprophytic functions, *C. Herbarum* engages in competitive interactions with bacteria, influencing microbial community dynamics and nutrient availability. The ability of *C. herbarum* to inhibit bacterial growth may provide it with a competitive advantage in nutrient-limited environments [19, 20].

Fungi are known to produce a diverse array of secondary metabolites with antimicrobial properties. These metabolites serve various ecological functions, including competition with bacteria and protection against pathogens [21]. *C. herbarum* has been shown to exhibit antibacterial activity against several pathogenic bacteria, including *Staph. aureus* and *E. coli* [22]. Research indicates that the antimicrobial effects of *C. herbarum* may be attributed to specific compounds, such as polyketides and terpenoids, [23] which have demonstrated inhibitory activity against bacterial growth. These compounds can disrupt bacterial cell walls, interfere with metabolic processes, and induce cell lysis [24].

Detection of active compounds in *Cladosporium herbarum*

Sumiki's acid and Acetyl Sumiki's acid were isolated and identified from the fungus *C. herbarum*. The extracted amount of Sumiki's acid and Acetyl Sumiki's acid[35] compounds after growing the fungus on the production medium for seven days was 0.32 and 0.97 mg per 100 ml, respectively (Table 2). The above-produced quantities may vary depending on the quality of the medium and other factors in development [25].

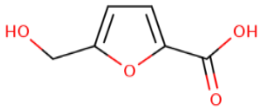
Sumiki acid is one of the natural human metabolites and has been identified in the urine of leukemia patients [26]. This compound belongs to the class of organic compounds known as furoic acid derivatives, which are organic compounds containing the furoic acid moiety, with a structure characterized by the presence of a furan ring bearing a carboxylic acid group at the C2 or C3 carbon atom [27]. It is usually known by its traditional name (5-hydroxymethyl-2-furoic acid). Both compounds belong to the furan carboxylic acid class, as shown in Figure (1-A & B). It has several synonyms [28]. It has also been isolated and identified from *C.herbarum* isolated from marine sponge [29, 30].

Conclusions:

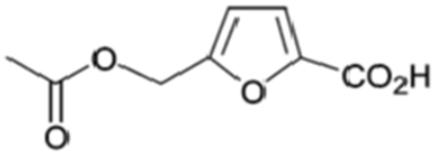
The following findings may be addressed:

1- *C. Herbarum* fungus has the ability to produce many metabolic compounds, the most important of which are Sumiki's acid and Acetyl Sumiki's acid. 35 Which has the ability to penetrate the cytoplasmic membrane and affect both Gram-positive and Gram-negative bacteria cells.

2- The extracts of the fungus *C.* Herbarum has the ability to inhibit some types of Gram-positive and Gram-negative bacteria. The growth inhibition may be due to their ability to produce furan carboxylic acid, and this has industrial chemical importance as a preservative in food sterilization and as an antibacterial, as well as a food colorant and flavoring agent.



A- Chemical structure of Sumiki's acid



B- Chemical composition of Acetyl Sumiki's acid

Figure.1. Chemical composition of the fungus extract *C. herbarum*

Table.1. Biological activity of secondary metabolites of *C. herbarum* against *E. coli* and *Staph. aureus* compared with the antibiotic Ciprofloxacin

Bacterial species	Concentration Used			Ciprofloxacin (control)
	50 mg\ml	100 mg\ml	250mg\ml	
E.coli	13 f	20 d	28 b c	30 a b
Staph.aureus	13 f	17 d e	28 b c	32 a

Table.2. Secondary metabolic products of *C. herbarum*

secondary metabolites	Concentration mg/100 ml	Filtrate volume (ml)	Biomass (g)
Sumiki's acid,	0.32	100	0.1
Acetyl Sumiki's acid	0.97	100	0.38

ETHICAL APPROVAL

The research protocol was approved by the Ethical Research Committee of the Kirkuk Education Authority.

INFORMED CONSENT

NA

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HUMAN AND ANIMAL RIGHTS

NA

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

AUTHORS CONTRIBUTION

Study conception and design: SN, AS

Data collection: SN, AS.

Analysis and interpretation of results: SN, AS

Draft manuscript: SN, AS.

All authors reviewed the results and approved the final version of the manuscript.

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