

CHARACTERIZE OF FUNGAL EXTRACT FROM *PENICILLIUM CITRINUM* ISOLATED FROM KOYA DISTRICT SEWAGE WATER

ABDULRASOL KH. ALBAYATY

University of Koya, Iraq /Dept. of Biology College of science

ABSTRACT

The study was carried out to isolate and identify the fungi from 10 samples which collected from Koya district sewage water in April-June 2010. The results showed that *Aspergillus* and *Penicillium* have 45% and 30% occurrence respectively, while the appearance of *Rhizopus*, *Alternaria*, *Fusarium* and *Cladesporium* were 9%, 0%, 4% and 1% respectively. *Penicillium citrinum* Were identified and isolated from sewage in Koya district by using production media of antibiotics. The fungal extract was obtained from the culture by solvent extraction. The extraction showed zone of growth inhibition activity against *Staphylococcus aureus*. IR and UV were used to identify the fungal extract wavelength.

INTRODUCTION

Fungi are native inhabitants of soil and water and some species behave as opportunistic pathogens in man. They are ubiquitous and no geographical area or any group of people is shared by these organism They can contaminate rivers, lakes, ground water, seas and oceans. They have been one of the problems facing society and the impact is acutely in all countries (Mbata; 2008). Several researches were carried out in order to isolate and identified filamentous fungi from aquatic ecology concerning the production of mycotoxin and secondary metabolism or antibiotics. A total of 10 strains of filamentous fungi were isolated from three different sources (wastewater, sewage sludge and leach) in Malasia, the strains isolated belonged to the genera of *Penicillium*, *Aspergillus*, *Trichoderma*, *Spicaria* and *Hyalofrae*. (Fakhrul-Razi *et al*; 2002).

Ana and Russell; (2006) indicated that fungi in drinking water are involved in the production of tastes and odours in water, a total of 34 taxa were isolated, like *Penicillium*, *Cladesporium*, *Rhizopus*, *Alternaria*, and others, these species were associated with the production of the mycotoxin, and *P. brevicompactum* was detected throughout sampling period and is known to produce the immunosuppressive drug. A marine strain of *Penicillium sp.* was isolated from a sample of sea water in France, and afforded the identification of the antifungal fraction as grisofulvin. (Petit *et al*; 2004)

Marine fungi have proved to be a rich source of new bioactive natural products (Jensen and Fenical 2000).

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Because of their particular living condition (Salinity, nutrition, higher pressure, temperature, variation, competition with bacteria, viruses and other fungi) they may have developed specific secondary metabolic pathway compared with terrestrial fungi (Liberra and Lindquist; 1990). Mbata *et al*; (2008) have isolated and characterized of filamentous fungi from Jordan river, the results showed that the most predominant genera were *Aspergillus spp*, *Penicillium spp* and *Candida* of the examined samples respectively, while Varo *et al*; (2007) isolated *Trichoderma sp.*, *Cladesporium sp.*, *Aspergillus sp.*, *Fusarium sp.*, respectively from water system of hemodialysis unit in the interior of the state of Sao-Paulo, Brazil. Many other workers have studied the ability of *Penicillium* which have been isolated from different sources to produce antibiotics (Al-Bayat; 2000, and Abdullah; 1999).

In the present study the main objectives were to isolate and characterize the prevalence fungi from sewage water in Koya district and ability of *P. citrinum* to induce antifungal compounds.

Materials and Methods

1. Study Area .

Fifty samples of Sewage water which used in this study were collected from three different parts of koya district situated at the north of Iraq , Kurdistan Region . These samples were collected in screw – capped bottles then transferred to the laboratory and mixed together until the analysis was done .

Isolation and identification of Fungi .

Twenty Petri dishes (9 cm) of potato dextrose agar media were prepared for the isolation of fungi , three drops of collected samples were placed on each Petri dish and the plates were moved clockwise to distribute the drops equally on the plate .

The plates were then incubated at 25 C° for 10 days preparing the growing colonies to macroscopic and microscopic characters of isolated fungi (Carlos; 1980 and Philip; 2004).

2. Production Medium.

Four flasks contain 20 ml of media production were prepared and sterilized, the media were inoculated with 10⁶ spore / ml of the *P. citrinum* isolate. The fungal extract was carried out for 4 days at 25 C° on a rotary shaker (200 rpm) . Acetone solvent was used for extraction of fungal extract from the fermented medium after the mixture had been vigorously stirred. To obtain the extracted fungal , methods of Egorove ; (1980) , and Yasuhiro. *et.al*; (2000) , were used .

3. Preparation of inoculums.

A loopful of mature slant of *Staphylococcus aureus* placed in 1 ml of nutrient broth, the mixture shaken slightly for distribution of bacteria equally in the medium, the inoculated medium streaked on nutrient agar medium .Disk plate technique used to determine susceptibility of microorganisms of the fungal extract concerning this study ,Michael *et.a*; (1986) was used.

4. Analysis of fungal extract by Infrared (IR) .

Infrared spectrophotometer (IR) was used for recording the spectrum of fungal extract in region 4000 cm to 600 cm using powder and dried potassium bromide .

Analysis of fungal extract by Ultraviolet (UV) Visible .

A quantity of 0.1 mg of the dried fungal extract was dissolved in distilled water and the absorption spectrum of the resulted solution was measured in the region between 200 nm -- 800 nm using 1 cm cubic quartz cell .

RESULTS AND DISCUSSION

1. Isolation of fungi

Filamentous fungi are everywhere with world wide occurrence but little attention has been given to the presence in aquatic environment and in highly contaminated of toxic materials and other minerals like sewage water, so in this study ,Table (1) illustrated that out of the total number of colonies were founded *Aspergillus* spp, followed by *Penicillium* spp have common appearance, and *Rhizopus*, *Fusarium*, *Alternaria* and *Cladesporium* respectively have frequent appearance, (Dowdes 1984). *Penicillium citrinum* was identified based on primarily on the macroscopic and microscopic morphology which revealed that the colony grow rapidly , attaining a diameter of (2-2.5)mm after 10 days forming velvety

,lanate or floccose texture , with blue – green changing to dark green colors. Reverse of colony is yellow , the colony uncolored or yellow droplets are produced on surface of the colony (Philip; 2008 , Carlos; 1980, and Mislivec; 2008), this isolate then was used for extraction of an inhibitory substance . The prevalence of filamentous fungi, *Aspergillus* and *Penicillium* in waste water probably due to presence of organic matter, and waste water heavily contaminated with water microorganisms or this water may be rich of other nutrient materials and the pH of the waste water in koya probably acidic which create a suitable environments for those fungi. The occurrence of fungi in this study were agreed with Mbala and Obeleagu; (2008) which mentioned that this fungi live in salty water, and Abdulla(1999) illustrated that this fungi common in soil near Dijlah river in Iraq. The results included in this study were moved parallel with results found by T.I.Mbata(2008),which mentioned that prevalence of filamentous fungi was found to be 68% in examined samples .The prevailing genera were *Aspergillus* 44,1% and *Chaetomium* 20,7% .

2. Production medium.

All the fermented broth (1 liter) added one liter of acetone to obtain the fungal extract, 0,1 mg of dried fungal extract was obtained. Fungal extracted activity was detected according to method Michael *et.al.* 1986 , using *staphylococcus aureus* as a test organism which obtain from college science laboratory, University of Koya,the production of an inhibitory substance by the fungus has created (0-6) mm a zone of growth inhibition of the bacterium. This result parallel to what Alexsander Fleming would have observed. Antibiotics are widely used in human , Agriculture and veterinary medicines for disease treatment. They are largely used in animal operations for growth promotion and for disease control. The residual antibiotics from human and animal use can enter the environment via various pathway, including waste water, (Mc Evoy; 2008), this point view is agreed with the present study which concern extraction of an inhibitory substance from *P.citrinum* isolated from sewage water in koya district. Other have been detected a variety antibiotics in drinking water (Miao; 2008) or from natural waters (Hilton; 2003), so it seems to indicate the impact of waste water discharge in koya. So and by the possibility of contamination of animal source which include a wide variety of microorganisms. In agreement with (Chiou ; 1989), the pH of waste water and extracted solutions determines the charge of ionizable antibiotics which in turn influence their solubility and diffusion into microorganisms, for this reason some antibiotic was detected in fungus which isolated from sewage water in koya district.

3. Analysis of fungal extract by infrared(IR).

Figure (1) showed the IR spectrum obtained for the potassium bromide (KBr) disc of the dried fungal extract. The spectrum indicate four characteristic bands which corresponding with (Albayaty; 2008) plus other band which due to the purity of the study sample.

4. Analysis of fungal extract by ultraviolet (UV) visible.

Figure(2) showed the UV visible spectra of 0,1 mg of dried fungal extract. This spectra indicate a wave length absorption in 260nm, 300nm and 360nm. This result need farther study to illustrate the U.V. absorption in this fungal extract.

Table (1): The occurrence and percentage of different Genera to a total number of fungal colonies.

Fungi isolate	Number of colonies	%
<i>Aspergillus spp.</i>	٢٢	٤٠,٧٤%
<i>Penicillium spp.</i>	١٩	٣٥,١٨%
<i>Rhizopus</i>	٥	٩,٢٥%
<i>Alternaria</i>	٣	٥,٥٥%
<i>Fusarium</i>	٤	٧,٤٠%
<i>Cladesporium</i>	١	١,٨٥%
Total	٥٤	

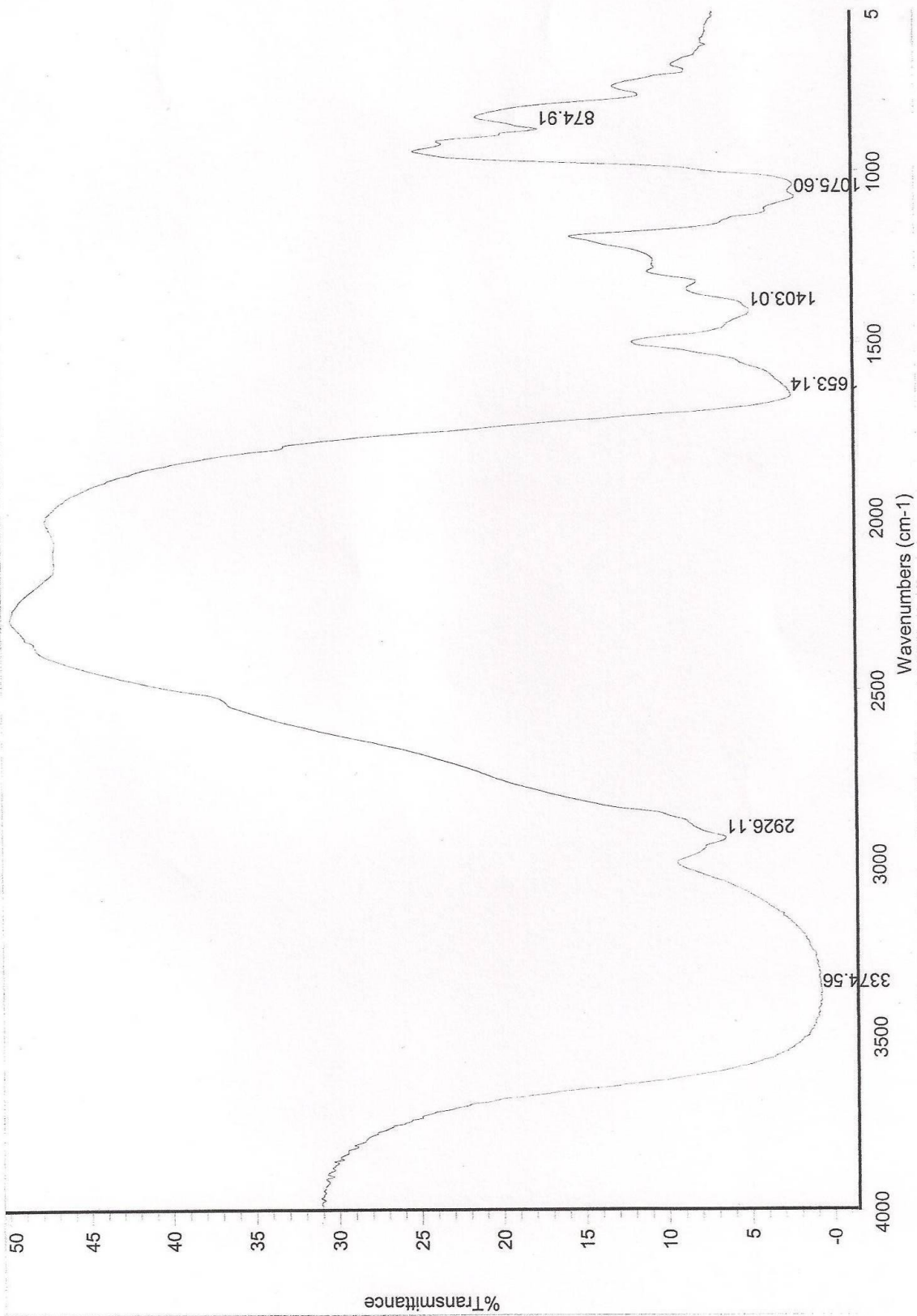


Figure (1): IR Spectrum of Dried Fungal Extract.

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توصيف المستخلص الفطري للفطر *Penicillium citrinum* المعزول من مياه مجاري منطقة كوية

عبدالرسول خضر البياتي
جامعة كوية/ كلية العلوم

الخلاصة

دراسة شملت ٥٠ نموذجاً لمياه مجاري منطقة كوية خلال شهري نيسان ومايس ٢٠١٠ حيث اشارت النتائج الى أن نسبة وجود الفطريات *Aspergillums* و *Penicillium* ٤٠,٧٤% و ٣٥,١٨% على التوالي بينما تواجد الفطريات *Rhizopus* و *Alternaria* و *Fusarium* و *Cladesporium* هي ٩,٢٥% و ٥,٥٥% و ٧,٤٠% و ١,٨٥% على التوالي. تم تشخيص الفطر *Penicillium citrinum* واستخدام لانتاج المضاد الحيوي منه باستخدام وسط غذائي سائل بطريقة الاستخلاص بالمذيب. اظهر المستخلص الفطري دائرة تثبيط للنمو البكتيري للبكتريا *Staphylococcus aureus*. استخدم القياس بالأشعة الحمراء IR والأشعة فوق بنفسجية UV لتحديد الطول الموجي للمستخلص الفطري.