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ISOLATION AND IDENTIFICATION OF ANTIBIOTICS PRODUCED BY *PENICILLIUM BRASILIANUM* BATISTA ISOLATED FROM SALAHADDIN PROVINCE SOILS

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ABSTRACT

This paper focused on the isolation of different genera of fungi from 80 soils samples in 9 stations in Salahaddin Province, north of Baghdad ,IRAQ. According to different environmental factors , these genera include *Aspergillus* ,*Penicillium, Rhizopus, Mucor, Botrytis,* and *Epicoccum.* They were isolated by dilution plate method and soil washing technique using the culture media viz. P.D.A., SAB. and MEA. The most widespread genera were *Aspergillus* and *Penicillium* followed by R*hizopus* , *Mucor* , *Botrytis,* and *Epicoccum* respectively. *P. brasilianum* Batista. was identified and used for antibacterial production. Other results in this paper were included.

INTRODUCTION

Penicillium was known scince 1809 , three species being recognized at that time (*Penicillium expansum, Penicillium candidum* and *Penicillium glaucum*) these species were related to *Penicillium expansum* which caused rotten fruit (Rot of apples) [(1)] . Robert *et al.*[(2)], Pitt [(3)] and Samson *et al.* [(4)].mentioned the features of the genus *Penicillium* , these include rapid growing colonies in different colours , consisting of a dense felt of conidiophore (stipe) .Conidiophores are either single (mononematous) or bundled (synnematous) consisting of a single stipe terminating in either a whorl of phialides (simple , monoverticillate) or in a pincillus (A branched part of the conidiophore above the stipe), so the branching pattern was either one stage branched (biverticillate – symmetrical) , two stage branched biverticillate – asymmetrical or three to more stages branched [(5,6)]. Genus *Penicillium* has been found the most abundant genus in the soil, Kwella and Weissbach [(7)] and Radwan *et al.*[(8)] had isolated the genus *Penicillium* from Egyptian soils. Larrond and Calvo [(9)] had isolated genus *Penicillium* in all soil samples in the Mediterranean beach in respective to the area and the season of the year. 3an8 isolates of *Penicillium* were identified from Japanees soil by Tseng *et al* [(10)] Nurettin and Usam [(11)] had isolated and identified genus *Penicillium steckii* from soil enrichment culture in Turkey. Ayse [(12)] claimed that the most widespread genusra were *Penicillium* in Harran plain in Turkey . Philip [(13)] isolated (12) species of *Penicillium* from the soil in U.S.A. Similar results were obtained by Ismail and Abdullah [(14)] in Iraq. From the 1940 the researches about antibiotics were increased and the pharmaceutical industries had developed more than 100 varieties of antibiotics .The researchers efforts are still focused on *Penicillium* species as asource of antibiotics production. Omura *et al.* [(15)] have

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isolated a new antifungal antibiotic complex named atpenin from soil *Penicillium sp* . Paloma *et al.* [16] isolate β-lactam from Eucaryotics organism , specially *Penicillium chrysogenum*. Elina *et al.* [17] isolated Galoctosidases enzymes of *Penicillium simplicissimam* . Federico *et al.* [18] isolated antibiotic s from *Penicillium nalgiovnse* . Nose *et al* [19] purified two novel antifungal antibiotics , PF 1163 A and B from the fermentation broth of *Penicillium sp* . which showed potent growth inhibitory effect on *Candida albicans*. Yasuhiro *et al.* [20] isolated antibiotic , showed an activity against Gram positive bacteria, from *Penicillium simplicissimum* . Maskey *et al.*[21] . It is believed there are few reports focusing on genus *Penicillium brasilianum* Batista. Tomoyuk *et al.* [22] isolated this genus from Japanies soil and he found that it produced new compounds with antibiotics activity . Henning *et al.* [23] used this genus for the production of enzymes .This study was designed to achieve the following objectives :

1. to determine the distribution of fungi in soil of Salahadden Province .
2. to determine the occurrence of *Penicillium* genus in a different stations in Salahaddin Province.
3. to test the antibacterial activity of the products of penicillium specieses
4. to Purify and identify the antibacterial products produced by *P. brasilianum* Batista isolate using UV, IR spectra and HPLC.

MATERIALS & METHODS

Isolation of Soil born Fungi: Before collection of samples , the surface of the profile soil was cleaned . The soil samples were collected from 10-15 cm depth from the soil surface using disinfected spatula, 8-10 samples were collected from each station viz. (Sharqat area, Baiji area, Tikrit area, AL-Alam area, Dozkhormato area, AL-Dour area, Samarra area, Balad area and Al-Dujail area) . Samples collected from several locations of each station were mixed together and stored in sterilized bottles until they reached the Laboratory . Ten grams of soil were added to 90ml of distilled water and were agitated on a shaker for 15 min. The sample was taken out of shaker and allowed to settle for 15 min. 1 ml of the supernatant liquid was used for the isolation of the fungi by applying the liquid on different media using a serial dilution plate technique . Other method was used for the same purpose such as , soil washing technique , in this method the soil samples were firstly placed on muslin cloth and washed with 2L of tap water . the procedure was then repeated using 2L of distilled water . After this treatment from muslin cloth the contents of soil particles were picked up with sterile loop or forceps and transferred on to plate of different media . **Identification of genus *Penicillium* :** For identification purposes , the genera of *penicillium* were allowed to grow on (SAB) . For microscopic diagnosis, the inoculated plates in both methods were incubated at 25C° for 10 days. For illustration, the soil dilution plate method was used to isolate the propaqules of microfungi occurring inactively in the soil , Whereas the reason for using the soil washing technique was to isolate active microfungus hypha [12,24,25].

reparation of Inoculum : Spore suspension was obtained by flooding 10-15 days old cultures of (SAB)with 50 ml of distilled water and the conidia were removed gently by sterile glass rode, filtered through two layers of sterile muslin cloth and the resulted spore suspension was adjusted to 10^6 spore / ml using counting chamber (Haemocytometer).

The number of spore / ml was calculated by application of the following equation : $X \times 25 \times 10^4$ = spore/ml , X = The total number of cells on the grid. 25 = Number

of the grids. 10^4 = Number of the cells in one ml on the grids [26,27].

Preliminary testing of *Penicillium* Isolates For Antibiotics Production : The method of Harvy and Mason [28] was used to study the ability of different isolates of *Penicillium* for production of antibiotics through measuring bacterial inhibition , which surrounded the colony of the *Penicillium.* The isolate was allowed to grow on SAB for 6-7 days at 28C°, then the plates were overlaid with a thin film of nutrient agar containing cells of bacteria . The production of antibiotic by the fungus has created a zone of growth inhibition of the bacterium. This demonstration parallels to what Alexander Fleming would have observed .Plates were incubated at 37C° for 24 hours .

Identification of Balad Area isolate: *Penicillium* isolate from Balad area (Station) was identified as *Penicillium brasilianum* Batista in Microbiology Department (College of Veterinary Medicine , Baghdad University).

Extraction of Antibiotic Produced by *Penicillium brasilianum* Batista Isolated from Balad Soil Filtrate:

Determination of the optimum conditions: Production medium was distributed in 10 (250ml conical flasks, 100 ml in each one) plugged with cotton wool and autoclaved at (121 C°) for (15min.)under(15 p.s.i) final pH of sterilized medium was adjusted to 6.0-6.1. The flasks were inoculated with spores suspension of *Penicillium brasilianum* Batista 10^5 spore/ml , 10^6 spore/ml , 10^7 spore/ml and 10^8 spore/ml respectively . Fermentation was carried out for 6-7 days (Lag phase) at 28C° on rotary shaker incubator (200 rpm) [20].

Effect of Immiscible Organic Solvents on Extracted Antibiotic.

At the end of the incubation period mycelia of *P. brasilianum* Batista.(Balad area isolate) were recovered by centerfugation (5000 rpm) for 5 min. and filtered by whatman No.1 filter paper dices , the mycelia were dried at 85C° for 24 hours to determine biomass weight .The broth (out of mycelia) of different conical flasks were extracted 3 times by different organic solvents , (Diethyl ether , Chloroform , Butyl alcohol , Butylacelate , and Amylacetate) . Each flask was mixed vigorously for 1 hour using separator funnel . The residue was evaporated by steam of air .Dried material were weighed and used to determine the antibacterial activity [29 , 30 , 31] .

Probability of Presence the Antibiotic Intracellulary in the Mycelia :

By using ultra sonic with high frequency sound waves to break up and to dislodge microbial cells walls. The sonication time which takes 2-5 min. has been reported to extract the optimum number of attached microorganisms . Then the mixture was centrifuged (3000 rpm) for 10min. .The residue was discharged , and the supernatant was tested for antibacterial activity [32] .

The Activity of the Extract in Comparison with Standard Antibiotics:

Antibiotic activity in fermentation broths and purified samples from *P. brasilianum* Batista. were evaluated by the conventional paper discs assay (whatman No.1) using isolates of bacteria : *Ps. aeruginosa* , *Klebsiella pneumonia* , *Staph. aureus, and E.coli* Inhibition zone formed was measured in millimeter (mm) [20,29] .

Analysis of Antibiotic Product:

Analysis of Antibiotic Product by HPLC Analysis: According to Luis. e*t al.* (2003) [33] a precise and sensitive High Performance Liquid Chromatography (HPLC) assay was used to determine the antibiotic sample extracted from *P. brasilianum* Batista. , three antibiotics , carbenicillin , ampicillin and benzylpenicillin were used as control . The assay was performed using 150 × 4.6 mm reversed phase column , 150mm column length . 4.6mm internal diameter

, mobile phase : deionized water 0.1 % (Phosphoric acid : methanol 80:20 v/v , detection U.V. set at 240 nm. flow rate 1 ml/min. at 30C° temperature . The analysis of the sample was carried out by dissolving 1 mg of the sample in 2 ml of the mobile phase . The same analysis was applied for the three standard antibiotics.

Analysis of Antibiotic Product by Infrared (IR) Analysis : Infrared spectrophotometer (IR) was used for recording the spectrum of fungal extracted in the region 4000 cm^{-1} to 400cm^{-1}(2.5Mm to 15 Mm). One mg of dried fungal extract fungal was examined with 300mg of powdered and dried potassium bromide. British pharmacopia.(2003) [34] .These quantities are usually sufficient to give a disc of 13 mm diameter and spectrum of suitable intensity .

Analysis of Antibiotic Product by Ultraviolet (UV)visible Analysis : A quantity of 0.1 mg of the dried fungal extract was dissolved 3ml of diethylether and the absorption spectrum of the resulted solution was measured in the region between 800-200nm using 1cm cubic quartiz cell, diethylether only was used as blank.

RESULTS

Isolation of Soil borne Fungi: A total of about (80) soil samples were taken from a (9) stations in Salahaddin Province from September – October 2003 . The samples were cultured to determine the presence of genus *Penicillium* . Table (1) illustrates the occurrence of different genera of fungi using different culture media in dilution (1/100).

Table (2) showed that the frequency of genus *Aspergillus* , *Penicillium* , *Rhizopus* and *Mucor* have common appearance . Whilst *Epicoccum* has very rare appearance. In soil washing technique it appeared that the number of isolates obtained from soil filtrate exceeded the number of isolates obtained from soil particles.

Identification of Genus *Penicillium* : *Penicillium* isolates were grown on the sabouraud dextrose agar with 0.05 g/l Chloramphenicol . The colony of *Penicillium* exhibited certain typical and striking characteristics , including colour changes and texture and the identification were carried out by using:

Light Dissective Microscope : After 10 days of incubation at 25C° the colony of the fungi was grown moderately to fairly rapidly attaining different diameter of (20-24 mm) The colony of the surface was radiant either regulary or irregularly wrinkled , with sometime thin margin white colour surrounded the colony , forming velvety , lanate , or floccose texture with blue gray , pale or deep gray – green or blue green to dark green colours.

Light Microscopic Characteristics of *Penicillium* : Septate conidiophores are rising from the substrate . the conidiophores grew perpendicular to the mycelium . The ends of these conidiophores are clusters of flask-shaped philiades . Conidia are small aseptate and shapes range from globose to cylindrical , with walls range from smooth to strongly roughened surfaces.

Preliminary Examination of *Penicillium* Isolates for Antibiotic Production :

***Penicillium* isolated from soil filtrate : As show in Table (3) Al-Dour isolate exerted no effect against *E. coli* ,while all other isolates were significantly inhibiting growth of *E. coli.* Statistical analysis (Duncan test) showed that there was no significant variations among the effectiveness of them.**

Against *Klebsiella pneumoma* Balad, Baiji , Sharqat , Al-Dijail and Doz isolates were significantly ($P<0.05$) the more effective . Against *Pseudomonas aeruginosa* , AL-Doure and Balad isolates were the most effective ($P<0.001$) followed by Baiji and AL-Dijail isolates.

Baiji and AL-Alam isolates appeared the most effect (P<0.05) against *Staphylacoccus aureus*.

***Penicillium* isolated from soil Particles : *Penicillium* isolated from all stations except Al-Dour isolate were exerted the same inhibiting activity against *E. coli* .It appeared that Doz isolate was the most effective isolate against *Kleb. pneumonia* (P<0.001) followed by Baiji, Balad, Sharqat, AL- Dijail and Tikrit isolates. On the other hand, Al-Dijail isolate was the most effective isolate against *Pseudomonas aeruginosa* , (P<0.05) followed by AL- Alam isolate, while Baiji and AL-Alam isolates were the most effective isolate (p< 0.05) against *Staphylococcus aureus.* Table (4).**

By using analysis of variance (ANOVA) it appeared that *Penicillium* isolated from soil filtrate was more effective than that isolated from soil particles (P<0.001). Analysis of variance also showed that *Penicillium* isolated from Balad soil filtrate is the most effective (P<0.001) in comparison with *Penicillium* isolates obtained from soil filtrate of the other 8 stations (Figure 1).

Identification of *Penicillium* species , (Balad area isolate) :-

According to the prilimenary study which showed that *Penicillium* isolate obtained from Balad soil filtrate was the most effective isolate against the test microorganism, this isolate was identified according to Carlos (1980) and the result was confirmed by the Department of Microbiology , College of Veterinary Medicine , Baghdad University , the isolate was diagnosed as *Penicillium brasilianum*. Batista .

Extraction of Antibiotic Produce by *Penicillium brasilianum* Batista isolated from Balad soil Filtrate :This process included the following :

1-Determination of the optimum conditions : Many cultural attempts were applied with different cultural conditions to obtain the best conditions which gave the best mycelial growth (dry weight). According to the result of these experiments , it appeared that the optimum conditions for antibiotic production, represented by using 10^6 spore/ml (spore concentration in production media) at pH 6.0-6.1 and 28C° , Table (5).

2-Using of Immiscible Organic Solvents in Extraction Antibiotic : Table (6) Showed the effect of different water immiscible organic solvents used for extraction of *Penicillium brasilianum* Batista product represented by the dry weight of extract (g/l) .It appeared that diethylether gave the highest weight of extract (0.151 g/l) .Furthermore , when equal amount of the products extracted by different solvents (0.1 ml of 100mg /ml) were compared with each other , it appeared that the products extracted by diethylether was the more effective one (P<0.01) (Duncan test)).

3- Probability of Presence of the Antibiotic Intracellularly in the Mycelia: Negligible values of inhibition zones (mm) were obtained from the extract of sonicated cells of *Penicillium brasilianum* Batista against all tested bacteria .

The Activity of the Obtained Extract in Comparison with Standard Antibiotics: A comparison was carried out by measuring the inhibition zones induced by ampicillin , carbenicillin and saturated paper discs Whatman No. 1 with soaked extracted antibiotic 0.1 ml of 100 mg /ml(100ug /disc) .Statistical analysis showed that there was no significant differences between the activity of ampicillin or carbenicillin and the extracted antibiotic against all the tested microorganisms (Table 7, Figure 2).

Analysis of Antibiotic Product :

The Following Results Obtained from the Analysis :-

1-Analysis Antibiotic Product by HPLC : Figure. (3) showed the chromotograph which was obtained by the separation of mixture of 3 standared compounds

ampicillin , carbincillin and benzylpencillin. On the other hand, the chromatogran obtained for the analysis of fungal extract is shown in Figure (4).

2- Analysis Antibiotic Product by Infrared Spectrum(IR) : Figure (5) showed the IR spectrum obtained for the Potassium bromide (KBR) disc of the dried fungal exract .

3-Analysis Antibiotic Product by Ultraviolet Visible. (UV) : Figure (6) A, B and C showed the uv-visible spectra of 1.O mg of dried fungal extract, ampicillin and carbenicillin in 3ml of dietheylether respectively against diethylether as blank.

DISCUSSION

Isolation of Soil borne fungi : *Penicillium* is commonly found in soil and can survive and even grow in low water activity environment. It also frequently inhibits soils that contain a high amount of organic matter, especially in soils contain aboundant of leaf litter on the surface [6] .But Pandey *et al.* [35] found that these species were sensitive to low temperatures although they preferred a mesophallic temperature ranged from 25-35C° . Most fungi were able to grow in a wide range of pH (4-12). Decline of soil pH was positively correlated with the development of specific microbial community . Furthermore Ingold [36] stated that the majority of known fungi are mesophillic growing between 25-37C° in cultural media. Garrett [37], by using dilution plate method found that *Aspergillus* is the dominant genus in the soil (13 species) followed by *Penicillium* (4 species). Pandey *et al.* [35] discussed the role of light and dark on the distribution of the fungi in soils sample collected in December 1997 from Indian caves, he found that the number of species of fungi obtained from light zone of soil sample were significantly more, compared to that of dark zone, so the distribution species of predominantly occurring genera (*Aspergillus* and *Penicillium*) decreased from light part to dark part of the caves, whereas there was an increase in the number of colonies of *Rhizopus* and *Mucor* toward the dark part (interior part) [37] Cubbon [38] claimed that this distribution is due to the higher temperature, lower humidity and availability of light . On the other hand, Waltham [39] mentioned the constancy of temperature and humidity in addition to darkness might presumably be conductive for the growth of fungi.Other researchers [12,25,40] found that *Penicillium* survive in different texture of soils either clay or sandy types . Abdullah [41] reported that *Aspergillus* and *Penicillium* were the prominent genus isolated from the edges of the river Tigris in Iraq.

In this study several genera of fungi were isolated from Salahaddin Province soil which consist of different pH, Temperature, humidity and organic matter under different climate . The most common microorganism isolated were genus *Aspergillus* and *Penicillium* .The results in this part of study agreed with many authors [12,13,41]. Other fungal genera were isolated in moderate and low occurance these included *Rhizopus* , *Mucor* , *Botrytis* and *Epicococcum* hence, the results agreed with Pandey *et al* [35] and Michael *et al.* [42]. . With the using of different media in this study , it appeared that the percentage of the presence of each genera were parallels to those recorded by Pandey *et al.* [38].

The result of this study showed that the number of isolates obtained from soil filtrate exceeded that isolated from soil particles. Similar results were also recorded by Ayse[12] .

Identification of genus *Penicillium* : Sabouraud dextrose agar (SAB) with 0.05 g/l chloramphenicol was used as selective media for isolation and identification of *Penicillium* because this media showed the minimum contamination with other

microorganisms because it contains chloramphenicol . Many other mycologist have used this media for this purpose [12,25] .Preliminary Examination of *Penicillium* Isolates for Antibiotic Production : The difference in the activity of *Penicillium* isolates extracts against tested bacteria could be attributed to different species according to different ecosystems which could produced different antibiotic [14,40]. This study showed that the most effective isolate on tested bacteria was Balad area isolate which was obtained from soil filtrate, therefore, this isolate is used in this study for extraction of antibiotic.

Extraction of Antibiotic Produce by *Penicillium brasilianum* Batista isolated from Balad Soil Filtrate using (CSL):Determination of the Optimum Conditions .According to our results, the best yield of mycelial growth was obtained when the production media inoculated with 10^6 spore/ml of *Penicillium brasilianum* and pH 6.0-6.1 and incubated at 28C for 6-7 days with agitation 200 (rpm) .This experiment was performed because the production of antibiotics from genus *Penicillium* is sensitive to these factors [29,43,44] . Using of Immiscible Organic Solvents on Extacted Antibiotic . Penicillins usually extracted from aqueous media by water immiscible solvents (Amylacetate , chloroform , butyl acetate , acetone , diethyl ether) . The principle underlying solvents extraction of penicillin (and many other antibiotics) consist in dissolution of penicillin in the form of three acid in a suitable solvent with subsequent dryness . In this study , the highest yield of antibiotic was obtained when the extraction method was carried out with diethyl ether . Corresponding to the result obtained from Egorov [29], the extract of penicillium with diethylether was found to be the most effective than others . These result reflects the difference in solubility of different extracted compounds in different solvents . The amount of antibiotic extracted by diethyl ether was (0.151 g/l) , it as similar to that obtained by Yasuhiro *et al.* [20] by using the same solvent. Analysis of Antibiotic Product. Analysis of Antibiotic Product by HPLC. Figure (3,4) showed two bands at retention times (3.977) and 5.52 min) which are in close identity with the first two bands. This could be attributed to the presence of ampicillin and carbenicillin respectively in the fungal extract . Moreover, the chromatogram figure (4) shows an additional band at retention time (2.81) min which indicates the presence of other additional compound in the fungal extract .

Analysis of Antibiotic Product by IR .

The spectrum of the dried fungal extract showed four characteristic bands at 3410 cm^{-1} (Λ OH) 1620cm^{-} (Λ C = O) ,1665 (C = C for conjugated system) and 1050 (Λ C- O) which agreed with the spectra found by Yashiro et al [20], for B- lactam antibiotics. Figure (5).

Analysis of Antibiotic Product by Ultraviolet (UV).

The U.V measurements of the fungal extract solution in Diethyether and for ampicillin and carbencillin showed similar spectra Figure(6, A, B, and C) respectively with wave lengths of maximum absorption illustrated in Table(8) [34]. These results clearly showed that the extract is a mixture of ampicillin , carbinicillin and an additional band of a new antibiotic.

ISOLATION AND IDENTIFICATION OF ANTIBIOTICS PRODUCED BY *PENICILLIUM BRASILIANUM* BATISTA ISOLATED FROM SALAHADDIN PROVINCE SOILS

Table (1) The Genera Isolated from Soil Samples in 9 Stations (1/100 dilution)
+ = Presence of the genus . - Absent of the genus . SAB = Sabouraud Dextrose Agar.

The genera	SAB	P.D.A	MEA	SAB with Chloramphenicol	MEA with Chloramphenicol
Aspergillus	+	+	+	+	+
Penicillium	+	-	+	+	+
Rhizopus	+	+	+	+	-
Mucor	+	+	+	-	-
Botrytis	-	+	-	-	-
Eprococcum	-	-	-	-	+

MEA = Malt Extract Agar. **P.D.A.= Potato Dextrose Agar.**

Table(2) The Occurance and Percentage of Difference Genera to The Total Number of Fungal Colonies in Different Stations Using SAB.

The stations	Number of colonies and their percentage %												
	Aspergillus		*Penicillium*		*Rhizopus*		*Mucor*		*Botrytis*		*Epicoccum*		Total of colonies
	Num. of colonies	%	Num.of colonies	%	Num.of colonies	%	Num.of colonies	%	Num.of colonies	%	Num.of colonies	%	
Sharqat	10	37.0	6	22.2	5	18.5	4	14.8	1	3.7	1	3.7	27
Baiji	8	28.5	7	25	5	17.8	5	17.8	2	7.1	1	3.5	28
Tikrit	9	34.6	7	26.9	4	15.3	5	19.2	1	3.8	-	0.0	26
Al-Alam	10	33.3	8	26.6	5	16.6	6	20	1	3.3	-	0.0	30
Al-Dour	9	36	8	32	4	16	4	16	-	0.0	-	0.0	25
Samarra	9	36	7	28	5	20	4	16	-	0.0	-	0.0	25
Balad	12	38.7	8	25.8	5	16.1	6	19.3	-	0.0	-	0.0	31
Al-Dijail	13	40.6	8	25	6	18.7	5	15.6	-	0.0	-	0.0	32
Doz.	8	40	6	30	3	15	3	15	-	0.0	-	0.0	20
Total	88	36.06	65	26.6	42	17.2	42	17.2	5	2	2	0.8	244

Table (3): The Mean of Zones (mm) of Inhibition Induced by *enicillium* Isolates Obtained from Soil Filtrate of Stations in Salahaddin Province Against Four Isolates of Bacteria.

Stations of *Penicillium* isolate	*E. coli.*	*Kleb. pneumonia.*	*Ps. aeruginosa.*	*Staph. aureus.*
Al-Dour	0.00 $^{a\pm}$ 0.00	8.00 $^{a\pm}$0.57	40.00 $^{d\pm}$0.00	6.00 $^{b\pm}$0.57
Baiji	4.00 ^{b} $^{\pm}$0.57	10.00 $^{c\pm}$0.57	19.33 $^{c\pm}$0.33	10.00 $^{c\pm}$0.57
Balad	4.00 ^{b} $^{\pm}$0.57	10.00 $^{c\pm}$0.57	40.33 $^{d+}$0.00	4.33 $^{ab\pm}$0.88
Sharqat	4.67 ^{b} $^{\pm}$0.33	10.00 $^{c\pm}$0.57	3.67 $^{a\pm}$0.33	3.33 $^{a\pm}$0.88
Al-Alam	4.67 ^{b} $^{\pm}$0.33	5.00 $^{a\pm}$0.57	9.67 $^{b\pm}$0.33	9.00 $^{c\pm}$0.57
Tikrit	5.00 ^{b} $^{\pm}$0.57	8.00 $^{b\pm}$0.57	9.67 $^{b\pm}$0.33	3.67 $^{a\pm}$0.33
Samarra	5.00 ^{b} $^{\pm}$0.57	7.00 $^{b\pm}$0.57	4.00 $^{a\pm}$0.57	3.00 $^{a\pm}$0.57
Al-Dijail	5.00 ^{b} $^{\pm}$0.57	10.00 $^{c\pm}$0.57	19.67 $^{c\pm}$0.33	3.00 $^{a\pm}$0.57
Doz.	5.00 ^{b} $^{\pm}$0.57	10.00 $^{c\pm}$0.57	4.00 $^{a\pm}$0.57	4.00 $^{a\pm}$0.57

The similar letters (Column) means no significant different between stations (Duncan test).

Table (4): The Mean of Zones (mm) of Inhibition Induced by *Penicillium* Isolates Obtained from Soil Particles of 9 stations in Salahaddin Province Against Four of Isolates of Bacteria.

Stations of *Penicillium* isolate	*E. coli.*	*Kl. Pneumonia*	*Ps. aeruginosa*	*Staph. aureus*
Al-Dour	0.00 $^{a\pm}$0.00	5.00 $^{a\pm}$0.0.57	5.00 $^{b\pm}$0.57	6.00 $^{b\pm}$0.57
Baiji	3.67 $^{bc\pm}$0.33	9.67 $^{e\pm}$0.33	3.67 $^{ab\pm}$0.33	10.00 $^{c\pm}$0.57
Balad	4.00 $^{bc\pm}$0.57	8.67 $^{de\pm}$0.33	2.67 $^{a\pm}$0.33	4.00 $^{a\pm}$0.57
Sharqat	4.33 $^{bc\pm}$0.33	8.67 $^{de\pm}$0.33	3.67 $^{ab\pm}$0.33	3.00 $^{a\pm}$0.57
Al-Alam	3.67 $^{bc\pm}$0.33	5.67 $^{ab\pm}$0.33	7.67 $^{c\pm}$0.33	10.00 $^{c\pm}$10.00
Tikrit	4.67 $^{c\pm}$0.33	7.67 $^{cd\pm}$0.33	6.67 $^{c\pm}$0.33	2.67 $^{a\pm}$0.33
Samarra	5.00 $^{cv\pm}$0.57	6.67 $^{bc\pm}$0.33	3.67 $^{ab\pm}$0.33	3.67 $^{a\pm}$0.33
Al-Dijail	4.67 $^{c+}$0.33	8.67 $^{de+}$0.33	10.00 $^{d+}$0.57	2.67 $^{a+}$0.33
Doz.	3.00 $^{b+}$0.57	40.00 $^{f+}$0.00	4.00 $^{ab+}$0.57	2.67 $^{a+}$0.33

The similar letters (Column) means no significant different between stations (Duncan test)

Table (5): Mycelial Growth (Dry Weight)g/l . Obtained by using Different PH , Temperature and spore/ml in Production Media

pH	Temperature	spore conc.			
		10^5	10^6	10^7	10^8
		Mycelial growth (Dry weight) gr/l			
6.0	28	6.0	13.75	4.0	1.5
	33	5.55	11.45	4.9	1.4
	38	5.2	10.85	4.5	1.5
	43	3.75	7.75	3.85	0.88
5.5	28	4.9	6.55	0.95	0.75
	33	4.3	6.0	0.5	0.2
	38	3.52	3.98	0.32	0.2
	43	1.2	1.58	0.15	0.0
5.0	28	1.99	2.48	1.0	0.1
	33	2.1	2.22	0.8	0.0
	38	2.0	3.0	0.5	0.0
	43	0.9	1.21	0.0	0.0
4.5	28	0.5	0.9	0.0	0.0
	33	0.3	1.25	0.3	0.0
	38	0.3	0.91	0.0	0.0
	43	0.2	0.9	0.0	0.0

Table(6):Effect of Organic Solvents on Extracted Dry Weight Determination of Activity of Extract (mm)

Organic solvent	Dry weight of extract(g/l)	*Ps.aeruginosa*	*Kleb.pnumonia.*	*Staph.aureus .*	*E. coli.*
Diethyether	0.151	13.06 d±1.15	7.33 d± 0.33	2.33 c± 0.33	1.33b± 0.33
Chloroform	0.110	4.33 c± 0.33	4.66 c± 0.33	-	-
Butanol 1	0.031	2.33 b± 0.33	2.33 b± 0.33	1.66 b± 0.33	-
Butylacetate	0.023	-	-	-	-
Amylacetate	0.024	-	-	-	-

(-) Uninhibited zone, the similar letters (Column) means no significant different between stations (Duncan test).

Table(7): Comparison of Inhibition Zone of Extracted Antibiotic with Standard Antibiotic Susceptibility Discs.

Bacteria isolates	Ampicillin 25 µg/disc	Carbenicillin100 µg/disc	Extracted antibiotic100 µg/disc
Ps. aeruginosa.	26[a] ± 0.57	26[a] ±0.57	26 [a] ±0.57
Kl.neumonia	25[a] ±0.57	23.6[a] ± 0.33	25 [a] ± 0.33
Staph. aureus.	3[a] ±0.57	3 [a] ±0.57	3.33 [a] ±0.88
E. coli.	1[a] ±0.57	1 [a] ±0.57	0.66 [a] ± 0.33

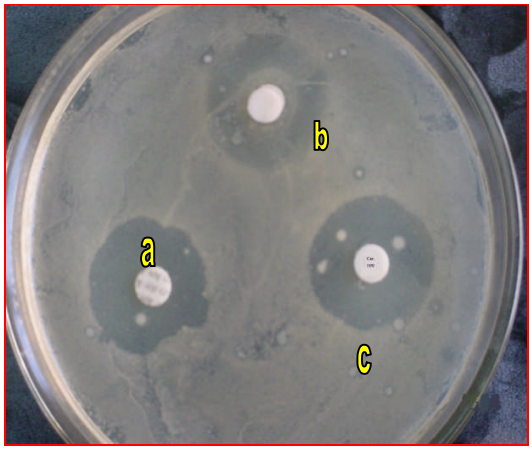
The similar letters (Rows) mean no significant differences (Duncan test).

Table (8) Characterstic U.V. Visible Absorption of the Fungal Extract and Controls Using Diethyl ether as Solvent System.

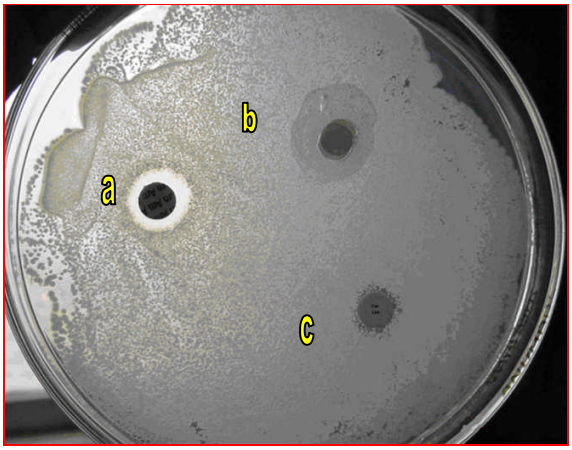
The compound	Λ max (nm)				
Fungal extract	242	250	272	330	604
Ampicillin	242	-	272	330	604
Carbenicillin	242	250	272	330	604

Colony **morphological character** **Conidia**

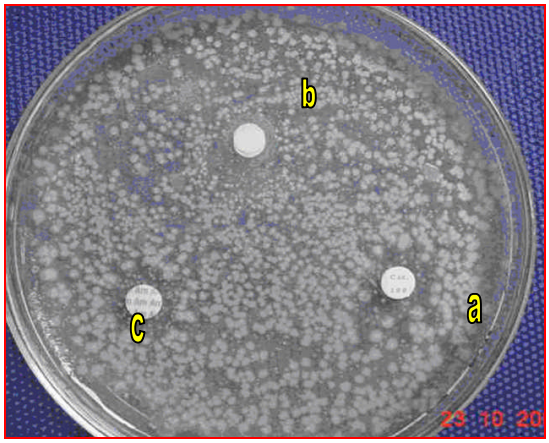
Figure (1):*Penicillium brasilianum* Batista (Balad isolate)of 8-10 days old culture on SAB



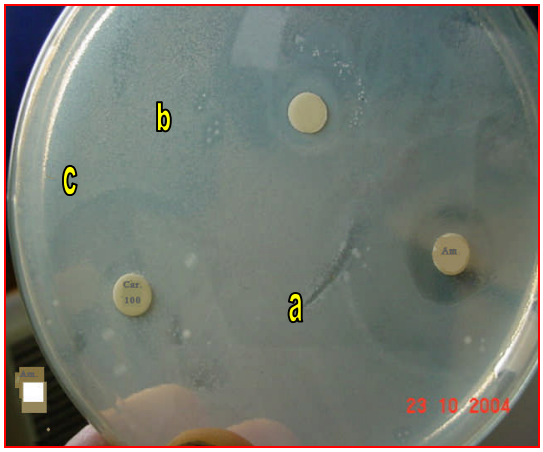
Effect of the extracted antibiotic on *Ps.aeruoginosa* compared with susceptibility to standard antibiotic discs.



Effect of the extracted antibiotic on *Kleb. pneumonia* compared with susceptibility to standard antibiotic discs.



Effect of the extracted antibiotic on Staph. aureus compared with susceptibility to standard antibiotic discs.



Effect of the extracted antibiotic on *E. coli* compared with susceptibility to standard antibiotic discs.

Figure (2) : The sensitivity of test microorganisms to the extracted antibiotic in comparison with the standard antibiotics , a : Ampicillin , b : Extracted antibiotic , c : Carbenicillin

Identity Peak

$t_{R.}$	Area	Compound
4.063	20424	Ampicillin
5.64	94874	Carbenicillin
9.713	11358	Benzylpenicillin

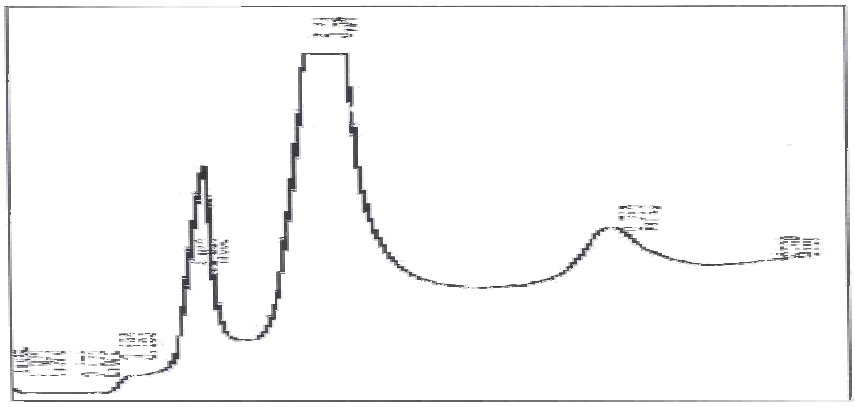


Figure (3) The Chromatogram of Mixture of Three Compounds , Ampicillin , Carbencillin and Benzylpenicillin.

Identity Peak

$t_{R.}$	Area	Compound
2.81	37254	Fungal extract
3.977	11198	Ampicillin
5.52	23173	Carbenicillin

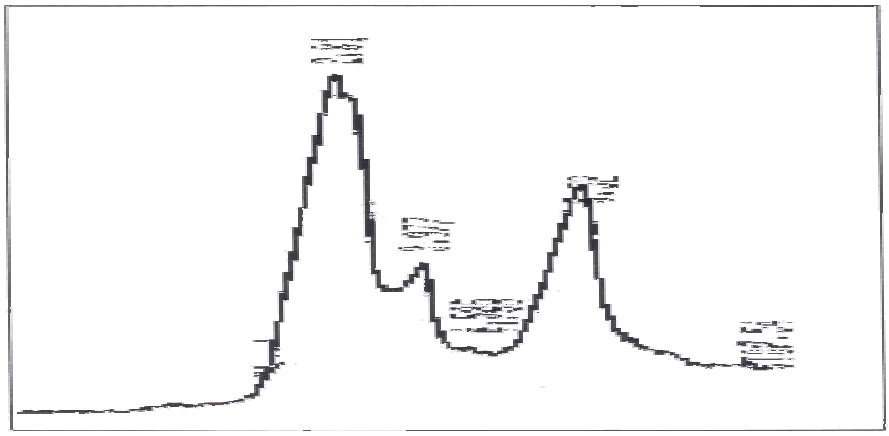


Figure (4): The Chromatogram of the Standard Additional band of the Fungal Extract

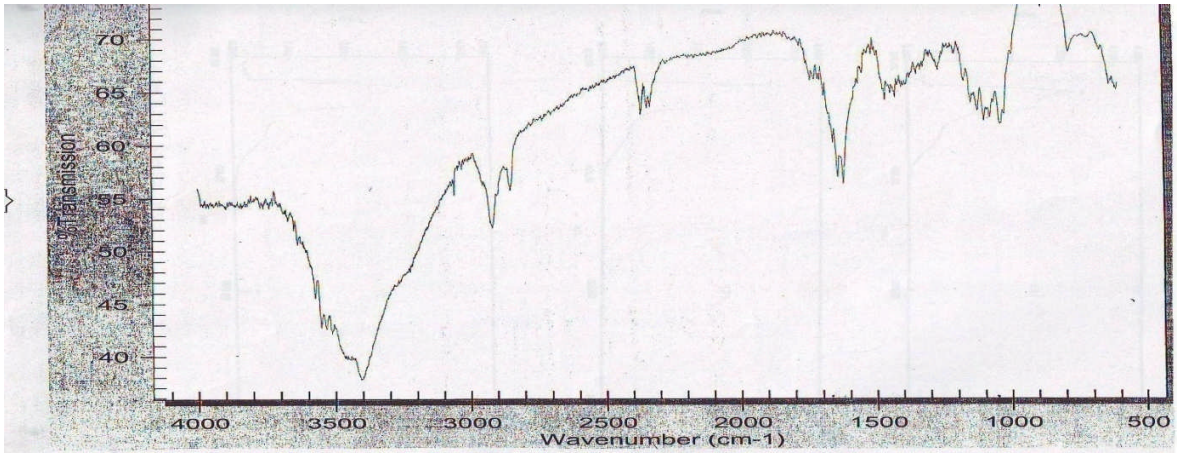


Figure (5) : IR spectrum of the dried fungal extract

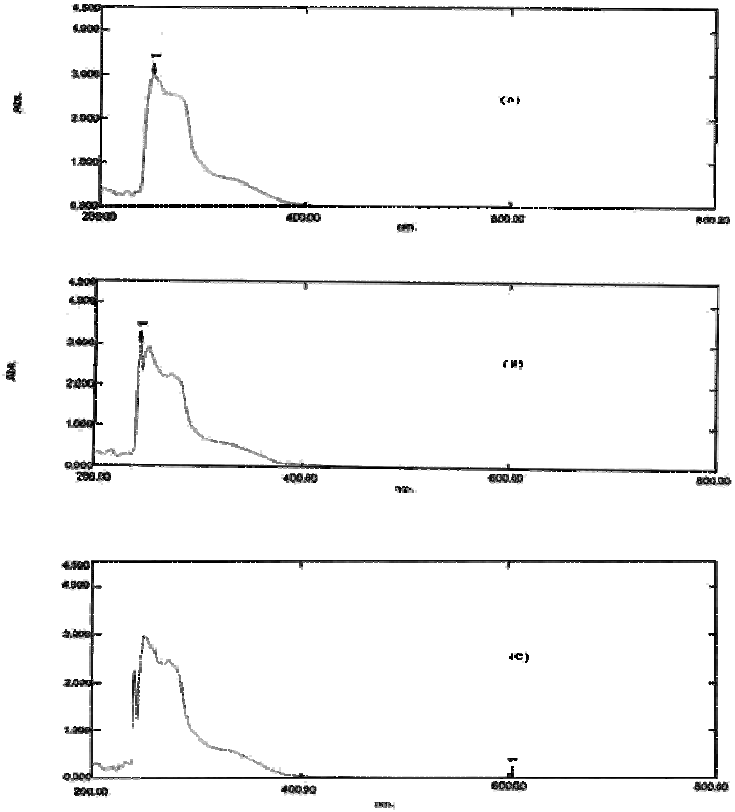


Figure (6): UV spectra of
a-Fungal extract of P.barasillianum Batista
b-Ampicillin
c- Carbenicillin

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