Evaluation antibacterial activity of compounds extracted from three Aspergillus species

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Abstract :

Three fungal species (A.niger A. terrus, and A .flavus) extracts were evaluated for their antibacterial activity by using Kirby Bauer diffusion test against Gram negative and Gram positive bacteria. All broth media of fungal species were extracted using ethyl acetate as organic solvent. Aspergillus niger extract showed a significantly antibacterial activity against Escherichia coli and staphylococcus aureus in compared with the ether fungal species, all the fungal species extract have the same chemical compounds represented by alkaloids, tannins, saponins and flavonoids.

Keywords: Evaluation , antibacterial activity, Extraction, Aspergillus species

Introduction :

Natural products are an important source of new bioactive compounds and for the drugs launched over the period 1981- 2002, (21) found that 40% were either natural products as is ,or modified natural products.

The search of new pharmacologically active agents obtained by screening natural source such as microbial fermentation and plant extracts had led to discovery of many clinically useful drugs that play a major role in the treatment of human disease . The extraction process is an important step in the investigation of biologically active compounds when extraction compounds from fungi or any living source the type of solvent used the extraction process employed and the age, part of cultivation of living tissue, can all have a marked effect on the type of compound that can be extracted (22).

Due to their Pharmaceutical potential secondary metabolites of fungi have been studied for more than 70 years. The search for new drugs from fungi started with the discovery of penicillin by Fleming in 1929 a potent antibiotic against Gram positive bacteria which was produced by Penicillium notatum. A further millstone in the history of fungal products for medicinal use was the discovery of the immunosuppressant cyclosporine which is produced,e.g. by Tolypocladium inflatum and Cylindrocarpon lucidum (8).

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The antifungal agent griseofulvin being isolated from Pencillium griseoflavin and the cholesterol biosynthesis lavastain isolated from Aspergillus terreus are tow further examples supporting today's great interest in new secondary metabolites from fungi(٦).

Most fungi studied to date have been isolated from soil and proven to have a highly creativity index,i.e.new and interesting secondary metabolites could be isolated genera as Aspergillus, Pencillium, Acermonium, Fusarium, typical soil isolates, are known for their ability to synthesis divers chemical structure. The genus Aspergillus is known to elaborate a number of secondary metabolites, some of which posses antibiotic activity.(15) .Table(1) provides some of metabolites isolates from Aspergillus.

Table(1)
some antibiotic isolated from Aspergillus species

Fungus species	Metabolire(s) isolated	Literature citation
Aspergillus Panamensis	Tetronic acid derivatives	4
A.aculeatus	Aculracin A	13
A. terrus	Tetracycline acid A	19
A. versicolor	Mycoversillin	24
A. fumigatus	Fumi fungi	18
A. sydowii	Mulumdo candin	23
A.sydowii Var.muludensis	Deoxymuludo candin	17

Material and methods

- Isolation of fungal species

The producing organism were isolated (A.niger A. terrus, and A .flavus) from soil using dilution method described by (14).

- Culture media

All media prepared as described by their company manufacturer instrucion

- Fermentation

The culture of Aspergillus species were maintained on potato dextrose agar. The stock cultures were inoculated in 250 ml flasks containing 100ml of potato dextrose agar and incubated for 7 days at 27 C. Preparation of spore suspension was performed by addition of 50 ml of sterilized distilled water to the 250ml flasks followed by vigorous of the water over the agar surface with a sterile loop, 2 ml of the spore suspension were used for the inoculation of a 2 liter conical flask each flask containing 1 liter of the following medium: g/100 ml glucose 1.0sucros 2.0gmNaNo30.2gmK_2HPO_4 0.1gmKCl 0.05gm$MgSO_4.7H_2O$ 0.05gm$FeSO_4.7H_2O$ 0.001gm corn step liquor 1.0gm .(PH adjusted to 6.0 prior to sterilization) The flask were incubated on a rotary shaker at 120 rpm at 27 C for 10 days for the production of organic materials.(25).

Isolation of compound

After 10 days old culture broth of each species of Aspergillus were filtered by whatman No.1 to separate mycelium. The both filtration of each species was adjusted to pH 3 with (1N HCl) and extracted with equal volume of ethyl acetate by separated funnel, the ethyl acetate layer was dried over Na_2SO_4 and concentrated in vacuum to dryness (organic matter). (26)

Antibacterial activity

The ethyl acetate extracte of three Aspergillus species were studied for anti bacterial activity against tow bacterial species E.coli and S.aureus by using Kirby Bauer diffusion test. Petri dishes with 20ml of Muller-Hinton agar were prepared , inoculated with 1*10^6 cell/ml. Sterile filter paper disc of 6mm in diameter were laoded with 150 ug/ml of each extracts using micro pipette and were dried under laminar air flow hood. The inoculated plates were inocubated for 24 hours at 37C. Standard antibiotic, Streptomycin, Erythromycin, Neomycin,Fusidic acid and Tetracycline were used as appositive control. After inocubation time, the diameter of inhibition zone diameter were measured in mm.(5)

Qualitative analysis of fungal extracts:

Preliminary qualitative analysis extracts were performed using the following tests:

1- **Carbohydrates test**. Carbohydrates were tested using Molish's reagent as follows: 1ml. of extract was mixed with 5 drops of alcoholic a-naphthol in test tube, with well shaking 2.5ml of sulfuric acid was added. Violet ring was formation, indicates the presence of carbohydrates.(11)

2- **Alkaloid test:**

Using meyer's reagent: it consists of:

Solution 1: 1.36gm. of mercury chloride ($HgCl_2$) dissolved into 60ml.distaled water

Solution 2 : 5gm. of potassium iodide dissolved in 10 ml.distaled water

Solution 1 mixed with solution 2 , 1ml. of extracts was added to 1 ml. of reagent, creamy precipitate indicates the presence alkaloid (10).

3- Flavnoid test:

1ml.of extract was mixed with 1ml. of alcoholic potassium hydroxide 0.5m. yellow precipitate indicate the presence of flavonoid (3)

4-Phenol's test

Ferric chloride test:

One gm. of ferric chloride dissolved in 100ml. D.W, then equal volume of extract and reagent 1:1 were mixed and blue or green color was formed. This indicates the presence of phenols.(6)

5- tannins test:

Ferric chloride test:

Five drops of 1% w/v ferric chloride in D.W were added into 1 ml. of extract, when blue- green color is formed this indicates the presence of tannins(7).

6- Saponin test:

1 ml. of extract was added to one ml. of (5%)$HgCl_2$ in D.W the formation of white precipitate indicates the presence of Saponins (9).

Statistical analysis :

Analysis of data used in this study dune by SSPS statistics 17.0

Result

Results showed that fungal species appear obvious significantly differences on their antibacterial activity against Gram negative and Gram positive bacteria. A.niger showed highly significant differences ($P<0.05$)in their antibacterial activity reach to 17 mm against E.coli followed by A.flavus 14mm A.terreus 9mm,while the fungus A.niger was showed significantly differences in their antibacterial activity on gram positive bacteria reach to 20mm followed by A.flavus 17.5mm and A.terreus 17mm, as in Table(2). while Table (3) showed antibacterial activity for some standard antibiotic activity presented by inhibition zone.

Table(2)
Antibacterial activity of Aspergillus species extracts

BACTRIA Bacteria Fungal Species extract	inhibition diameter mm	
	E.coil	S.aureus
A.flavus	14	17
A.niger	17	20
A.terreus	9	17

Qualitative Chemical Analysis of fungal extracts showed differences in their chemical compound in three aspergillus species A.flavus contains flavonoid, phenols,tannins and saponins without alkaloids where as the extract of A.niger had alkaloids,flavonoids, saponins,where as the extract of A.terreus had all compounds without flavnoids.

Table(3)
preliminary Qualitative chemical Analysis of Bioactive extracts

Compounds	Test	A. flavus	A.niger	A.terreus	Indicator (+ve)
Carbohydrate	Molish reagent	+	-	+	Violet ring
Alkaloids	Meyer's reagent	-	+	+	Creamy precipitate
Flavonoids	Alchoholic OH	+	+	-	Yellow precipitate
Phenols	Ferric chloride	+	-	+	Blue or green color
Tannins	Ferric chloride	+	-	+	Blue green color
saponins	Hg Cl2 5%	+	+	+	White precipitate

(+)positive (-) negative

Table(4)
Antibacterial Activity of standard Antibiotics presented by inhibition zone diameter (mm)

standard Antibiotic	Concentration	E.coli	S.aureus
Erythromycin	15ug	40	23
Fusidic acid	10ug	-	10
Neomycin	30ug	-	18
Streptomycin	10ug	25	20
Tetracycline	30ug	31	25

Discussion

The result reported in this study show that three Aspergillus species extracts process antibacterial activity against two bacteria E.coli and S.aureuss .

the antibacterial activity of Aspergillus extracts are similar to a number of secondary metabolites activity isolated from A.nidulans,Adeflectus and terreus(6,19)

Antibacterial activity of three fungal extracts may be done as reason of chemical compounds that investigated in our studies such as alkaloids,tannins,phenols,saponins and flavonoids. The presence of this compounds in the extracts shows that the extracts were of pharmacological importance (1).

(20)record that the presence alkaloid, tannis and saponind enhanced the antibacterial activity against the broad spectrum of organisms.

Many interpene saponins and their glycones have been repoted by (12)to have vaied uses as antiukerogenic, antiinflamotary,fibrinolytic and anti-edematous in action

Conclusion

Fungal species produce compound with antibacterial activities.The extracts of fungal Aspergillus species have bioactive compounds such as alkaloids, phenols, tannins and flavonoids.The organic extract of A.niger was showed higher antibacterial activity against tow bacteria compared with another tow species used in this study.

Recommendation

Purification of the secondary metabolites and study the Characterization of the purified active compound by 1- Infra Red spectra 2- Mass spectroscopy .

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تقيم الفعالية ضد البكتيريه لبعض المركبات المستخلصة من ثلاثة انواع من فطر Aspergillus

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الخلاصة :

تم تقيم الفعالية ضد البكتيرية لعبض المركبات المستخلصة من ثلاثة انواع من فطرAspergillus وهي (A.niger A.terrus, and A.flavus) باستخدام Kirby Bauer diffusion test ضد نوعين من البكتيريا السالبه والموجبه لصبغة غرام (Escherichia coli and staphylococcus aureus) حيث تم استخلاص المركبات من المزرعة الفطرية باستخدام الاثيل استيت كمذيب عضوي ،اظهر مستخلص (A.niger) فرقا معنويا بامقارنة مع بقية الانواع ،كما بينت الدراسة ان كل المستخلصات الفطرية كان لها نفس التركيب الكيمياوي والمتمثل بالالكولويد،التانين،الصابونين والفلافونويد.