

Isolation and Identification of two bioactive compounds from basidiomycetes fungus *Coprinus* sp.

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Abstract

The fungus *Coprinus* sp. was isolated and cultivation in laboratory on two PDA medium with some growth induction .Two bioactive chemical compounds were isolated and purified from mycelial culture of *Coprinus* sp. by using potato dextrose agar and potato dextrose broth . The two compounds were identified by using GC-mass technique .The molecular weight of purified compounds 1 and 2 were 352 KD and 388 KD respectively and chemical formula of compound 1 is C₂₁H₂₄N₂O₃ which isolated from solid medium while compound 2 is C₂₄H₂₆O₄ which isolated from broth medium . The antibacterial activity of the purified compounds against three bacterial species *E. coli* , *Proteus* sp. and *S. aureus* were tested by using a disk diffusion agar method reaching to 40 , 30 , 20 for compound 1 and 37, 25 , 17 mm for compound 2.

Key words: Isolation , Identificaton , Bioactive comp. , *Coprinus* sp.

عزل وتشخيص اثنين من المركبات الفعالة من الفطر البازيدي *Coprinus* sp.

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الخلاصة

عزل الفطر البازيدي *Coprinus* sp وزرع مختبريا على وسط PDA باستخدام محفزات نمو . تم عزل وتنقية اثنين من المركبات الكيميائية الفعالة من مزرعة الفطر باستخدام وسط البطاطا والدكستروز الصلب ووسط البطاطا و الدكستروز السائل . شخص المركبان باستخدام تقنية طيف الكتلة GC-mass . الوزن الجزيئي للمركبين 1 و 2 بلغ 352 Kd و 388 Kd على التوالي . الصيغة الكيميائية للمركب الاول (1) C₂₁H₂₄N₂O₃ والمعزول من الوسط الصلب بينما الصيغة الكيميائية للمركب الثاني (2) C₂₄H₂₆O₄ والمعزول من الوسط السائل . أختبرت الفعالية الحيوية للمركبين ضد بعض الانواع البكتيرية *S. aureus*، *E. coli*، و *Proteus* sp باستخدام طريقة الأقراص والبالغة 40 ، 30 و 20 ملليمتر للمركب الاول و 37 ، 25 و 17 ملليمتر للمركب الثاني(2) على التوالي .

كلمات المفتاح : عزل،تشخيص ، مركبات فعالة ، الفطر البازيدي.

Introduction

The researches interest to explore new antimicrobial agents from fungi is continued ,fungi are a rich source of bioactive secondary metabolites and mushroom –forming fungi are especially known for the synthesis of numerous bioactive compounds (Anke et al., 2004 ; Keller et al., 2005 ; Agger , 2009 ; Muhsin et al., 2011).

Coprinus belongs to mushrooms , which is a black spored family coprinaceae (all species go through an auto digestion at maturity in which the cap forms black spores).The genus *Coprinus* is considered to be more than 100 species distributed from the northern hemisphere to south African (Keirle et al ., 2004). The classification of the *Coprinus* species is still unclear because it was only based on morphological

characteristics without molecular analysis (Mwita et al., 2010).Extracts from several members of the genus *Coprinus* are known in many part of the world to exhibit among others , antimicrobial , antitumor , hypoglycemic , antinematodes and antioxidant effects . (Ndyetabura et al., 2010).Examples of the bioactive compounds that have been reported present in *Coprinus* mushrooms extracts are : abroad spectrum bioactive indole compound tryptamine (Worthen et al., 1962) , Miaceol (Asterol) with antibacterial activity against the bacteria *Corynebacterium xerosis* and *Staphylococcus aureus* (Zahid et al., 2006) and (2,2)-4-oxo-2,5- heptadienedioic acid , which has inhibitory activity against glutathione S- transferase an enzyme that have been implicated in the resistance of cancer cells against chemotherapeutic agents especially alkylating drugs (Zahid et al., 2006).According to our knowledge so far a little information is available about the production of secondary bioactive metabolites by this fungus , this report elucidates interesting chemical compound, extracted , purified and identified from mycelial culture of *Coprinus* sp. As a bioactive agents tested against a selected species of bacteria .

Material and Methods

1: Fungal Mushroom culture

Fruiting bodies were collected from palm trees (*Phoenix dactylifera*) southern of Iraq . In the laboratory , small Pieces (0.5 cm long) were cut from the fruiting body surface , sterilized with 10% sodium hypochlorate for 3 min. , washed with sterile distilled water and placed on potato dextrose agar (PDA) with growth indicated (Asparagine and thiamine) in petri dishes , plates were incubated at 25 ± 1 Co for two weeks . After cultivation , the mycelium was removed from the agar medium surface and amended into a liquid culture medium potato dextrose broth (PDB) in 1 L conical flasks and incubated at 25 Co in a rotary shaker incubator for 3 weeks .

2: Extraction , Isolation and Purification

The fungal culture in broth medium was filtered on Watmann No. 1 filter paper , the filtrate was extracted three time with ethyl acetate (1:1 v/v) using separating funnel , while fungal culture on the solid media prior to extraction with ethylacetate,the solid medium were diluted with H₂O and blended using blender (Zur , 2001).Organic layer was collected and dehydrated with Na₂SO₄ then placed in Petri dishes and dried at room temperature , thin layer chromatography (TLC) was applied for the isolation of extracted metabolites using silica gel of 2×10 cm (Silica gel

GF2 Merck) and Rf value (Rate flow) were measured . Purification of extracted compounds was made on silica gel column chromatography (silica gel mesh 60) (Column 1.5×50 cm and elution with methanol - ethyl acetate (1:1). a further purification of fraction compound were made by using another column 1.5 × 50 cm and using eluent cyclohexane and ethyl acetate (1: 1) . The identification of the purified compounds was made by using Gas chromatography GC-Mass technique type (Simadzu . GCMS-QP2010 Ultra)

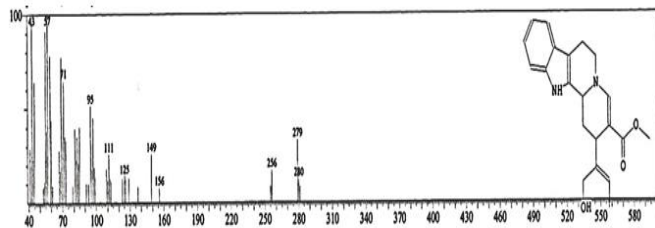
3: Bioactive test

Disc diffusion agar method (Casals , 1979) was used to examine the antimicrobial activity of the purified compounds , Three strains of pathogenic bacteria from (Lab. of Pharmacy college) , *Escherichia coli* , *Proteus* sp. and *Staphylococcus aureus* were used for this purpose . 2.5 mg of the dried fungal extract was dissolved in 1 ml of dimethyl sulfoxide (DMSO) solvent used as stock solution for this test . Disk of 0.6 mm diameter Whatmann No. 1 filter paper was sterilized and soaked in the fungal extract solution and placed on plates containing Muller –Hinton agar (MHA) medium inoculated with 0.1 ml suspension of bacterial strains by streaking method. The test was carried out in triplicates . Antimicrobial activity was assayed by measuring the inhibition zone around the disk in mm.

Results :

The fungus *Coprinus* sp. Which isolated in this study has some characters such as : Basidiocarp brown in color , brown cap with brown –black spores (4.5 - 5.5 × 4 – 7.5) μm stalk diameters (5 × 0.25) cm and spores are brown in color . Mycelium white in color when grow on the solid media and grow up on the media surface . Two compounds were isolated and purified from solid and broth culture of *Coprinus* sp. Mycelium. The compound (1) isolated from solid culture , while compound (2) isolated from broth culture . Based on Gas chromatography (GC-mass) apparently that the molecular formula of compound (1) is C₂₁H₂₄N₂O₃with molecular weight 352Kd and its chemical name is 2-(1-hydroxymethyl - propenyl) - 1,2,6,7,12,12b-hexahydro-indo [2,3-a] quinolizine -3-carboxylic methyl ester showed in fig (1) , While the molecular formula of compound (2) is C₂₄H₃₆O₄and its name is Cycloheptylnonphthallate with molecular weight 388Kd fig (2) . The purified compound 1and 2 appeared antibacterial activity against bacterial species . The inhibition zone diameter of compound (1) reach to

(40 , 30 , 20) mm for *S. aureus* , *E. coli* and *Proteus* sp. respectively and (37 , 25 , 17) mm of compound (2) for three bacterial species respectively fig (3) .



Fig(1): Chemical structure and GC –Mass spectroscopy of compound -1 (2-(1-hydroxymethyl-propenyl)-1,2,6,7,12,12b-hexahydro-indo [2,3-a] quinolizine -3-carboxylic methyl ester) isolation from solid media

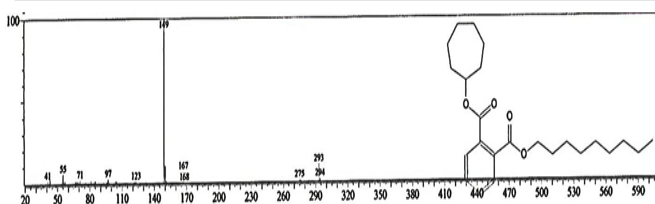


Fig (2): Chemical structure and GC –Mass spectroscopy of compound- 2(Cycloheptyl nonphthalate) isolation from broth media

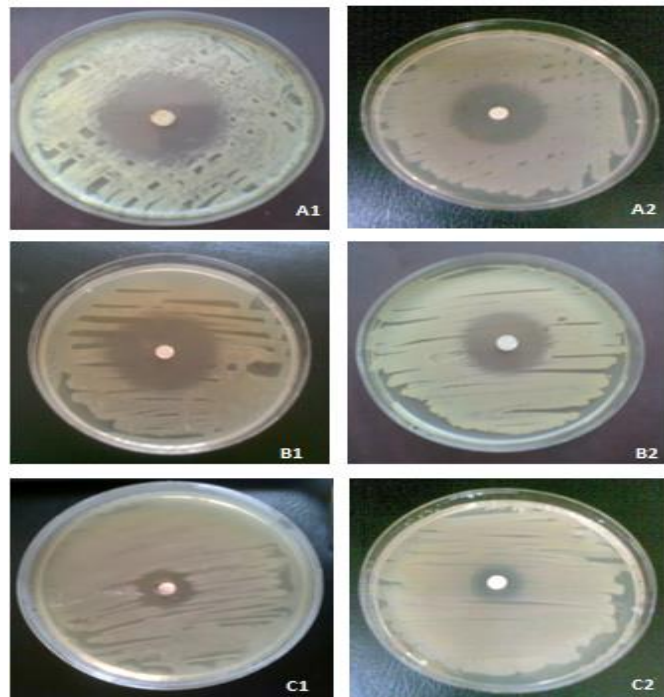


Fig (3): Inhibition zone diameter (mm) :- 1: compound No. (1) and 2: Compound No(2)
A = *E. coli* B= *S. aureus* C= *Proteus* sp.

Discussion

Fungi in general and mushroom in particular are a good source for antimicrobial products (Janes *et al.*, 2007) . Results showed the activity of two compounds purified from *Coprinus* sp. (1,2) against gram positive and gram negative bacteria , so this activity may be due to active substances present in the *Coprinus* sp. tissues (Ndyetabura *et al.*, 2010) , also these results agreed with Zenkora *et al.*, (2003) whom reported that the *Coprinus representatives* produces compounds which are able to inhibit growth of all common gram-negative and gram positive bacteria and fungal strains .The antimicrobial activity of compound (1) against bacterial strains may be due to the quinolizine and indole group in its structure , also this groups have different biological activity such as antimicrobial , anti-inflammatory , antituberculosis , cytotoxicity (Thakur *et al.*, 2010) , while the antimicrobial activity of compound (2) may be due to its one of the phthalide derivatives which are compounds of polyketide metabolism , they are produced by a wide range of organisms , i.e marine and terrestrial fungi (Almedia *et al.*, 2011) . Phthalides exhibit equally broad spectrum of bioactivity , including modulation of central nervous system protection against brain ischemia , as well as antibacterial , antifungal and phytotoxic activity. Moreover, the rings compounds acts as a protoplasm toxin to destroy the cell wall system and to precipitate protein in cells (Gayon , 1972). The compound produced from solid media have more biological activity against bacteria than liquid media , several researches showed that produced through solid media are more stable and produced in higher quantities than liquid . In this fermentation technique , the substrate can be used for long fermentation periods , hence , this technique supports controlled release of nutrients (Subramaniyam and Vimala , 2013). The role of the physiological and genetic properties of the microorganism used during growth on solid substrates compared with aqueous solution has so far been all but neglected , despite the fact that it may be the microbiology that makes solid state fermentation advantageous against submerged fermentation biotechnology (Acura-Arguelus *et al.*, 2004).The fact basidiomycetes have been insufficiently investigated coupled with broad range of structural types of antibiotics . However basidiomycetes may become source of new and useful bioactive compound (Srivastava and Sharma , 2011) . To our knowledge , no investigation has been performed for comparing

antimicrobial activity potential of basidiomycetes strains . Further studies on isolation and identification of the active compounds may provide a better source for developing new therapeutic agents .

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