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Original article

Hepatoprotective evaluation and isolation of the major secondary metabolites from the ethyl acetate extract of liquid culture filtrate of *Chaetomium globosum*

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ABSTRACT

The aim of the present study was to evaluate the hepatoprotective activity of ethyl acetate extract of the liquid culture filtrate of *Chaetomium globosum* fungus (family Chaetomiaceae). Rats were intraperitoneally injected by CCl4 (0.5 ml/kg) twice a week for six consecutive weeks. Treatment tacks (250 mg/kg) place at the same time of CCl4 induction and with the same duration. The evaluation was done through determination of liver function indices; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total serum protein content. In addition, the oxidative stress markers; hepatic glutathione content (GSH), hepatic malondialdehyde (MDA), hepatic superoxide dismutase (SOD), and hepatic total protein were estimated. Moreover, the liver architectures were also examined. Isolation and identification of the main secondary metabolites were identified. Seven volatile compounds were identified from the plain chloroform fraction where, 1-Cyclopentyl-2,2-dimethyl-1-propanol (54.63%) was presented as the major compound. Eleven compounds were also identified from the fraction eluted by chloroform: methanol (85:15). 1,5,5-Trimethyl-6-methylene-1-cyclohexene (25.79%) and Norbornan-2-one (26.84%) are presented as the major compounds of this fraction. In conclusion, the extract recorded hepatoprotective effect by ameliorating the biochemical parameters under investigation. The liver histopathological pictures confirmed our results.

1. Introduction

Endophytes are considered as one of the essential interesting topics in the field of natural product chemistry and one of the major clades of life [1]. *Chaetomium globosum*, the type species of the genus*Chaetomium* belonging to Phylum *scomycota*. Class *Sordariomycetes*(FamilyChaetomiaceae), spread all over the worldin soil, water, plant material and other cellulosic substances [2].

This fungusproduces diverse groups of secondary metabolites with several biological activities such as antimicrobial, immunomodulatory, and anticancer [3], where ithas also been proven to be an essential source of diverse bioactive constituentssuch ascytoglobosins, azaphilones, chaetoviridins, pyrones, orsellides and globosumones [4].

Prenisatin, chrysophanol, chrysazin,chaetoviridin A and B were isolated from the ethyl acetate extract of the liquid culture filtrate of *Chaetomiumglobosum*, where recorded remarkable antioxidant andantimicrobial activity on different bacteria and fungi [5].

The aim of the present study is to evaluate the ethyl acetate extract of the liquid culture filtrate of *Chaetomium globosum* fungus as antioxident and hepatoprotective agent on liver injury induced by CCl4 in rats.

2. Material and methods

2.1. Fungal isolation

During autumn and winter growing season of 2014/2015, a surveystudy was conducted at the major cucumber (*Cucumissativus*L.) growingareas under plastic greenhouse conditions. Samples of cucumber plants exhibiting damping-off, root-rot, stem-rot and wilt symptoms were collected and immediately transferred to Plant Pathology laboratory for isolation procedures. The pathogens were

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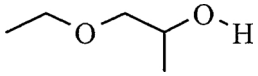
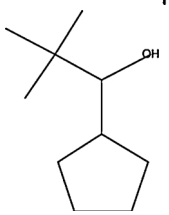
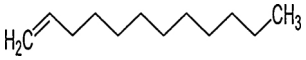
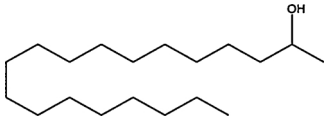
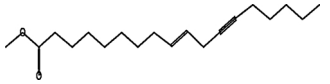
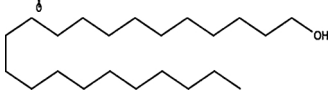
Table 1
GC/MS analysis of the fraction 1 eluted with 100% chloroform.

No	RT	Mol. Formula	Mol. weight	BP	Compound	%	Structure
1	15.17	$C_5H_{12}O_2$	104	43	3-Ethoxy propanol	2.59	
2	27.60	$C_{10}H_{20}$ O	156	57	1-Cyclopentyl-2,2-dimethyl-1-propanol	54.63	
3	30.23	$C_{12}H_{24}$	168	41	Dodecene	3.15	
4	34.71	$C_{18}H_{32}O_2$	280	55	17-Octadecynoic acid	4.44	
5	42.62	$C_{19}H_{40}$ O	284	57	2-Nonadecanol	4.19	
6	56.99	$C_{19}H_{32}O_2$	292	43	Methyl octadeca-9-ene-12-ynoate	1.74	
7	62.02	$C_{22}H_{46}$ O	326	55	1-Docosanol	5.07	

isolated by tissue segment method [6]. The root pieces were cultured onto the surface of sterilized Petriplates containing freshly preparing Potato Dextrose Agar (PDA) medium. After 10 days of incubation at 25 ± 1 °C, the frequency occurrence (%) of isolated fungi was recorded. The fungal hyphal tip of developed colonies around the root pieces were transferred onto PDA medium and incubated for10 days at 25 ± 1oC for further studies [6].

2.2. Fungal identification

Isolated fungi were identified at the Plant PathologyDepartment, National Research Centre (NRC), Egypt, and confirmed byFungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt according to the morphological and culture characters using the methods previously described by Barnett and Hunter [7], and Ramirez [8]. Stock cultures were maintained on PDA slants and kept in a refrigerator at 5 °C for further studies.

2.3. Preparation of the ethyl acetate extract of liquid culture filtrate of C. globosum

Twenty liters of the liquid culture filtrate of *C. globosum* have been extracted by ethyl acetate. The extract has been concentrated in a rotary evaporator at 45 °C under reduced pressure.

2.4. Identification of the volatile secondary metabolites from the ethyl acetate extract

The dried ethyl acetate extract of the liquid culture filtrate of *C. globosum* was dissolved in the least amount of ethyl acetate, loaded onto a silica gel column (120 cm height × 2.5 cm i.d.) containing 175 g activated silica (70–230 mesh; E. Merck, Darmstadt, Germany).

The elution was done using the organic solvents; chloroform and chloroform: methanol (85:15). Collection of the eluted fractions were done, then evaporated under reduced pressure and subjected to TLC examination using toluene: ethyl acetate (8:2). Similar fractions were collected together. Characterization of the compounds were carried out by GC/MS analysis (Shimadzu GC/MS −QP5050A, 70 eV) for of the volatile constituents.

2.5. Biological study

2.5.1. Acute toxicity

Male Wistar albino rats (120–140 g) were obtained from the Animal House, National Research Center, Egypt and kept in controlled environment of air and temperature with access of water and diet. One oral dose of 5, 10, 50 and 100 mg of the ethyl acetate extract body weight. Rats were observed after 24 h of administration and along the following fourteen days. Dead rats were counted and the mortality rate was calculated. Lethal dose that killed 50% of animals (LD_{50}) was monitored.

The lethal dose that killed 50% of rats (LD50) along fourteen days was 100 mg/kg b.wt. Therefore, the selected dose is 20 mg/kg b.wt representing 1/5 of the lethal dose.

2.5.2. Doses and route of administration

Administration regimen was twice a week for six consecutive weeks. CCl_4 (0.5 ml/kg) was suspended in olive oil (1:9 v/v) and injected intraperitoneally [9]. The ethyl acetate extract was administrated orally at a dose 20 mg/kg. Silymarin; a reference herbal drug was orally administered at a dose 100 mg/kg [10]. Blood samples and slices of liver tissues were taken from the eviscerated animals and kept for the biochemical assays. Handling of the rats was obeyed the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt (Approval no: 134, 2011).

2.5.3. Experimental design

Forty eight rats were divided into six groups of eight rats each. Group 1 served as normal healthy control rats orally vehicle with 0.5 ml

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