Chemical Composition of Aspergillus creber Extract and Evaluation of its Antimicrobial and Antioxidant Activities

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Abstract

Among the species belonging to the Aspergillus section Versicolores, Aspergillus creber has been poorly studied and still unexplored for its biological activities. The present study was undertaken to analyze A. creber extract and to evaluate its in vitro antimicrobial and antioxidant activities. UHPLC-MS/MS analysis of A. creber extract allowed the characterization of five known fungal metabolites including: asperlactone, emodin, sterigmatocystin, deoxybrevianamide E, and norsolorinic acid. The highest antimicrobial activity was displayed against Candida albicans, with a mean strongest inhibition zone of 20.6 ± 0.8 mm, followed by Gram-positive drug-resistant bacteria. The MIC values of A. creber extract varied from 0.325 mg/ml to 5 mg/ml. A. creber extract was shown a potent antioxidant activity and a high level of phenolic compounds by recording 89.28% scavenging effect for DPPH free radical, 92.93% in ABTS assay, and 85.76 mg gallic acid equivalents/g extract in Folin-Ciocalteu assay. To our knowledge, this is the first study concerning biological and chemical activities of A. creber species. Based on the obtained results, A. creber could be a promising source of natural antimicrobial and antioxidant compounds.

Key words: Antimicrobial activity, antioxidant activity, Aspergillus creber, UHPLC-MS/MS, Versicolores

Introduction

Fungal secondary metabolites represent a diverse group of bioactive natural products often produced at a restricted part of the life cycle (Keller et al. 2005). Aspergillus species are among the major contributors to the secondary metabolites of fungal origin (Dewi et al. 2012; Bai et al. 2014). Although the functions of these compounds for the producing fungi are obscure or unknown, they confer important benefits to human-kind as many of them have antibiotic and pharmacological activities (Yu and Keller 2005; Siddiquee et al. 2015). Isolation of a new fungal strain is often conducted to the identification of new natural products (Brakhage and Schroechk 2011).

Aspergillus species assigned to section Versicolores are among the most ubiquitous fungi, frequently isolated from environmental samples (Gautier et al. 2016). The important characteristics that render these species of interest to the scientific community are their prevalent in indoor environments, their capacity to produce sterigmatocystin (STC), a carcinogenic mycotoxin, and their diverse biotechnological applications (Siqueira et al. 2016). Before using molecular methods for species identification, isolates belonging to section Versicolores were commonly reported as A. versicolor (Despot et al. 2016). In 2012, Jurjevic et al. revised the section Versicolores and accepted 13 species; among them, Aspergillus creber was described for the first time as a new species. Currently, the section Versicolores comprises 17 distinct species (Despot et al. 2017); among them, A. versicolor and A. sydowii are still the most commonly reported and studied species (Siqueira et al. 2016). However, very few reports are available concerning the species A. creber and those reports were only concerned with contamination by A. creber and its ability to...
produce STC. *A. creber* was reported as the most prevalent species in indoor air environments in USA (Jurjevic et al. 2012) and in Italian libraries (Micheluz et al. 2015). *A. creber* was also reported as among the lower producers of STC (Jurjevic et al. 2013).

Therefore, the lack of knowledge of this species recently assigned to section Versicolores has attracted our attention to more explore and study this species. In our research *A. creber* was assayed for its secondary metabolites diversity using UHPLC-MS/MS technique. Then, the antimicrobial activity of *A. creber* against some human pathogens and its ability to scavenge different free radicals, and to produce antioxidant compounds were also evaluated to improve the knowledge of this species, which has never been tested before for its biological activities.

**Experimental**

**Materials and Methods**

**Fungal strain.** The strain of *A. creber* employed in this study was isolated from maize grains collected from Batna region, Northeast Algeria. Morphological identification of the selected strain was made following the recommended method and media (Samson et al. 2014). Then, the identity was confirmed by the German Collection of Microorganisms and Cell Cultures (DSMZ) using the analysis of the Internal Transcribed Spacer rDNA region (ITS) and the calmodulin gene (CaM) sequences. The strain was kept on Potato Dextrose Agar (PDA) slants at 4°C in the fungal collection of the Laboratory de Mycologie, de Biotechnologie et de l’Activité Microbienne (LaMyBAM), University des Frères Mentouri Constantine-1.

**Production and extraction of *A. creber* secondary metabolites.** Under sterile conditions, a small amount of *A. creber* was transferred onto new PDA plates and incubated at 25°C until sporulation. The fungal spores were harvested by pouring 9 ml of sterile distilled water, containing 0.1% Tween 80, on the agar plate surfaces and transferred into sterile Falcon tubes. The prepared fungal suspension was inoculated aseptically in five 1000 ml Erlenmeyer flasks [5% (v/v)], each containing 250 ml of Czapek-Dox broth supplemented with yeast extract (Slack et al. 2009). The Erlenmeyer flasks were covered with the aluminum foil and incubated at 28 ± 2°C with shaking at 125 rpm for two weeks.

The culture broth was filtered, using Whatman filter paper number 1, to remove the mycelia. The filtered broth was extracted three times with equal volumes of ethyl acetate (EtOAc) and evaporated until dryness by using a rotary evaporator at 45°C. The resulted ethyl acetate extract of the filtrate (EAF) was reconstituted in methanol to get a concentrated stock solution of 200 mg/ml, which was used for further analysis.

**Ultrahigh-performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) analysis of *A. creber* extract.** A Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, USA) coupled to a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, USA) via a HESI-II electrospray ionization (ESI) source was used for the analysis of EAF. A volume of 1 μl of EAF was injected on a Luna Omega C18 column (50 × 2.1 mm, 1.6 μm particle size). The column thermostat was maintained at 25°C. A mobile phase consisting of eluent A (0.1% formic acid in water, 5 mM ammonium formate) and eluent B (0.1% formic acid in methanol, 5 mM ammonium formate) was used at a flow rate of 0.4 ml/min. The gradient elution was performed as follows: 0 min 20% eluent B; 0.5 min 40% eluent B; 6 min 100% eluent B; 8 min 20% eluent B; 10 min 20 % eluent B.

The Orbitrap mass spectrometer was operated in both positive and negative mode with the use of the following parameter settings: spray voltage, 4 kV; sheath gas (N2 > 95%), 35 arbitrary units; auxiliary gas (N2 > 95%), 10 arbitrary units; capillary temperature, 290°C; S lens RF level, 50; heater temperature, 305°C. Two scan events were carried out, the LC-MS was used in full scan mode at a resolution of 70 000 fwhm (full width at half maximum) in the range m/z 90–1000 without the use of any lock masses. The maximum injection time (MIT) was 100 ms with one micro scan, and the automatic gain control (AGC) target was set to 1e6. The MS/MS was performed in parallel reaction monitoring (PRM) mode, in order to obtain two product ions for each target compound, at a resolution of 35 000 fwhm with collision energy (CE) of 30, the AGC and MIT were set at 2e5 and 200 ms, respectively. The instrument control and data analysis were performed by Thermo Fisher Xcalibur v. 3.0.63 software.

**Antimicrobial activity assay**

**Test microorganisms.** Eight clinically isolated microorganisms, obtained from the laboratory of Clinical Microbiology, University of Federico II Napoli-Italy, were used to evaluate the antimicrobial activity of EAF. Four Gram-negative pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella Typhi*) and two Gram-positive drug-resistant bacteria (DRB, *Staphylococcus aureus* producing beta-lactamase (SABL) and methicillin-resistant *Staphylococcus epidermidis* (MRSE)) were used for the antibacterial tests. Two pathogenic yeasts (*Candida albicans* and *Candida glabrata*) were used for antican didal tests. Pathogenic bacteria were inoculated into the nutrient broth and incubated at 37°C for 24 h. *Candida* species were inoculated into Sabouraud Dextrose broth at 28°C for 48 h.
Antimicrobial activity determination. Antimicrobial activity of EAF against the test organisms was examined using disc diffusion method according to Turkoglu et al. (2007) with slight modification. Suspension of the organisms tested (0.5 McFarland standards) was spread on the sterile growth media plates containing Nutrient agar and Sabouraud Dextrose agar for bacteria and yeasts, respectively. Sterilized paper disks (6 mm) were impregnated with 20 µl of EAF and dried aseptically. The impregnated discs were placed on the surface of plates seeded with the test organisms and incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for yeasts. Both chloramphenicol (10 µg/disk) and ketoconazole (30 µg/disk) were employed as positive controls for pathogenic bacteria and Candida species, respectively. Similarly, 20 µl of methanol and clean disks (without solvent) were used as negative controls. All experiments were carried out in triplicate. After incubation, antimicrobial activity was determined by measuring the diameter of inhibition zones around the discs.

Determination of minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) of EAF was carried out against the sensitive test microorganisms by using broth microdilution method following the recommendations of Clinical and Laboratory Standards Institute (CLSI) protocols for bacteria (CLSI 2012) and for yeasts (NCCLS 2002). Chloramphenicol and ketoconazole were used as a positive control. The MIC was taken as the lowest concentration at which no visible growth was observed.

Antioxidant analysis
2,2-diphenyl-1-picryl hydrazyl (DPPH) assay. The DPPH radical scavenging activity was determined according to Jakovljević et al. (2014). Different concentrations of EAF were prepared: 12.5, 25, 50, 100, 200, 400 µg/ml. To 1 ml of DPPH solution, 1 ml of each concentration was added. After incubation for 30 min at 37°C, the absorbance was measured at 517 nm against the blank. Ascorbic acid was used as positive control. The percentage of the DPPH scavenging activity was calculated using the formula:

\[ \% \text{ inhibition} = \left( \frac{A_0 - A_f}{A_0} \right) \times 100 \]

where \( A_0 \) represents the absorbance of the blank sample, and \( A_f \) represents the absorbance of the mixture.

2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay. Using the same concentrations, ABTS assay was used as reported by Dudonné et al. (2009). The ABTS radical cation (ABTS•+) stock solution was prepared by mixing 7 mM of ABTS with 2, 45 mM of potassium persulfate. The mixture was left in the dark at room temperature for 12–16 h. For the study of EAF, the ABTS•+ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. To 3 ml of ABTS•+ diluted solution, 100 µl of each concentration was added, and the absorbance reading at 734 nm was taken at the end of the tenth minute. Percent inhibition of ABTS•+ was calculated using the previous formula.

Total phenolic content (TPC) assay. TPC of A. creber was determined using the Folin-Ciocalteu method. To 0.5 ml of EAF, 2.5 ml of Folin-Ciocalteu reagent was added. After 4 min, 2 ml of sodium carbonate (7.5%, w/v) were added, and the absorbance was measured at 760 nm after incubation for 2 h at room temperature (Gan et al. 2010). The TPC of the extract was expressed as mg Gallic acid equivalent per g of extract (mg GAE/g extract).

Results

Fungal strain. Aspergillus creber isolate showed the typical macro- and micro-morphological features described for section Versicolores. For species designation, sequencing of the ITS rDNA and CaM gene followed by a BLAST search revealed that the isolated strain had a high identity (99%) with A. creber. Thus, according to these results, the strain was identified as A. creber and its sequences data were submitted at GenBank with accession numbers: MH796366 and MH796367.

UHPLC-MS/MS analysis. After two weeks of growth in the fermentation broth, the secondary metabolites of A. creber were extracted, through EtOAc, filtered and concentrated in vacuo to give EAF, which was subjected to UHPLC-MS/MS analysis. On the basis of the fungal metabolites list established by Lehner et al. (2011), molecular mass data, MS/MS analysis and references, five known fungal metabolites were tentatively identified as asperlactone (1), emodin (5), sterigmatocystin (7), deoxybrevianamide E (9) and norsolorinic acid (10) (Table I) whereas five metabolites remained unidentifiable. The Total Ion Current (TIC) chromatograms of the ten peaks of detected compounds are shown in Fig. 1.

Antimicrobial activity. Results obtained for the antimicrobial activity of EAF are shown in Table II. In the case of bacteria, the highest zone of inhibition was displayed against Gram-positive DRB, including MRSE and SABL, followed by Gram-negative bacteria K. pneumoniae and S. typhi with an inhibition zone ranged from 8.5 ± 0.6 to 14.0 ± 0.2 mm. No activity was observed against E. coli and P. aeruginosa at the concentration used. In the case of yeasts, the EAF inhibited the growth of all tested yeasts (C. albicans and C. glabrata) with maximum inhibitory activity against C. albicans, which displayed an important zone of inhibition (20.6 ± 0.8 mm).

Table II showed also the MIC values of A. creber extract against the tested organisms, which showed an inhibition zone in the disc diffusion assay. The EAF of A. creber showed MIC values ranging from
The higher MIC values were recorded against *C. albicans* (0.325 mg/ml) and Gram-positive DRB (0.625 mg/ml) whereas the lower MIC values were recorded against *C. glabrata* (1.25 mg/ml) and Gram-negative bacteria *K. pneumoniae* (2.5 mg/ml) and *S. typhi* (5 mg/ml).

**Antioxidant analysis.** In the DPPH assay, the EAF of *A. creber* extract demonstrated a dose-dependent scavenging activity and the highest decolorization was recorded at 400 µg/ml (Fig. 2a). In the ABTS assay, like the DPPH assay, the EAF exhibited a rich scavenging effect and the highest decolorization was also recorded at a concentration of 400 µg/ml (Fig. 2b). In both assays, the scavenging activity of EAF was slightly lower than that of ascorbic acid. In the Folin-Ciocalateu assay, the EAF showed a high level of total phenols (Table III).

| Peak No. | Putative compound name | Adduction | Measured mass (m/z) | Productions (m/z) | t<sub>R</sub> (min) | Ref<sup>b</sup> |
|----------|------------------------|-----------|---------------------|------------------|------------------|-------------|
| 1        | Asperlactone           | [M+H]+    | 185.08011           | 141.05444, 113.05948 | 1.73            | Vishwanath et al. 2009 |
| 2        | NI                     | [M+Na]+   | 211.06121           | 195.03455, 133.02816 | 1.78            | –           |
| 3        | NI                     | [M+H]+    | 308.10992           | 280.95387, 145.10104 | 3.38            | –           |
| 4        | NI                     | [M+H]+    | 327.04734           | 309.18594, 191.15396 | 4.28            | –           |
| 5        | Emodin                 | [M+H]−    | 269.04590           | ND               | 5.42            | Sulyok et al. 2007; Lehner et al. 2011; Micheluz et al. 2016 |
| 6        | NI                     | [M+H]+    | 251.09145           | 233.09256, 204.09319 | 5.49            | –           |
| 7        | Sterigmatocystin       | [M+H]+    | 325.07053           | ND               | 6.62            | Lehner et al. 2011; Micheluz et al. 2016 |
| 8        | NI                     | [M+Na]+   | 423.25041           | 405.27911, 239.14821 | 7.27            | –           |
| 9        | Deoxybrevianamide E    | [M+H]+    | 352.20352           | ND               | 8.04            | Lehner et al. 2011; Micheluz et al. 2016 |
| 10       | Norsolorinic acid      | [M+H]−    | 369.09892           | ND               | 8.24            | Micheluz et al. 2016 |

<sup>a</sup> Retention time; <sup>b</sup> Reference; ND – not detected; NI – not identified

| Zone of inhibition (mm)<sup>a</sup> | MIC of ethyl acetate extract (mg/ml) |
|-------------------------------------|-------------------------------------|
| Ethyl acetate extract              | Positive control<sup>b</sup>       |
| **Bacteria**                        |                                     |
| *Escherichia coli*                  | 0                                   | 23.4 ± 0.3 | –         |
| *Klebsiella pneumoniae*             | 10.0 ± 0.3                          | 25.0 ± 0.4 | 2.5       |
| *Pseudomonas aeruginosa*            | 0                                   | 20.8 ± 0.4 | –         |
| *Salmonella Typhii*                 | 8.5 ± 0.6                           | 21.5 ± 0.3 | 5         |
| *SABL*<sup>c</sup>                 | 12.8 ± 0.3                          | 19.8 ± 0.2 | 0.625     |
| *MRSE*<sup>d</sup>                 | 14.0 ± 0.2                          | 19.2 ± 0.2 | 0.625     |
| **Yeasts**                          |                                     |
| *Candida albicans*                  | 20.6 ± 0.8                          | 22.8 ± 0.4 | 0.325     |
| *Candida glabrata*                  | 13.0 ± 0.3                          | 25.7 ± 0.5 | 1.25      |

<sup>a</sup> Mean of three replicates (±) SD
<sup>b</sup> Chloramphenicol and ketoconazole were used as the positive control for bacteria and yeasts respectively
<sup>c</sup> *Staphylococcus aureus* producing beta lactamase
<sup>d</sup> Methicillin resistant *Staphylococcus epidermidis*
0 – no zone of inhibition

**Table III**

| Activity | Extract | Ascorbic acid<sup>d</sup> |
|----------|---------|---------------------------|
| DPPH     | 89.28 ± 0.32 | 91.39 ± 0.39 |
| ABTS<sup>c</sup> | 92.93 ± 0.30 | 93.03 ± 0.45 |
| Total phenolic content (mg GAE/g) | 85.76 ± 0.96 | – |

<sup>c</sup> Percentage of inhibition at a concentration of 400 µg/ml.
Values are mean of three replicates (±) SD
Analysis and bioactivities of *Aspergillus creber* extract

Discussion

Fungi are major producers of secondary metabolites with different biological activities and various chemical structures (Abo-Elmagd 2014). Until today, except for *A. versicolor* and *A. sydowii*, there is no thorough study on metabolic profiles for the *Aspergillus* species belonging to section *Versicolores* (Despot et al. 2017). Moreover, to our knowledge, there are no published reports recorded the antimicrobial and the antioxidant activities of *A. creber*.

Our study demonstrated that *A. creber* is able to produce a variety of secondary metabolites previously reported as potent biologically active compounds. The biological activity of asperlactone, a polyketide metabolite, had been established for the first time by Balcells et al. (1995). It was the first fungal compound that exhibited insect growth regulating activity in *in vivo* tests. Asperlactone has also been reported as a potent antibacterial and antifungal metabolite (Chen et al. 2014). Emodin is the most studied anthraquinone derivative for its diverse biological activities, including antibacterial (Hatano et al. 1999), antifungal (Kim et al. 2004), antioxidant (Izhaki 2002), and anticancer properties (Cheshmi et al. 2017; Zhao et al. 2017). STC is a polyketide mycotoxin and a precursor of Aflatoxin B1 (AFB1), however, its toxicity is lower than that of AFB1 (Piontek et al. 2016). Deoxybrevianamide E is a prenylated indole alkaloid that belongs to the family of brevianamides. Although a variety of biological activities were exhibited by many brevianamides including antibacterial, anti-insect pests and antitubercular properties (Xu et al. 2017) there are no reports in the literature concerning the bioactivities of deoxybrevianamide E. Norsolorinic acid is an anthraquinone derivative fungal metabolite. Wang et al. (2008) reported that norsolorinic acid has antiproliferative activity on T24 human bladder cancer cells.

![Fig. 1. Total Ion Current chromatogram of *Aspergillus creber* extract obtained with UHPLC-MS/MS. The retention times of the peaks (1–10) and their corresponding molecules names are presented in Table I.](image)

![Fig. 2. Free radical-scavenging activities of *Aspergillus creber* extract and ascorbic acid (400 µg/ml) measured (a), in DPPH assay and (b), in ABTS assay.](image)
The ability of *A. creber* to produce STC is in accordance with previous studies demonstrating that most of *Aspergillus* section *Versicolores* are STC producers (Jurjević et al. 2013, Despot et al. 2016). Moreover, our findings are in agreement with the study of Micheluz et al. (2016) reported that *A. creber* is able to produce emodin, STC, deoxybrevianamide E and norsolorinic acid. However, to our knowledge, this is the first report concerning the production of asperlacone from the species *A. creber*.

In the antimicrobial assays, our study indicated that *A. creber* exhibited more potent antibacterial activity against Gram-positive than Gram-negative bacteria. This variation of susceptibility could be attributed to the morphologic difference in the composition of their cellular membranes, which influences their reaction to antibacterial compounds (Valle Jr et al. 2015). In addition to its antibacterial activity, the EAF of *A. creber* exhibited higher antifungal activity against *Candida* species. In the case of *Candida* species, ergosterol is the main target of antifungal drugs (Martins et al. 2015). These results are in contrast to the previous studies reported that the metabolites produced by *A. versicolor*, the most studied species of *Aspergillus* section *Versicolores*, exhibited lower or no antibacterial and antifungal activities (Zhuang et al. 2011; Song et al. 2012; Ebada et al. 2018). Another attractive finding on *A. creber* was the higher MIC values of its ethyl acetate extract against *C. albicans* (0.312 mg/ml) and Gram-positive DRB (0.625 mg/ml) since these species are the most common opportunistic pathogens (Martins et al. 2015; Knafl et al. 2017). Moreover, our findings can be considered very promising since the extract was crude and obtained by using non-optimized fermentation, which usually produces a poor yield of active compounds (Noor Ifatul et al. 2016).

Our study also brings additional data on *A. creber*, since the antioxidant activity and the TPC of this species were evaluated for the first time. The antioxidant ability of *A. creber* was tested using two different procedures, DPPH and ABTS assays. The DPPH assay is a widely used method that based on the reduction of the purple DPPH free radical to a yellow colored ABTS form (Re et al. 1999). Thus, the higher scavenging activities of the EAF indicated its bioactive potential to neutralize the DPPH free radicals and ABTS+•. These results are in agreement with many other studies reported the antioxidant potency of filamentous fungi from different sources (Abo-Elmagd 2014; Kumaresan et al. 2015; Sharma 2015; Smith et al. 2015; Sugiharto et al. 2016). The slight difference between the antioxidant capacities of EAF and ascorbic acid may be explained either by the purity of ascorbic acid, whereas the extract of *A. creber* is a mixture of multiple compounds or by the capacity of some compounds in the fungal extract to neutralize or to inhibit the effect of active compounds (Dhankhar et al. 2012). Moreover, our data revealed that *A. creber* is able to produce much higher content of phenols than the other filamentous fungi in the literature (Jakovljević et al. 2014; Smith et al. 2015; Sugiharto et al. 2016). Many previous studies attributed the antioxidant activity of filamentous fungi to their TPC (Abdel-Monem et al. 2013; Smith et al. 2015, Nwobodo et al. 2017). In accordance with this, the higher percentages of inhibition of DPPH radical and ABTS+• in our fungal extract might contribute to the high level of phenolic compounds.

In conclusion, UHPLC-MS/MS analysis revealed that *A. creber* could produce a variety of secondary metabolites including polyketides, anthraquinones, and alkaloids. Our findings suggest that *A. creber* might represent a novel source of natural bioactive products since we have demonstrated its ability to inhibit the growth of many pathogen microorganisms, mainly *C. albicans* and Gram-positive DRB, and its high antioxidant activity. Our work will increase the knowledge about the species *A. creber* newly described and serve as a prelude to a better understanding of the biology and the chemistry of its metabolites.

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**Conflict of interest**

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

**Literature**

Abdel-Monem N, Abdel-Azeem AM, El Ashy ESH, Ghareeb DA, Nabil-Adam A. Assessment of secondary metabolites from marine-derived fungus as antioxidant. Open J Med Chem. 2013;03(03):60–73. https://doi.org/10.4236/ojmc.2013.33009

Abo-Elmagd HI. Evaluation and optimization of antioxidant potentiality of *Chaetomium madrasense* AUMC 9376. J Genet Eng Biotechnol. 2014;12(1):21–26. https://doi.org/10.1016/j.jgeb.2014.03.002

Bai ZQ, Lin X, Wang Y, Wang J, Zhou X, Yang B, Liu J, Yang X, Wang Y, Liu Y. New phenyl derivatives from endophytic fungus *Aspergillus flavipes* AIL8 derived of mangrove plant *Acanthus ilicifolius*. Fitoterapia. 2014;95:194–202. https://doi.org/10.1016/j.fitote.2014.03.021.
Species diversity and cytotoxic activity of the toxin-producing fungus Aspergillus sp. 16-02-1. Mar Drugs. 2014;12(6):3116–3137.

Cheshmi F, Kazerouni F, Omrani MD, Rahimpour A, Shanaki M, Dehghan-Nayeri N, Younesian O, Rezapour Kalkhoran M. Effect of emodin on expression of VEGF-A and VEGFR-2 genes in human breast carcinoma MCF-7 Cell. Int J Cancer Manag. 2017;10(7):e8095.

https://doi.org/10.5812/ijcm.8095

CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard – Ninth Edition. CLSI document M07-A9. Wayne (USA): Clinical and Laboratory Standards Institute; 2012.

Despot DJ, Kocubš S, Bencic O, Kecksmetı A, Sazeres A, Vagvolgyi C, Varga J, Klarič MŠ. Diversity and cytotoxic potency of airborne sterigmatocystin-producing Aspergillus from the section Versicolores. Sci Total Environ. 2016;562:296–304.

https://doi.org/10.1016/j.scitotenv.2016.03.183

Despot DJ, Kocubš S, Bencic O, Kecksmetı A, Sazeres A, Vagvolgyi C, Varga J, Klarič MŠ. New sterigmatocystin-producing species of Aspergillus section Versicolores from indoor air in Croatia. Mycol Prog. 2017;16(1):63–72.

https://doi.org/10.1007/s11557-016-1250-4

Dewi RT, Tachibana S, Itoh K, Ilyas M. Isolation of antioxidant compounds from Aspergillus terreus LS01. J Microbial Biochem Technol. 2012;4:100–104.

Dhankhar S, Kumar S, Dhankhar S, Yadav JP. Antioxidant activity of fungal endophytes isolated from salvadora oleoides descn. Int J Pharm Pharm Sci. 2012;4:380–385.

Dudonné S, Vitrac X, Coutière P, Woillez M, Mérillon JM. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, ORAC assays. J Agric Food Chem. 2009;57(5):1768–1774.

https://doi.org/10.1021/jf803011r

Ebada SS, El-Neketi M, Elsharief W, Starzyk JP, Lechów H. Analysis and bioactivities of fungal secondary metabolites from indoor air in Croatia. Mycopathologia. 2012;181(1):57–64.

https://doi.org/10.1007/s11046-011-9413-3

Fleогel А, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPHH assays to measure antioxidant capacity in popular antioxidants: Part A. 2011;28(10):1457–1468.

https://doi.org/10.3920/WMJ2015.1958

Garcinia kola. Study on the final stage of the production of the food additive. J Farg Food Compos Anal. 2011;24(7):1043–1048.

https://doi.org/10.1016/j.jfca.2011.01.008

Gan RY, Kuang L, Xu XR, Zhang Y, Xia EQ, Song FL, Li HB. Screening of natural antioxidants from traditional Chinese medicinal plants associated with treatment of rheumatic disease. Molecules. 2010;15(9):3988–3997.

https://doi.org/10.3390/molecules15093988

Gautier M, Normand AC, Ranque S. Previously unknown species of Aspergillus. Clin Microbiol Infect. 2016;22(8):662–669.

https://doi.org/10.1016/j.cmi.2016.05.013

Hatanaka T, Uebayashi H, Ito H, Shiota S, Tsuchiya T, Yoshida T. Phenolic constituents of Cissus seeds and antimicrobial effect of some naphtalenols and anthraquinones on methicillin-resistant Staphylococcus aureus. Chem Pharm Bull (Tokyo). 1999;47(8):1121–1127.

https://doi.org/10.1248/cpb.47.1121

Izhaki I. Emodin – a secondary metabolite with multiple ecological functions in higher plants. New Phytol. 2002;155(2):205–217.

https://doi.org/10.1046/j.1469-8137.2002.00459.x

Jakovljević V, Milićević J, Stojarović J, Solujić S, Vrvić M. Anti-oxidant activity of ethanolic extract of Penicillium chrysogenum and Penicillium funiculosum. Hem Ind. 2014;68(1):43–49.

https://doi.org/10.2298/HEMIND121102027J

Jurjević Z, Peterson SW, Horn BW. Aspergillus section Versicolores: nine new species and multicopy DNA sequence based phylogeny. IMA Fungus. 2012(3):1–59.

https://doi.org/10.5598/imafungus.2012.03.01.07

Jurjević Z, Peterson SW, Solfrizzi M, Peraica M. Sterigmatocystin production by nine newly described Aspergillus species in section Versicolores grown on two different media. Mycotoxin Res. 2013;29(3):141–145.

https://doi.org/10.1007/s12550-013-0160-4

Keller NP, Turner G, Bennett JW. Fungal secondary metabolism – from biochemistry to genomics. Nat Rev Microbiol. 2005;3(12):937–947.

https://doi.org/10.1038/nrmicro1286

Kim YM, Lee CH, Kim HG, Lee HS. Anthraquinones isolated from Cassia tora (Leguminosae) seed show an anti-oxidant property against phytotoxicogenic fungi. J Agric Food Chem. 2004;52(20):6096–6100.

https://doi.org/10.1021/jf049379p

Knall D, Tobudic S, Cheng SC, Bellamy DR, Thalhammer F. Dalbavancin reduces biofilms of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus epidermidis (MRSE). Eur J Clin Microbiol Infect Dis. 2017;36(4):677–680.

https://doi.org/10.1007/s10096-016-2845-z

Kumaraseelan, Karthi, Venthilikkumar, Valakumar S, Stephen A. Biochemical constituents and antioxidant potential of endophytic fungi isolated from the leaves of Azadirachta indica A. Juss (Neem) from Chennai, India. J. Acad. Ind. Res. 2015;3:355–361.

Lehner SM, Neumann KKN, Sulyok M, Lemmens M, Krksa R, Schuhmacher R. Evaluation of LC-high-resolution FT-Orbitrap MS for the quantification of selected mycotoxins and the simultaneous screening of fungal metabolites in food. Food Additives & Contaminants: Part A. 2011;28(10):1457–1468.

https://doi.org/10.1080/19440049.2011.599340

Martins N, Barros L, Henriques M, Silva S, Ferreira ICFR. Activity of phenolic compounds from plant origin against Candida species. Ind Crops Prod. 2015;74:648–670.

https://doi.org/10.1016/j.indcrop.2015.05.067

Micheluz A, Manente S, Tignini V, Prigione V, Pinzari F, Ravagnan G, Varese GC. The extreme environment of a library: xerophilic fungi inhabiting indoor niches. Int Biodeterior Biodegradation. 2015;99:1–7.

https://doi.org/10.1016/j.ibiod.2014.12.012

Micheluz A, Sulyok M, Manente S, Krksa R, Varese GC, Ravagnan G. Fungal secondary metabolite analysis applied to Cultural Heritage: the case of a contaminated library in Venice. World Myco-toxin J. 2016;9(3):397–407.

https://doi.org/10.1007/s12550-013-0160-4

NCCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard – Second Edition. NCCCLS document M27-A2. Wayne (USA): National Committee for Clinical Laboratory Standards; 2002.

Noor Ifatul HMD, Lee HY, Nazamid S, Norhana W, Mahyudin NA. In vitro antibacterial activity of marine-derived fungi isolated from Pulau Redang and Pulau Payar Marine Parks, Malaysia against selected food-borne pathogens. Int Food Res J. 2016;23:2681–2688.

Nwodo BC, Ugwu MC, Okoye FBC. Screening of endophytic fungal secondary metabolites from Garcinia kola and Cola nitida for antioxidant properties. J Pharma Res. 2017;10:00136.

Piontek M, Łuszczyńska K, Lechów H. Occurrence of the toxin-producing Aspergillus versicolor Tiraboschi in residential buildings. Int J Environ Res Public Health. 2016;13(9):862.

https://doi.org/10.3390/ijerph13098662

Re R, Pellegrini N, Protegante A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231–1237.

https://doi.org/10.1016/S0891-5849(98)00315-3
Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CHW, Perrone G, Seifert KA, Susca A, Tanney JB, et al. Phylogeny, identification and nomenclature of the genus Aspergillus. Stud Mycol. 2014;78:141–173. https://doi.org/10.1016/j.simyco.2014.07.004

Sharma SK. Optimized extraction and antioxidant activities of polysaccharides from two entomogenous fungi. J Bioanal Biomed. 2015;07(06):180–187. https://doi.org/10.4172/1948-593X.1000141

Siddiquee S, Azad SA, Abu Bakar F, Naher L, Vijay Kumar S. Separation and identification of hydrocarbons and other volatile compounds from cultures of Aspergillus niger by GC-MS using two different capillary columns and solvents. J Saudi Chem Soc. 2015;19(3):243–256. https://doi.org/10.1016/j.jsca.2012.02.007

Siqueira JPZ, Sutton DA, García D, Gené J, Thomson P, Wiedenholt N, Guarro J. Species diversity of Aspergillus section Versicola in clinical samples and antifungal susceptibility. Fungal Biol. 2016;120(11):1458–1467. https://doi.org/10.1016/j.funbio.2016.02.006

Slack GJ, Puniani E, Frisvad JC, Samson RA, Miller JD. Secondary metabolites from Eurotium species, Aspergillus calidoustus and A. insuetus common in Canadian homes with a review of their chemistry and biological activities. Mycol Res. 2009;113(4):480–490. https://doi.org/10.1016/j.mycres.2008.12.002

Smith H, Doyle S, Murphy R. Filamentous fungi as a source of natural antioxidants. Food Chem. 2015;185:389–397. https://doi.org/10.1016/j.foodchem.2015.03.134

Song F, Liu X, Guo H, Ren B, Chen C, Piggott AM, Yu K, Gao H, Wang Q, Liu M, et al. Brevianamides with antitubercular potential from a marine-derived isolate of Aspergillus versicolor. Org Lett. 2012;14(18):4770–4773. https://doi.org/10.1021/ol302051x

Sugiharto S, Yudiarti T, Isroli I. Assay of antioxidant potential of two filamentous fungi isolated from the Indonesian fermented dried cassava. Antioxidants. 2016;5(1):6. https://doi.org/10.3390/antiox5010006

Sulyok M, Krinka R, Schuhmacher R. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. Anal Bioanal Chem. 2007;389(5):1505–1523. https://doi.org/10.1007/s00216-007-1542-2

Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K. Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murrill. Food Chem. 2007;101(1):267–273. https://doi.org/10.1016/j.foodchem.2006.01.025

Valle DJ Jr, Andrade JJ, Puzon JMJ, Cabrera EC, Rivera WL. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. Asian Pac J Trop Biomed. 2015;5(7):532–540. https://doi.org/10.1016/j.apjtb.2015.04.005

Vishwanath V, Sulyok M, Labuda R, Bicker W, Krksi R. Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/tandem mass spectrometry. Anal Bioanal Chem. 2009;395(5):1355–1372. https://doi.org/10.1007/s00216-009-2995-2

Wang CCC, Chiang YM, Kuo PL, Chang JK, Hsu YL. Norsesolonic acid inhibits proliferation of T24 human bladder cancer cells by arresting the cell cycle at the G0/G1 phase and inducing a Fas/membrane-bound Fas ligand-mediated apoptotic pathway. Clin Exp Pharmacol Physiol. 2008;35(11):1301–1308. https://doi.org/10.1111/j.1440-1681.2008.05007.x

Xu X, Zhang X, Nong X, Wang J, Qi S. Brevianamides and myco-phenolic acid derivatives from the deep-dea-derived fungus Penicillium brevicompactum DFSCS025. Mar Drugs. 2017;15(2):43. https://doi.org/10.3390/md15020043

Yu JH, Keller N. Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol. 2005;43(1):437–458. https://doi.org/10.1146/annurev.phyto.43.040204.140214

Zhao C, Wu Y, Li F, Jin X. Emodin inhibits proliferation and invasion, and induces apoptosis in human esophageal cancer cell line ECA109. Trop J Pharm Res. 2017;16(4):781–785. https://doi.org/10.4103/tjpr.v16i4.6

Zhuang Y, Teng X, Wang Y, Liu P, Wang H, Li J, Li G, Zhu W. Cyclopeptides and polyketides from coral-associated fungus, Aspergillus versicolor LCJ 5–4. Tetrahedron. 2011;67(37):7085–7089. https://doi.org/10.1016/j.tet.2011.07.003