International Food Research Journal 26(2): 595-598 (April 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Antifungal activity of castor (*Ricinus communis* L.) leaves methanolic extract on *Aspergillus niger*

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Article history

Received: 16 January, 2017
Received in revised form: 1 July, 2017
Accepted: 21 November, 2018

Keywords

Aspergillus Niger
Castor
Food Spoilage
Seed-Borne Fungi
Ricinine.

Abstract

Aspergillus niger is the most common fungus causing food spoilage and bio-deterioration. It is also a type of seed-borne fungus that can lower the seed viability. *A. niger* is very fast growing and difficult to control. Usually, fungicides are used to protect plants from fungal attacks. In the present work, the potential use of the methanolic extract of castor (*Ricinus communis* L.) leaves as a natural antifungal compound against *A. niger* isolated from stored groundnuts was investigated. The bioactive compound was also determined by gas chromatography-mass spectroscopy (GC-MS). Extraction of castor leaves was done by maceration using methanol as solvent. The yield of the extract was 10.64%. The antimicrobial activity against *A. niger* was determined by biomass growth inhibition test. The results showed that the extract at 500 µg/mL inhibited the fungal growth (71.46%). The presence of compounds in the methanolic extract of castor leaves was assumed to be toxic against *A. niger*. Based on GC-MS results, ricinine was the main compound in the methanolic extract of castor leaves. The potency of antifungal activity of castor methanolic extract may support its usage as renewable fungal toxicant in stored food.

Introduction

Storing food commodities such as peanuts for long periods could lead to fungal contamination. There are several types of seed-borne fungi found to be predominant in peanut storage namely *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, *Aspergillus flavus* and *A. niger* (Rasheed *et al*., 2004). Some of these fungi produce mycotoxins e.g. aflatoxin and fumonisin that can contaminate the seeds. These mycotoxins have been shown to be co-produced and exhibited synergistic effect which subsequently aggravated the toxicity in contaminated food products (Maryam, 2006).

The presence of *A. niger* in the seeds during storage could reduce their viability. *A. niger* is a type of fungus that is very fast growing and difficult to control. Noonimabe *et al*. (2009) reported that the mycotoxin fumonisin B2 was produced by *A. niger* in coffee beans. Moreover, *A. niger* also involved in fruit spoilage. Its presence in stored products will affect the food quality, safety and lead to substantial economic loss.

The most important method to protect crops and foodstuff against fungal attacks is the use of fungicides. However, many fungicides are toxic to the environment. Furthermore, halogenated hydrocarbons in fumigant (insecticide, fungicide) such as methyl bromide (EFSA, 2011) and several non-biodegradable synthetic fungicides can accumulate in soil, plants and water. This may later exert negative consequences to humans through the food chain. The utilisation of essential oils and medicinal plants as a renewable fungal toxicants and environmentally friendly thus appears as a promising natural fungicide (Shahi *et al*., 2012) due to their minimal environmental impact.

Previous study has shown the potency of castor (*Ricinus communis* L.) as an effective larvicidal agent (Ladda and Kamthane, 2014). In Indonesia, castor oil is mainly used to produce biodiesel from its seeds which yield 67.7% oil (Haque *et al*., 2009).

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Meanwhile, the utilisation of the leaves, roots and seed oil have also been shown in medication. Hexane and methanolic extracts of 200 mg/mL of castor root exhibited minimum antimicrobial activity ($p < 0.0001$) both to *A. niger* and *Escherichia coli* (Mathur *et al*., 2011). In the present work, the potential use of the methanolic extract of castor leaves as a natural antifungal against *A. niger* isolated from stored groundnuts was investigated.

Materials and methods

Preparation of raw materials and extraction

Six kilograms of castor leaves were air-dried until reached the moisture content of ±10%. The dried leaves were milled to powders. The extraction process was conducted by maceration using methanol:water 1:4 (v/v) for 48 h at room temperature. The same amount of fresh mixture solvent was re-added and the extraction was continued for another 48 h. This was referred from Handayani and Nurcahyanti (2015).

Isolation and visually observation of A. niger

A. niger was isolated from stored groundnuts, and then identified according to Barnett and Hunter (1998).

Preparation of medium

Potato Dextrose Broth (PDB) medium was made by boiling 200 g potatoes in 1 L distilled water added with 20 g dextrose. The prepared medium was sterilised by autoclave for 15 min at 121°C and 15 psi.

Preparation of inoculum

Aspergillus niger hyphal plug (6 mm in diameter) was inoculated onto sterilised PDB medium, and incubated at 27°C for 5 d with vigorous shaking at 100 rpm.

Antifungal assay

The experiment was conducted in three repetitions for each treatment. *A. niger* spores were taken using a spatula and then put in a bottle containing PDB medium with different levels of methanolic extract concentration (0, 0.5, 5, and 7.5 mg/mL). Each treatment was incubated at 27°C for 7 d with vigorous shaking at 100 rpm. Next, the *A. niger* mycelia were separated from the media by vacuum filtration, and the mycelia were oven-dried for 24 h at 60°C. The dried mycelia were weighed. The relative inhibition level was determined according to Achmad (1997) with this formula:

$$HR = \frac{(B1-B2)}{B1} \ 100$$

where HR was relative inhibition (%), B1 was control colony biomass (g) and B2 was treatment colony biomass (g).

Analysis of the chemical components

The chemical component of the extract was analysed using GC-MS instrument Agilent Technologies 6890N series.

Results and discussion

Visual observation of Aspergillus niger

Aspergillus niger is a fungus belonging to the Deuteromycota. It is an imperfect fungus which is only known to have the anamorph/asexual phase. The hyphae are well developed and have bulkhead. This class has the asexual spores called conidia. *A. niger* belongs to a class of artificial Hyphomycetes and artificial order of Moniliales (Barnett and Hunter, 1998).

The observation on *A. niger* isolated from stored groundnuts was conducted macro- and microscopically. Initially, the colonies were yellowish in colour, and then turned black three to seven days following inoculation (Figure 1A). The observed hyphae had diameter of 7.66–12.53 µm Figure 1B), while the conidia was about 4.31–5.8 µm (Figure 1C).

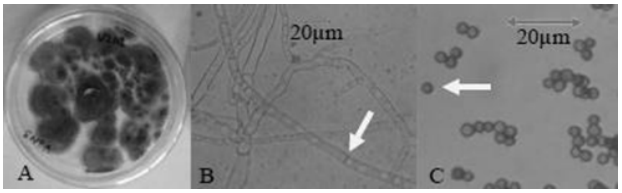

Figure 1. Macroscopic observation of *Aspergillus niger* colony (A), *A. niger* mycelia with septate (arrow sign) (B), and conidia of *A. niger* (arrow sign) (C).

Macroscopic observation of *A. niger* colonies showed that the colony was of spherical shape and black colour, while the lower surface of the compact colonies appeared white and yellow. The *A. niger* isolate exhibited large round heads, densely packed with black, brown or purple brown colour. The hyphae appeared to have septates. The conidia grew above the stigma. Microscopic structures of *A. niger* fruiting bodies was characterised by semi-round to round vesicles (Wangge *et al*., 2012). The conidia were brown in colour and round in shapes.

Previous studies have reported that numerous plants and other food products were contaminated by *A. niger*. This fungus has been identified as the cause of illness in *Alstonia scholaris* seeds (Rustam *et al*., 2013). In addition, this fungus also caused root rot in the base of peanut sprouts (Ayu *et al*., 2012). *A. niger* has also been reported as the causal agent of spoilage of mangos (Prakash and Raoof, 1989) and tomatoes (Sinha and Saxena, 1987)

Castor leaves extract in methanol

The maceration was conducted twice in methanol. This solvent is able to dissolve the polar to non-polar compounds in the leaves (Houghton and Raman, 1998). The methanolic extract of castor leaves was greenish-black and had distinctive smell. The yield of the methanolic extract of castor leaves was about of 10.64%.

Antimicrobial activity

Aspergillus niger grown on media containing different concentrations of methanolic extract showed different amounts of fungal biomass (Figure 2). The highest biomass was found in the control medium. *A. niger* mycelia following seven days incubation were weighed after oven-dried at 60°C. There are several factors that affect the growth of the mycelia in the media containing bioactive compound extracts. One of them is the shaking treatment. Shaking increases the oxygen level, which is useful for the aerobic *A. niger*. The rate of shaking at 100 rpm was optimum for the fungal growth and mycelial formation (Achmad *et al*., 2013).

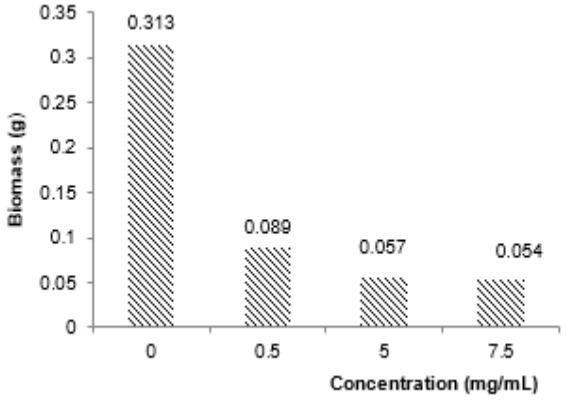

Figure 2. *A. niger* biomass on PDB media containing various concentrations of castor leaves methanolic extract following seven days incubation.

The addition of castor extracts to the *A. niger* on PDB media resulted in the inhibition of its cell biomass growth (Figure 3). The present work showed that the higher the bioactive concentration the higher the growth inhibition. Previous study stated that castor extracts in methanol inhibited the growth of *A. fumigatus* and *A. flavus* more effective than in water and ethanol (Naz and Bano, 2012).

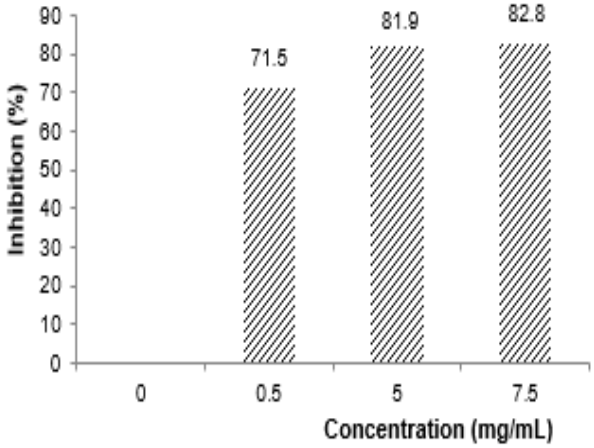

Figure 3. *A. niger* growth inhibition on PDB media containing various concentrations of castor leaves methanolic extract following seven days incubation.

The chemical components of methanol extract

GC-MS analysis of the methanolic extract of leaves of castor showed that ricinine was the main component. The peak of ricinine appeared at the retention time of 15.71 min. Peng *et al*. (2014) reported that ricinine is a poisonous alkaloid derived from the leaves and seeds of castor. It can cause vomiting and various other toxic reactions. Therefore, in the present work, ricinine might be the compound that inhibited A. niger.

Previous research also stated that the castor leaves ethanolic extract consisted of *n*-hexadecanoic acid, octadecanoic acid, 1-hexadecanoic acid, 2,4a.7 trihydroxy-1-methyl-8methylene, 1,4-α-lactone-10 -methyl, L-valine, ethyl ester, hexadecamethyl, tetradecamethyl, octadecamethyl, butanadioic acids, hydroxyl and diethyl ester (Hussein *et al*., 2015). Sandam and Su (2015) reported that the GC-MS analysis of the castor leaves methanolic extract produced eight compounds that exhibited antimicrobial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Conclusion

Methanolic extract of castor leaves showed antifungal activity. This was indicated by the biomass reduction of *A. niger* isolated from stored groundnuts. At 0.05%, the extract showed 71.46% inhibition. GC-MS analysis showed the presence of ricinine which could be responsible in *A. niger* inhibition.

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