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FULL LENGTH ARTICLE

Assessment of antimycotoxigenic and antioxidant activity of star anise (*Illicium verum*) *in vitro*

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Abstract In recent years scientists have focused on the identification and the application of natural products for inactivation of mycotoxins. Essential oils with antimicrobial properties are probably the most promising method for the prevention of potentially toxigenic fungi. Thus the aim of this work is to characterise star anise (*Illicium verum*) and to assess its antioxidant and antifungal and antimycotoxigenic properties using different methods. Results revealed that the major components of star anise essential oil identified by GC/MS were trans-anethole (82.7%), carryophyllene (4.8%) and limonene (2.3%). Total phenolics of ethanol and methanol extracts recorded 112.4 and 96.3 mg GAE/g DW respectively, whereas higher total flavonoid content was recorded for the ethanol extract than the methanol extract. Star anise essential oil showed lower antioxidant activity (55.6 mg/mL) than the extracts using DPPH-scavenging and β-carotene/linoleic acid assays. Results revealed growth reduction of *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* by 83.2%, 72.8% and 65.11%, respectively when using 100 ppm of the star anise essential oil, where a complete inhibition was achieved at 200 ppm for *A. flavus* and *A. parasiticus* respectively. Aflatoxin B1 and Fumonisin B1 production were inhibited completely at 100 and 200 ppm respectively. It could be concluded that star anise extracts could be considered an important substance that should be explored for the discovery and development of newer and safer food supplements as well as drug products.

1. Introduction

In areas, such as Egypt, harvested grains are infected by various species of fungi, such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* (*syn. Fusarium moniliforme*) leading to deterioration and mycotoxin production (Reddy and Raghavender, 2007). These mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha and Muthomi, 2008). Aflatoxins (AFs) and Fumonisins (FUM) are the most

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important mycotoxins (Liu et al., 2006; Mangala et al., 2006). AFs induce mutagenic, teratogenic and carcinogenic effects (Rustom, 1997), whereas FUM have been found to be associated with several animal diseases such as leukoencephalomalacia in horses (Kellerman et al., 1990), pulmonary edema in pigs (Harrison et al., 1990), while in humans, their occurrence has been associated with high incidences of oesophageal cancer (Rheeder et al., 1992; Chu and Li, 1994) and liver cancer (Ueno et al., 1997).

Current protective measures rely heavily on the chemical control of pathogens, with severe and undesirable environmental consequences (Chen and Zhou, 2009). Thus there is an increasing demand for safe and organic foods, without chemical preservatives. Most plants produce antimicrobial secondary metabolites, either as part of their normal programme of growth and development or in response to pathogen attack or stress (Bajpai et al., 2008). A novel way to reduce the proliferation of microorganism and/or their toxins production is the use of essential oils. They are composed mainly of terpenoids and phenylpropanoids, including polyketides and very few alkaloids. The terpenoids are classified depending on the number of isoprene units as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes and tetraterpenes (de Sousa, 2011).

Phenolic compounds are ubiquitously distributed throughout the plant kingdom (Naczk and Shahidi, 2004). Phenolic phytochemicals are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor, and antimicrobial (Middleton et al., 2000). The wide spectrum of bioactivities displayed by phenolic compounds isolated from different foods or food products has dictated a demand for accurate determination of phenolic compounds in different food matrices (Luthria et al., 2008). Inhibition of fungal growth and AF production by phenol compounds has been a subject of many studies (Kim et al., 2005, 2006).

Star anise (*Illicium verum*) is an aromatic evergreen tree bearing purple-red flowers and anise-scented star-shaped fruit. It grows almost exclusively in southern China and Vietnam. Its fruit is an important traditional Chinese medicine as well as a commonly used spice (Loi and Thu, 1970; Jiangsu New Medical College, 1977). Few reports were published for characterisation of star anise and its efficiency as antifungal activity especially against mycotoxigenic fungi. Therefore, the aim of the current study is to identify the chemical composition and determine total phenolic and flavonoid content, as well as investigate the antioxidant and antimicotoxigenic activity of star anise against toxigenic strains of *Aspergillus* and *Fusarium* species and discuss the mechanism of action with references to its main components.

2. Materials and methods

2.1. Chemicals and reagents

Aflatoxin B_1 (AFB_1) and Fumonisin B_1 (FB_1) were purchased from Sigma–Aldrich Co. (St. Louis, MO 63103, USA). Solvents were HPLC grade (Merck, Germany). Folin–Ciocalteau reagents, 2,2-diphenylpicrylhydrazyl (DPPH), β-carotene, Tween 40, linoleic acid, sodium carbonate, sodium sulphate anhydrous, aluminium chloride and sodium nitrite were obtained from TCI AMERICA (Portland, OR 97203, USA). The authentic compounds were obtained from Takata Koryo Co. Ltd. (HYOGO 6610001, Osaka, Japan). Clean, mature star anise was purchased from local market (Haraz, Egypt).

2.2. Fungal cultures

Fusarium species (*F. verticillioides*, *F. solani F. oxysporum* and *F. graminearum*) and *Aspergillus* species (*A. Parasiticus, A. flavus*) used in this study were isolated in a previous study from maize samples collected in Egypt. All cultures were maintained on Potato Dextrose Agar (PDA, Difico).

2.3. Preparing essential oil and extracts

Essential oil was prepared by hydro-distillation using a Clevenger type apparatus, and was dried over anhydrous sodium sulphate. Essential oil was kept in a closed dark glass bottle and stored at 4 °C until use.

Ethanol and methanol extracts were prepared according to the method of Shyamala et al. (2005) with some modifications. Briefly, 15 g of dried star anise fruits were extracted with 100 mL of ethanol and methanol for 24 h with occasional shaking. Both extracts were filtered and evaporated to dryness in vacuum.

2.4. Gas chromatography–mass spectrometry analysis (GC/MS)

The essential oil was analysed by gas chromatography (Perkin Elmer Auto XL GC, Waltham, MA, USA) equipped with a flame ionisation detector, and the GC conditions were EQUITY-5 column (60 m × 0.32 mm × 0.25 μm); H2 was the carrier gas; column head pressure 10 psi; oven temperature programme isotherm 2 min at 70 °C, 3 °C/min gradient 250 °C, isotherm 10 min; injection temperature, 250 °C; detector temperature 280 °C. The identification of individual compounds was based on their retention times relative to those of authentic samples and matching spectral peaks available with the published data (Adams, 2007).

2.5. Determination of total flavonoid content

Total flavonoid content of star anise extracts was determined using the aluminium chloride colorimetric method as described by Willet (2002), with some modifications. Methanol and ethanol extracts (0.5 mL), 10% aluminium chloride (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (4.3 mL) were mixed, and the absorbance was measured at 415 nm using a Spectrophotometer (Shimadzu, Japan). Catachine was used to make the calibration curve. The calculation of total flavonoids in the extracts was carried out in triplicate.

2.6. Determination of total phenolic compounds

Total phenolic content was determined by the Folin–Ciocalteau method (Ordonez et al., 2006). Star anise extracts (100 μl) were mixed with 4 mL of diluted Folin–Ciocalteau reagent (1:9, v/v) for 5 min and 2.0 mL of 75 g/L Na_2CO_3 was then added. The absorbance was measured at 760 nm after