Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00050


# Chemical components and antifungal and anti-mycotoxigenic (aflatoxin $b_1$ and fumonisin $b_1$) activity of star anise (*Illicium verum*) essential oils in Yen Bai Province, Vietnam

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**Abstract**

In Vietnam, star anise is grown in the provinces of Yen Bai, Ha Giang, Lao Cai, Lang Son, Bac Kan, and Cao Bang. The study aimed to analyze the chemical components, and antifungal and anti-mycotoxigenic (aflatoxin $B_1$ and fumonisin $B_1$) activity of star anise essential oils. By the gas chromatography mass spectrometry (GC-MS) method, 15 chemical components were predicted in the star anise essential oils. The major components were trans-anethole (67.05%), caryophyllene oxide (12.73%), limonene (4.27%), α-terpineol (2.43%), and myrcene (2.39%). The antifungal activity at 100 ppm recorded 85.24 ± 0.05%, 76.73 ± 0.06%, 69.37 ± 0.02%, and 67.96 ± 0.04% inhibition for *Aspergillus flavus, Aspergillus parasiticus, Fusarium verticillioides*, and *Penicillium*, respectively. Complete inhibition of 100% was observed at 200 ppm and 300 ppm for both *A. flavus* and *A. parasiticus* and at 400 ppm for *F. verticillioides* and *Penicillium*. Complete aflatoxin $B_1$ inhibition was achieved at 100 and 200 ppm for *A. flavus* and *A. parasiticus.*

**Keywords:** aflatoxin $B_1$; antifungal; chemical components; fumonisin $B_1$; star anise essential oil.

**Practical Application:** This is the scientific basis for the application of the star anise essential oil in food processing.

## 1 INTRODUCTION

The scientific name of the star anise is *Illicium verum,* which is native to southwest China and Vietnam and is mainly distributed in the tropical and subtropical areas of Asia (Wei et al., 2014). In Vietnam, star anise is grown in Yen Bai, Ha Giang, Lao Cai, Lang Son, Bac Kan, and Cao Bang provinces. It is an important essential oil tree cultivated in Vietnam and is one of the signature flavors of Chinese savory cooking. It is also used in dietary supplements and to flavor curries, tea, coffee, candy, bakery products, and pickles (Gholivand et al., 2009). Star anise fruits could be considered a good source of natural compounds with significant antioxidant and antimicrobial, mainly antifungal, activity, which can be attributed to the high percentage of the main constituents or to the synergy among the different oil constituents, which provokes a biocide effect against pathogenic fungi and mycotoxin production. The antifungal activity of the oil can be attributed to its high content of trans-anethole, which was confirmed as the main active component among the volatile compounds in the essential oil (Aly et al., 2016). Trans-anethole, the major compound of star anise, is largely used as a substrate for the synthesis of various pharmaceutical substances such as chloral, an anticonvulsive agent, and phenobarbital. Antimicrobial and antioxidant activity have been strongly revealed in star anise essential oils (Della Porta et al., 1998). The antioxidant activity of star anise essential oil is due to the high percentage of trans-anethole (> 80%) (Besharati-Seidani et al., 2005). Star anise essential oil contains many chemical components: the major chemical components were trans-anethole (85.69%), limonene (2.92%), chavicol (2.69%), and anisaldehyde (1.79%) (Gholivand et al., 2009). Few reports were published on the characterization of star anise and its efficiency as an antifungal agent, especially against mycotoxigenic fungi (Aly et al., 2016). Vietnam is a country with a large area and a large production of star anise. But, up to this point, there have been very few studies on the chemical composition and biological activity of star anise essential oil in Vietnam. Therefore, this study aims to analyze the chemical components, and antifungal and anti-mycotoxigenic (aflatoxin $B_1$ and fumonisin $B_1$) activity of star anise essential oils. This is the scientific basis for the application of star anise essential oil in food processing and preservation in Vietnam.

## 2 MATERIALS AND METHODS

### *2.1 Materials*

The star anise was harvested from the Tran Yen district of the Yen Bai Province, Vietnam, in 2022. The essential oil was obtained by steam distillation after drying with $Na_2SO_4$. The sample was stored in the Department of Food Science and Technology at the University of Science, Vietnam National University, Hanoi.

The tested bacterial strains (*Aspergillus flavus, Aspergillus parasiticus, Fusarium verticillioides,* and *Penicillium*) were obtained from the Institute for Quality Testing and Inspection.

*Received Jun. 2, 2023.*
*Accepted Aug. 13, 2023.*
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### *2.2 Methods*

#### *2.2.1 The essential oil of star anise was analyzed by gas chromatography mass spectrometry*

The GC used was a Perkin Elmer Auto XL GC (Waltham, MA, USA) equipped with a flame ionization detector. The GC conditions were an EQUITY-5 column (60 m x 0.32 mm x 0.25 $\mu$m) with $H_2$ as the carrier gas, a column head pressure of 10 psi, an oven temperature program of 2 min at 70°C, a 3°C/min gradient to 250°C, and an isotherm of 10 min. The injection temperature was 250°C, and the detector temperature was 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 (Aly et al., 2016).

#### *2.2.2 Method to determine the antifungal activity of star anise essential oils*

Star anise essential oils at concentrations of 50, 100, 200, 300, and 400 ppm were screened for their ability to inhibit the growth of *A. flavus, A. parasiticus, F. verticillioides*, and *Penicillium*. In 96-well microplates, 160 $\mu$L of potato dextrose broth and 20 $\mu$L of different concentrations of star anise essential oils were mixed with 20 $\mu$L of four different strains, namely, *A. flavus, A. parasiticus, F. verticillioides*, and *Penicillium* spores (at a concentration of 106 CFU per well) and shaken overnight at 28°C. The fungal growth was determined by measuring the absorbance at 600 nm of the fungal culture in 96-well microplates using a microplate reader for 24 and 48 h. All assays were performed in triplicate. The concentration of star anise essential oil with the best inhibition of mold effect was chosen for the next trials (Han et al., 2022). The percentage of inhibition of fungal growth was calculated using the Equation 1 (Deabes et al., 2011):

$$\% \text{ inhibition} = (\text{control - treatment / control x } 100) \quad (1)$$

#### *2.2.3 Method to determine the inhibition of aflatoxin $B_1$ and fumonisin $B_1$ by star anise essential oils*

Aflatoxin $B_1$ was extracted from the cultures that showed mycelia growth by taking 25 mL of liquid media and using 25 mL of chloroform twice. After shaking for 30 min, the lower layer was filtered through filter paper containing anhydrous sodium sulfate and then the filtrate was evaporated to dryness under a stream of nitrogen. The filtrate was passed through an immunoaffinity column for cleanup with 5 mL of purified water at a rate of about 2 drops/s. Aflatoxin $B_1$ was eluted with 1.0 mL of methanol at a rate of 1–2 drops/s and then evaporated to dryness under a stream of nitrogen. Fumonisin $B_1$ was extracted by taking 50 g corn sample with 100 mL of acetonitrile/water (1:1 v/v) for 30 min. Portion of the extract was filtered through a filter paper. Notably, 2 mL of the filtered extract was combined with 5 mL of aqueous 1% potassium chloride and applied to a preconditioned C18 Sep-Pak column. The column was washed with 5 mL of 1% aqueous potassium chloride, followed by 5 mL of acetonitrile (Aly et al., 2016). The inhibitory ability of aflatoxin $B_1$ and fumonisin $B_1$ in anise essential oil was arranged according to the method of making samples in conical flasks. The star anise essential oil was used in the concentrations of 50, 100, 200, 300, and 400 ppm. The percentage of inhibition of aflatoxins $B_1$ and fumonisin $B_1$ was calculated using the Equation 2 (Deabes et al., 2011):

$$\text{Inhibition (\%)} = (\text{control - treatment/control x } 100) \quad (2)$$

## 3 RESULTS AND DISCUSSION

### *3.1 The chemical components of star anise essential oils*

The chemical components of star anise essential oils, identified by GC-MS and quantified by GC-FID, are presented in Table 1 and Figure 1.

Chemical analysis of the components of the star anise essential oil by GC-MS led to the identification of 15 chemical components, representing 98.94% of the hydro-distilled essential oil. Table 1 shows that 15 chemical components were present in the star anise essential oil. Of these, six were hydrocarbons such as monoterpenes (7.64%) and sesquiterpenes (2.39%), and the rest were oxygenated hydrocarbons such as alcohols (72.86%), aldehydes (2.08%), esters (0.63%), acids (0.61%), and oxides (12.73%). These results show that star anise varieties grown under different ecological and edaphoclimatic conditions have a direct influence on the composition of essential oils. The major chemical components were trans-anethole (67.05%),

**Table 1**. The chemical components of star anise essential oils.

| No. | Chemical components | Retention time (min) | Proportion (%) |
|---|---|---|---|
| | **Monoterpenes** | | **7.64** |
| 1 | Myrcene | 10.92 | 2.39 |
| 2 | α-Phellandrene | 12.23 | 0.98 |
| 3 | Limonene | 12.32 | 4.27 |
| | **Sesquiterpenes** | | **2.39** |
| 4 | α-Cubebene | 14.81 | 0.87 |
| 5 | α-Humulene | 17.29 | 0.79 |
| 6 | β-Bisabolene | 17.66 | 0.73 |
| | **Alcohols** | | **72.86** |
| 7 | Linalool | 12.94 | 2.06 |
| 8 | α-Terpineol | 13.84 | 2.43 |
| 9 | Trans-anethole | 14.16 | 67.05 |
| 10 | Elmol | 18.93 | 0.67 |
| 11 | Farnesol | 19.77 | 0.65 |
| | **Aldehydes** | | **2.08** |
| 12 | Acetaldehyde | 16.06 | 2.08 |
| | **Esters** | | **0.63** |
| 13 | Geranyl acetate | 16.24 | 0.63 |
| | **Acid** | | **0.61** |
| 14 | Hexadecanoic acid | 20.46 | 0.61 |
| | **Oxides** | | **12.73** |
| 15 | Caryophyllene oxide | 15.10 | 12.73 |
| | **Total** | | **98.94** |

(%): calculated by chromatographic peak area.

caryophyllene oxide (12.73%), limonene (4.27%), α-terpineol (2.43%), and myrcene (2.39%). The components trans-anethole, caryophyllene oxide, and α-terpineol are all oxygenated hydrocarbons. The results of this study are also consistent with those of Aly et al. (2016) and Zhai et al. (2009). Our results are in agreement with those who reported that the major compound of star anise oil is trans-anethole, which ranged from 86 to 93% (Aly et al., 2016). In the same trend, it was reported that trans-anethole is the major component in star anise oil and ranged between 86.66% and 94.21% (Cai et al., 2013; Padmashree et al., 2007).

### *3.2 Antifungal activity of star anise essential oil*

The determination of the antifungal activity of star anise essential oil has high scientific and practical significance, which serves as the scientific basis for the application of star anise essential oil in food processing and preservation. The results of this determination are shown in Table 2.

Data in Table 2 illustrate the antifungal activity at 50 ppm, which recorded 51.18 ± 0.07%, 39.25 ± 0.04%, 38.62 ± 0.03%, and 34.16 ± 0.02% inhibition for *A. flavus, A. parasiticus, F. verticillioides*, and *Penicillium*, respectively. The antifungal activity at 100 ppm recorded 85.24 ± 0.05%, 76.73 ± 0.06%, 69.37 ± 0.02%, and 67.96 ± 0.04% inhibition for *A. flavus, A. parasiticus, F. verticillioides*, and *Penicillium*, respectively. Complete inhibition of 100% was observed at 200 ppm and 300 ppm for both *A. flavus* and *A. parasiticus*, and at 400 ppm for *F. verticillioides* and *Penicillium*. The results of this study are consistent with those of Aly et al. (2016). These results were confirmed by Singh et al. (2006), who reported that the volatile oil completely inhibited the growth of *F. verticillioides*. Research results show that inhibition of *Aspergillus flavus* by phenolic compounds, such as salicylic acid, thymol, vanillyl acetone, and vanillin, and cinnamic acid is due to the mitochondrial oxidative stress system defense (Kim et al., 2006). The antifungal activity of star anise essential oils can be actively attributed to their components such as anethole. Star anise is one of many spices that contain bioactive compounds, as well as a number of phenolic and flavonoid compounds, which have antioxidant, preservative, and antimicrobial properties (Shobana & Naidu, 2000).

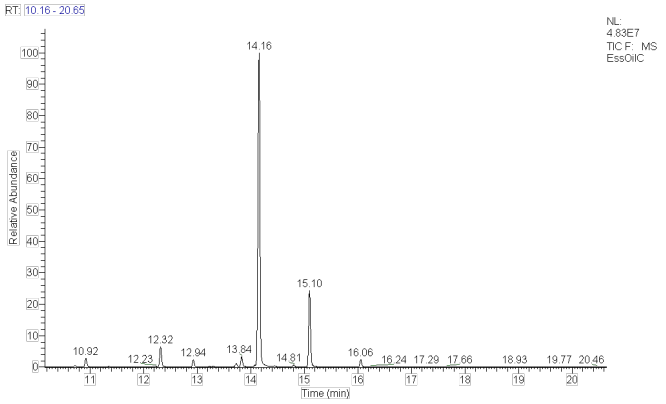


**Figure 1**. The content of the chemical components is calculated as a percentage of the chromatographic peak's area.

### *3.3 The anti-mycotoxigenic (aflatoxin $B_1$ and fumonisin $B_1$) activity of star anise essential oil*

In addition to determining the antifungal ability, the determination of the inhibition of aflatoxin $B_1$ and fumonisin $B_1$ by star anise essential oil is also very important, which is the scientific basis for applying star anise essential oil to food processing and preservation. The results of determining the anti-mycotoxigenic (aflatoxin $B_1$ and fumonisin $B_1$) activity of star anise essential oil are shown in Table 3.

Investigations revealed that the mycotoxin inhibition differed according to the producing fungi, with aflatoxin B1 production inhibited by 74.56 ± 0.08% when produced by *A. flavus* and inhibited by 67.24 ± 0.06% when produced by *A. parasiticus*. Complete aflatoxin $B_1$ inhibition was achieved at 100 and 200 ppm for *A. flavus* and *A. parasiticus*, respectively. Furthermore, the inhibition of fumonisin $B_1$ production increased from 54.13 ± 0.04 to 100% with increasing star anise essential oil concentrates from 50 to 200 ppm, as shown in Table 3. These results are consistent with those of Hua et al. (2014), who found that 500 kg/g of clove, star anise, and mountain

**Table 2**. Antifungal activity (%) of star anise essential oil.

| No. | Fungus species | Star anise essential oil concentration (ppm) | | | | |
|---|---|---|---|---|---|---|
| | | 50 | 100 | 200 | 300 | 400 |
| 1 | *A. flavus* | 51.18 ± 0.07 | 85.24 ± 0.05 | 100 | 100 | 100 |
| 2 | *A. parasiticus* | 39.26 ± 0.04 | 76.73 ± 0.06 | 100 | 100 | 100 |
| 3 | *F. verticillioides* | 38.62 ± 0.03 | 69.37 ± 0.02 | 92.28 ± 0.04 | 98.13 ± 0.05 | 100 |
| 4 | *Penicillium* | 34.16 ± 0.02 | 67.96 ± 0.04 | 87.07 ± 0.06 | 93.05 ± 0.03 | 100 |

**Table 3**. The anti-mycotoxigenic (aflatoxin $B_1$ and fumonisin $B_1$) activity of star anise essential oil.

| No. | Mycotoxins | Star anise essential oil concentration (ppm) | | | | |
|---|---|---|---|---|---|---|
| | | 50 | 100 | 200 | 300 | 400 |
| 1 | Aflatoxin $B_1$ | 74.56 ± 0.08 | 100 | 100 | 100 | 100 |
| | | 67.24 ± 0.06 | 95.02 ± 0.07 | 100 | 100 | 100 |
| 2 | Fumonisin $B_1$ | 54.13 ± 0.04 | 82.27 ± 0.02 | 100 | 100 | 100 |

thyme were necessary to reduce aflatoxin production by 85 to 100% (Bluma et al., 2008). Some research results have identified and applied natural products for the inactivation of aflatoxins, and reported that some essential oils and other extracts (several flavonoids and phenolic compounds) of plants could potentially provide protection against aflatoxin $B_1$ (Rasooli et al., 2008). The anti-aflatoxigenic activity of essential oils may be related to the inhibition of the ternary steps of aflatoxin biosynthesis involving lipid peroxidation and oxygenation. It is clear that phenolic compounds inhibit one or more steps in the aflatoxin $B_1$ biosynthesis pathway (Hua et al., 2014). The results of this study are also consistent with those of Aly et al. (2016).

## 4 CONCLUSION

Using GC-MS, 15 chemical components were predicted in the star anise essential oils. Of these, six were hydrocarbons such as monoterpenes (7.64%) and sesquiterpenes (2.39%), and the rest were oxygenated hydrocarbons such as alcohols (72.86%), aldehydes (2.08%), esters (0.63%), acid (0.61%), and oxides (12.73%). The major components were trans-anethole (67.05%), caryophyllene oxide (12.73%), limonene (4.27%), α-terpineol (2.43%), and myrcene (2.39%). At 100 ppm, the antifungal activity was 85.24 ± 0.05%, 76.73 ± 0.06%, 69.37 ± 0.02%, and 67.96 ± 0.04% inhibition for *A. flavus, A. parasiticus, F. verticillioides*, and *Penicillium*, respectively. Complete inhibition of 100% was observed at 200 and 300 ppm for both *A. flavus* and *A. parasiticus*, and at 400 ppm for *F. verticillioides* and *Penicillium*. Complete aflatoxin $B_1$ inhibition was achieved at 100 and 200 ppm for *A. flavus* and *A. parasiticus*, respectively. On the contrary, the inhibition of fumonisin $B_1$ production increased from 54.13 ± 0.04 to 100% with increasing star anise essential oil concentrates from 50 to 200 ppm.

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