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In-Vitro Antibacterial and Antifungal Effects of High Levels of Chinese Star Anise

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■Author(s)

Alhajj MS[I] https://orcid.org/0000-0001-7471-9008
Qasem MAA[I] https://orcid.org/0000-0002-0939-6078
Jar El Nabi AR[I] https://orcid.org/0000-0003-1500-9289
Al-Mufarrej SI[I] https://orcid.org/0000-0002-9126-0654

[I] Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.

■Mail Address

Corresponding author e-mail address
Mohamed Abd Elmohsen Elsayed
Department of Chemistry, University of Malakand Chakdara Dir Lower KPK, Pakistan.
Phone: 00201141190052
Email: mohamedmabrouk341@yahoo.com

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ABSTRACT

This study investigated the *in-vitro* antibacterial and fungicidal activities of three crude extracts of *Illicium verum* (absolute methanol, 50% methanol and aqueous extracts) against two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), two Gram-negative bacteria (*Escherichia coli* and *Salmonella* Arizona), and two fungi (*Aspergillus fumigatus* and *Aspergillus niger*). The antibacterial action was measured using an agar disk diffusion test, and the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined. The fungicidal activity of the star anise extracts was assessed by the agar disk diffusion test, and poisoned food techniques, and the MICs and minimum fungicidal concentrations (MFCs) were determined. The Chinese star anise extracts showed antibacterial and antifungal activities against A. *fumigatus*. The results of this study suggest that alcoholic extracts are more active than aqueous extracts and that star anise could be used as a natural antimicrobial agent.

INTRODUCTION

There are growing concerns about the possible hazards of antibiotic residues in animal products to public health, and the indiscriminate use of antibiotics has contributed to the emergence of antibiotic-resistant microbes (Pelicano *et al*., 2004; Edens, 2003). Therefore, the higher demands on the current antimicrobials have led to renewed efforts to seek new agents that are effective against pathogenic bacteria resistant to current antimicrobials (Yang *et al*., 2010). Furthermore, foodborne diseases are a major challenge because of the high costs of treating these pathological conditions. Recently, the report by Painter *et al*. (2013) showed that among 17 commodities, poultry products accounted for 22% of all diseases and that more deaths (19%) attributed to poultry compared with other commodities. These deaths were generally due to *Listeria monocytogenes* (63%) and *Salmonella* spp. (26%). Therefore, it is necessary to find new sources that can be utilized to counteract pathogens. Medicinal plants and spices have received significant attention in this respect. Various drugs are extracted and produced from traditional herbal medicines (Falodun *et al*., 2006). Many plants have therapeutic potential, including *Illicium verum*, which is also known as star anise. It has received significant attention after shikimic acid extracted from star anise and it is used to manufacture Tamiflu®, which is a drug used to treat influenza (Wang *et al*., 2011).

The tree bears star-shaped fruits, which are rich in oils, particularly anethol (74-94%), and contain some polyphenols, including flavonols (quercetin and kaempferol), anthocyanins, tannins and phenolic acids, such as shikimic and gallic acid (Wang *et al*., 2011). Traditionally, the fruits are used as a spice and a pharmaceutical treatment for flatulence,

spasmodic pain, and colic. Furthermore, the star anise oil is used topically for rheumatism and as an antiseptic (Verghese, 1988). The plant has shown antioxidant activity (Singh *et al*., 2006), antibacterial effects (Iauk *et al*., 2003), antiviral activity (Song *et al*., 2007), antifungal properties (Mugnaini *et al*., 2012), insecticidal activity (Park and Shin, 2005), and anticancer activity (Yadav & Bhatnagar, 2007).

Therefore, in the present study, Chinese star anise extracts using hot water, 50:50% water and methanol and 100% methanol were screened for their antimicrobial action against bacteria and fungi.

MATERIAL AND METHODS

The plant

Chinese star anise (*Illicium verum*) dry fruits were purchased from a local herb store in Riyadh, Saudi Arabia.

Preparation of star anise extracts

The extraction of Chinese star anise was carried out using three solvents: absolute methanol (100MOH), 50:50% methanol (50MOH) with sterile distilled water, or pure distilled water alone (WA). The dry fruits of star anise were ground into a fine powder. For each extract, 100g of anise powder was added separately to 500mL of the corresponding solvent (100MOH, 50:50% MOH with WA and WA) and mixed well in tightly sealed flasks; the flasks were then placed in a water bath at 37°C for 24 hours with intermittent shaking. The supernatant was collected and filtered (Whatman filter paper No. 1). The residue was kept in the flasks, and the extraction process was repeated 3-5 times until a clear solution was obtained. Subsequently, the extract solutions were collected and filtered again (Whatman filter paper No. 1). The extract was allowed to dry in an oven at 45°C. The dried extract was weighed and diluted to a final concentration of 500mg/mL or stored in sealed capped bottles in a refrigerator until use. The diluted extracts were centrifuged and filtered through a 0.22µm Millipore membrane filter (Whatman, Kent, UK).

Microorganisms

The tested microbes were supplied by IDAC Laboratories (Saudi Arabia): two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 7644), two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella enterica* subsp. arizona (*S. arizona*) ATCC 13314), and two fungal strains (*Aspergillus fumigatus* ATCC 28282 and *Aspergillus niger* ATCC 16404.

Antibacterial activity of herbal extracts

Preparation of bacterial inoculum

The bacterial inocula were produced following the standard protocol of the Clinical and Laboratory Standards Institute (CLSI, 2006). The bloodline of bacteria was activated by suspending the bacteria in tryptone soy broth for 24-48h; then, bacteria were subcultured into sterile tryptone soy agar plates. Sub-culturing was repeated three times to ensure that the bacteria were completely activated. The inoculum suspension for each experiment prepared from the fresh culture by reading four or five pure colonies from an overnight growth. These colonies were emulsified in sterile normal saline, and the turbidity of the suspensions was read using a spectrophotometer at 625nm (DRLANG, Germany). The suspensions were sequentially diluted until the turbidity was comparable to a 0.5 McFarland turbidity standard. This resulted in an inoculum density of approximately 108 colony forming units (CFU)/mL.

Agar disk diffusion test

The antibacterial action of Chinese star anise extracts (absolute, 50% methanol, and aqueous extract) against bacteria (*S. aureus, L. monocytogenes, E. coli,* and *S. arizona*) was assessed using agar disk diffusion methods, following the standard protocol of the CLSI (2006), with minor modifications. Briefly, after adjusting the turbidity of the inoculum suspension, the adjusted suspension was inoculated into sterile tryptone soy agar plates. The inoculum was allowed to dry at room temperature in a safety cabinet (NUAIRE Class II biological safety cabinet, PLYMOUTH MN 55447, USA). The disks (5mm diameter), which had soaked with 40µL of each extract, were placed on the previously inoculated agar plates. The plates were set aside at 4°C for 1h and then incubated at 37°C for 18h. The diameters of the zones of complete inhibition were measured, including the diameter of the disks. Colistin and erythromycin used as active controls, and paper disks soaked with the corresponding extraction solvent were used as negative controls. The tests were carried out in triplicate.

Determination of minimum inhibitory concentrations (MICs)

In this experiment, the MICs of star anise extracts were determined by the microdilution method according to the CLSI protocol (CLSI, 2012). The adjusted inoculum suspensions of bacteria were further diluted (1:100) to obtain 10^6CFU/mL. Sterile

96-well flat microtiter plates (Nunclon, Denmark) were used in this experiment. Each well contained 50µL of sterile tryptone soy broth. Then, serial twofold dilutions were performed (the obtained concentration range was from 250 to 0.24mg/mL) from each of the extract stock solutions (concentration of 500mg/cc). Then, 50µL of the adjusted inoculum (10^6CFU) was added to each well, except for the negative control wells (inoculum contained approximately 10^5CFU/mL). The adjusted inoculum suspensions without extract were used as positive controls, whereas medium only was used as negative control. The plates incubated at 37°C for 18h. After 18h, 20µL of P-iodonitrotetrazolium violet (INT), an indicator of bacterial growth, was added to each well, and the plates further incubated for 30 min. The result was visually read because the colorless tetrazolium salt is reduced to a red color by biologically-active bacteria. Wells showing a red color indicated bacterial growth, whereas wells with the lowest extract concentration with no color change (clear) indicated complete growth inhibition and were considered as the MIC. Wells that showed no color change were used to determine the minimum bactericidal concentration (MBC). The experiment performed in triplicate.

Determination of minimum bactericidal concentrations (MBCs).

The MBC is defined as the lowest concentration of an antimicrobial agent that is required to completely prevent bacterial growth. In order to determine the MBC, 10µL from the wells that showed no bacterial growth was taken and inoculated in sterile tryptone soy agar plates. The plates inoculated with bacteria applied as active control, and the plates with only tryptone soy agar used as negative control. The plates were incubated at 37°C for 48 hours, and bacterial growth was measured. The lowest extract concentration with bacterial growth was considered the MBC. The experiment performed in triplicate.

Antifungal activity

The activity of the star anise extracts against *A. niger* and *A. fumigatus* was assessed by the agar disk diffusion method and the poisoned food technique. The MIC and MFC values are also determined.

Preparation of inoculum

Pure fungal strains of *A. niger* and *A. fumigatus* were used in this study. The fungi suspension was prepared following the National Committee for Clinical Laboratory Standard protocol (NCCLS, 2002.

Agar disk diffusion test

The disk diffusion method was used to study the inhibitory activity of star anise extracts against *A. niger* and *A. fumigatus* according to Naeini *et al*. (2009). In brief, 10µL of an adjusted suspension of *A. niger* or *A. fumigatus* were evenly streaked on sterile potato dextrose agar plates. The inoculated plates were allowed to dry at room temperature in a safety cabinet. Then, empty paper disks (5mm in diameter) saturated with 40µL of star anise extracts were placed on the surface of the previously inoculated plates. The plates incubated at 35°C for 48-72h, and all plates sealed with parafilm. After 48-72h of incubation, each plate was examined. The diameters of the inhibition zone were measured, including the diameter of the disk. Itraconazole used as an active control, and paper disks soaked with the solvents as negative controls. The experiment performed in triplicate.

Determination of minimum inhibitory concentrations (MICs)

The MIC of star anise extracts against *A. niger* and *A. fumigatus* performed according to the National Committee for Clinical Laboratory Standard protocol (NCCLS, 2002) using the broth microdilution method. The adjusted inoculum suspensions were mixed using a vortexer (0.4-5 x 10^4CFU/mL). The anise extracts were dissolved in DMSO (10% of the final volume) and diluted to a concentration of 500mg/mL. Sterile 96-well flat microtiter plates (Nunclon, Denmark) were used in this test. Each well contained 100µL of sterile potato dextrose broth. Then, serial two-fold dilutions were performed (the obtained concentration range was from 250 to 0.24mg/mL) from each of the stock solutions of the extracts (concentration of 500mg/mL). Then, 100µL of the adjusted inocula were added to each well, except for the negative control well. The diluted inoculum suspensions without extracts were used as active controls, and the medium without extracts used as negative control. The plates incubated at 35°C for 48h. The lowest concentration of the extracts in the wells that showed no visible growth was considered as MIC. Wells showing no growth were used to determine the minimum fungicidal concentration (MFC). The experiment performed in triplicate.

Determination of minimum fungicidal concentrations (MFC)

In order to determine the MFC, 10µL from wells that showed no growth were collected and inoculated onto potato dextrose agar plates. The plates inoculated with

fungi were used as active controls and the plates with only potato dextrose agar used as negative control. The plates were incubated at 35°C for seven days, and fungal growth evaluated. The highest dilution (lowest concentration of the extracts) with no growth on the plates was considered as MFC. The test performed in triplicate.

Poisoned food method

The antifungal activity of the extracts by were evaluated by the poisoned food method, according to Mohana & Raveesha (2007). Different concentrations of each extract (25, 12.5 and 6.2mg/mL) were aseptically mixed with sterile potato dextrose agar medium to a final volume of 20mL and then poured onto the plates. After solidification, 5mm-diameter disks of *A. niger* or *A. fumigatus* were inoculated at the center of the plates and incubated at 30°C for seven days. The potato dextrose medium plates with no extracts were used as negative controls and plates with itraconazole were used as active control. Subsequently, the fungal growth diameters were measured and used to calculate the percentage of inhibition. The percentage (%) of growth inhibition was determined as [(C–T) /C] ×100, where C is the average increase of fungal growth on the negative control plates and T is the average increase of fungal growth on plates treated with the evaluated extracts.

RESULTS AND DISCUSSION

Antibacterial activity

Three extracts obtained from the fruits of star anise (*Illicium verum*) were evaluated in this study, and included methanol (absolute and 50%) and aqueous extracts. The antibacterial effect of star anise extracts against four bacteria (*S. aureus, L. monocytogenes, E. coli,* and *S. arizona*) using the agar disk diffusion test is presented in Figure 1 (a-d). The results showed that star anise extracts exhibited inhibitory effects against all tested bacteria. Significant differences were found among the extracts, with absolute methanol extract being more active than the other extracts, with an inhibition zone diameter of 13.7mm against *S. arizona* compared with the 50% methanol and aqueous extracts, which inhibition zones presented diameters of 10.8 and 8.6mm, respectively. The absolute methanol extract presented higher activity than the 50% methanol and aqueous extracts against *S. aureus*, *L. monocytogenes*, and *E. coli*. Absolute methanol extract showed the highest inhibitory activity against *S.*

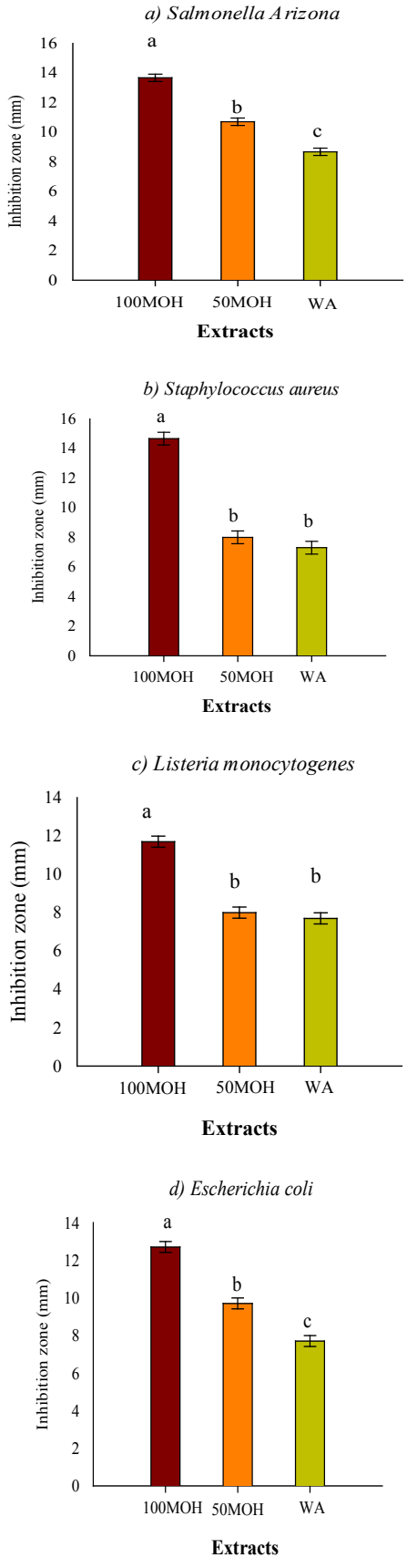

Figure 1 – Inhibition zone of *Illicium verum* extracts against bacteria by the agar disk diffusion test. (100MOH) 100% methanol, (50MOH) 50% methanol and (WA) water extracts.

aureus, with an inhibition zone diameter of 14.7mm. In contrast, the 50% methanol extract showed greater inhibitory effect against *S. arizona* (10.7mm) than the aqueous extract (7.8mm). The 50% methanol extract and aqueous extract showed the same trend of inhibitory activity against *E. coli*, with inhibition zones diameters of 9.7 and 7.7mm, respectively. Both extracts showed similar inhibitory activity against *S. aureus* and *L. monocytogenes*.

The MIC and MBC results of the evaluated star anise extracts against *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. arizona* are summarized in Table 1. The MIC of the absolute methanol extract against *E. coli* (15.6mg/mL) was higher against *L. monocytogenes*, *S. arizona*, and *S. aureus* (7.8mg/mL for each). The MICs of the 50% methanol extracts against all tested bacteria were similar, of 15.6mg/mL. The MICs of aqueous extracts against both *S. arizona* and *E. coli* were similar (15.6mg/mL) to the MICs obtained with the 50% methanol extract, as well as against *L. monocytogenes* and *S. aureus* (31.2mg/mL) as the aqueous extract.

The MBCs of the absolute methanol extract on *E. coli*, *L. monocytogenes*, and *S. arizona* were similar at 15.6mg/mL and lower than the MBC on *S. aureus* (31.2mg/mL). All of the tested bacteria showed similar MBCs at 31.2mg/mL for 50% methanol and aqueous extracts; however, in both of those extracts, *S. arizona* exhibited the lowest MBC (15.6mg/mL). Overall, *E. coli* showed a similar MIC and MBC (15.6mg/mL) for the absolute methanol extract, which indicated that the extract had a bactericidal effect on *E. coli* at this concentration. Similarly, the absolute methanol extract had bactericidal activity against *S. arizona*. Additionally, the aqueous extract exhibited similar MICs and MBCs on *L. monocytogenes*, *S. arizona*, and *S. aureus*.

Traditionally, plant extracts are obtained primarily with water, but there are several studies in which organic solvents showed better activity than aqueous extracts (Rakholiya *et al*., 2014). In this study, the star anise extracts were prepared with methanol (100% and 50%) and water to compare the three extracts and determine the optimal extract. The antibacterial activity of absolute methanol extracts was superior to those of the other extracts, with inhibition zones that ranged from 12 to 15mm. Water extracts exhibited the lowest antibacterial activity among the extracts, with inhibition zones that ranged from 7 and 9mm. However, it is usually expected that the sensitivity of Gram-positive bacteria will be higher than Gram-negative bacteria (Shan *et al*., 2007; Mohamed *et al*., 2010). However, in the present study, no clear difference between Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*S. arizona* and *E. coli*) bacteria was observed.

The inhibition zones ranged from 8 to 14mm for Gram-positive bacteria and from 7 to 15mm for Gram-negative bacteria. Besides, the MICs and MBCs were similar for both Gram-negative and Gram-positive bacteria and ranged from 7.8 to 31.2mg/mL. Singh *et al*. (2006) found similar results using agar well diffusion technique, with the antimicrobial activity of *Illicium verum* extracted using acetone exhibiting significant inhibitory activity against *Staphylococcus aureus*. Also, the results of the present study agree with those of Khesorn Nanthachit (2002), who found that *Illicium verum* ethanol extracts exhibited antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and that the crude dichloromethane and crude methanol extracts showed antibacterial activity against *S. aureus*. It was also reported that star anise essential oils were active against *S. aureus* and *E. coli*, but had no activity against *Salmonella typhi* (Singh *et al*., 2007).

Recently, Yang *et al*. (2010) revealed that supercritical CO_2 (SFE) and ethanol extracts of *Illicium verum* exhibited considerable antibacterial activity against 67 clinical drug-resistant isolates, including 27 strains of *Acinetobacter baumannii*, 20 strains of *Pseudomonas aeruginosa*, and 20 strains of methicillin-resistant *Staphylococcus aureus*. Furthermore, the diethyl ether (EE) fraction obtained from partition extraction and SFE revealed antibacterial activity with MIC values of 0.15–0.70 and 0.11mg/cc, respectively. The EE fraction showed synergistic effects with some commercial antibiotics. E-anethole, anisyl acetone, anisyl alcohol,

Table 1 – Minimum inhibitory concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) values for *Illicium verum* extracts against bacteria using the micro-dilution method.

Microorganism	MIC (mg/mL)			MBC (mg/mL)		
	100MOH	50MOH	WA	100MOH	50MOH	WA
E. coli	15.6	15.6	15.6	15.6	31.2	31.2
L. monocytogenes	7.8	15.6	31.2	15.6	31.2	31.2
S. arizona	7.8	15.6	15.6	15.6	15.6	15.6
S. aureus	7.8	15.6	31.2	31.2	31.2	31.2

100% Methanol (100MOH), 50% Methanol (50MOH) and Water extraction (WA)

and anisyl aldehyde extracted from *Illicium verum* also examined for their antibacterial action. E-anethole had a higher antibacterial activity than did the other constituents (anisyl aldehyde, anisyl acetone, and anisyl alcohol) against *A. baumannii*, with an MIC value of 0.11mg/mL. Anisyl aldehyde and anisyl alcohol showed broader antimicrobial activity against all of the tested microbes, with MIC ranges of 1–2mg/mL and 3–6mg/mL, respectively. Anisyl acetone showed antimicrobial activity against *A. baumannii* and *P. aeruginosa*, with MIC values of 2 and 4mg/mL, respectively.

The antimicrobial activity of star anise extract may be attributed to its essential oils, particularly the anethole present in the dried fruit (Singh *et al*., 2006; Mimica-Dukic *et al*., 2003). Also, other components of the star anise extracts that are present in small amounts could be considered for their potential synergistic and antagonistic properties (De *et al*., 2002). The mode of action of E-anethole is still not completely understood, but it has been suggested that bacterial membrane integrity may be disrupted by lipophilic compounds (Yang *et al*., 2010). This hypothesis is confirmed by the observations obtained from scanning electron microscopy analysis, whereby chemical components were shown to exert their toxic effects against the test strains by disrupting bacterial membrane integrity. These ingredients were able to destroy the cellular integrity and thereby inhibit respiration and iron transport processes. They might also increase membrane permeability in bacterial mitochondria (Baik *et al*., 2008).

By contrast, Shan *et al*., (2007) found that *Illicium verum* methanol extract showed low activity against *Escherichia coli* and *Salmonella anatum* and no activity against *Listeria monocytogenes* and *Staphylococcus aureus*. However, there is no clear explanation for the mode of action of star anise extracts, particularly because limited studies have been conducted on the antimicrobial effects of star anise. However, it is likely that the underlying mechanism is one of the following previously reported suggestions: prevention of cell wall synthesis (Marcucci *et al*., 2001), accumulation in the bacterial membranes causing energy depletion (Conner, 1993), or interference with the permeability of the cell membrane, causing an increase in permeability and loss of cellular contents, disintegration of the cytoplasmic membrane and changes in the structure and function of cellular constituents that result in mutation, cell damage, and death (Kim *et al*., 1995).

Antifungal activity

The antifungal effect of anise extracts against two types of fungi (*A. fumigatus* and *A. niger*) using agar disk diffusion test are presented in Table 2. The absolute and 50% methanol extracts showed inhibitory activity against *A. fumigatus*, but the aqueous extract did not exhibit any inhibitory activity against *A. fumigatus*. The absolute methanol extract showed a stronger effect than the other extracts, with an inhibition zone of a diameter of 14.33mm. By contrast, none of the extracts showed any effect on *A. niger*.

Table 2 summarizes the MIC and MFC results. The MIC values confirmed the inhibitory activity of the absolute and 50% methanol extracts on *A. fumigatus*, with MIC values of 31.2 and 62.5mg/mL, respectively. The MFC values for the absolute and 50% methanol extracts are 31.2 and 250mg/mL, respectively. The absolute methanol extract showed the lowest MIC and MFC values. Moreover, the absolute methanol extract showed fungicidal effect, as reflected by the similar MIC and MFC values.

The results of the inhibitory effect of star anise extract on *A. fumigatus* by the poisoned food method are presented in Fig. 2. The absolute methanol extract showed high inhibitory activity ($p<0.05$) against *A. fumigatus* at all concentrations; the level of inhibition ranged from 52 to 71%, and the highest inhibitory concentration was 25mg/mL (71%). The 50% methanol extract showed a moderate inhibition effect that ranged from 32 to 46%, whereas the water extract showed the lowest ($p<0.05$) inhibitory effect on *A. fumigatus*, with inhibition values ranging from 15 to 28%. By contrast, the absolute methanol extract showed moderate inhibitory activity against *A. niger*

Table 2 – Inhibition zone obtained by the agar disk diffusion test and the Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC) values for *Illicium verum* extracts against fungi.

	Aspergillus fumigatus			*Aspergillus niger*		
Extracts	Inhibition zone (mm)	MIC (mg/mL)	MFC (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)	MFC (mg/mL)
100MOH	14.33 ± 0.88[a]	31.2	31.2	_	_	_
50MOH	10.00 ± 0.58[b]	62.5	250	_	_	_
WA	_	_	_	_	_	_
P-value	<.0001					

(-) no effect

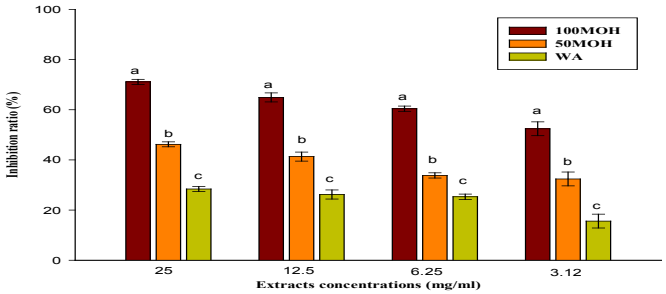

Figure 2 – Inhibition ratio of different concentrations of *Illicium verum* extracts against *A. fumigatus* using the poisoned food method. (100MOH) 100% methanol, (50MOH) 50% methanol and (WA) water extracts.

at the concentrations of 25mg/mL and 12.5mg/mL, with an inhibitory rate of 50% and 26%, respectively, whereas no inhibition was observed at other concentrations (Fig. 3). The 50% methanol extract showed an inhibitory effect of 26% at a concentration of 25mg/mL and an inhibitory effect of 3.6% at a concentration 12.5mg/mL, but there was no inhibition at the other concentrations.

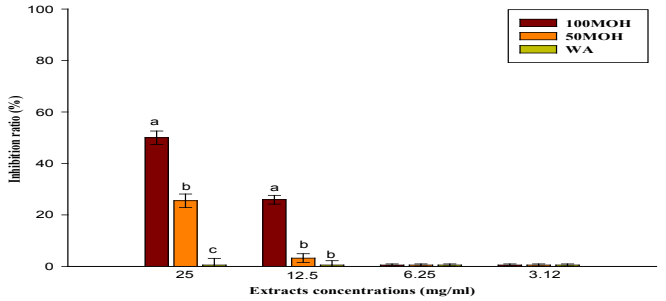

Figure 3 – Inhibition ratio of different concentrations of *Illicium verum* extracts against *Aspergillus niger* using the poisoned food method. (100MOH) 100% methanol, (50MOH) 50% methanol and (WA) water extracts.

In general, the absolute methanol extracts showed the highest (p<0. 05) inhibitory effect (71%) among all of the extracts on the growth of *A. fumigatus*, whereas the aqueous extract showed the lowest effect. For the interaction between fungi and concentrations, only the absolute methanol extract inhibited the growth of *A. fumigatus* by more than 50% at all concentrations, and the other extracts inhibited the growth of *A. fumigatus* by less than 50%. The antifungal activity may be attributed to the active constituents of star anise, specifically essential oils, such as anethol, as previously reported (De *et al.*, 2002; Singh *et al.*, 2006; Huang *et al.*, 2010).

The water extract showed the lowest activity against the tested microbes. The superiority of the antimicrobial activity of methanol extracts may be due to the presence of more lipid-soluble components. The high inhibitory activity of essential oils and trans-anethole against the tested fungi may be attributed to the presence of trans-anethole in the oil, which could be developed as a natural fungicide to combat plant disease and to aid fruit and vegetable preservation Huang *et al*. (2010).

However, it difficult to compare the results obtained in this study with previously published data for several reasons, including variations in the composition of plant extracts due to the extraction method, environmental conditions, microbial strains, test procedures, assay conditions and concentrations used.

CONCLUSION

The Chinese star anise extracts showed antibacterial activity against all the tested bacteria and antifungal activity against *A. fumigatus*. The results of this study indicate that the alcoholic extracts are better than aqueous extract and may be a promising natural antimicrobial agent.

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