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Original Article

Chemical Composition and Antifungal Activities of Cinnamon Bark Essential Oil on *Aspergillus Niger*

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Abstract *- Aspergillus niger is a highly virulent and environmentally resistant fungus that often causes spoilage in fruits and vegetables and can also cause disease in humans. Therefore, there is a need to find natural extracts to combat this fungus. This research found that cinnamon bark essential oil has significant antifungal activity against Aspergillus niger. GC-MS analysis of cinnamon bark essential oil revealed 15 volatile compounds, with cinnamaldehyde being the dominant component. Antifungal activity was tested using the disc diffusion method and dilution technique to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). The results showed that cinnamon bark essential oil exhibited a large inhibitory zone of up to 12.29 mm, demonstrating significant antifungal activity against A. niger. These results indicate that cinnamon bark essential oil, with cinnamaldehyde as the main component, is a highly potent natural extract for preventing fungal infections.*

Keywords - *Cinnamon bark essential oil, Aspergillus niger, GC–MS analysis, Antifungal activity, Natural extract.*

1. Introduction

Aspergillus niger is a very widespread fungus, found worldwide. It is frequently found contaminating food and animal feed, as well as in the environment [1-2]. Spores can be found in a variety of environments, including organic material [3]. Conidiophores produce asexual spores, allowing them to survive in immobile phases and become resistant to various environmental conditions [3-4]. *A. niger* is a common fungal contamination found in some fruits and vegetables, including peanuts, onions, and grapes [5]. It can be pathogenic in humans and may cause otomycosis or mild respiratory illnesses [6]. The current global warming is one of the causes of environmental changes that affect the ecological suitability of various living things [7]. Global models predict that by 2100, the prevalence of *A. niger* could increase by 16% or more, raising public health concerns such as glycotoxin contamination in food and airborne transmission [8-9]. Adding preservatives is important for extending product shelf life, but it can compromise food safety, taste, and nutritional value [10]. Although synthetic substances such as sodium benzoate and potassium sorbate can be applied to inhibit the growth of *A. niger* and are commonly found in food and other processing, excessive use raises significant toxicological concerns, leading to allergic reactions, genetic damage, and an increased risk of cancer [11-12]. Currently, health food products are a very popular trend among consumers. Therefore, scientists are seeking natural substitutes for synthetic preservatives. Essential oils from spices are considered one such natural substitute, as effective as synthetic preservatives and with strong microbial elimination capabilities [13-14].

Due to numerous bioactive compounds, spice essential oils are now highly significant in the biotechnology industry, including functional food development [15-16]. Cinnamon is recognized as one of the most globally significant and frequently utilized spices [17-18]. The nomenclature of cinnamon originates from the Greek kinnamon, which was used to describe spices of Arabian origin. The cinnamon genus is currently recognized as being comprised of approximately 250 species [19-20]. It is regarded as an important medicinal spice because of its demonstrated antifungal, antibacterial, antiviral, anti-inflammatory, anticancer, and antioxidant effects [21-22]. Extracts achieved from various parts of the cinnamon, such as the bark, leaves, and branches, vary considerably in their chemical composition [19, 23]. The bark of the cinnamon tree is considered a spice that is used as a flavoring agent in the food industry. Widely employed in cuisine for its desirable sensory attributes, cinnamon bark is increasingly validated by research for its positive effects on human health [22-24]. Cinnamon Bark Essential Oil (CBEO) demonstrates superior antimicrobial efficacy against common foodborne pathogens compared to other essential oils [25]. It was found to contain mainly cinnamaldehyde [26]. Cinnamaldehyde has been shown to suppress the proliferation of various microorganisms and their ability to synthesize

toxins [27-28]. In the United States, the Food and Drug Administration (FDA) has classified cinnamon oil as generally safe (GRAS) for use in food preservation [29-30]. This substance is suitable for use as an antimicrobial additive in food, helping to protect against a wide variety of pathogenic microorganisms. [28, 31].

From the previous studies, Pawar and Thaker (2006) discovered that cinnamon oil was most potent in restraining the fungi *Aspergillus flavus* and *Penicillium expedition* compared to cloves and lemongrass, when using lower concentrations (lower MIC value) of cinnamon oil [32]. Pozzatti *et al.* (2008) found that although oregano oil yielded very similar results, cinnamon oil remained the most effective at destroying fungal cell walls rapidly among the phenolic oils [33]. Xing et al. (2010) stated that, compared to thyme and clove oils, cinnamon oil in vapor form was the most effective in inhibiting fungal spore germination. Cinnamaldehyde had a more suitable vapor pressure for diffusion into fungal cells than thymol or eugenol [34]. Angelini *et al.* (2006) conducted research and discovered that cinnamon oil (*Cinnamomum* sp.), whose main component is cinnamaldehyde, has antifungal properties [35]. Zhang *et al.* (2016) studied that cinnamon essential oil interrupts the bacterial membrane integrity, causing intracellular components to leak out [36]. Cava-Roda *et al.* (2021) found that oils extracted from vanillin, cloves, cinnamon bark, and cinnamon leaves have different antibacterial capabilities [37]. Li *et al.* (2022) noted that clove essential oil has antimicrobial and anti-spoilage properties, which can be applied to pork without affecting its texture [38]. Thi Thanh Nguyen *et al.* (2023) showed that eucalyptus leaves (*Eucalyptus globulus*) and cinnamon bark oil (*Cinnamomum cassia*) can be used effectively as a therapeutic agent for bacterial infections [39].

Despite the extensive literature on the antibacterial efficacy of essential oils, there is a notable lack of studies exploring their potential as antifungal agents [36-39]. Therefore, in the present work, aim to characterize the chemical profile and investigate the antifungal efficacy of CBEO on *Aspergillus niger*. Consequently, this research can be used as information for applying CBEO to inhibit fungi.

2. Materials and Methods

2.1. Strains preparation

Aspergillus niger TISTR 3012 was collected from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. Following TISTR protocols, the strain was inoculated by applying drops of the suspension onto Potato Dextrose Agar (PDA) plates. The strain was incubated in an incubator at 25 ± 2°C for 3 days [40]. Fungal spores were suspended by adding sterile normal saline (0.85% w/v, 1% Polyoxyethylene (20) sorbitan monooleate) to the plates [41]. The spore suspension was harvested via elution and filtration, then standardized to 1 × 10^6 CFU/mL using a hemocytometer.

2.2. Solvents and Chemicals

Polyoxymethylene (20) sorbitan monooleate, potato dextrose broth, and potato dextrose agar were acquired from Sino-Japan Chemical (Japan), Sisco Research Laboratories (India), and HiMedia Laboratories (India), respectively. Amphotericin B for injection was procured from Biolab (Thailand). CBEO was obtained from CT Chemical (Thailand). Dimethyl sulfoxide was obtained from Merck (USA).

2.3. Gas Chromatography (GC)

Separation of substances was performed using chromatography with a Shimadzu GC-8A system supplied with a Flame Ionization Detector (FID). Chromatographic separation was performed using an HP-5 fused-silica capillary column, featuring dimensions of 30 m × 0.25 mm (i.d.) and a 0.25 μm stationary phase film thickness, as well as a Porapak Q packed column with a mesh size of 80/100. An isothermal temperature condition was maintained for the oven temperature throughout the run. The oven temperature was initially set to 50°C for 2 minutes, then the temperature was increased by 5°C/minute until it reached 110°C, and maintained that temperature for another 8 minutes. The temperature was then increased in increments of 10°C/minute until it reached 220°C (maintained for 2 minutes), followed by a final temperature increase of 25°C/minute until it reached 250°C, which was then maintained for 12 minutes. Helium served as the carrier gas, maintained at a steady flow rate of 1.0 milliliter/minute throughout the analysis. A sample volume of 0.2 μL was injected through a temperature-controlled inlet at 230°C using a sample split ratio of 1:100. The detector temperature was kept constant at 250°C throughout the experiment. Quantitative results were electronically derived from FID peak area percentages without the application of correction factors.

2.4. Gas Chromatography–Mass Spectrometry (GC–MS)

GC-MS serves as the definitive analytical tool for characterizing the chemical profile of CBEO, which effectively separates volatile components from non-volatile ones. GC-MS was utilized for the identification of the CBEO's chemical profile. Chemical analyses were conducted using an Agilent 7890B Gas Chromatograph (GC) connected to an Agilent 5977B Mass Selective Detector (MSD), utilizing the same capillary GC parameters detailed above. Using helium as the carrier gas, the mass spectrometer was utilized in Electron Ionization (EI) mode at 70 eV. The ion source temperature was maintained at 230°C. Data were acquired over a scan range of 35–500 m/z.

2.5. Identification of Chemical Components

Constituent identities were confirmed by comparing their mass spectra to reference standards from the National Institute of Standards and Technology (NIST) MS Library. Identities were further substantiated by comparing experimental Retention Indices (RI) with those of authentic reference standards.

2.6. Antifungal Activity Test of CBEO using the Dilution Method

The dilution method is the most effective method for measuring MIC. Samples were serially diluted in PDB medium supplemented with 1% Dimethyl Sulfoxide (DMSO). CBEO in two-fold serial dilutions was sequentially done for each tube, ranging from 1% (v/v) to 0.008% (v/v). Amphotericin B was prepared in a twofold serial dilution series, with concentrations ranging from 16 to 0.13 ppm (v/v) in the medium. The final volume in each tube was adjusted to 2 mL by inoculating 1 mL of the respective treatment with 1 mL of fungal suspension (approximately 10^6 CFU/mL). A negative control was assembled by incorporating 1 mL of sterile normal saline with 1 mL of the fungal suspension. All tubes underwent incubation for 48 h under controlled conditions (25 ± 2°C). The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the sample that prevented visible microbial growth in the liquid medium [42]. The Minimum Fungicidal Concentration (MFC) was defined as the lowest concentration of the sample that resulted in no visible fungal growth upon subculturing [43].

2.7. Antifungal Activity Test of CBEO using the Disc Diffusion Method

The antifungal activity of CBEO was assessed using the disc diffusion method, as follows by Miller *et al.* (1984). Efficacy was determined by measuring the resulting zones of inhibition [44]. Adjust the initial *A. niger* culture concentration to approximately 1 x 10^6 CFU/mL. A 100 µL aliquot of the fungal inoculum was uniformly distributed over the Potato Dextrose Agar (PDA) surface using a sterile cotton swab. The plates were rotated 60° between repeated swabbings to guarantee an even lawn of growth. For the antifungal assay, sterile 5.5 mm discs were impregnated with 60 µL of CBEO, while 10 µL of amphotericin B served as the positive control. After inoculating the PDA, the treatments were applied to the agar surface [45]. The samples were plated at 25 ± 2°C for 72 h. Before preparing the agar medium, 1% (w/v) DMSO must be added as an emulsifier to allow the oil to dissolve in the water. To quantify antifungal activity, the inhibition zone diameters were evaluated with a Vernier caliper. All experimental procedures were carried out in three independent replicates.

2.8. Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$). level. All statistical procedures were performed using SPSS software (Version 20.0).

3. Results and Discussion

3.1. Chemical Composition

The chemical profile and relative concentrations of CBEO components were determined via GC–MS. The chemical profile of CBEO identified via GC–MS, is summarized in Table 1. The chromatogram of CBEO is shown in Figure 1.

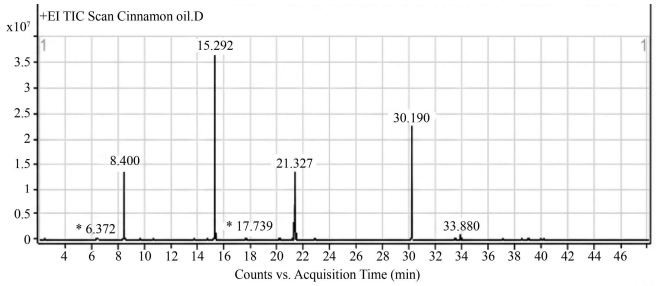

Fig. 1 GC chromatogram of CBEO

The chemical profile of the CBEO, as determined by GC–MS, comprised 15 individual components. Cinnamaldehyde was identified as the major constituent of CBEO, accounting for 48.49% of its total composition, followed by cis-2-methoxycinnamic acid with 26.10%, benzyl benzoate with 13.72%, and benzyl alcohol with 9.99%, respectively. Furthermore, benzyl cinnamate, benzaldehyde, estragole, 2-propenal, 3-phenyl-, phenylmethyl ester, 2-propenoic acid, benzaldehyde, benzaldehyde dimethyl acetal, methyl ester and 2,2-dimethoxybutane amounted only to 0.86%, 0.29%, 0.15%, 0.11%, 0.08%, 0.07%, 0.04%, 0.03%, 0.03%, 0.03% and 0.02%, respectively (Table 1). The percentage of missing cinnamaldehyde may be due to the high yield of cis-2-methoxycinnamic acid. The presence of significant 26.10% cis-2-methoxycinnamic acid could indicate that some of the volatile aldehydes may have undergone oxidation during the extraction process (likely steam distillation) or due to storage conditions. When considering aldehydes and related acid derivatives together, the total amount exceeds 74%, which is consistent with the standard value for *Cinnamomum cassia*. According to Senanayake et al. (1977), cinnamaldehyde is the major compound in the cinnamon bark oil [46]. Cinnamaldehyde is a primary bioactive constituent of cinnamon essential oil and is responsible for its diverse range of biological activities [47-48]. Previous studies have reported its antifungal, antibacterial, antiviral, anti-ulcer, antioxidant, anti-inflammatory, antidiabetic, and anticancer properties [49-53].

Table 1. Chemical constituents in CBEO

No.	Compounds Name	Retention time (min)	Peak Area (%)
1	2,2-Dimethoxybutane	2.42	0.02
2	Benzaldehyde	6.37	0.29
3	Benzyl alcohol	8.40	9.99
4	Phenylmethyl ester	9.67	0.07
5	Benzaldehyde dimethyl acetal	10.64	0.03
6	2-Propenal	13.73	0.11
7	Benzaldehyde	14.78	0.03

8	Cinnamaldehyde	15.29	48.49
9	2-Propenoic acid	17.74	0.04
10	Methyl ester	20.27	0.03
11	Cis-2-methoxycinnamic acid	21.33	26.10
12	3-phenyl-	22.96	0.08
13	Benzyl Benzoate	30.19	13.72
14	Estragole	33.43	0.15
15	Benzyl cinnamate	33.88	0.86

Cinnamaldehyde is a viscous, pale-yellow substance that occurs naturally within cinnamon tree bark [54]. Eugenol is the main component of cinnamon leaf oil, while cinnamaldehyde is the main compound in cinnamon bark oil [55]. Cinnamon leaves (*Cinnamomum verum*) yield an essential oil with a high eugenol content, while the bark yields an oil with a high concentration of cinnamaldehyde. [56]. Cinnamon (*Cinnamomum zeylanicum*) is found to have cinnamaldehyde as the main component in the oil extracted from its bark, while eugenol, benzyl benzoate, and β-caryophyllene are components obtained from the oil extract from its leaves. Several studies have shown that cinnamaldehyde exhibits significant antifungal activity against several human pathogens [57-58]. All these studies show that cinnamaldehyde, the main component of the oil extracted from cinnamon bark, has been found to be usable as an antifungal agent, which can inhibit β-(1,3)-glucan and chitin synthase in filamentous fungi [59]. The essential oil extracted from the bark of *C. zeylanicum* is characterized by a high concentration of trans-cinnamaldehyde and exhibits broad-spectrum antimicrobial activity against pathogens in animals and plants, including fungi that cause spoilage and food contamination [60,34]. Consequently, we hypothesized that cinnamaldehyde serves as the primary constituent responsible for the antifungal activity of CBEO.

3.2. Antifungal activity test of CBEO

The inhibitory effects of CBEO on *A. niger* are investigated in this research. In antifungal susceptibility testing, the MIC indicates the point at which the concentration of an active agent becomes sufficient to inhibit visible hyphal growth or spore germination. To ensure a reliable quantification of antifungal potency, the dilution method was selected as the most robust approach for establishing the MIC. The MFC is characterized as the minimum concentration of essential oil required to achieve a 99.9% reduction in the initial fungal population, signifying a potent fungicidal effect [61]. The MICs and MFCs of CBEO against the *A. niger* were validated using the dilution method. In this experiment, CBEO concentrations of 1%, 0.5%, 0.25%, 0.125%, 0.063%, 0.031%, 0.016% and 0.008% (v/v) were prepared by serial dilutions. Amphotericin B functioned as the positive control, and fungi without extract was utilized as the negative control. Each test tube used to determine MIC and MFC values was filled with 1 milliliter of broth to ensure that the conditions were standardized. The MIC and MFC were determined to be 0.25% and 0.5% (v/v), respectively.

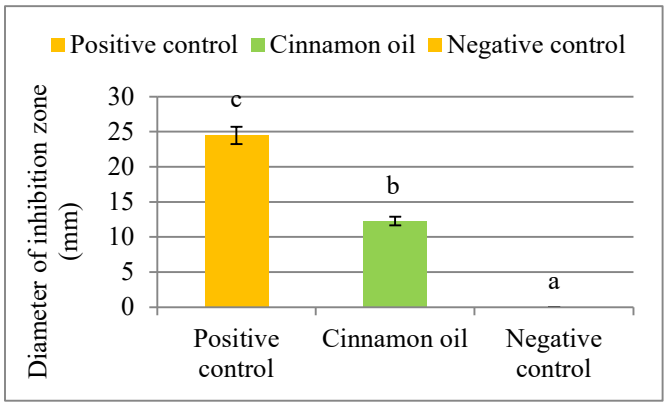

Fig. 2 The diameter of the inhibition zone (mm) against A. niger growth using the disc diffusion method. Different letters on bars display a statistically significant difference at P < 0.05

The mean diameters of the inhibition zones for *A. niger* are demonstrated in Figure 2, reflecting the antifungal potency of the tested samples. The inhibition zone diameters for *A. niger* showed significant differences across the tested groups, as established by the one - way ANOVA analysis ($P < 0.05$). Statistical analysis revealed that the inhibition zone diameters for the CBEO were significantly different from those noted for both the positive and negative control groups. Experimental data demonstrated that CBEO presented a statistically significant inhibitory effect on the proliferation of *A. niger* compared to the negative control ($P < 0.05$). Therefore, CBEO demonstrates a high level of antimicrobial potency. This may be attributed to its chemical composition. As illustrated in Figure 2, the zones of inhibition were 12.29 mm against *A. niger*.

Cinnamaldehyde possesses significant antimicrobial properties, effectively inhibiting the growth of microorganisms [62]. Cinnamaldehyde is a lipophilic substance, meaning it readily dissolves in the double phospholipid layer of the fungal cell membrane. Ergosterol, similar to human cholesterol, helps maintain the cell membrane's flexibility. Cinnamon oil inhibits ergosterol synthesis; without it, the cell membrane becomes brittle and leaky. When the cell membrane loses its integrity, many intracellular components leak out. In particular, studies have shown significant leakage of potassium and ATP ions, leading to immediate metabolic failure [63-64]. The antimicrobial mechanism of cinnamon involves inhibiting ATPase activity, inhibiting cell wall synthesis, and destabilizing the cell membrane. This allows cinnamaldehyde and its derivatives to exhibit antifungal activity against a wide range of fungal species [65,59,66].

Cinnamon oil alters chemical reactions within cells by generating free radicals (ROS). These free radicals damage mitochondria, disrupt electron transport, and lead to the

excessive production of other free radicals such as superoxide anion and hydrogen peroxide. These free radical molecules break down remaining fatty acids in the cell membrane, a process called lipid oxidation, ultimately leading to the death of *A. niger* cells [67-68].

Recent transcriptomic studies have found that genes involved in ergosterol synthesis (e.g., ERG3 and ERG11) are inhibited, causing membrane damage, resulting in a decrease in genes involved in chitin synthesis and β-(1,3)-glucan, weakening the fungus and making it susceptible to osmotic rupture, thus placing the cells in a state of starvation and dormancy [69-71]. These findings are consistent with the research of Liu et al. (2024), which found that cis-2-methoxycinnamic acid has innate antimicrobial properties [66]. This process occurs due to increased relative electrical conductivity, leading to cytoplasmic leakage and damage to the fungal cell membrane.

Although cinnamon oil is a highly effective bioactive compound containing cinnamaldehyde as its main ingredient, it has impressive antibacterial and antioxidant properties. But their actual use often faces high sensitivity to environmental factors. This can lead to rapid deterioration and loss of therapeutic value. This is the insoluble properties of cinnamon oil and its potential to be corrosive. Therefore, advanced delivery methods are required for industrial applications. One of the most successful in the real world is mixing oils into food film.

4. Conclusion

GC-MS analysis of the study revealed the chemical details of essential oils from CBEO. Chemical analysis identified cinnamaldehyde (48.49%) as the major constituent of the essential oil extracted from cinnamon bark, a major antifungal component. The antifungal properties of CBEO against *A. niger* were characterized in this study. Disc diffusion method shows that the inhibition zone diameter of 12.29 ± 0.52 mm was recorded for CBEO when tested against *A. niger*, yielding results that are more significant ($P < 0.05$) than the negative control. CBEO exhibited the Minimum Inhibitory Concentration (MIC) of *A. niger* at 0.25 % (v/v), corresponding to the optical density of 0.008. The Minimum Fungicidal Concentration (MFC) of the CBEO was established at 0.5% (v/v). Consequently, these findings suggest that CBEO represents a promising source of natural antifungal agents for use as bio-preservatives within the food industry and as botanical materials for medicinal properties.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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