

Short communication

Cinnamon bark oil, a potent fungitoxicant against fungi causing respiratory tract mycoses

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Cinnamic aldehyde has been identified as the active fungitoxic constituent of cinnamon (*Cinnamomum zeylanicum*) bark oil. The fungitoxic properties of the vapours of the oil/active constituent against fungi involved in respiratory tract mycoses, i.e., *Aspergillus niger*, *A. fumigatus*, *A. nidulans*, *A. flavus*, *Candida albicans*, *C. tropicalis*, *C. pseudotropicalis*, and *Histoplasma capsulatum*, were determined *in vitro* as minimum inhibitory concentration (MIC), minimum lethal concentration (MLC), inoculum density sustained, and exposure duration for fungicidal action at MIC and higher doses, as well as effect of incubation temperatures on fungitoxicity. It is concluded that these inhalable vapours appear to approach the ideal chemotherapy for respiratory tract mycoses.

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Plant volatiles exert potent bioregulatory action, particularly on microorganisms (1, 2). In recent years, volatile constituents of various higher plants, i.e., many essential oils and their constituent terpenoids, have shown potent fungitoxic activity in their vapours (3–7). Use of such volatiles for protection of stored foods against fungal infestation and also for controlling fungal diseases of crops has been suggested (8, 9).

The ubiquity of the aspergilli in the air leads to almost constant human respiratory tract exposure to the organisms. The most frequent mechanism of entry of aspergilli into the respiratory tract is through inhalation of spores. However, fungitoxic volatiles from higher plants are potentially effective in the chemotherapy of human and avian respiratory tract mycoses since their fungitoxic vapours can be administered through inhalation.

Earlier screening of an array of essential oils against many fungi causing human respiratory tract mycoses revealed that vapour of cinnamon bark oil is an effective fungitoxicant, active at comparatively low doses (10).

Therefore, the present study was undertaken to investigate the *in vitro* fungitoxic properties of cinnamon (*Cinnamomum zeylanicum* Breyn) bark oil and

its active constituents against some fungi involved in respiratory tract mycoses, i.e., *Aspergillus fumigatus* Fres., *A. niger* von Tieghem, *A. nidulans* (Eidam.) Wingate, *A. flavus* Link, *Candida albicans* (Robin) Berkhout, *C. tropicalis* (Cast.) Berkhout, *C. pseudotropicalis* (Cast.) Basgel, and *Histoplasma capsulatum* Darling.

Material and methods

Essential oil of cinnamon bark was obtained by hydrodistillation followed by drying over anhydrous calcium sulphate. Physicochemical properties of the oil were determined according to Langenau (11).

Fungitoxic activity of vapour of oil and its active constituent was assessed by the inverted Petri dish technique. A 5-mm diameter inoculum disc of the test fungus, cut from the periphery of the mycelial colony of a 7-day-old culture, was inoculated on 10 ml Saboraud agar medium in an 80-mm diameter Petri dish. The dish was then inverted, and the requisite amount of oil/active constituent in 0.5 ml acetone, soaked on a 25-mm diameter sterile filter paper disc, was placed inside the dish on its lid. Sterile distilled water, taken in place of oil in 0.5 ml acetone, was used as control. Every experiment was

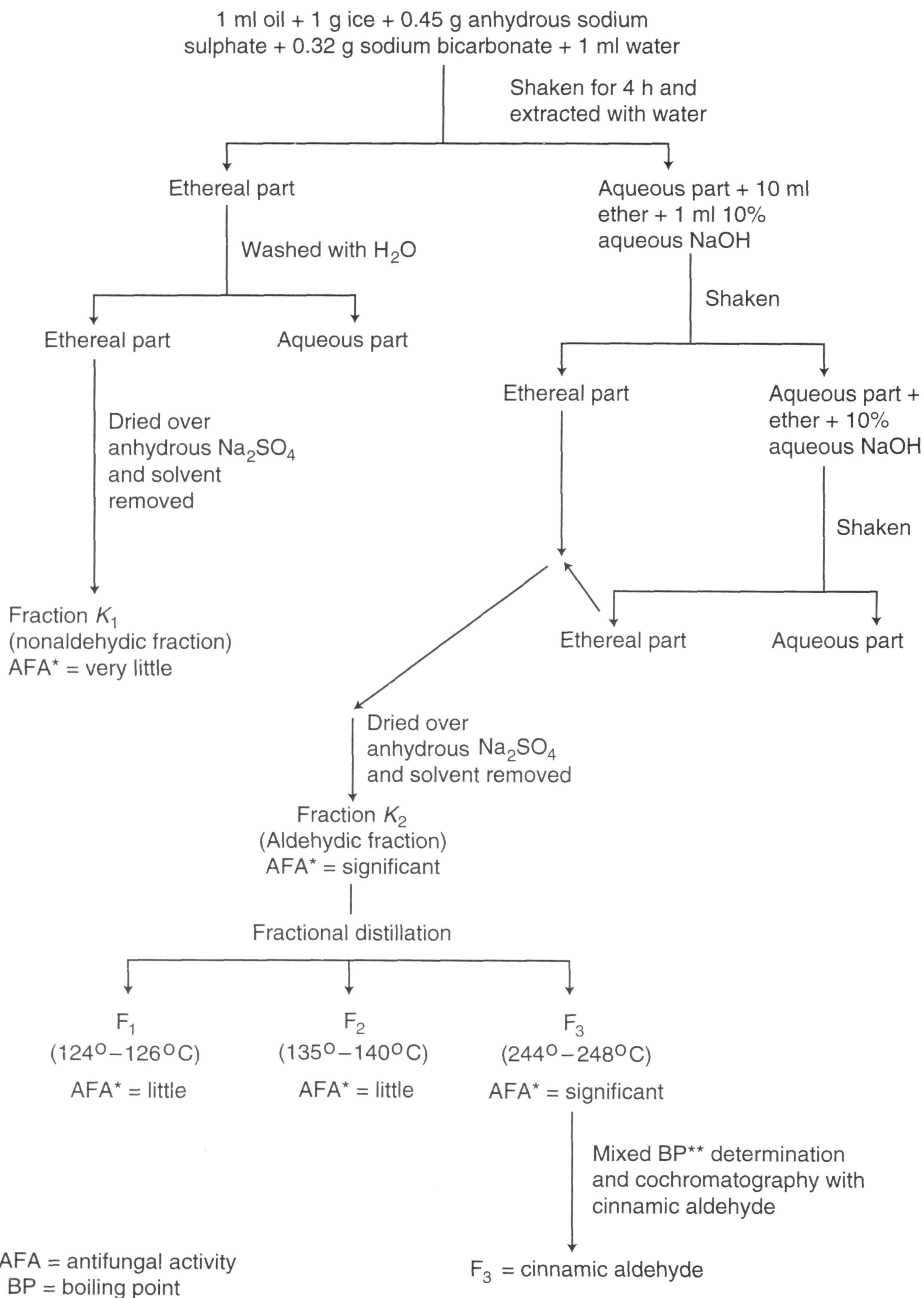


Fig. 1. Fractionation of oil and identification of fungitoxic constituents.

replicated 10 times and the average of results was recorded. The dishes were incubated at $25^{\circ} \pm 1^{\circ}\text{C}$, and on day 7 fungitoxicity was recorded as per cent inhibition of mycelial growth, calculated by the formula $(gc - gt/gc) \times 100$, where gc and gt are average diametric growth of mycelial colony of control and treatment sets, respectively.

The dose of vapours of oil/active constituent was expressed as ppm (parts per million), i.e., parts (volume) of oil per million parts of aerial volume inside the Petri dish available for diffusion of oil vapour, arbitrarily assuming that the given volume of oil volatilizes to produce an equal volume of vapour (9). The Corning glass Petri dish (80-mm diameter) used in this study had an average inner volume of 60 ± 2 ml, of which 10 ml was occupied by the medium and 50 ml medium-free aerial space was available for diffusion of oil vapour. The ppm dose of oil was calculated by progression as the amount of oil (μl) used per litre of medium-free aerial space available for diffusion of oil vapour.

Fractionation of cinnamon oil into nonaldehydic and aldehydic fractions, K_1 and K_2 , respectively, and identification of cinnamic aldehyde as the active fungitoxic constituent were carried out by the method as shown in the flow chart (Fig. 1).

The fungitoxic properties of oil and its active constituent were studied as follows. The minimum inhibitory concentration (MIC) was determined by observing per cent inhibition of mycelial growth of the test fungus by progressively lower doses of oil/constituent, in the range of 1000–10 ppm. The minimum dose required for 100 per cent inhibition (fungistatic/fungicidal) was recorded as the MIC. The fungistatic/fungicidal nature of fungitoxicity was observed (12) at the MIC and higher doses for determining the minimum lethal concentration (MLC), which was recorded as the minimum dose required for fungicidal action. Inoculum density sustained by vapours of oil/active constituent at the MIC and higher doses was determined by increasing the number of inoculum discs in each assay dish of the treatment set in arithmetic progression of two, up to a maximum of 24 discs (9). The exposure duration for fungitoxic action was determined as the minimum duration of exposure to the MIC and higher doses of the oil vapour required for fungicidal action on one inoculum disc of the test fungus (9). The effect of incubation temperature on the fungitoxicity of the oil was assessed by incubating the assay dishes at desired temperatures (10° , 20° , 30° , and 40°C).

Results and discussion

The concentration of various ingredients in essential oil from the same plant species may vary due to ecologic and plant growth factors (13, 14). There-

fore, cinnamon bark oil in this study was standardized by determining its physicochemical properties. Cinnamon bark yielded 0.3 ml essential oil/100 g on hydrodistillation. Physicochemical properties were observed as specific gravity (1.033 at 25°C), specific rotation ($-2^{\circ}44'$ at 25°C), refractive index (1.401 at 20°C), acid number (9.61), and carbonyl content (64.6%). We recommend that cinnamon bark oil of similar physicochemical properties should be used for faithful reproduction of our results.

Isolation and identification

The fungitoxic activity of essential oils is due to their diverse volatile constituents (1). Various terpenes and other volatile contents of essential oils have been reported as their active constituents (15, 16). In this study, cinnamon bark oil was fractionated by solvent extraction into nonaldehydic (K_1) and aldehydic (K_2) fractions, of which K_2 was found to possess fungistatic activity against test fungi. This fraction was fractionally distilled to yield a small fraction (F_1) at 124° – 126°C , another small fraction (F_2) at 135° – 140°C , and a large fraction (F_3) at 244° – 248°C . Fungitoxicity was found to be present in F_3 . The boiling point of F_3 was determined to be $246 \pm 2^{\circ}\text{C}$, i.e., to be identical with that of cinnamic aldehyde, which is already known to be present in large amounts (up to 76%) in cinnamon bark oil (14). The fraction F_3 was conclusively determined as cinnamic aldehyde by determination of the boiling point of the mixture of equal amounts of F_3 and a known sample of cinnamic aldehyde which was equal to that of the latter. Further confirmation was done by cochromatography of F_3 with a known sample of cinnamic aldehyde on thin layer dishes, using benzene:ethyl acetate (9:1) as solvent, and developing the chromatogram in an iodine chamber, whereupon both the samples showed the same RF (Fig. 1).

Fungitoxic properties

The MIC of vapours of cinnamon bark oil and cinnamic aldehyde varied from 16 to 40 ppm for different test fungi (Tables 1 and 2). Generally, aspergilli were inhibited at a lower MIC than that needed for inhibition of other fungi. *A. fumigatus* appeared most vulnerable and was inhibited by a 16-ppm dose of cinnamic aldehyde vapour. Vapours of oil and cinnamic aldehyde proved fungicidal at the MIC (MLC = MIC) for aspergilli under study. However, for *Candida* spp. and *H. capsulatum*, both the vapours were fungistatic at the MIC (MLC > MIC). The literature shows that the MIC and the MLC of vapours of cinnamon bark oil and cinnamic aldehyde are considerably lower than those of other

Table 1. *In vitro* fungitoxic properties of vapours of essential oil of *C. zeylanicum* bark against respiratory tract fungi

Test fungi	Fungitoxic properties of essential oil						
	MIC* (ppm = µl/l)	MLC* (ppm = µl/l)	Inoculum density sustained**		Exposure duration for fungicidal action (h/min)		
			At MIC dose	At 5 × MIC dose	At MIC dose	At 5 × MIC dose	At 100 × MIC dose
<i>Aspergillus fumigatus</i>	20	20	2	24	36 h	10 h	30 min
<i>A. niger</i>	20	20	1	20	48 h	12 h	50 min
<i>A. nidulans</i>	20	20	1	22	48 h	12 h	50 min
<i>A. flavus</i>	20	20	2	24	48 h	12 h	45 min
<i>Candida albicans</i>	40	160	2	8	+	24 h	2 h
<i>C. tropicalis</i>	40	120	2	10	+	24 h	2 h
<i>C. pseudotropicalis</i>	40	120	2	10	+	24 h	2 h
<i>Histoplasma capsulatum</i>	40	120	1	20	48 h	12 h	1 h

* MIC = minimum inhibitory concentration (fungistatic/fungicidal).

MLC = minimum lethal concentration (fungicidal).

** Number of inoculum discs (5-mm diameter) inhibited.

+ Not fungicidal at MIC.

– No effect on fungitoxicity.

Table 2. *In vitro* fungitoxic properties of vapours of cinnamic aldehyde against respiratory tract fungi

Test fungi	Fungitoxic properties of essential oil						
	MIC* (ppm = µl/l)	MLC* (ppm = µl/l)	Inoculum density sustained**		Exposure duration for fungicidal action (h/min)		
			At MIC dose	At 5 × MIC dose	At MIC dose	At 5 × MIC dose	At 100 × MIC dose
<i>Aspergillus fumigatus</i>	16	16	2	24	30 h	8 h	30 min
<i>A. niger</i>	18	18	2	24	36 h	10 h	50 min
<i>A. nidulans</i>	18	18	2	24	36 h	10 h	50 min
<i>A. flavus</i>	18	18	2	24	36 h	10 h	45 min
<i>Candida albicans</i>	35	160	2	24	+	22 h	2 h
<i>C. tropicalis</i>	30	100	2	24	+	20 h	1 h 30 min
<i>C. pseudotropicalis</i>	30	100	2	24	+	20 h	1 h 30 min
<i>Histoplasma capsulatum</i>	30	100	1	24	36 h	8 h	50 min

* MIC = minimum inhibitory concentration (fungistatic/fungicidal).

MLC = minimum lethal concentration (fungicidal).

** Number of inoculum discs (5-mm diameter) inhibited.

+ Not fungicidal at MIC.

– No effect on fungitoxicity.

fungitoxic essential oils reported earlier (3, 4, 8, 9, 16).

Vapours of cinnamon bark oil and cinnamic aldehyde could inhibit no more than two inoculum discs of the test fungi at the MIC. However, at a higher dose (5 × MIC), these vapours retained fungitoxicity for appreciably higher inoculum density. Cinnamic aldehyde vapour sustained higher inoculum density than that of the oil (Tables 1 and 2).

The duration of exposure of the target fungus to a fungicide for lethal action of the latter is important for its employment as a chemotherapeutic agent; the quicker this action, the better the prospects of the agent. In this study, vapours of the oil and cinnamic

aldehyde required a long exposure duration (10–48 h) for their fungicidal action at the MIC and moderately higher (5 × MIC) doses. However, at a very high dose (100 × MIC), these vapours proved fungicidal at exposure durations of 30 min – 2 h for different test fungi. *Candida* spp. sustained longer exposure durations than aspergilli and *H. capsulatum*. The limited amount of research done has looked at the exposure time of contact between oil and fungi; vapours have not been studied (17–19).

Fungitoxicity of vapours of essential oils may be affected by incubation temperature in two ways: firstly, the incubation temperature may affect the rate of volatilization of the fungicide, thereby influencing

the concentration of fungitoxic vapour in the environment; secondly, the rate of growth of the fungus itself may be affected by the incubation temperature (20). Our study showed that the fungitoxicity of vapours of cinnamon bark oil and cinnamic aldehyde, at their MIC, remained unaffected by incubation temperatures ranging from 10°C to 40°C.

Standard antifungal compounds such as amphotericin B, nystatin, and 5-fluorocytosine have limited efficacy against the fungal pathogens in general and would be of little value in the treatment of either allergic bronchopulmonary aspergillosis or aspergilloma. Several years' investigation of the mode of action of antifungal compounds in medically important fungal pathogens has shown that effective treatment of aspergillosis is the result of limiting the growth of the fungus until the host immune system can eliminate it (21).

We conclude that vapours of cinnamon bark oil and cinnamic aldehyde are effectively toxic at very low doses and at high inoculum density against the test fungi causing respiratory tract mycoses. These vapours appear to be almost the ideal chemotherapy for respiratory tract mycoses since they can be administered through inhalation, and, as they are lipophilic, the vapours can penetrate deep into the host tissues, unbarred by the plasma membrane. The oil and cinnamic aldehyde are thus suggested for *in vivo* trials in order to explore the possibility of their use as chemotherapeutic agents against respiratory tract mycoses.

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