TOXIC AND ANTIFUNGAL PROPERTIES OF THE ESSENTIAL OILS OF *CINNAMOMUM* SPECIES FROM PENINSULAR MALAYSIA

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IBRAHIM JANTAN, RASADAH MAT ALI & GOH SWEE HOCK. 1994. Toxic and antifungal properties of the essential oils of *Cinnamomum* species from Peninsular Malaysia. The essential oils of seven *Cinnamomum* species from Peninsular Malaysia (*C. pubescens, C. javanicum, C. iners, C. impressicostatum, C. mollissimum, C. porrectum* and *C. camphora*) were investigated for their toxic and antifungal properties. Brine shrimp lethality bioassay on the essential oils revealed that samples distilled from the leaf of *C. mollissimum, C. iners* and *C. impressicostatum* were very toxic to the brine shrimp with a LC_{50} of 1.6, 5.1 and 11.6 *ug ml*$^{-1}$ respectively. The essential oils from the other species also showed high toxicity values with LC_{50} mostly < 100 *ug ml*$^{-1}$. The inhibitory study of the essential oils on the growth of three fungi species, *viz. Gloeophyllum trabeum, Coriolus versicolor* and *Bostryodiplodia theobromea* by the agar dilution technique indicated that the essential oils were effective. The leaf oil of *C. pubescens* was the most effective, exhibiting ED_{50} of 60.3 *ug ml*$^{-1}$ for *C. versicolor*, 58.8 *ug ml*$^{-1}$ for *G. trabeum* and 48.0 *ug ml*$^{-1}$ for *B. theobromea*. The bark oil of *C. javanicum* also exhibited significant inhibitory activity against the three fungi with ED_{50} ranging from 84.4 to 324.0 *ug ml*$^{-1}$.

Key words: Essential oils - *Cinnamomum* species - bioassay - antifungal activity - toxicity - brine shrimp

IBRAHIM JANTAN, RASADAH MAT ALI & GOH SWEE HOCK. 1994. Ciri-ciri toksik dan antikulat minyak pati spesies *Cinnamomum* dari Semanjungan Malaysia. Ciri-ciri toksik dan antikulat minyak pati tujuh spesies *Cinnamomum* dari Semenanjung Malaysia (*C. pubescens, C.javanicum, C.iners, C.impressicostatum, C. mollissimum, C. porrectum* dan *C. camphora*) telah di kaji. Biocerakinan maut brine shrimp keatas minyak-minyak tersebut menunjukkan sampel yang di suling daripada daun *C. mollissimum, C.iners* dan *C. impressicostatum* adalah sangat toksik keatas brine shrimp dengan LC_{50} pada 1.6, 5.1 dan 11.6 *ug ml*$^{-1}$ tiap-tiap satu. Minyak-minyak pati dari spesies yang lain juga menunjukkan nilai toksik yang tinggi, kebanyakkanya dengan LC_{50} yang kurang dari 100 *ug ml*$^{-1}$. Kajian aktiviti rencatan minyak pati ke atas tumbuhan tiga spesies kulat, iaitu *Gloeophyllum trabeum, Coriolus versicolor* dan *Bostryodiplodia theobromea* menerusi teknik pencairan agar menunjukkan minyak-minyak pati tersebut adalah berkesan. Minyak pati daripada daun *C. pubescens* adalah yang paling efektif, menunjukkan ED_{50} 60.3 *ugml*$^{-1}$ keatas *C. versicolor*, 58.8 *ugml*$^{-1}$ keatas *G. trabeum* dan 48.0 *ugml*$^{-1}$ keatas *B. theobromea*. Minyak pati dari kulit *C. javanicum* juga menunjukkan aktiviti rencatan yang tinggi keatas ketiga-tiga kulat dengan ED_{50}berkisar dari 84.4 ke 324.0 *ug ml*$^{-1}$.

Introduction

The genus *Cinnamomum* belongs to the family Lauraceae. Cinnamon, the most important commercial product obtained from the bark of four *Cinnamomum* species, *viz. C. zeylanicum, C. loureirii, C. burmannii* and *C. cassia,* depends on cinnamaldehyde for its characteristic taste (Lawrence 1967). The bark oil of *C. zeylanicum* is used as flavour in foods and drinks, as a component of perfumes and in many pharmaceutical preparations (Reynolds 1989). The leaf oil from the same species is an important natural source of eugenol (Guenther 1975).

The essential oils of other *Cinnamomum* species have been the subject of some study. For example, linalool, camphor, safrole, cinnamaldehyde, 1, 8 - cineole and terpinen - 4 - ol have been found as major components of the essential oils of various species (Fujita & Fujita 1972, Yuangzheng *et al.* 1986, Fang *et al.* 1989, Biyao *et al.* 1986). There are 21 species of *Cinnamomum* in Peninsular Malaysia and several of the species have been investigated for their chemical components (Ibrahim & Goh 1992).

Knowledge on the chemical composition of the essential oils of *Cinnamomum* species and biological testing to study the toxicity of the oils are essential in appraising their suitability as raw materials in foods, pharmaceutical and industrial products. The pharmacology of cinnamon oil from *C. zeylanicum* has been studied fairly extensively (Lawrence 1967). However, biological studies on the other species have not been fully established. In our effort to identify bioactive natural products which have the potential to be developed into new fungicides, pesticides, insecticides and other pharmacologically useful compounds, toxic and antifungal properties of the essential oils of seven *Cinnamomum* species, *viz. C. pubescens, C. mollissimum, C. porrectum, C. iners, C. impressicostatum, C. javanicum* and *C. camphora,* against brine shrimp (*Artemia salina*) and three wood-rotting fungi, namely, *Coriolus versicolor* (white rot fungus), *Gloeophyllum trabeum* (brown rot fungus) and *Bostryodiplodia theobromea* (blue stain fungus) respectively were investigated.

Materials and methods

The ground plant samples (200 *g*) (mesh size 40 - 60) were water distilled for 8 *h*. The aqueous layer from the distillate was extracted with diethyl ether. The ether layer was dehydrated with anhydrous sodium sulfate and the solvent was distilled at slightly reduced pressure to recover the dissolved oil.

Brine shrimp lethality test

Shrimp eggs were allowed to hatch and mature as nauplii in two days in a hatching tank filled with artificial seawater. The free-swimming nauplii were attracted by a light to a compartment from which they could be collected for the assay proper. Vials containing 1-1000 $ug\,ml^{-1}$ samples were prepared by dissolving the oils in acetone and transferring the solution to each vial. The solvent was evaporated at room temperature and sea water was added to achieve the correct concentration. Ten shrimps were added to three vials for each dose via a disposable pipette. The number of deaths out of 30 shrimps per dose was recorded after 24 *h* and LC_{50} values with 95% confidence intervals were determined for each compound by a Finney computer programme (Finney 1971).

The control solution consisted of 30 nauplii in the artificial seawater. Solutions of potassium dichromate dissolved in the brine medium at 1 - 1000 *ug ml* $^{-1}$ concentrations were used as standard toxicant. For acceptable readings, the LC_{50} for the toxicant should fall within 27-35 *ug ml*$^{-1}$ range (Sam *et al.* 1986).

Determination of antifungal activity

A 2% solution of malt extract agar (MEA) was autoclaved and then cooled to approximately 50°*C*. The essential oils dissolved in acetone (1 *ml* solution containing 0.01 to 2 *mg* sample) were aseptically added to the agar and the mixtures were vigorously shaken. Each aliquot was poured into a series of sterile petri dishes (9 *cm* diameter). Each dish was inoculated at the centre with a 4 *mm* - disc cut from the vegetative growing margin of 4 to 14 - day - old cultures of the fungi *C. versicolor, G. trabeum,* and *B. theobromea* maintained on MEA. The fungi inoculated on 2% MEA amended with acetone served as control. Testing was carried out in triplicates for each concentration of the tested samples. The dishes were incubated at 28 °*C* for 4 to 14 days. The concentrations of the oils which suppressed the growth of the microorganism on the agar medium were determined. The colony diameter taken as the mean of two diameters at right angle to each other was measured. The percentage inhibition of growth compared to maximum growth on control plate was calculated. A probit-log concentration analysis was carried out to determine ED_{50} values.

Results and discussions

The bioassay of the essential oils of *Cinnamomum* species to determine their toxicities against brine shrimp demonstrated that they were active against the tiny crustacean (Table 1). Most of the oils were as toxic as the standard toxicant, potassium dichromate, towards the brine shrimp. The leaf oils of *C. mollissimum, C. impressicostatum* and *C. iners* showed very high toxicity effect on the test organism. Amongst these the leaf oil of *C. mollissimum* with a LC_{50} of 1.6 *ug ml*$^{-1}$ was the most toxic. The high levels of benzyl benzoate (77.7% in *C. mollissimum,* 34.6% in *C. impressicostatum,* 32.7% in *C. iners*) and in combination with other major compounds such as benzyl alcohol, geraniol and (E)-methyl cinnamate (Ibrahim & Goh 1992), could be responsible for the high toxicities of the leaf oils. The essential oils from the other species also showed high toxicity values with LC_{50} mostly less than 100 *ug ml*$^{-1}$. The high toxicity values of the oils suggested that the combination of compounds of diverse structures (nonterpenes, monoterpenoids and sesquiterpenoids) could exhibit different mode of action towards the test organism, resulting in high activities. The results should encourage further testing of the oils for specific pharmacological properties.

Table 1. Toxicities of essential oils of *Cinnamomum* species to brine shrimp (*Artemia salina*)

Species	LC50 in *ug ml*$^{-1}$	95% confidence intervals
C. pubescens		
leaf	68.9	60.5 - 75.3
bark	63.7	58.2 - 68.8
wood	92.9	86.1 - 104.6
C. javanicum		
leaf	73.9	63.5 - 85.8
bark	46.2	35.6 - 56.6
wood	115.8	108.7 - 127.5
C. iners		
leaf	5.1	3.1 - 7.4
bark	83.2	72.1 - 95.0
wood	118.7	104.3 - 130.7
C. impressicostatum		
leaf	11.6	8.6 - 13.3
bark	57.8	48.2 - 65.9
wood	41.4	32.3 - 47.9
C. mollissimum		
leaf	1.2	0.7 - 1.7
bark	40.4	35.3 - 45.3
wood	48.1	35.9 - 50.6
C. porrectum		
leaf	26.8	17.9 - 38.6
bark	52.8	45.4 - 70.8
wood	36.4	26.6 - 43.2
C. camphora		
leaf	190.8	158.4 - 227.9
bark	130.6	102.5 - 163.0
wood	326.3	292.4 - 372.9
potassium dichromate	30.6	24.4 - 36.9

The antifungal activity of each essential oil is described by the ED_{50} value which is the concentration causing a 50% reduction in fungal growth measured in *ug ml*$^{-1}$ (Luken 1971). A probit-log concentration analysis was carried out to determine the ED_{50} values (Finney 1971). The slope of the probit-log concentration regression line of each fungus and toxicant (Figure 1) is a characteristic of the toxicant as it measures potency with changing concentration of the toxicant (Horsfall & Dimond 1957).

Table 2 shows the ED_{50} values obtained from the probit-log concentration analysis for each fungus with the corresponding oil sample tested. All the oils tested exhibited some degree of inhibitory activity towards the three fungi. The leaf oil of *C. pubescens* was the most effective against the three fungi as indicated by its low ED_{50} values of 60.3 *ug ml*$^{-1}$ for *C. versicolor*, 58.7 *ug ml*$^{-1}$ for *G. trabeum* and 48.0 *ug ml*$^{-1}$ for *B. theobromea.* The bark oil of *C. javanicum* also showed strong inhibition against the three fungi with ED_{50} values ranging from 84.4 to 324.0 *ug ml*$^{-1}$. *C. versicolor* and *G. trabeum* were also significantly affected by the leaf oils

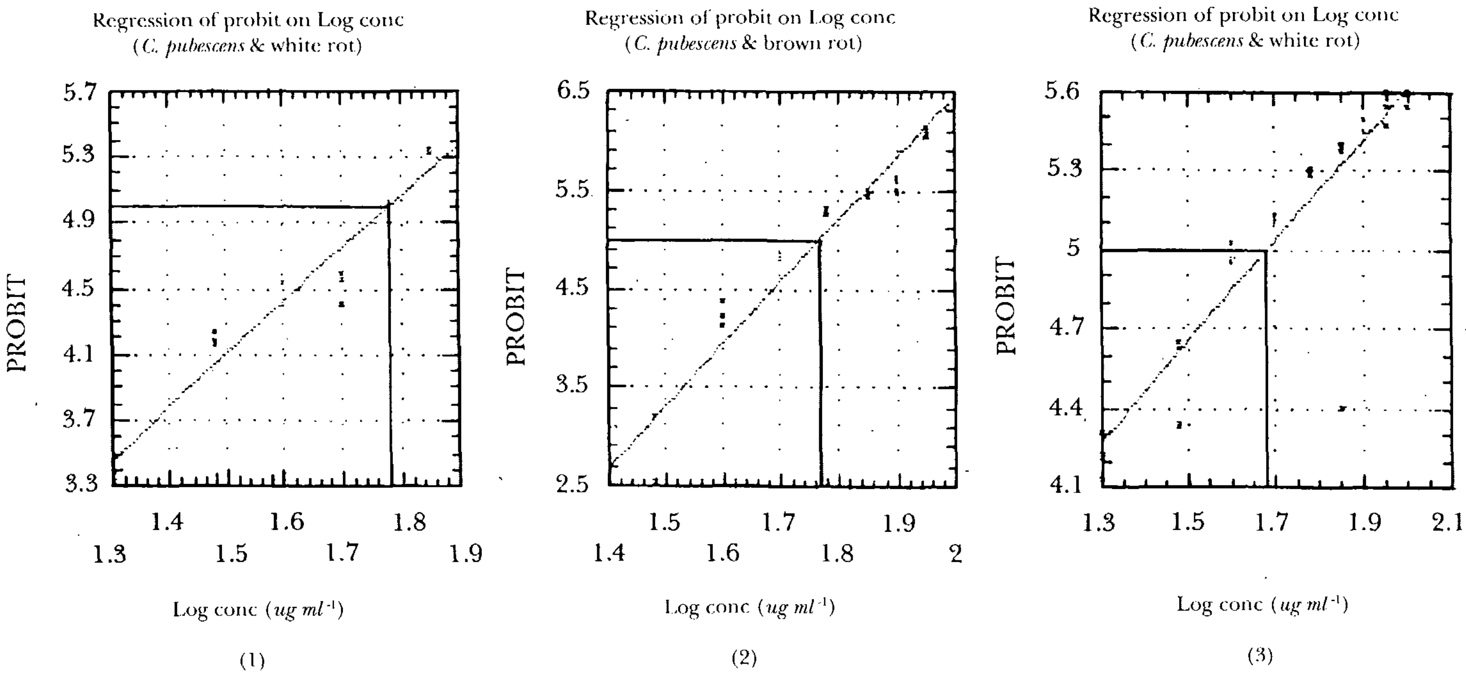

Figure 1. Probit - log concentration analysis for *C. pubescen*
1. *Coriolus versicolor* 2. *Gleoephyllum trabeum* 3. *Bostryodiplodia theobromea*

of *C. mollissimum*, *C. impressicostatum* and *C. iners* which gave ED_{50} values varying from 139.4 to 325.3 *ug ml* $^{-1}$, 277.3 to 300.9 *ug ml* $^{-1}$ and 225.3 to 368.3 *ug ml* $^{-1}$ respectively. The leaf oil of *C. camphora* showed significant inhibitory activity against two of the fungi with ED_{50} values ranging from 348.9 to 382 *ug ml* $^{-1}$. The ED_{50} values of the other oils were relatively higher than those mentioned above, ranging from 417.5 to 1241.9 *ug ml* $^{-1}$.

Table 2. Effective dose at 50% inhibition (ED_{50}) (*ug ml* $^{-1}$) for the essential oils of *Cinnamomum* species on different fungi

Essential oils of *Cinnamomum* spp.	White rot	Brown rot	Blue stain
C. pubescens			
leaf	60.3	58.8	48.0
C. mollissimum			
leaf	325.3	139.4	1281.4
wood	486.9	1118.2	550.6
C. iners			
leaf	225.3	368.3	985.8
C. porrectum			
leaf	578.1	1241.9	817.1
C. camphora			
bark		788.9	630.5
wood		838.3	417.5
leaf		382.2	349.0
C. impressicostatum			
leaf	277.3	300.9	1096.0
C. javanicum			
bark	324.0	147.9	84.4

White rot fungus - *Coriolus versicolor*, Brown rot fungus - *Gloeophyllum trabeum*,
Blue stain fungus - *Bostryodiplodia theobromea*

The relative antifungal activity of the essential oils could not be easily correlated with any individual component. The inhibitory activity of the oils may be due to the different modes of action of the total components of the oils towards the fungi.

Conclusion

The high toxicities of the essential oils against brine shrimp, especially the benzyl benzoate-containing leaf oils of *C. mollissimum*, *C. impressicostatum* and *C. iners*, suggested that the oils are potential source of useful bioactive materials. The rapid and inexpensive but reliable toxicity study served as a prescreening for bioactive compounds which may then be subjected to more elaborate bioassays for specific pharmacological activities. The significant effect of the essential oils, especially the leaf oil of *C. pubescens* and the bark oil of *C. javanicum*, against the wood-rotting fungi suggested that the oils may possibly be used as wood preservatives or fungicides.

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