APPLICATION OF IONIC LIQUIDS IN THE ULTRASOUND-ASSISTED EXTRACTION OF ANTIMICROBIAL COMPOUNDS FROM THE BARK OF CINNAMOMUM CASSIA

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ABSTRACT

This study compares the chemical constituents of extracts from the bark of *Cinnamomum cassia* obtained with the use of ionic liquids and organic solvents, as well as investigates their antifungal and yeasticidal activity. The type of ionic liquid was found to play a significant role in this process. We found that the ionic liquid tris (2-hydroxyethyl) methylammonium methylsulfate is a most efficient extracting agent, superior to classical solvents such as ethanol, ethyl acetate, hexane, dichloromethane. The obtained extract revealed high antimicrobial activity against yeasts, fungi pathogenic for plants and bees, dermatophytes, filamentous moulds with similar potencies as the standard fungicides.

Keywords: *Cinnamomum cassia*, Ionic liquids, Antifungal activity, Chemical composition

INTRODUCTION

Cinnamomum cassia (syn. *C. aromatica*), also named Chinese cinnamon is an evergreen tropical tree, belonging to the *Lauraceae* family. It is one of the most important spices which has been traditionally used in treatment of gastritis, blood circulation disorders, dyspepsia, inflammatory diseases^{1,2} such as influenza and arthritis³. The essential oil and extracts obtained from the bark, young branches and leaves of this plant have excellent inhibitory effects against bacteria⁴ and fungi⁵⁻⁹. In addition, extracts from *C. cassia* bark inhibited skin and pulmonary tumours as well as the growth of gastric, colon and cervical cell cancer lines¹⁰. The oil and extracts from *C. cassia* exhibited high toxicity to adult beetles of the stored product pests, *Lasioderma serricorne*, *Sitophilus oryzae* and *Callosobruchus chinensis*¹¹ and can also be used as potential repellents against *Aedes aegypti*¹², *Delia radicum*¹³ and *Alphitobius diaperinus*¹⁴. These unique properties of extracts and oil of cinnamon are associated with their chemical composition. The major bioactive components of *C. cassia* are as follows cinnamaldehyde, terpenes, aromatic compounds and esters.

A variety of developed extraction techniques play a key role in the separation and analysis of bioactive compounds from plant material. The extraction of antimicrobial components from *C. cassia* bark has been carried out by several extraction methods. These include heating reflux extraction¹⁵, maceration at room temperature¹⁶ and Soxhlet extraction¹⁷ with water, methanol, ethanol, petroleum ether, hexane, dichloromethane and some mixtures such as solvents. The applied organic solvents are usually flammable, toxic and volatile, so they are extremely dangerous for the environment. In this work, we propose ionic liquids as a green alternative to conventional organic solvents.

Ionic liquids (ILs) also known as molten salts, composed of organic cations and inorganic or organic anions are liquid at approx room temperature (or by convention below 100°C)¹⁸. Ionic liquids have unique properties such as negligible vapor pressure, good thermal stability, relatively low toxicity, good ultrasonic absorbing ability. In addition, ionic liquids have a large electrochemical window and excellent solvent properties for a wide range of inorganic and organic materials¹⁹, which makes them a very interesting subject of research. Over the last few years, ionic liquids have been offered as extractants of ions of various metals from alkali through heavy²⁰⁻²² to radioactive metals²³. Phenolic compounds, amino acids, hydrocarbons in mixtures and food dyes in water solutions can be also effectively extracted to ionic liquid. Moreover, desulfurization of gas oils and gasoline by extraction with these salts is also possible^{24,25}.

Recently, ionic liquids (ILs) have been used as solvents for the effective extraction of active metabolites from plants, for instance to obtain: polyphenolic compounds such as; trans-resveratrol from *Rhizma Polygoni Cuspidati*²⁶, saponins from *Dioscorea zingiberensis*²⁷, rutin from *Saururus chinensis*²⁸ and piperine from white pepper²⁹, three alkaloids N-nornuciferine, O-nornuciferine, and nuciferine from lotus leaf³⁰, insecticidal as well as microbiocidal compounds of the fungus *Cantharellus cibarius*³¹. The purpose of this study was to use ammonium-based ionic liquids for the extraction of secondary metabolites from the bark of *Cinnamomum cassia*. The chemical composition of the obtained extracts and the activity against fungi and yeast were determined.

MATERIALS AND METHODS

Plant material

The dried bark of *Cinnamomum cassia* produced in China was purchased from a local market in Poland. A voucher of specimen (38 GS15) was stored in the laboratory for further reference. The bark was ground to powder in a sterile coffee mill before extraction.

Chemicals

Figure 1. Structures of ionic liquids used in the extraction process; Benzalconium lactate- **A**, Didecylmethyl lactate- **B**, Benzalconium nitrate- **C**, Didecylmethyl nitrate- **D**, Tris(2-hydroxyethyl)methylammonium methylsulfate- **E**.

Figure 1 lists the four ammonium-based ionic liquids 1-4 used in this work, which were prepared in high yields (efficiency of over 95%) according to the procedure presented in the literature^{32, 33}. Tris (2-hydroxyethyl) methylammonium methylsulfate (5), cinnamaldehyde, coumarin, ketoconazole were purchased from Aldrich (USA).

Extraction of plant material

The plant material underwent extraction using a classic method of isolating active substances sensitive temperature, called maceration. In order to increase the efficiency of obtaining active compounds, the ultrasonic bath *Sonorex Super RK 512 H* generating ultrasounds of 35 Hz frequency was used. The extraction was carried out using equal amounts of powdered biological material (25 g). The evaporation of organic solvents from the extracts was carried out using a vacuum evaporator at 30-40°C.

Extraction with organic solvents

First, 50 ml of organic solvent (ethyl acetate, hexane, methylene chloride, methanol) was poured over the dried and ground plant material in the amount of 25 g, the material was then placed in an ultrasonic bath for 2 x 10 minutes and put away for 3 days (stirred occasionally). The whole extraction process took place at ambient temperature (20 °C). After filtering the constant residues in a Schott funnel, the filtrate was dried by vaporization of solvents with a vacuum evaporator. The obtained extracts were then used in chemical and biological analyses.

Extraction with ionic liquids

First, 50 ml of ionic liquid was poured over a specified amount of dried and powdered bark of the cinnamon tree, then the bark was placed in the ultrasonic bath for 2 x 10 minutes and put away for 3 days (stirred occasionally). The whole extraction process took place at ambient temperature (20 °C). At the subsequent stage, the constant residues were filtered in a Schott funnel. The ionic liquid solution was then mixed up with hexane, its layers were separated and the solvent was vaporized from the layer of hexane using an evaporator. The obtained extracts were then used in chemical and biological analyses.

GC-MS analysis conditions

The analyses of the extracts of *C. cassia* bark were performed using GC/MS system consisting of a Varian 450-GC gas chromatograph (Varian, USA), Varian 320 MS mass detector (Varian USA) and CP-8400 auto sampler. A VF-5MS capillary column (Varian, USA) (30mx0.25 mm, 0.25µm film thickness) was used with helium as the carrier gas, at a flow rate of 0.5 ml/min at a split ratio of 1:100. The GC oven temperature was programmed at an initial value of 50 °C for 1min, then heated up to 250°C at 4°C/min, and kept at 250°C for 10 min. The injector and detector temperatures were set at 250°C. Diluted samples (1:100 v/v, in hexane) of 1.0 were injected automatically. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The qualitative analysis was carried out on the basis of MS spectra which were compared with the spectra of the NIST (2002), LIBR (TR) (1990) database and the data available in the literature^{34, 35}. Retention indices for all the compounds were determined according to the Kovats method^{36, 37} using n-alkanes as standards (C₆-C₄₀). The compounds were identified by comparing the Kovats indices with those reported in the literature^{37, 38}.

Evaluation of antimicrobial activity

The antimicrobial activity of the *C. cassia* extracts and its major components: cinnamaldehyde and coumarin were tested against yeasts and fungi.

Microbial strains and culture media

The activity of *C. cassia* extracts was evaluated against the following fungal and yeasts species: **plant pathogenic fungi**: *Alternaria alternata* (Fries) Keissler, *Fusarium culmorum* (Smith) Saccardo, *Gaeumannomyces Graminisvar tritici*, *Rhizoctonia solani* Kuhn, *Phytophthora cactorum* (Lebert et Cohn) Schroeter, *Fusarium graminearum* Schwabe, **fungus causing ascosphaeriosis in bees**: *Ascosphaera apis* ATCC 13785, **dermatophytes causing human and animal skin dermatosis**: *Trichophyton mentagrophytes* ATCC 9533, *Microsporum gypseum* ATCC 6231, *Microsporum cookei* ATCC 13275, **fungi causing microbial corrosion of materials (filamentous)**: *Aspergillus niger* (LOCK 0439), *Chaetomium globosum* (LOCK 0476), *Paecilomyces variotii* (LOCK 0525), *Scopulariopsis brevicaulis* (LOCK 0553), **yeasts**: *Candida albicans* ATCC 10231 and *Rhodotorula rubra* (syn. *R. mucilaginosa*) ATCC 20129, *Malassezia furfur* ATCC 14521.

The dermatophytes, yeasts and *Ascosphaera apis* were supplied by the American Type Culture Collection (ATCC), USA. The plant pathogenic fungi were obtained from the Department of Phytopathology and Entomology, University of Warmia and Mazury in Olsztyn, Poland. Fungi causing microbial corrosion of materials were purchased from the Institute of Fermentation Technology and Microbiology, Łódź University of Technology. All fungal strains (with the exception of plant pathogenic fungi) were cultured on

Sabouraud Dextrose Agar (SDA, Oxoid) at 28°C for 4-7 days. The strains that cause plant diseases were kept on potato dextrose agar (PDA, Oxoid) at 25°C for 7-10 days. The test strains were kept on agar slants, stored in a refrigerator at 4°C and were transferred every 5-8 weeks to a fresh medium.

Yeasticidal activity

The assays against yeasts were carried out using the disc diffusion method which was applied for the determination of antibacterial and yeasticidal activity of the essential oil and the plant extracts^{39, 40}. The extracts from *C. cassia*, cinnamaldehyde and coumarin were dissolved in acetone to obtain the concentration of 5mg/ml. Sterile filter paper discs (5 mm in diameter) were impregnated with 50 µl of prepared extracts or reference substances and were then dried in a laminar flow cabinet at room temperature to evaporate the acetone. The dried paper discs were placed in 9 cm Petri dishes containing Sabouraud dextrose agar (SDA, Oxoid) cultured with the appropriate yeast strains. Petri dishes were placed at 4°C for 2 h to allow the extracts and constituents to diffuse into the medium and then incubated at 28°C for 48 hours. The disc soaked in 50 µl acetone and then dried, served as the negative control. As the positive control, ketoconazole (5 mg/ml), a commercially available fungicide was used. The diameters of the inhibition zones were measured in millimetres. The size of the inhibition zone was proportional to the inhibitory activity of the tested sample. The experimental studies were repeated three times.

Antifungal activity

The experiments were carried out according to a slightly modified standard method used in laboratory investigations of fungicides⁴¹. The tested compounds were dissolved in acetone and added to a sterile Sabouraud Dextrose Agar (SDA) or Potato Dextrose Agar (PDA) medium to obtain the concentration of 5 mg/ml. The control sample contained acetone only. The media were poured onto 90 mm Petri dishes. When acetone evaporated, 5mm discs of 5-7 -day-old test fungi were placed in the middle of the Petri dishes. The dishes were incubated in the dark at 25 ± 1°C (plant pathogenic fungi, *Ascosphaera apis*, dermatophytes) and 28 °C (filamentous fungi) for a period of 4-7 days. As a reference, ketoconazole was used. After incubation, the diameters of the cultures were measured. The growth of fungi on media containing the tested compound was compared to the control ones. The percentage of fungal growth inhibition was calculated according to the Abbot formula:

$$I (\%) = \frac{d_c - d_t}{d_c} \times 100$$

d_c : the diameter of mycelium growth in the control plate

d_t : the diameter of mycelium growth in the experimental dish (mm)

The following evaluation scale was used:

1-efficacy	0-20%	-not active
2-efficacy	21-50%	-weak activity
3-efficacy	51-80%	-medium activity
4-efficacy	81-100%	-good activity

Statistical analysis

The Scheffé method of SAS was used to analyse the difference of yeasticidal and antifungal properties among nine *C. cassia* bark extracts and their main components ($p < 0.05$)

RESULTS AND DISCUSION

Yields and chemical composition of the extracts from *C. cassia* bark

The effectiveness of active compounds extracted from *C. cassia* bark was found to be dependent on the kind of ionic liquids applied (Table 1). The highest amount of extract was obtained with the classical solvents: ethanol, ethyl acetate, hexane and dichloromethane. Slightly lower amounts of extract were obtained when using ionic liquids (E) or (A) and (D). The lowest amounts were obtained with the ionic liquid (B) which contains lactate as anion and didecylidimethyl as cation.

Table 1. Effectiveness of various ionic liquids and classical solvents for extraction of active compounds from *Cinnamomum cassia* bark.

Extract	Extraction solvent	Yield of extraction [g]
I	A	0.244
II	B	0.118
III	C	0.138
IV	D	0.236
V	E	0.35
VI	Hexane	0.405
VII	Ethyl acetate	1.237
VIII	Dichloromethane	0.65
IX	Ethanol	2.2

The chemical composition of extracts from the bark of *C. cassia* was analysed using GC-MS. The percentage content of each component in the analyzed extracts with the retention times is shown in Table 2.

Table 2. Chemical composition of the extracts from bark of *Cinnamomum cassia* prepared by different solvents (%).

Compounds	RT/min	Concentration (%)								
		Extract (see Table 1)								
		I	II	III	IV	V	VI	VII	VIII	IX
α -pinene	7.37	0.12	-	0.09	-	-	-	-	-	-
Benzaldehyde	8.34	0.32	0.52	0.1	-	-	0.21	-	-	-
1,8-cineole	10.69	0.3	0.22	0.6	0.05	-	0.19	-	-	-
Linalool	14.24	-	-	-	-	0.14	0.2	-	-	-
Terpinen-4-ol	16.06	-	0.07	0.21	-	0.56	0.58	0.38	-	-
Borneol	17.03	-	-	0.05	-	0.37	0.46	0.1	0.11	-
α -Terpineol	17.27	-	0.26	0.14	-	0.78	0.79	0.22	0.08	0.31
Cis-Cinnamaldehyde	18.77	-	-	-	-	2.06	0.68	0.13	0.06	-
Trans-Cinnamaldehyde	19.41	12.51	29.29	50.33	10.19	80.72	75.79	84.04	87.66	84.42
Bornyl acetate	19.73	0.52	2.17	1.24	-	-	-	-	-	-
Trans-Cinnamyl alcohol	20.53	0.16	-	-	-	0.58	0.54	-	-	-
Cubebene	21.84	0.13	0.38	0.17	0.3	-	-	0.06	0.07	0.16
α -Copaene	22.83	2.23	2.24	3.16	0.42	0.14	2.01	1.24	1.17	1.13
Bergamotene	24.03	0.45	0.45	0.59	0.08	0.07	0.72	0.31	0.3	0.67
β -Caryophyllene	24.30	1.94	1.19	1.26	0.18	0.13	0.83	0.75	0.81	0.97
Coumarin	24.88	0.35	1.31	1.80	0.98	11.77	8.25	10.80	7.10	5.99
α -Humulene	25.43	0.54	0.22	0.21	-	-	-	-	-	-
α -Amorphene	27.39	0.27	0.34	0.53	0.05	0.07	0.63	0.42	0.39	0.52
Trans -Calamenene	27.494	0.23	-	0.22	-	0.07	0.51	0.23	0.26	0.39
Trans-o-Metoxy cinnamaldehyde	27.76	-	-	-	-	0.36	0.42	-	-	-
Murrolene	27.63	-	0.07	0.28	-	0.06	0.79	0.35	0.48	0.3
Cadina-1,4-diene	27.85	0.18	0.06	0.13	-	-	0.11	-	-	-
Cubenol	30.74	-	0.2	0.24	-	0.2	0.4	0.13	-	0.08

- not detected

The chemical structures of the above-mentioned compounds are shown in Figure 2.

It was found that the main components in all the analyzed extracts are as follows: *trans*-cinnamaldehyde and coumarin, whose content in the extracts is (12.51% - 87.66%) and (0.35 % - 11.77%), respectively. There also is α -copaene (0.13% -3.16%) and β - caryophyllene (0.13% - 1.94%) present in large quantities.

The major constituents identified in this study have also been reported in some previous studies related to the chemical analyses of *C. cassia*. For instance, major compounds present in the ethyl acetate extract from the bark of *C. cassia* were cinnamaldehyde (38.81 mg/g), coumarin (6.5 mg/g), and copaene (5.27mg /g)⁴². Yang et al.⁴³ (2012) found that the major compounds isolated from the bark of *C.cassia* were cinnamaldehyde (57–69%), coumarin (4–21%), and naphthalene (3–14%). In another study, Jayatilaka et al.⁴⁴

(1995) also reported 25 compounds from the bark of cinnamon with *trans*-cinnamaldehyde (92–98%), *cis*-cinnamaldehyde (0.8–2.7%), β -caryophyllene (0.4–3.6%), coumarin (0.1–1.6%) and α -ylangene (0.1–2.7%) as their major compounds.

Figure 2. Chemical structures of the four major compounds identified in extracts from the bark of *Cinnamomum cassia*; (E)-cinnamaldehyde (F), coumarin (G), α -copaene (H), β -caryophyllene (I).

It was found that the extracts obtained with tris (2-hydroxyethyl) methylammonium methylsulfate (**E**) and organic solvents (hexane, ethyl acetate, dichloromethane, ethanol) contained the largest amount of *trans*-cinnamaldehyde. Furthermore, it was shown that the contents of coumarin in the extracts obtained with tris (2-hydroxyethyl) methylammonium methylsulfate (**E**) and ethyl acetate were higher than in other compositions. The best extractants of caryophyllene and copaene proved to be ionic liquids with benzalkonium cation (**A** and **C**). Slightly worse results in the extraction of terpenes were obtained using didecylmethyl lactate (**B**). *Trans*-cinnamaldehyde and coumarin belong to alkyl aryl carbonyl compounds and the other compounds belong to terpenes. The results of the experiment indicate that tris (2-hydroxyethyl) methylammonium methylsulfate (**E**) and organic solvents are the preferred option for extracting alkyl aryl carbonyl compounds and ionic liquids (**A**, **B**, **C**) are more suitable for extracting terpene compounds.

Antifungal and yeasticidal activity

The yeasticidal and antifungal activities of test extracts were examined at the concentration of 5mg/ml, and the results were presented in Table 3 and Table 4, respectively.

In general, extracts **V-IX**, revealed yeasticidal activities against *Candida albicans*, *Rhodotorula rubra* and *Malassezia furfur*. However, extracts **V** and **VI**, exhibited higher activities than extracts **VII-IX**. Extracts **I** and **IV** did not reveal any activity against the tested yeast strains. The most potent activity was revealed by extract **V** with the values higher than found for ketoconazole and the other reference substances.

Table 3. Diameter of growth inhibition zone (in mm) of yeasts strains treated with different extracts from *C. cassia* and reference substances.

Extract (see Table 1)	Zone of inhibition (mm)		
	Test strain		
	<i>Candida albicans</i>	<i>Rhodotorula rubra</i>	<i>Malassezia furfur</i>
I	0	0	0
II	12.73 $\pm$ 2.3	13.2 $\pm$ 2.8	14.5 $\pm$ 0.5
III	14.5 $\pm$ 0	15.0 $\pm$ 1.7	14.5 $\pm$ 0.2
IV	0	0	0
V	50 $\pm$ 0	50 $\pm$ 0	45.5 $\pm$ 0.9
VI	37.7 $\pm$ 0.96	39.0 $\pm$ 0	35.0 $\pm$ 0
VII	28.6 $\pm$ 0.34	30.5 $\pm$ 1.8	29.0 $\pm$ 0.4
VIII	25.0 $\pm$ 1.5	23.5 $\pm$ 0	23.5 $\pm$ 0.3
IX	22.2 $\pm$ 0.3	19.5 $\pm$ 1.5	23.0 $\pm$ 0
CA ^{a)}	20.75 $\pm$ 2.98	23.7 $\pm$ 2.5	20.0 $\pm$ 0
CU ^{b)}	26.0 $\pm$ 1.4	10.0 $\pm$ 0	25.0 $\pm$ 1.15
KET ^{c)}	37.5 $\pm$ 2.9	36.25 $\pm$ 2.5	18.3 $\pm$ 0.58

Reference substance; CA^{a)} - Cinnamaldehyde, CU^{b)} - Coumarin KET^{c)} - Ketoconazole

It was found that extracts **I** and **IV** had the weakest activity against plant and bee pathogenic fungi, as well as to fungi responsible for degradation of technical materials. The highest activity plant and bee pathogenic fungi, fungus of bees and corrosion fungal species was shown by extracts obtained with tris (2-hydroxyethyl) methylammonium methylsulfate (**E**) and hexane. Extracts obtained using ionic liquids with (**B**) and (**C**) showed slightly weaker activity against fungal plant pathogens and pathogens causing biodeterioration of technical materials than extracts obtained by tris (2-hydroxyethyl) methylammonium (**E**) and organic solvents. Extracts **III**, **V**, **VI-IX** showed strong activity against dermatophytes, while extracts **I** and **IV** showed weak or moderate effect. The excellent activity against the most tested fungi was also demonstrated by *trans*-cinnamaldehyde and coumarin. Previous literature has reported that cinnamaldehyde and coumarin were equally effective in inhibiting the growth of various isolates of filamentous moulds, yeasts, dermatophytes and plant pathogenic fungi⁴⁵⁻⁴⁷. The results showed that extracts from the bark of *C. cassia* **V-IX** demonstrate excellent antifungal activity associated with the presence of *trans*-cinnamaldehyde, coumarin as the main constituents. Furthermore, it was observed that the biological activity is directly proportional to the contents of the above-mentioned components in the extracts. Extracts **I-IV**, which are characterized by a relatively low content of coumarin and *trans*-cinnamaldehyde in their composition, exhibited the weakest biological activity. Although the **V-IX** extracts contain approximately equal amounts of *trans*-cinnamaldehyde and coumarin in their composition, extract **V** showed the strongest biological activity among them all. It is believed that this may be due to the presence of small amounts of other compounds, e.g. o-methoxy cinnamaldehyde and *cis*-cinnamaldehyde, or other compounds that may have the effect of increasing the fungicidal and yeasticidal activity of the extract. There are indications that these trace compounds play a key role in the antimicrobial properties, most likely due to synergistic effects with other components of the extracts. However, it is extremely difficult to investigate the synergistic effects and interdependencies between all the compounds present in the extracts.

CONCLUSION

Based on the results, it can be concluded that the extract from the bark of *C. cassia* obtained with tris (2-hydroxyethyl) methylammonium methylsulfate as solvent had a stronger antimicrobial activity than the other tested extracts. This study also demonstrated that tris (2-hydroxyethyl) methylammonium methylsulfate is the best solvent for the extraction of the main antimicrobial constituents *trans*-cinnamaldehyde, coumarin from cinnamon. Furthermore, it was found that ionic liquids with benzalkonium cation are the best solvents for extraction of terpene compounds (monoterpenes and sesquiterpenes). This *in vitro* study supports the role of the extracts from *C. cassia* as efficient, natural yeasticidal and antifungal agents.

Table 5. Effect of extracts from *C. cassia* and reference substances against fungi.

Test strain	Inhibition of test fungi growth (%)											
	Extract (see Table 1)									Reference substances		
	I	II	III	IV	V	VI	VII	VIII	IX	CAN ^{a)}	CU ^{b)}	KET ^{c)}
<i>Fusarium culmorum</i>	17±4.2	35±0.3	42±5.0	21±4.5	100	71±9.5	51±9.5	48±4.0	45±1.4	100	64±0.9	80±3.05
<i>Phytophthora cactorum</i>	0	50±1.3	57±9.3	16±4.0	68±5.4	60±1.3	61±13	67±1.5	50±1.8	78±2.9	58±0.7	68±7.5
<i>Fusarium graminearum</i>	15±2.0	32±0.9	40±3.2	30±5.0	100	69±4.5	58±2.5	60±3.2	55±2.4	27±2.0	67±0.7	51±2.8
<i>Rhizoctonia solani</i>	5±1.2	45±0.5	55±2.0	0	90±0	78±1.3	65±1.8	68±2.0	60±1.4	55±1.3	45±0.9	30±8.0
<i>Alternaria alternata</i>	5.4±0.5	35±0	49±9.0	11±6.0	88±0	70±0	63±0.4	67±2.0	58±2.5	59±4.7	68±0.5	68±2.51
<i>Geumannomyces graminis</i>	20±4.0	52±0.6	100	35±0	100	100	100	100	100	100	100	100
<i>Trichophyton mentagrophytes</i>	51±0.2	82±0.9	100	50±0	100	100	100	100	100	100	100	100
<i>Microsporum gypseum</i>	30±0.5	79±1.3	100	48±7.5	100	100	100	100	100	100	100	73±2.8
<i>Microsporum cookei</i>	32±0.9	70±0.7	100	50±6.0	100	100	100	100	100	100	100	75±2.6
<i>Scopulariopsis brevicalis</i>	10±3.6	30±1.4	32±1.5	22±1.5	93±1.0	72±2.3	42±2.8	48±2.3	45±1.3	100±0	30±2.9	50±2.3
<i>Aspergillus niger</i>	9±2.5	15±0.7	18±2.3	4.5±1.2	40±2.3	25±2.2	20±2.0	25±1.3	28±2.2	50±0.8	36±2.9	21±2.9
<i>Chaetomium globosum</i>	29±1.7	45±5.7	45±1.5	35±0.5	100	75±2.5	68±1.5	60±1.5	62±1.8	52±2.1	60±3.9	53±1.0
<i>Paecilomyces varioth</i>	0	29±3.5	30±3.2	25±2.8	68±5.0	46±3.4	36±3.4	40±3.5	42±1.8	50±2.0	60±3.9	66±4.0
<i>Ascosphaera apis</i>	0	20±4.9	31±0.3	0	100	77±14	67±14	60±1.0	55±0.5	100	100	45±2.9

Reference substance; CA^{a)} - Cinnamaldehyde, CU^{b)} - Coumarin KET^{c)} - Ketoconazole

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