Antifungal activity of essential oils from *Cinnamomum cassia*, *Myristica fragrans* and *Syzygium aromaticum* against *Rhodotorula mucilaginosa*

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Rhodotorula genus comprises yeasts from Sporidiobolaceae family. Considered as non-pathogenic until the last two decades, different species of *Rhodotorula* are emerging as human pathogens. *Rhodotorula mucilaginosa* is the most prevalent species commonly involved in infections which range from simpler clinical conditions such as skin manifestations to more severe cases as meningitis and endocarditis. The major facilitating agents for the emergence of these infections are invasive procedures such as catheter implants. The primary drugs of choice used to treat these infections are amphotericin B and fluconazole. However, some strains of this yeast show different degrees of resistance to these substances, thus justifying the search for new therapeutic agents. Considering this, the present study aims the investigation of the antifungal activity of the essential oils of *Cinnamomum cassia* (cinnamon), *Syzygium aromaticum* (clove) and *Myristica fragrans* (nutmeg) against clinical isolates of *R. mucilaginosa*. The essential oils were obtained by hydrodistillation and characterized by GC-MS. The investigation of the antifungal activity was performed by the agar diffusion test followed by the Minimum Inhibitory Concentration (MIC) determination. All the essential oils are characterized by the presence of phenylpropanoids, with eugenol (77.6 to 94.4%) as the main compound of clove and *E*-cinnamaldehyde (90.4 to 100%) of cinnamon. Nutmeg oil is characterized by the presence of the phenylpropanoids myristicin (1.8 to 12.8%) and elemicin (4.3 to 11.1%), besides the monoterpenes sabinene (28.2 to 44.4%) and terpinen-4-ol (16.0 to 19.5%). In the investigation of antifungal activity, all the oils showed potential action against clinical isolates of *R. mucilaginosa*, with MICs ranging from 8 to 500 µg/mL. The results demonstrate that these oils are promising candidates in the search for new anti-*Rhodotorula* agents, enabling the treatment of aforementioned infections.

Keywords: *Rhodotorula mucilaginosa*, essential oils, antifungal.

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Introduction

Rhodotorula is a yeast fungus commonly found in nature. It can be found in different types of samples, such as soil, water, milk, fruit juices, and in the air. Although it has been considered a non-pathogenic microorganism until the last two decades, different species of *Rhodotorula* are emerging as human pathogens. Invasive procedures such as catheter implantation, especially in immunosuppressed patients, are a major factor in the emergence of secondary infections, such as fungemias, endocarditis, meningitis, peritonitis, among others (1).

Rhodotorula spp. appear to be more frequent in tropical regions (2). The first case of fatal endocarditis attributed to species of this genus was published in the 1960s in a 47-year-old rheumatic woman with heart disease (3). Since then, other reports of infection in humans have been described in the scientific literature, mainly in the last two decades (4). *Rhodotorula mucilaginosa* is the most commonly involved in human infections, followed by *R. glutinis* and *R. minuta* (2). The first choice of treatment involves the administration of amphotericin B and fluconazole. A study conducted by Franconieri et al. (5), including eleven case studies of peritonitis caused by *R. mucilaginosa*, found that amphotericin B and fluconazole

had the strongest anti-*Rhodotorula mucilaginosa* activity. The tests performed to check its presence allow the visualization of the growth of its colonies after 24 to 48 hours of incubation, commonly showing smooth and mucous, reddish-orange appearance in Sabouraud dextrose agar culture medium with chloramphenicol. The maximum growth temperature varies from 25 to 37 °C (6,7).

Cinnamomum cassia (L.) J. Presl (cinnamon-of-china) is an aromatic plant, belonging to the Lauraceae family widely used in cooking and fragrance production (8). It originates in China and is widely cultivated in Indonesia, India, Malaysia, Laos, Taiwan, Thailand, and Vietnam (9). In folk medicine, it is used to treat inflammatory processes, fever, and hypertension. The essential oil has exhibited antifungal and antibacterial activity, in addition anti-inflammatory, antioxidant, anti-tyrosinase actions, among others (10–15). Regarding *E*-cinnamaldehyde, the main component of essential oil, the anti-inflammatory and antioxidant (16), antifungal (17) and antibacterial (18) activities have been proven.

Syzygium aromaticum L. Merr. & L.M. Perry (clove) is a large tree (12 to 15 meters tall), belonging to the Myrtaceae family and widely cultivated in Tanzania, Brazil, Sri Lanka, Indonesia, and Madagascar (19). It has

been popularly used due to its flavoring properties, in food preservation, oral hygiene products, and also in gastrointestinal disorders. Several studies show its importance as a bactericidal and fungicidal, antioxidant, and anti-inflammatory agent, among others (20–23). The antimicrobial activity observed may be related to eugenol, the main constituent of the essential oil of the floral bud of *S. aromaticum*, a compound for which antimicrobial activities have already been proven (24–26), in addition to anti-inflammatory and antioxidant (16).

Myristica fragrans Houtt. (nutmeg) is a tree in the Myristicaceae family that can reach up to 12 meters in height as an adult (27). The largest producer is Indonesia, followed by Granada, Sri Lanka and Trinidad and Tobago (28). This species has been used in cooking as a food spice, as a flavoring and also in treatments for diseases such as stomach pain, rheumatism and vomiting during pregnancy (29). For the nutmeg essential oil, antimicrobial, antiseptic, antiparasitic, anti-inflammatory and antioxidant activities have been demonstrated (29–32). To date, there are no reports in the specialized literature describing the action of these species against *Rhodotorula mucilaginosa*. Thus, this work aims to extract the essential oils from *Cinnamomum cassia*, *Syzygium aromaticum* and *Myristica fragrans*, perform their chemical characterization through GC-MS and evaluate their antifungal activity against *R. mucilaginosa*, looking for new antifungal prospects for the market.

Materials and Methods

Samples

Samples of *Cinnamomum cassia* (barks), *Syzygium aromaticum* (floral bud), and *Myristica fragrans* (fruits) were commercially acquired in the state of Rio Grande do Sul. The oils were obtained by hydrodistillation in a Clevenger type apparatus, according to the methodology recommended by the Brazilian Pharmacopoeia 6th ed. (2019).

The microorganisms used in this study are clinical isolates of *R. mucilaginosa* resistant to classic antifungals (approval by the Ethics Committee No. 4.069.576, CEP-UFRGS), provided by the Biomicolab Laboratory (Mycology Laboratory of the Federal University of Rio Grande do Sul). Five clinical isolates of *R. mucilaginosa* were selected. The stock cultures were kept in freezing soybean trypticase broth (TSB; Himedia®) plus 10% glycerol at -18 °C. To reactivate the strains, 10 µL of the stock culture were inoculated into tubes containing Sabouraud dextrose agar with chloramphenicol (Kasvi®) and incubated at 30 °C for 48-h. As positive controls, fluconazole (Polydrug Laboratoire Private Limited/India), ketoconazole (Aairt Drugs Limited/India), amphotericin B (North China Pharmaceutical/China) and itraconazole (Cecon/Brazil) were used.

Essential Oils Chemical Analysis

For chromatographic analysis, the oils obtained were diluted to 2% in ethyl ether (v/v) (Tédia®). The analysis was performed using a gas chromatograph coupled to a mass detector (GC-MS), model Shimadzu QP5000, equipped with a capillary column of fused silica Durabond-DB-5 (John Wiley & Sons Scientific, US, 30 m x 0.25 mm x 0.25 µm) for separating the constituents. The source temperature, quadrupole and injector were set at 230 °C, 150 °C and 220 °C, respectively. Detector temperature was set at 250 °C. The column temperature was programmed with a heating ramp from 60 °C to 300 °C with an increase of 3 °C / min, using ultrapure helium as the carrier gas at 80 kPa and flow of 1 mL/min. The mass detector was operating at 70 eV.

The components were identified by comparing their relative retention index, calculated by relative linear interpolation for the retention time of a series of *n*-alkanes (C₈ to C₃₂), and their mass spectrum, with data obtained in the literature (33), as well as, by comparison with mass spectrums of the acquisition spectrotheca (NIST 62 and 12 - National Institute of Standards and Technology, Kyoto, JP) and standards from our research group repository. Relative amounts of each compound were calculated from the peak areas by normalization.

Antifungal Activity

Initially, antifungal activity against *R. mucilaginosa* was investigated by the paper disc diffusion technique (Kirby Bauer method). The tests were performed on sterile Petry plates containing Sabouraud agar. In summary, a suspension in yeast Sabouraud agar (90%) was spread on the plate agar. Paper discs were impregnated with 15-µL of the essential oil and placed on the surface of the previously inoculated medium. The plates were incubated in an oven at 37 °C and the inhibition halos were read after 48-h. The measurement scale was as follows: the zone of inhibition >15 mm was considered to be strongly inhibitory; the zone of inhibition 10-15 mm moderately inhibitory and <10 mm non-inhibitory (34). The test was performed in duplicate. The essential oils that exhibited strong inhibition were subjected to the test to determine the Minimum Inhibitory Concentration (MIC). As for antifungals, it was performed with the itraconazole disc to verify the antifungal action of this compound on the yeast *R. mucilaginosa*.

The determination of the Minimum Inhibitory Concentration (MIC) against *R. mucilaginosa* was carried out by the broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute - CLSI (35). The oils were dissolved in dimethyl sulfoxide - DMSO (Nuclear®). The concentration range used for essential oils was 0.488 to 1000 µg/mL (36). The *R. mucilaginosa* culture (103 CFU/mL) in RPMI 1640 (Sigma Aldrich®) was added to the microplate wells to detect the action of the oil. The microplates were incubated at 30 °C and inspected after 24 and 48 h for visual verification of growth (35,37,38). The experiment

was carried out in duplicate. MIC was considered to be the lowest concentration of oil capable of completely inhibiting microbial growth visible to the naked eye. For results evaluation, the following cut-off points were used for the antifungals tested according to CLSI: fluconazole: sensitive (S) $\leq 8 \mu\text{g/mL}$, dose-dependent sensitive (SDD) $16\text{--}32 \mu\text{g/mL}$, resistant (R) $> 64 \mu\text{g/mL}$; itraconazole: S $\leq 0.12 \mu\text{g/mL}$, S-DD $0.25\text{--}0.5 \mu\text{g/mL}$, R $> 1 \mu\text{g/mL}$; amphotericin B: R $\geq 1 \mu\text{g/mL}$ (37). For ketoconazole, the following cut-off point S $\leq 1 \mu\text{g/mL}$, S-DD $2\text{--}4 \mu\text{g/mL}$, R $> 4 \mu\text{g/mL}$ was used.

Results and Discussion

The data regarding the samples are shown in Table 1. Three samples of cloves (identified by OSA1 to OSA3), five samples of cinnamon (OCC1 to OCC5) and two of nutmeg (OMF1 and OMF2) were considered. The percentage yield was calculated by the relationship between the volume of oil obtained by the dry mass of the plant. Considering the levels of essential oil obtained, there are some peculiarities, as in the case of *Syzygium aromaticum*, with a high variation in the oil content obtained from the three commercial samples (average of $9.8 \pm 4.03\%$). This result may be related to the supplier, as the samples OSA1 and OSA2 are from the same supplier and have the highest levels. Here, different suppliers may be associated with the variation of producers, of the storage conditions, among other factors that can be related to the yield variation. Regarding the average value of yield obtained, Kapadiya and Desai (39) obtained 11.35% yield for the essential oil extracted from the referred plant, corroborating the average value obtained in the present study.

Table 1. Percentage yield of essential oils obtained by hydrodistillation from *Syzygium aromaticum* (floral bud), *Cinnamomum cassia* (barks) and *Myristica fragrans* (fruits).

Species	Supplier	Acquisition date	Expiration date	Yield [%; p/v]
<i>S. aromaticum</i>				
OSA1	A	18/09/2018	19/03/2020	14.1
OSA2	A	02/10/2018	11/03/2020	9.3
OSA3	B	03/10/2018	Not informed	6.1
<i>C. cassia</i>				
OCC1	A	12/09/2018	20/07/2020	0.5
OCC2	C	14/09/2018	01/08/2020	0.2
OCC3	A	26/09/2018	11/06/2020	0.3
OCC4	A	27/09/2018	11/06/2020	0.2
OCC5	A	28/09/2018	20/07/2020	0.5
<i>M. fragrans</i>				
OMF1	A	24/09/2018	12/06/2020	1.7
OMF2	D	25/09/2018	06/07/2019	2.6

For the samples of *Cinnamomum cassia*, it is observed that the variation was not so great, presenting an average yield of $0.3 \pm 0.14\%$. This value is close to that found in the literature. Tao et al. (40) obtained an average yield of 1.48% in the essential oil extracted from *Cinnamomum*

cassia. Considering the suppliers there is no difference among them. Finally, for *Myristica fragrans*, the two samples showed a difference in their essential oil yield value with an average of $2.1 \pm 0.64\%$, which may be related also to the supplier. This yield value is slightly below that reported in the literature for that plant (41). Regarding the expiration date, all samples were within the validity period (the ones that had this information).

The chemical characterization of essential oils obtained by hydrodistillation was performed by GC-MS. The results are described in Table 2. All the species selected for this study are characterized by the presence of phenylpropanoids. The analysis of the *Syzygium aromaticum* samples highlighted the majority of eugenol (77.6 to 94.4%) followed by its eugenyl acetate derivative (4.8 to 20.7%). The samples of essential oil of *Cinnamomum cassia* presented *E*-cinnamaldehyde as the main constituent, sometimes accounting for the totality of the oil (90.4 to 100%). Finally, the essential oil of *Myristica fragrans* was characterized by the presence of myristicin (1.8 to 12.8%) and elemicin (4.3 to 11.1%). In the oil of this species, monoterpenes were also present in large quantities, represented mainly by sabinene (28.2 to 44.4%) and terpinen-4-ol (16.0 to 19.5%).

Souza et al. (42) studied the chemical composition of 16 essential oils, including those extracted from *Syzygium aromaticum*, *Cinnamomum cassia* and *Myristica fragrans*. The results associated with the composition of *S. aromaticum* oil reported in that study showed that it was mainly composed of eugenol (80.67%) and eugenyl acetate (11.92%). These values corroborate the analysis carried out in the present study and reported in Table 2, where an average concentration of 84% eugenol and 15% eugenyl acetate was found in clove oils. Still, according to these same authors, the essential oil of *Cinnamomum cassia* is composed mainly of *E*-cinnamaldehyde (84.52%), also in agreement with the results found in this study (Table 2), which verified an average value of 96.9% for the concentration of *E*-cinnamaldehyde. For oil extracted from *Myristica fragrans*, these authors also reported the presence of sabinene (16.54%), terpinen-4-ol (5.44%) and myristicin (15.1%), among other constituents, however it was not reported the presence of elemicin, a compound that also characterizes the essential oil of *M. fragrans*. On the other hand, in a recent work involving the characterization of essential oil of this species (43) the presence of this compound with an average content of 9.93% was observed. According to Table 2, the *M. fragrans* oils analyzed showed average concentrations of 36.3% sabinene, 17.8% terpinen-4-ol, 7.3% myristicin and 7.7% for elemicin, which is consistent with the literature data.

Table 2. Chemical characterization of essential oils obtained from different samples of *Syzygium aromaticum*, *Cinnamomum cassia* and *Myristica fragrans* by GC-MS.

RRI	Constituents	<i>Syzygium aromaticum</i>			<i>Cinnamomum cassia</i>					<i>Myristica fragrans</i>	
		OSA1	OSA2	OSA3	OCC1	OCC2	OCC3	OCC4	OCC5	OMF1	OMF2
923	tricycle	-	-	-	-	-	-	-	-	0.5	0.9
926	α -thujene	-	-	-	-	-	-	-	-	1.5	3.8
928	α -pinene	-	-	-	-	-	-	-	-	1.9	4.0
962	benzaldehyde	-	-	-	-	-	-	-	1.7	-	-
967	sabinene	-	-	-	-	-	-	-	-	28.2	44.4
971	β -pinene	-	-	-	-	-	-	-	-	3.3	5.0
990	mycrene	-	-	-	-	-	-	-	-	2.3	2.6
1003	α -felandrene	-	-	-	-	-	-	-	-	0.5	0.7
1008	δ -3-carene	-	-	-	-	-	-	-	-	1.2	1.7
1014	α -terpinene	-	-	-	-	-	-	-	-	2.0	2.2
1022	p-cimene	-	-	-	-	-	-	-	-	0.9	0.8
1025	limonene	-	-	-	-	-	-	-	-	7.0	7.9
1055	γ -terpinene	-	-	-	-	-	-	-	-	3.9	3.9
1085	terpinolene	-	-	-	-	-	-	-	-	1.2	-
1184	terpinen-4-ol	-	-	-	-	-	-	-	-	19.5	16.0
1281	<i>E</i> -cinnamaldehyde	-	-	-	98.0	93.2	100.0	100.0	90.4	-	-
1365	eugenol	77.6	80.0	94.4	-	-	-	-	-	-	-
1406	β -caryophyllene	1.6	0.5	0.9	-	-	-	-	-	1.9	trace
1447	cinamyl <i>E</i> -acetate	-	-	-	2.0	0.2	-	-	-	-	-
1487	cinnamic acid	-	-	-	trace	-	trace	Trace	7.9	-	-
1519	myristicin	-	-	-	-	-	-	-	-	12.8	1.8
1528	eugenyl acetate	20.7	19.6	4.8	-	-	-	-	-	-	-
1538	<i>E</i> -o-methoxy cinnamaldehyde	-	-	-	trace	-	-	-	-	-	-
1556	elemicin	-	-	-	-	-	-	-	-	11.1	4.3

Compounds listed in order of elution in DB5 column; RRI: relative retention index. OSA: essential oil of *Syzygium aromaticum*, OCC: essential oil of *Cinnamomum cassia*, OMF: essential oil of *Myristica fragrans*.

The investigation of antifungal activity was performed to verify the susceptibility of *R. mucilaginosa* yeast to *Syzygium aromaticum* (OSA), *Cinnamomum cassia* (OCC) and *Myristica fragrans* (OMF) oils. Pooling was performed to determine the samples used in the test. Initially, a screening was carried out to select the samples that demonstrated action on yeast. For this purpose, the Kirby Bauer method was used which consists of impregnating paper discs with the oil samples to be tested. Itraconazole was used to check the susceptibility of the microorganism. The results obtained in this stage are shown in Figure 1, and Table 3 describes the diameters of the inhibition halos for each essential oil and for itraconazole. It can be seen that *R. mucilaginosa* was resistant to itraconazole, but sensitive to the three oils used. Thus, the three oils were sent to the next step, which consisted of determining the minimum inhibitory concentration (MIC). At this time, the antifungal agents itraconazole (ITR), amphotericin B (AMP), ketoconazole (KET), fluconazole (FLU) were also used to check yeast susceptibility.

The MIC for each sample was determined in duplicate and on two different days. The concentration ranges tested were: 0.488 - 1000 $\mu\text{g/mL}$ for essential oil samples; 0.0312 - 16 $\mu\text{g/mL}$ of ANF; 0.03 - 16 $\mu\text{g/mL}$ CET; 0.125 - 64 $\mu\text{g/mL}$ of FLU and 0.0312 - 16 $\mu\text{g/mL}$

of ITR. MICs values were defined as the lowest concentration of oil and amphotericin B samples in which *R. mucilaginosa* did not show visible growth. For itraconazole (ITR), ketoconazole (KET), fluconazole (FLU) it was defined as being 50% of the positive control. This value represents the drug concentration that inhibits the growth of 50% of the isolates. In general, all essential oils tested showed promising activity, with MICs ranging from 8 to 500 $\mu\text{g/mL}$ (Table 3).

Table 3. Inhibition halos size (values in mm) of the *Syzygium aromaticum*, *Cinnamomum cassia* and *Myristica fragrans* essential oils and Itraconazole against five clinical isolates of the yeast *R. mucilaginosa*.

Clinical isolate	OSA	OCC	OMF	ITR
#1	28	40	15	R
#2	25	40	18	R
#3	25	40	10	R
#4	30	40	15	R
#5	35	40	20	R

OSA: essential oil of *Syzygium aromaticum*, OCC: essential oil of *Cinnamomum cassia*, OMF: essential oil of *Myristica fragrans*. ITR: itraconazole. R: resistant.

Considering the classic antifungals, it was observed that all clinical isolates were resistant. It is interesting to note

the difference in response according to the clinical isolate, which can be related to variations in intrinsic or acquired resistance, possibly induced by the environment. Among the essential oil samples tested, the one that showed the best result was *Cinnamomum cassia*, for which all clinical isolates tested were susceptible,

with a MIC value of 8 $\mu\text{g/mL}$ being observed. The oil of *Myristica fragrans* showed the best result for isolate 4 (MIC 8 $\mu\text{g/mL}$). The oil of *Syzygium aromaticum* showed action against all isolates, with MIC ranging from 62 to 125 $\mu\text{g/mL}$.

Figure 1. A) Results associated with clinical isolate 1 from essential oil of *Syzygium aromaticum* L. Merr. (OSA), *Cinnamomum cassia* (L.) J. Presl (OCC), *Myristica fragrans* Houtt (OMF) and itraconazole (ITR), from left to right, respectively; B) results associated with clinical isolate 2 from OSA, OCC, OMF and ITR; C) results associated with clinical isolate 3 from OSA, OCC, OMF and ITR; D) results associated with clinical isolate 4 from OSA, OCC, OMF and ITR; E) results associated with clinical isolate 5 from OSA, OCC, OMF and ITR.

The essential oil extracted from *Cinnamomum cassia* has been the subject of scientific studies due to its biological properties. In a study conducted by Cavalcanti and Almeida (10), the oil of this species demonstrated antifungal activity against strains of *Candida albicans*

isolated from HIV positive patients and standard strain (ATCC 76845), with a MIC value of 64 $\mu\text{g/mL}$ being observed for the most part of the strains analyzed. MA et al. (9), have already proved the antifungal property of *C. cassia* oil against pathogenic fungi present in *Panax*

notoginseng, a plant widely used in traditional Chinese medicine. Research specifically focused on the action of cinnamaldehyde has shown action against *Malassezia pachydermatis* with MIC of up to 2.5 µg/mL (44) and against clinical isolates of *Candida* resistant to fluconazole, presenting MIC of 90 up to 100 µg/mL (45). Such results corroborate those presented in this work, where there was a potential antifungal action of the essential oil of *C. cassia* against *R. mucilaginosa*. Considering that some of the tested samples present the oil composed of 100% E-cinnamaldehyde, it can be said that this phenylpropanoid is responsible for the action observed against *R. mucilaginosa*.

Several scientific studies have reported the antifungal activity of the essential oil obtained from *Syzygium aromaticum* (46–50). Rajkowska et al. (50), for example, evaluated the antifungal action of *Syzygium aromaticum* against nine species of *Candida*, with MIC values varying between 0.03 and 8%. In the research conducted by Pinto et al. (48), antifungal activity of *S. aromaticum* oil was confirmed against strains of some species of *Candida*, *Aspergillus* and dermatophytes. As reported, these results were related to the high concentration of eugenol present in the essential oil. In another study, the action of eugenol alone against *Candida albicans* and in combination with amphotericin B was demonstrated, showing synergistic potential, enabling the use of lower concentrations of this substance in the treatment of *C. albicans* (51). Such findings are in agreement with the results presented in this work, demonstrating the potential antifungal effect of the oil against several species of fungi.

The antifungal action of *Myristica fragrans* essential oils has also been demonstrated against strains of *Aspergillus niger*, *Fusarium axysporum*, *Pinicillium glabrum*, *Rhizopus oryzae* and *Mucor recemosus* (28) as well as against *Candida albicans* (52). These results corroborate the present study, where antifungal activity against *Rhodotorula mucilaginosa* was also proven (Table 4).

Table 4. Minimum Inhibitory Concentration (MIC) (values in µg/mL) of the *Syzygium aromaticum*, *Cinnamomum cassia* and *Myristica fragrans* essential oils against five clinical isolates of the yeast *R. mucilaginosa*.

Clinical isolate	OSA	OCC	OMF	AMP	KET	FLU	ITR
#1	250	62	500	16	16	64	16
#2	62	125	500	64	16	64	16
#3	125	NT	500	64	16	64	16
#4	62	31	8	64	16	64	16
#5	125	8	31	16	16	64	16

OSA: essential oil of *Syzygium aromaticum*, OCC: essential oil of *Cinnamomum cassia*, OMF: essential oil of *Myristica fragrans*. AMP: amphotericin B, KET: ketoconazole, FLU: fluconazole; ITR: itraconazole. NT: not tested.

Some studies have shown that essential oils that contain cinnamaldehyde, thymol, carvacrol or eugenol have high antimicrobial activity (42,53). Thus, the presence of E-cinnamaldehyde and eugenol identified in the chemical

composition of the *Cinnamomum cassia* and *Syzygium aromaticum* essential oils, respectively, may be associated with the potential antifungal activity against *R. mucilaginosa* observed in the present study, aiming at the prospect of new therapeutic agents.

It has already been found that the same essential oil can present different antifungal activity and mechanism of action when it is tested against different pathogens (54,55). Such a result may be associated with the complexity and diversity of antifungal mechanisms (9). According to studies in the field, the mechanism of antifungal action of the *Cinnamomum cassia* essential oil may be associated with the CHO group present in the molecule of the cinnamaldehyde. Thus, considering the fact that CHO group is hydrophilic, the cinnamaldehyde can easily absorb the hydrophilic constituents of the surface of the fungi, facilitating its penetration into the cell wall and, consequently, causing the death of the cell (9). For the essential oil of *Syzygium aromaticum*, the mechanism of antifungal action may be related to eugenol, its main constituent, and its ability to disturb the integrity and permeability of the fungal cell membrane, which may cause irreversible damage to the cell. In addition, such a constituent can destroy yeast proteins and inhibit their synthesis (56). The presence of phenylpropene terpinen-4-ol and phenylpropanoid elemicin among the main constituents of *Myristica fragrans* essential oil may be related to the antifungal action verified for this substance in the present study. According to results reported in the literature, the mechanism of antifungal action of phenylpropenes is potentially associated with their lipophilicity, which allows them to act on the permeability of the cell membrane, in addition to inhibiting specific cellular processes or enzymes (57).

Conclusions

This study reports for the first time the activity of the essential oil extracted from *Syzygium aromaticum*, *Cinnamomum cassia* and *Myristica fragrans* against clinical isolates of *R. mucilaginosa*, demonstrating the originality of the study. The observed results allowed to verify the potential effect of the tested oils against this yeast, presenting MIC of up to 8 µg/mL. Chemically, the essential oils of these species are characterized by the presence of phenylpropanoids such as eugenol, E-cinnamaldehyde, myristicin and elemicin, and phenylpropene as terpinen-4-ol, compounds with recognized antifungal action and that are probably responsible for the observed action. Thus, the results presented in this article are promising and justify the continuation of the work in this area. As a proposal for future work is the analysis of the genotoxicity of oils extracted from these plants.

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Conflict of interest

The authors declare no conflicts of interest.

References

1. Wirth F, Goldani LZ. Epidemiology of *Rhodotorula*: An Emerging Pathogen. *Interdiscip Perspect Infect Dis*. 2012;1–7.
2. Alves KP. Realidade e desafios de infecções emergentes por *Rhodotorula* spp. em pacientes hospitalizados. Universidade Federal de Santa Catarina; 2019.
3. Louria DB, Greenberg SM, Molander DW. Fungemia caused by certain nonpathogenic strains of the family Cryptococcaceae. *N Engl J Med*. 1960;263(25):1281–4.
4. Tuon FF, Duboc de Almeida GM, Costa SF. Central venous catheter-associated fungemia due to *Rhodotorula* spp. - A systematic review. *Med Mycol*. 2007;45(5):441–7.
5. Franconieri F, Bonhomme J, Doriot A, Bonnamy C, Ficheux M, Lobbedez T, et al. Fungal peritonitis caused by *Rhodotorula mucilaginosa* in a CAPD patient treated with liposomal amphotericin B: A case report and literature review. *Perit Dial Int*. 2018;38(1):69–73.
6. Rippon JW. *Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes*. Philadelphia: W B Saunders Co; 1992. 707-729 p.
7. De Hoog GS, Guarro J, Gene J, Figueras MJ. *Atlas of clinical fungi*. 2nd ed. Amer Society for Microbiology; 2001. 1160 p.
8. Gomes MC, Firmino AV, de Almeida SSM da S. Efeito inibitório in vitro de extratos de *Cinnamomum zeylanicum blume* no controle de *Cylindrocladium candelabrum*. *Ciência Florest*. 2018;28(4):1559–67.
9. Ma YN, Chen CJ, Li Q, Wang W, Xu FR, Cheng YX, et al. Fungicidal activity of essential oils from *Cinnamomum cassia* against the pathogenic fungi of *Panax notoginseng* diseases. *Chem Biodivers*. 2019;16(11).
10. Almeida LFD, Cavalcanti YW, Castro RD, Lima EO. Atividade antifúngica de óleos essenciais frente à *Candida albicans* isoladas de pacientes HIV positivos. *Rev Bras Plantas Med*. 2011;(Atcc 76845):649–55.
11. Chang CT, Chang WL, Hsu JC, Shih Y, Chou ST. Chemical composition and tyrosinase inhibitory activity of *Cinnamomum cassia* essential oil. *Bot Stud*. 2013;54(1):2–8.
12. Chou ST, Chang WL, Chang CT, Hsu SL, Lin YC, Shih Y. *Cinnamomum cassia* essential oil inhibits α -MSH-induced melanin production and oxidative stress in murine B16 melanoma cells. *Int J Mol Sci*. 2013;14(9):19186–201.
13. Sun L, Zong SB, Li JC, Lv YZ, Liu LN, Wang ZZ, et al. The essential oil from the twigs of *Cinnamomum cassia* Presl alleviates pain and inflammation in mice. *J Ethnopharmacol* [Internet]. 2016;194:904. Available from: <http://dx.doi.org/10.1016/j.jep.2016.10.064>
14. Raffaella C, Casettari L, Fagioli L, Cespi M, Bonacucina G, Baffone W. Activity of essential oil-based microemulsions against *Staphylococcus aureus* biofilms developed on stainless steel surface in different culture media and growth conditions. *Int J Food Microbiol* [Internet]. 2017;241:132–40. Available from: <https://doi.org/10.1016/j.ijfoodmicro.2016.10.021>
15. Veloso DJ, Abrão F, Martins CHG, Bronzato JD, Gomes BPFA, Higino JS, et al. Potential antibacterial and anti-halitosis activity of medicinal plants against oral bacteria. *Arch Oral Biol*. 2020;110.
16. Mateen S, Shahzad S, Ahmad S, Naeem SS, Khalid S, Akhtar K, et al. Cinnamaldehyde and eugenol attenuates collagen induced arthritis via reduction of free radicals and pro-inflammatory cytokines. *Phytomedicine* [Internet]. 2018;53:70–8. Available from: <https://doi.org/10.1016/j.phymed.2018.09.004>
17. Sun Q, Li J, Sun Y, Chen Q, Zhang L, Le T. The antifungal effects of cinnamaldehyde against *Aspergillus niger* and its application in bread preservation. *Food Chem* [Internet]. 2020;317:1–31. Available from: <https://doi.org/10.1016/j.foodchem.2020.126405>
18. Kenawy E, Omer AM, Tamer TM, Elmeligy MA, Eldin MSM. International Journal of Biological Macromolecules Fabrication of biodegradable gelatin / chitosan / cinnamaldehyde crosslinked membranes for antibacterial wound dressing applications. *Int J Biol Macromol* [Internet]. 2019;139:440–8. Available from: <https://doi.org/10.1016/j.ijbiomac.2019.07.191>
19. Ferrao JEM. *Especiarias : cultura, tecnologia, comercio*. Lisboa: Instituto de Investigacao Cientifica Tropical; 1993.
20. De Oliveira RA, Reis TV, Do Sacramento CK, Duarte

- LP, De Oliveira FF. Constituintes químicos voláteis de especiarias ricas em eugenol. *Brazilian J Pharmacogn*. 2009;19(3):771–5.
21. Beltrán-Villalobos KL, Déciga-Campos M, Aguilar-Mariscal H, González-Trujano ME, Martínez-Salazar MF, Ramírez-Cisneros M de los Á, et al. Synergistic antinociceptive interaction of *Syzygium aromaticum* or *Rosmarinus officinalis* coadministered with ketorolac in rats. *Biomed Pharmacother* [Internet]. 2017;94:858–64. Available from: <http://dx.doi.org/10.1016/j.biopha.2017.07.166>
22. Ma YN, Xu FR, Chen CJ, Li QQ, Wang MZ, Cheng YX, et al. The beneficial use of essential oils from buds and fruit of *Syzygium aromaticum* to combat pathogenic fungi of *Panax notoginseng*. *Ind Crops Prod* [Internet]. 2019;133(November 2018):185–92. Available from: <https://doi.org/10.1016/j.indcrop.2019.03.029>
23. Radünz M, da Trindade MLM, Camargo TM, Radünz AL, Borges CD, Gandra EA, et al. Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil. *Food Chem* [Internet]. 2019;276:180–6. Available from: <https://doi.org/10.1016/j.foodchem.2018.09.173>
24. Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, et al. Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*. 2019;24:1–12.
25. Ju J, Xie Y, Yu H, Guo Y, Cheng Y, Zhang R. Synergistic inhibition effect of citral and eugenol against *Aspergillus niger* and their application in bread preservation. *Food Chem* [Internet]. 2020;310:1–7. Available from: <https://doi.org/10.1016/j.foodchem.2019.125974>
26. Qian W, Sun Z, Wang T, Yang M, Liu M, Zhang J, et al. Antimicrobial activity of eugenol against carbapenem-resistant *Klebsiella pneumoniae* and its effect on biofilms. *Microb Pathog*. 2020;139:1–9.
27. Cho JY, Choi GJ, Son SW, Jang KS, Lim HK, Lee SO, et al. Isolation and antifungal activity of lignans from *Myristica fragrans* against various plant pathogenic fungi. *Pest Manag Sci*. 2007;63:935–940.
28. Rodianawati I, Hastuti P, Cahyanto MN. Nutmeg's (*Myristica Fragrans* Houtt) oleoresin: Effect of heating to chemical compositions and antifungal properties. *Procedia Food Sci* [Internet]. 2015;3:244–54. Available from: <http://dx.doi.org/10.1016/j.profoo.2015.01.027>
29. Muchtaridi, Subarnas A, Apriyantono A, Mustarichie R. Identification of compounds in the essential oil of nutmeg seeds (*Myristica fragrans* Houtt.) that inhibit locomotor activity in mice. *Int J Mol Sci*. 2010;11(11):4771–81.
30. Baser KHC, Buchbauer G. *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press; 2009. 991 p.
31. Pillai S, Mahmud R, Lee WC, Perumal S. Anti-parasitic activity of *Myristica fragrans* Houtt. essential oil against *Toxoplasma gondii* parasite. *APCBEE Procedia* [Internet]. 2012;2:92–6. Available from: <http://dx.doi.org/10.1016/j.apcbee.2012.06.017>
32. Das S, Kumar Singh V, Kumar Dwivedy A, Kumar Chaudhari A, Upadhyay N, Singh A, et al. Assessment of chemically characterised *Myristica fragrans* essential oil against fungi contaminating stored scented rice and its mode of action as novel aflatoxin inhibitor. *Nat Prod Res* [Internet]. 2018;1–5. Available from: <https://doi.org/10.1080/14786419.2018.1519826>
33. Adams RP. *Identification of Essential Oils by Ion Trap Mass Spectroscopy*. New York: Academic Press; 1989.
34. Carović-Stanko K, Orlić S, Politeo O, Strikić F, Kolak I, Milos M, et al. Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. *Food Chem*. 2010;119(1):196–201.
35. CLSI. *Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing*. 2007.
36. Magiatis P, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S. Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. chia. *Planta Med*. 1999;65(8):749–52.
37. CLSI. *Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard — third edition*. M27-A3. Wayne. PA, USA; 2008.
38. Oliveira LBS, Batista AHM, Fernandes FC, Sales GWP, Nogueira NAP. Atividade antifúngica e possível mecanismo de ação do óleo essencial de folhas de *Ocimum gratissimum* (linn.) sobre espécies de *Candida*. *Rev Bras Plantas Med*. 2016;18(2):511–23.
39. Kapadiya S, Desai MA. Isolation of Essential Oil From Buds of *Syzygium aromaticum* Using Hydrodistillation: Multi-Response Optimization and Predictive Modelling. *Int J Adv Res Sci Eng*. 2017;11:405–18.
40. Tao D, Li Y, Lu D, Luo Y, Yu S, Ye S. The essential

- oil components of *Cinnamomum cassia*: an analysis under different thinning models of plantation *Pinus massoniana*. J For Res. 2015;
41. Matulyte I, Jekabsone A, Jankauskaite L, Zavistanaviciute P, Sakiene V, Bartkiene E, et al. The essential oil and hydrolats from *Myristica fragrans* seeds with magnesium aluminometasilicate as excipient: Antioxidant, antibacterial, and anti-inflammatory Activity. Foods. 2020;37(9).
 42. Souza AA, Dias NAA, Piccoli RH, Bertolucci SKV. Composição química e concentração mínima bactericida de dezesseis óleos essenciais sobre *Escherichia coli* enterotoxigênica. Rev Bras Plantas Med. 2016;18(1):105–12.
 43. Zhao X, Wu H, Wei J, Yang M. Quantification and characterization of volatile constituents in *Myristica fragrans* Houtt . by gas chromatography-mass spectrometry and gas chromatography quadrupole-time-of-flight mass spectrometry. Ind Crop Prod [Internet]. 2019;130(151):137–45. Available from: <https://doi.org/10.1016/j.indcrop.2018.12.064>
 44. Schlemmer KB, Jesus FPK, Tondolo JSM, Weiblen C, Azevedo MI, Machado VS, et al. In vitro activity of carvacrol, cinnamaldehyde and thymol combined with antifungals against *Malassezia pachydermatis*. J Mycol Med [Internet]. 2019;29(4):375–7. Available from: <https://doi.org/10.1016/j.mycmed.2019.08.003>
 45. Shreaz S, Bhatia R, Khan N, Muralidhar S, Basir SF, Manzoor N, et al. Spice oil cinnamaldehyde exhibits potent anticandidal activity against fluconazole resistant clinical isolates. Fitoterapia [Internet]. 2011;82(7):1012–20. Available from: <http://dx.doi.org/10.1016/j.fitote.2011.06.004>
 46. Ranasinghe L, Jayawardena B, Abeywickrama K. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M.Perry against crown rot and anthracnose pathogens isolated from banana. Lett Appl Microbiol. 2002;35(3):208–11.
 47. Park M, Gwak K, Yang I, Choi W, Jo H, Chang J, et al. Antifungal activities of the essential oils in *Syzygium aromaticum* (L.) Merr. Et Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. J Microbiol. 2007;45(5):460–5.
 48. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and *dermatophyte* species. J Med Microbiol. 2009;58(11):1454–62.
 49. Nardoni S, Najar B, Fronte B, Pistelli L, Mancianti F. In vitro activity of essential oils against *Saprolegnia parasitica*. Molecules. 2019;24(7):6–13.
 50. Rajkowska K, Nowicka-Krawczyk P, Kunicka-Styczynska A. Effect of clove and thyme essential oils on *Candida* biofilm formation and the oil distribution in yeast cells. Molecules. 2019;24(10):1–12.
 51. Khan SN, Khan S, Misba L, Sharief M, Hashmi A, Khan AU. Synergistic fungicidal activity with low doses of eugenol and amphotericin B against *Candida albicans*. Biochem Biophys Res Commun [Internet]. 2019;518(3):459–64. Available from: <https://doi.org/10.1016/j.bbrc.2019.08.053>
 52. Iyer M, Gujjari AK, Gowda V, Angadi S. Antifungal response of oral-associated candidal reference strains (American Type Culture Collection) by supercritical fluid extract of nutmeg seeds for geriatric denture wearers: An in vitro screening study Meenakshi. J Indian Prosthodont Soc. 2019;17(3):267–72.
 53. Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol. 2005;22(4):273–92.
 54. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem. 2005;90:333–40.
 55. Miguel MG. Antioxidant and Anti-Inflammatory activities of essential oils: A short review. Molecules. 2010;15:9252–87.
 56. Cai R, Hu M, Zhang Y, Niu C, Yue T, Yuan Y. Antifungal activity and mechanism of citral , limonene and eugenol against *Zygosaccharomyces rouxii*. LWT - Food Sci Technol [Internet]. 2019;106(January):50–6. Available from: <https://doi.org/10.1016/j.lwt.2019.02.059>
 57. Sukcharoen O, Sirirrote P, Thanaboripat D. Management of aflatoxigenic strains via vapor and contact treatment with essential oil obtained from *Myristica Fragrans*. Biotropia (Bogor). 2019;26(1):16–22.