ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS FROM NATIVE TREE SPECIES IN SOUTHERN BRAZIL

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Resumo

Atividade antifúngica de óleos essenciais de espécies arbóreas nativas do sul do Brasil. O objetivo deste trabalho exploratório foi analisar o rendimento e composição química dos óleos essenciais (OE) de *Blepharocalyx salicifolius* [BS] e de dois *pools* de OE obtidos de *Nectandra megapotamica* [NM_1 e NM_2], bem como avaliar suas propriedades antifúngicas frente aos fungos *Pycnoporus sanguineus* (podridão-branca) e *Gloeophyllum trabeum* (podridão-parda). Os OE foram obtidos por hidrodestilação e seus componentes foram identificados por cromatografia gasosa acoplada à espectrometria de massas (CG-EM) e quantificados com detecção por ionização em chama (CG-DIC). Para a avaliação da atividade antifúngica, foi determinado o índice de crescimento micelial (ICM) e a inibição do crescimento micelial (IC%) de BS nas concentrações de 1, 2 e 5 $\mu L.mL^{-1}$, e 0,25; 0,5 e 1 $\mu L.mL^{-1}$ para MN_1 e NM_2. O rendimento dos OEs foi de 2,29% (BS), 0,14 % (NM_1) e 0,17 % (NM_2). Os componentes majoritários do OE de BS foram eucaliptol (63,44%) e β-cariofileno (13,68%), de NM_1 foram biciclogermacreno (16,14%) e α-pineno (15,93%), e para NM_2, α-pineno (19,04%) e sabineno (16,66%). O OE NM_1 ($1\mu L.mL^{-1}$) apresentou o melhor resultado para a atividade antifúngica, inibindo cerca de 57,55 ± 0,68 % do crescimento de *P. sanguineus* e 58,82 ± 0,78 % no teste com *G. trabeum.*

Palavras-chave: extrativo vegetal, Lauraceae, Myrtaceae, fungo apodrecedor da madeira.

Abstract

The objective of this exploratory work was to analyze the yield and chemical composition of essential oils (EO) from *Blepharocalyx salicifolius* [BS] and from two pools of EO obtained from *Nectandra megapotamica* [NM1 and NM2], as well as to evaluate their antifungal properties against the fungi *Pycnoporus sanguineus* (white-rot) and *Gloeophyllum trabeum* (brown-rot). The EO were obtained by hydrodistillation and its components were identified by gas chromatography coupled to mass spectrometry (GC-MS) and quantified with flame ionization detection (GC-FID). For the evaluation of antifungal activity, the mycelial growth index (MGI) and the mycelial growth inhibition (GI) of BS were determined at concentrations of 1, 2 and 5 $\mu L.mL^{-1}$, and 0.25; 0.5 and 1 $\mu L.mL^{-1}$ for MN1 and NM2. The yield of EOs was 2.29% (BS), 0.14% (NM1) and 0.17% (NM2). The major components of BS EO were eucalyptol (63.44%) and β-caryophyllene (13.68%), from NM1 were bicyclogermacrene (16.14%) and α-pinene (15.93%), and for NM2, α-pinene (19.04%) and sabinene (16.66%). EO NM1 ($1\mu L.mL^{-1}$) showed the best result for antifungal activity, inhibiting about 57.55 ± 0.68 % of *P. sanguineus* growth and 58.82 ± 0.78 % in the test with *G. trabeum*.

Keywords: Plant extractive, Lauraceae, Myrtaceae, Wood rotting fungus.

INTRODUCTION

Essential oils (EOs) stand out as an effective and environmentally safe alternative to control fungal agents. There are several studies in the literature that report the activity of numerous plant extractives against wood-deteriorating microorganisms (SILVA *et al.*, 2016; TCHINDA *et al.*, 2018; XIE *et al.*, 2017). Essential oils are complex mixtures of volatile organic substances, consisting of oxygenated components and hydrocbons, such as sesquiterpenes and monoterpenes (NERIO *et al.*, 2010). Among the EO-producing species are *Nectandra megapotamica* (Spreng.) Mez. (*N. megapotamica*) and *Blepharocalyx salicifolius* (Kunth) O. Berg. (*B. salicifolius*). These tree species belong to the Lauraceae and Myrtaceae families, respectively, and are native to the southern region of Brazil.

Wood is a material of biological origin, consisting basically of natural polymers of cellulose, hemicellulose and lignin. Once it is an organic, heterogeneous and biodegradable material, it is highly susceptible to attack by xylophagous organisms, such as wood-rotting fungi, which use natural polymers from the cell wall as a source of nutrition. Among the wood-rotting fungi, those belonging to the class of basidiomycetes stand out.

Among the phytopathogens that cause brown-rot, *Gloeophyllum trabeum* (Pers.: Fr) Murrill (*G. trabeum*) is highlighted, and *Pycnoporus sanguineus* (L.: Fr.) Murr, as a representative of white-rot. (*P. sanguineus*) (STANGERLIN *et al.*, 2013; BENTO *et al.*, 2014; CAI *et al.*, 2019). There are several chemicals that are effective in combating these pathogens. However, most of them contain heavy metals in their composition and are highly toxic to both the environment and animals, which led to the ban on the use of this class of wood preservatives in several countries (CHITTENDEN; SINGH, 2011; XIE *et al.*, 2017).

Considering the need to develop wood preservatives with less toxicity compared to products conventionally used by the wood industry, the antimicrobial potential of EO makes this class of plant extractives good candidates to meet this demand. Thus, hypothesizing that the forest species *B. salicifolius* and *N. megapotamica* have potential for the production of EO with fungitoxic activity on the in vitro growth of rotting fungi, the objective of the present work was to evaluate the potential of leaves of these two species for the EO production and test its fungitoxic activity against wood-rotting fungi.

MATERIALS AND METHODS

Obtaining the essential oil

Leaves of *B. salicifolius* and *N. megapotamica* were collected during the summer, between 9:00 and 11:00, in Santa Maria, Rio Grande do Sul state, Brazil, in fragments of a Seasonal Deciduous Forest. The trees showed no flowering or fruiting. To obtain the EO, immediately after collection, fresh leaves collected from trees of both species were fractionated and submitted to hydrodistillation in a modified Clevenger-type apparatus for 3 hours. After obtaining the extractive, the extraction yield (%) and density ($g.mL^{-1}$) of the EO were calculated. The extractives were stored in amber glass vials, sealed and kept at -4 °C until the bioassays were carried out and the chemical composition analyzed.

The EO of *N. megapotamica* used in this work are the result of two pools, composed of EO samples from leaves collected in the same city mentioned above, but from different individuals and in different locations. The two pools will be named NM1 and NM2, and the EO of *B. salicifolius* will be named BS. The plant material used to obtain the EO NM1 was collected in a fragment of native forest near km 318 of the BR 158 road, while the collection of plant material to obtain the EO NM2 was carried out in Morro do Elefante. The *N. megapotamica* collection sites are approximately 5 km apart. The plant material to obtain the EO BS was collected in a fragment of native forest near RS 509 road, km 06. The plant material used to obtain the plant extracts was randomly collected from different trees at each collection site.

Chemical analysis of essential oils

The chemical composition of the EO was determined by gas chromatography-mass spectrometry (GC-MS) in an Agilent 7890A hyphenated system equipped with a 5975C series mass selective detector. Parsing parameters: split inlet 1:100; carrier gas: He (1 $mL.min^{-1}$); HP-5MS column (30 m x 0.25 mm x 0.25 µm film thickness); analysis program: 40°C for 4 min, 40 – 320 °C at 4 $°C.min^{-1}$; temperatures: injector: 250 °C, interface: 280 °C; ionization energy: 70 eV.

The quantification of the constituents was performed using the GC coupled to a flame ionization detector (GC-FID). The parameters used were the same as above, except for the split inlet 1:50 mode; inlet and detector temperature: 300 ºC. The constituents were identified by comparing retention indices and mass spectra with the device database (NIST-EPA-NIH) and specific literature (ADAMS, 2009).

Bioassay of antifungal activities

The mycelial growth inhibition tests were performed at the Phytopathology Laboratory, Department of Plant Protection (CCR/UFSM). The plant extractives were tested against the fungi *P. sanguineus* and *G. trabeum*. Aliquots of both EO were incorporated into potato-sucrose-agar medium (PSA: 200 g potato, 20 g sucrose and 18 g agar in 1 L of distilled water). To determine the concentrations to be tested, pilot tests were carried out. The final concentrations evaluated for the EO of *N. megapotamica* (NM1 and NM2) were 0.25, 0.50 and 1.0 $µL.mL^{-1}$, and for the EO of *B. salicifolius* (BS), 1.0, 2.0 and 5.0 $µL.mL^{-1}$. The extractives were previously diluted in ethanol in a 1:1 ratio, aiming at better homogenization of the EO in the culture medium. In a laminar flow chamber, the supplemented medium was poured into sterilized Petri dishes (9.0 cm in diameter).

For the negative control, plates containing only PSA and ethanol at the highest concentration used as diluent were used. After solidification of the medium, 12 mm discs containing mycelium from each of the isolated fungi were transferred to the center of the plates. Subsequently, they were sealed and incubated in a BOD chamber with a controlled temperature of 25 ± 3°C and a photoperiod of 12 h (light/dark). The evaluation of the experiment started after 24 h by diametrically opposite measurements of the diameter of the colonies, carried out every two days, until the fungal colonies of the control treatments reached ¾ of the diameter of the plate (approximately 7 days). The mycelial growth index (MGI) was calculated according to Equation. 1:

$$MGI = [\left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \left(\frac{Gn}{Nn}\right)]$$

Where: G1, G2, Gn= mycelial growth of the fungus in the first, second and last evaluation; N1, N2, Nn= number of days after inoculation.

The percentage of mycelial growth inhibition (GI) was calculated using Equation 2:

$$GI(\%) = \left[\frac{(GT - Gt)}{GT}\right] * 100$$

Where: GT and Gt correspond to mycelial growth in the control and in the treatment, respectively (BADAWY, ABDELGALEIL, 2014).

Statistical analysis

The data were previously submitted to tests of homogeneity of variances and normality. Analysis of variance was used to compare the MGI and GI (%) data obtained and between the concentrations evaluated. Tukey and Kruskall-Wallis post-tests were used to verify statistical difference, at the minimum level of probability (P<0.05). All analyzes were performed using SigmaPlot software, version 11.0.

RESULTS

Yield and chemical composition

The EO BS showed a yield of 2.29% and a density of 0.89 g.mL^{-1}. The analysis of the chemical composition allowed the identification of 19 constituents, representing 94.55% of the total composition. Among them, 8.0% are monoterpene hydrocarbons, 66.77% are oxygenated monoterpenes and 18.88% are sesquiterpene hydrocarbons, while phenylpropanoids are represented by a single component (0.90%). As can be seen in Table 1, the major components were eucalyptol (63.44%) and β-caryophyllene (13.68%).

Table 1. Chemical composition of essential oil obtained from leaves of *Blepharocalix salicifolius*.
Tabela 1. Composição química do óleo essencial obtido de folhas de *Blepharocalix salicifolius.*

RT	Constituent	Class	RI calc	RI ref	% (Average)
10.02	*E*-tujene	MH	925	925^{N}	0.36
10.24	α-pinene	MH	931	937^{N}	1.71
11.87	sabinene	MH	971	975^{A}	0..26
12.65	β-pinene	MH	991	981^{N}	1.94
13.09	α-phelandrene	MH	1002	1007^{N}	1.02
13.59	α-terpinene	MH	1015	1016^{N}	0.81
14.15	eucalyptol	OM	1029	1031^{N}	63.44
14.94	β-*E*-ocyene	MH	1049	1050^{N}	0.28
15.28	γ-terpinene	MH	1058	1060^{N}	1.10
16.42	terpinolene	MH	1087	1088^{N}	0.52
19.78	terpinen-4-ol	OM	1177	1177^{A}	1.11
20.27	α-terpineol	OM	1190	1190^{N}	2.22
26.85	2-propenoic acid 3-phenyl-methyl ester	PP	1383	1378^{N}	0.90
28.01	β- caryophyllene	SH	1420	1420^{N}	13.68
29.07	humulene	SH	1455	1452^{N}	1.96
29.79	germacrene D	SH	1478	1480^{N}	0.74
30.36	guayan	SH	1496	1503^{A}	0.25
30.73	σ-cadinene	SH	1509	1519^{N}	1.24
31.20	δ-cadinene	SH	1525	1526^{N}	1.01
	Total identified				94.55

RT= Retention Time; RI calc = calculated Retention Index; R ref = Reference Retention Index; N: NIST-EPA-NIH; A: Adams, 2009; MH = monoterpene hydrocarbon; OM = Oxygenated monoterpene; SM = Sesquiterpene Hydrocarbon; PP: Phenylpropanoid.

Regarding to EO NM1, the extractive showed a yield of 0.14% and density of 0.85 g.mL^{-1}, while for EO NM2, the yield was 0.17% and density of 0.83 g.mL^{-1}. As can be seen in Table 2, the chemical analysis of EO NM1 allowed the identification of 28 constituents, representing 75.68% of the total composition. Of these compounds, 30.02% are monoterpene hydrocarbons, 0.33% oxygenated monoterpenes, 34.59% sesquiterpene hydrocarbons, 7.13% oxygenated sesquiterpenes and 3.47% are phenylpropanoids. According to Table 2, the major components of EO NM1 were bicyclogermacrene (16.14%), α-pinene (15.93%), β-pinene (13.58%) and germacrene-D (9.44%).

The chemical analysis of EO NM2 allowed the identification of 39 constituents, representing 80.99% of the total composition (Table 2). Of these, 45.59% are monoterpene hydrocarbons, 2.31% oxygenated monoterpenes, 19.44% sesquiterpene hydrocarbons, 10.92% oxygenated sesquiterpenes and 2.43% are phenylpropanoids. As can be seen in Table 2, the major components were α-pinene (19.04%), sabinene (16.66%) and β-cymene (7.74%).

Table 2. Chemical composition of the essential oil obtained from *Nectandra megapotamica* leaves (NM1 and NM2).

Tabela 2. Composição química do óleo essencial obtido de folhas de *Nectandra megapotamica* (NM1 e NM2).

Constituent	Class	RI calc	RI ref	% (Average)	
				NM1	NM2
α-pinene	MH	931	930[N]	15.93	19.04
camphene	MH	945	946[N]	–	1.47
sabinene	MH	971	970[N]	–	16.66
β-pinene	MH	974	975[N]	13.58	–
β-cymene	MH	1023	1021[N]	–	7.74
D-limonene	MH	1028	1028[N]	0.51	–
eucalyptol	OM	1030	1031[N]	–	0.15
Z-β-ocimene	MH	1049	1049[N]	–	0.52
β-linalool	OM	1102	1100[N]	0.33	0.55
1,3,8-*p*-menthatriene	MH	1106	1105[A]	–	0.16
L-pinocarveol	OM	1138	1138[N]	–	0.31
camphor	OM	1144	1145[N]	–	0.21
pinocarvone	OM	1163	1162[N]	–	0.19
4-terpineol	OM	1177	1163[N]	–	0.21
α-terpineol	OM	1191	1190[N]	–	0.13
myrthenol	OM	1196	1196[N]	–	0.42
1,3,5,8-undecathetraene	OC	1197	1180[N]	0.14	–
bornyl acetate	OM	1287	1286[A]	–	0.14
benzyl isobutanoate	OC	1299	1298[A]	–	0.30
δ-elemene	SH	1337	1338[A]	–	1.06
cyclosativene	SH	1366	1371[A]	–	0.17
α-cubebene	SH	1377	1379[N]	0.37	0.37
β bourbon	SH	1386	1385[N]	0.21	0.18
β-cubene	SH	1391	1391[N]	–	1.02
β-elemene	SH	1393	1394[N]	1.46	–
eugenol methyl ether	PP	1406	1405[N]	0.26	–
α-gurjunene	SH	1411	1409[N]	0.30	–
caryophyllene	SH	1421	1420[N]	3.14	2.61
β-copaene	SH	1429	1430[A]	–	0.25
E-α-bergamotene	SH	1437	1438[N]	–	0.43
α-guayene	SH	1440	1441[N]	0.61	0.73
α-caryophyllene	SH	1455	1455[N]	0.72	1.34
γ-gurjunene	SH	1462	1465[N]	0.65	1.12
α-copaene	SH	1378	1476[N]	0.44	–
γ-muurolene	SH	1478	1479[N]	–	0.57
germacrene D	SH	1482	1483[N]	9.44	4.61
bicyclogermacrene	SH	1498	1500[A]	16.14	4.14
γ-cadinene	SH	1515	1513[N]	0.35	0.39
δ-cadinene	SH	1525	1526[N]	0.76	0.45
elemicin	PP	1559	1559[N]	1.95	–
nerolidol	OS	1565	1565[N]	0.40	1.24
spathulenol	OS	1579	1578[N]	4.78	6.36
viridiflorol	OS	1593	1593[N]	0.87	0.80
dillapiole	PP	1627	1628[N]	0.48	2.43
τ-cadinol	OS	1644	1646[N]	0.46	–
isoelemycin	PP	1654	1657[N]	0.78	–
δ-cadinol	OS	1656	1655[N]	–	1.38
aromadendrene oxide (1)	OS	1674	1672[N]	0.38	–
corymbolone	OS	1787	1785[N]	0.24	1.14
Total identified				75.68	80.99

RI calc = calculated Retention Index; RI ref = Reference Retention Index; N: NIST-EPA-NIH; A: Adams, 2009; MH = monoterpene hydrocarbon; OM = Oxygenated monoterpene; SH = Sesquiterpene Hydrocarbon; OS = Oxygenated Sesquiterpene; PP: Phenylpropanoid; OC = Other class.

Evaluation of antifungal activity

In the antifungal bioassay against the fungus *P. sanguineus* using the EO BS, the data did not show homogeneity of variances, and were analyzed by the Kruskal-Wallis test. However, there was no statistical difference between treatments. In the bioassay to assess the susceptibility of the fungus *G. trabeum* to EO BS, the parametric data showed a statistically significant difference between the three concentrations analyzed (F = 847.71; $P < 0.001$; Figure 1).

In the antifungal bioassay using the EO NM1 against the white-rot fungus *P. sanguineus*, the data showed homogeneity of variances and normality. Through ANOVA, there was a significant difference between the treatments evaluated (F = 28.47; $P < 0.001$).

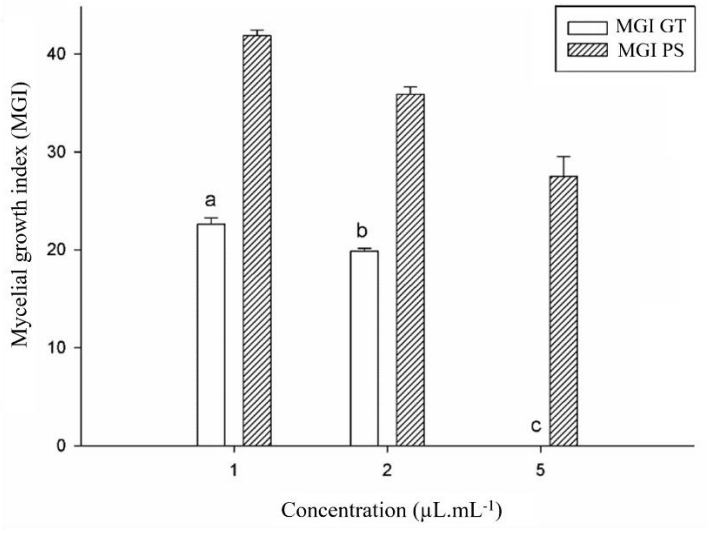

Figure 1. Antifungal bioassay of *Blepharocalyx salicifolius* essential oil against wood rot fungi. Where MGI GT = Mycelial growth index of the fungus *Gloeophyllum trabeum*; MGI PS = Mycelial growth index of the fungus *Pycnoporus sanguineus*; Lower case letters indicate statistical difference using the Tukey test (P <0.05).

Figura 1. Bioensaio antifúngico do óleo essencial de *Blepharocalyx salicifolius* frente a fungos de podridão da madeira. Onde MGI GT = Índice de crescimento micelial do fungo *Gloeophyllum trabeum*; MGI PS = Índice de crescimento micelial do fungo *Pycnoporus sanguineus*; Letras minúsculas indicam diferença estatística através do teste Tukey (P<0,05).

The Tukey test showed a significant difference between concentrations of 1 and 0.5 µL.mL^{-1} for the lowest concentration evaluated, 0.25 µL.mL^{-1} (P< 0.05), as can be seen in Figure 2A. In the bioassay to evaluate the effect of EO NM1 against the fungus *G. trabeum*, there was no statistically significant difference between the concentrations analyzed (F = 4.050; $P = 0.077$) (Figure 2A).

In the bioassay aimed at evaluating the activity of OE NM2 against the fungus *P. sanguineus*, the data were parametric. According to Figure 2B, there was no significant difference between the different concentrations (F = 5.293; $P = 0.047$). In relation to the fungus *G. trabeum*, when confronted with the OE NM2, the parametric data showed a significant difference (F = 43.267; $P < 0.001$). The post-test revealed that the treatments with EO at 0.25 and 0.50 µl.mL^{-1} are the same and differ from the treatment with extractive at 1.0 µL.mL^{-1}, in relation to the MGI data (Figure 2B). The comparison of the parametric values obtained with the results of the tests with the EOs BS and NM2, both at 1 µL.mL^{-1}, against the fungus *G. trabeum*, the results showed a significant difference (F = 37.807; $P < 0.001$) for the MGI variable. Along with the performance of the Tukey test, it was verified that there was a significant difference between both tested EOs.

The same happened with the data obtained with the EOs BS and NM1 at 1 µL.mL^{-1} against the fungus *P. sanguineus*. The parametric values of MGI showed a significant difference (F = 39.135; $P < 0.001$). When comparing the results obtained for the EOs NM1 and NM2 against the fungus *G. trabeum*, it was verified that there was a significant difference between the groups. However, there is no statistically significant interaction between sample and concentration ($P = 0.625$). In the comparison between the results of the bioassay performed with the EO of NM1 and NM2 against *P. sanguineus*, there was a significant difference in the mean values between the different levels of sample and concentration. However, there is no statistically significant interaction between sample and concentration ($P = 0.562$). Comparing the concentrations used in the EO of NM1 and NM2 against the fungus *P. sanguineus*, the Tukey test showed that there was no significant difference between the concentrations of 0.5 µL.mL^{-1} and 1.0 µL.mL^{-1} of the extractives.

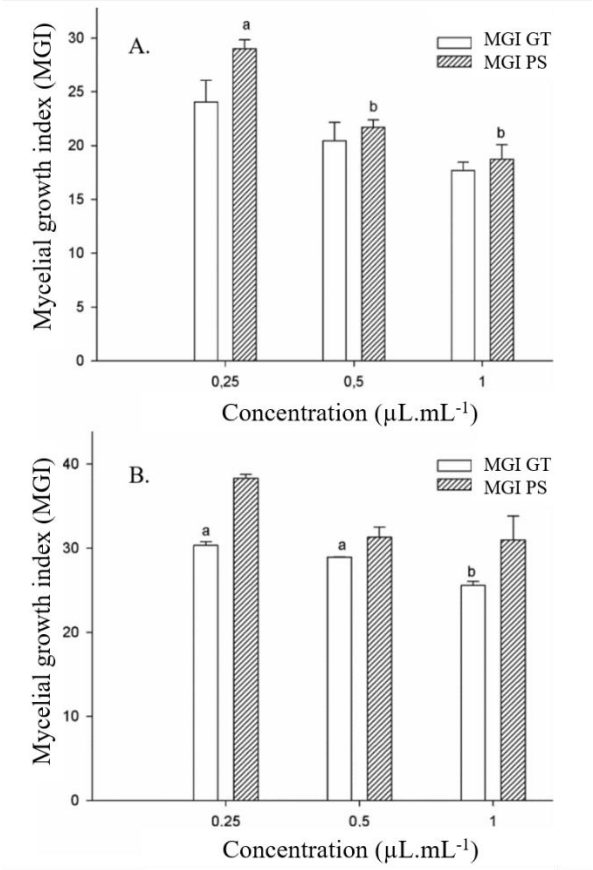

Figure 2. Antifungal bioassay of essential oil of *Nectandra megapotamica* NM1 (2A) and NM2 (2B) against wood rot fungi. Where MGI GT = Mycelial growth index of the fungus *Gloeophyllum trabeum*; MGI PS = Mycelial growth index of the fungus *Pycnoporus sanguineus*; Lower case letters indicate statistical difference through the Tukey test (P <0.05).

Figura 2. Bioensaio antifúngico do óleo essencial de *Nectandra megapotamica* NM_1 (2A) e NM_2 (2B) frente a fungos de podridão da madeira. Onde MGI GT = Índice de crescimento micelial do fungo *Gloeophyllum trabeum*; MGI PS = Índice de crescimento micelial do fungo *Pycnoporus sanguineus*; Letras minúsculas indicam diferença estatística através do teste de Tukey (P<0,05).

As for the percentage of mycelial growth inhibition (GI), for *P. sanguineus*, the highest percentage of inhibition occurred with EO NM1 at the highest concentration evaluated, with 57.55%. For the fungus *G. trabeum*, the best result was with the EO BS, which completely inhibited the growth of the fungus at a concentration of 5 µL.mL^{-1} (Table 3). Because the EOs NM1 and NM2 suggest a more intense biological activity due to the presence of phenylpropanoids (CARSON; HAMMER, 2011) and also because they present lower yields in relation to the EO of *B. salicifolius*, smaller aliquots of the EO of *N. megapotamica* were used *i*n antifungal bioassays. For this reason, a comparison of the results was performed considering only the concentration of 1 µL.mL^{-1}, which was the tested concentration of all EOs against all fungi. As can be seen in Table 3, in the analysis of antifungal activity against the fungus *G. trabeum*, for the variable %GI of EOs NM1 (58.82 ± 0.78%), NM2 (24.81 ± 0.46%) and BS (14.17 ± 0.66%), all at a concentration of 1 µL.mL^{-1}, the Tukey test showed that there was a statistical difference between all extractives evaluated (P < 0.050).

According to Table 3, the Tukey Test performed for the %GI variable of the OEs NM1 (57.55 ± 0.68%), NM2 (26.68 ± 2.85%) and BS (0.89 ± 0 .57%) at a concentration of 1 µL.mL^{-1} against the fungus *P. sanguineus*, there was also a statistical difference between the antifungal activity of all extractives evaluated (P < 0.050). The comparison of the percentage of mycelial growth inhibition (%GI) of equivalent concentrations (1 µL.mL^{-1}) of the three EO evaluated (Table 3), indicates that the EO of NM1 has a greater inhibitory potential against both fungal species evaluated. On the other hand, the NM2 EO showed an intermediate inhibition potential, while the BS EO was the least active.

Table 3. Percentage of mycelial growth inhibition (%GI) of the fungi *Pycnoporus sanguineus* and *Gloeophyllum trabeum* by the action of the essential oils of *Nectandra megapotamica* (NM1 and NM2) and *Blepharocalyx salicifolius* (BS).

Tabela 3. Porcentagem de inibição de crescimento micelial (%GI) dos fungos *Pycnoporus sanguineus* e *Gloeophyllum trabeum* pela ação dos óleos essenciais de *Nectandra megapotamica* (NM1 e NM2) e *Blepharocalyx salicifolius* (BS).

EO	GI (%)	
	P. sanguineus	*G. trabeum*
BS	Mean ± SEM	Mean ± SEM
1.0 µL.mL^{-1}	0.89 ± 0.57	14.17 ± 0.66
2.0 µL.mL^{-1}	15.07 ± 0.76	41.70 ± 0.32
5.0 µL.mL^{-1}	34.95 ±2.06	100 ± 0
NM_1	Mean ± SEM	Mean ± SEM
0.25 µL.mL^{-1}	30.76 ± 0.11	44.00 ± 2.01
0.5 µL.mL^{-1}	43.32 ± 0.83	52.37 ± 1.70
1.0 µL.mL^{-1}	57.55 ± 0.68	58.82 ± 0.78
NM_2	Mean ± SEM	Mean ± SEM
0.25 µL.mL^{-1}	9.28 ± 0.47	10.83 ± 0.45
0.5 µL.mL^{-1}	25.82 ± 1.16	14.92 ± 0.05
1.0 µL.mL^{-1}	26.68 ± 2.85	24.81 ± 0.46

GI: Mycelial Growth Inhibition (%); SEM: Standard Error of the Mean.

DISCUSSION

Regarding the EO BS, the extraction yield values described in the literature showed lower values than those ones found in the present study. Castelo *et al.*, (2010) obtained a yield of 0.12%, and Godinho *et al.* (2014) found a value of 0.70%. As for the chemical composition of EO BS, the same major constituents of this study were found by Limberger *et al.* (2008), who also found 1,8-cineole (25.2%) and β-caryophyllene (22.9%) as the major compounds. It should also be noted that the chemical composition and extraction yield of EOs are directly related to the genetic characteristics of different populations of a species. However, factors such as plant nutritional stress, fertilization, nutrients available in the soil and climate, as well as biotic components, such as attack by microorganisms, insects and other plants can also interfere with the production of EO by the plant (XIE *et al.*, 2012; SHARIFI-RAD *et al.*, 2017).

Romoff *et al.* (2010) evaluated the chemical composition of EO from leaves of *N. megapotamica* and reported the predominance of oxygenated sesquiterpenes, with α-bisabolol (65.85%) and δ-elemene (15.40%) being the major compounds in the extract. On the other hand, Torres *et al.* (2014) observed higher contents of sesquiterpenoids in relation to monoterpenoids in the EO of *N. megapotamica* leaves. These results are similar to those found for EO NM1 and NM2, since for both the content of sesquiterpenoids was higher in relation to monoterpenoids. Amaral *et al.* (2015) detected the occurrence of two major chemical groups (CG) in the EO of *N. megapotamica*, which major constituents were α-pinene and bicyclogermacrene. The CG of α-pinene had higher levels of monoterpenoids, while that of bicyclogermacrene had higher levels of phenylpropanoids. The extractive with the highest percentage of phenylpropanoids showed better results in allelopathic and insecticide tests compared to the first group, demonstrating greater biological activity. Based on the above, we emphasize that the results obtained in this study corroborate this statement, since the EO NM1, with a greater amount of phenylpropanoids compared to the NM2, showed better results in terms of antifungal activity.

Analyzing the concentration of 1 µL.mL^{-1}, which was the lowest concentration evaluated for BS and the highest for NM1 and NM2, it was found that EO NM1 caused greater inhibition of fungal growth, with 57.55 ± 0.68% for P. *sanguineus* and 58.82 ± 0.78% for *G. trabeum.* However, the EO BS, at a concentration of 1 µL.mL^{-1}, showed a growth inhibition of only 0.89 ± 0.57 % for *P. sanguineus* and 14.17 ± 0.66 % for *G. trabeum*, revealing to be less promising at lower concentrations (Table 3).

The water solubility of the chemical components can also influence the results of in vitro antifungal tests, since the low water solubility of compounds such as non-oxygenated terpenes can hinder their diffusion in the culture medium, which is one of the factors that can generate false-negative results. However, other characteristics also influence water solubility, such as the size of the carbon chain. Taherpour *et al.* (2011) calculated the

theoretical solubility of some terpenes in water and showed, as expected, that sesquiterpenoids are much less soluble than monoterpenoids. Thus, we emphasize that this may be one of the explanations for the total inhibition of the growth of *G. trabeum* by OE BS at the highest concentration evaluated, since this extractive presented in its composition a greater amount of monoterpenoids in relation to sesquiterpenoids.

Regarding their mode of action, EO components, mainly terpenoids, can act as direct plant defenders through their generalized toxicity, acting as antimicrobials through interference with the selective permeability of the plasma membrane (DAS *et al.*, 2013; MOORE *et al.*, 2014). Chittenden and Singh (2011) performed durability tests with wooden blocks using some phenylpropanoids, including eugenol, and the results obtained indicate that this compound prevents the action of rotting fungi. According to Carson and Hammer (2011), phenylpropanoids and terpenoids with a phenolic structure are an interesting class of extractives to be evaluated for their wood preservative properties. The results obtained with the EO NM1 corroborate this statement, since the NM1 was more efficient in relation to the EOs NM2 and BS. Thus, we emphasize that EO NM1 showed greater antifungal activity, possibly because it has a higher percentage of phenylpropanoids in its composition.

CONCLUSIONS

- Among the EOs whose antifungal activity was evaluated in this exploratory work, the one obtained from leaves of *N. megapotamica* called EO NM1 showed greater antifungal efficacy against both tested fungi.
- Economic feasibility must be analyzed to assess the cost-effectiveness of using its potential in the future.
- Subsequently, it is suggested to verify how these plant extractives act on wood-rotting fungi, through accelerated decay tests.
- It is also suggested that studies be carried out aiming at the development of the most appropriate formulations, among others using nanotechnology to reduce the volatility of EOs.

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REFERENCES

ADAMS, R. P. **Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy**. Illinois: Allured Publishing Corporation. 2009, 804p.

AMARAL, L. P.; SCHINDLER, B.; BIANCHINI, N. H.; LONGHI, S. J.; ALMEIDA, C. A. A.; MALLMANN, C. A.; HEINZMANN, B. M. Variabilidad de la composición química del aceite esencial de *Nectandra megapotamica* (Spreng.) Mez (Lauraceae). **Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas**, v. 14, n. 3, p. 190 - 205, 2015. http://www.redalyc.org/articulo.oa?id=85638535005.

BADAWY, M. E. I.; ABDELGALEIL, S. A. M. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. **Industrial Crops and Products**, v. 52, p. 776 – 782, 2014. https://doi.org/10.1016/j.indcrop.2013.12.003.

BENTO, T. S.; TORRES, L. M.; FIALHO, M. B.; BONONI, V. L. Growth inhibition and antioxidative response of wood decay fungi exposed to plant extracts of *Casearia* species. **Letters in Applied Microbiology**, v. 58, n. 1, p. 79 – 86, 2014. https://doi.org/10.1111/lam.12159.

CAI, L.; JEREMIC, D.; LIM, H.; KIMET, Y. β-Cyclodextrins as sustained-release carriers for natural wood preservatives. **Industrial Crops and Products**, v. 130, p. 42 – 48, 2019. https://doi.org/10.1016/j.indcrop.2018.12.061.

CARSON, C. F; HAMMER, K. A. Chemistry and bioactivity of essencial oils. In: THORMAR, H. **Chemistry and bioactivity of essencial oils**. West Sussex: John Wiley & Sons, 2011. p. 203 – 238.

CASTELO, A. V. M.; DEL MENEZZI, C.; RESCK, I. S. Yield and spectroscopic analysis (1H, 13 C NMR; IR) of essential oils from four plants of the Brazilian Savannah. **Cerne**, v. 16, n. 4, p. 573–584, 2010. https://www.researchgate.net/publication/289047506_Yield_and_spectroscopic_analysis_1H_13_C_NMR_IR_of_essential_oils_from_four_plants_of_the_Brazilian_Savannah. 04 Dez. 2020.

CHITTENDEN, C.; SINGH, T. Antifungal activity of essential oils against wood degrading fungi and their applications as wood preservatives. **International Wood Products Journal**, v. 2, n. 1, p. 44–48, 2011. https://doi.org/10.1179/2042645311Y.0000000004.

DAS, A.; LEE, S. E.; HYUN, T. K.; KIM, S. W.; KIM, J. Y. Plant volatiles as method of communication. **Plant Biotechnology Reports**, v. 7, n. 1, p. 9–26, 2013. http://dx.doi.org/10.1007/s11816-012-0236-1.

GODINHO, W. M.; FARNEZ, M. M.; PEREIRA, I. M.; GREGÓRIO, L. E.; GRAEL, C. F. F. Volatile constituents from leaves of *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae**). Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas**, v. 13, p. 249 – 253, 2014. http://www.redalyc.org/articulo.oa?id=85631010004. 26 Ago. 2020.

LIMBERGER, R. P.; SOBRAL, M. E. G.; ZUANAZZI, J. A. S.; MORENO, P. R. H.; SCHAPOVAL, E. E. S.; HENRIQUES, A. T. Biological Activities and Essential Oil Composition of Leaves of *Blepharocalyx salicifolius*. **Pharmaceutical Biology**, v. 39, p. 308 – 311, 2008. https://doi.org/10.1076/phbi.39.4.308.5915.

MOORE, B. D.; ANDREW, R. L.; KÜLHEIM, C.; FOLEY, W. J. Explaining intraspecific diversity in plant secondary metabolites in an ecological context. **The New Phytologist**, v. 201, n. 3, p. 733–750, 2014. https://doi.org/10.1111/nph.12526.

NERIO, L. S.; OLIVERO-VERBEL, J.; STASHENKO, E. Repellent activity of essential oils: A review. **Bioresource Technology**, v. 101, n. 1, p. 372 - 378, 2010. https://doi.org/10.1016/j.biortech.2009.07.048.

ROMOFF, P.; FERREIRA, M. J. P.; PADILLA, R.; TOYAMA, D. O.; FÁVERO, O. A.; LAGO, J. H. G. Chemical composition of volatile oils from leaves of *Nectandra megapotamica* Spreng. (Lauraceae). **Química Nova**, v. 33, n. 5, p. 1119–1121, 2010. http://dx.doi.org/10.1590/S0100-40422010000500021.

SHARIFI-RAD, J.; SUREDA, A.; TENORE, G. C.; DAGLIA, M.; SHARIFI-RAD, M.; VALUSSI, M.; TUNDIS, R.; SHARIFI-RAD, M.; LOIZZO, M. R.; ADEMILUYI, A. O.; SHARIFI-RAD, R.; AYATOLLAHI, S. A.; IRITI, M. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. **Molecules**, v. 22, n. 1, p. 1 – 55, 2017. https://doi.org/10.3390/molecules22010070.

SILVA, D. T.; BIANCHINI, N. H.; MUNIZ, M. F. B.; HEINZMANN, B. M.; LABIDI, J. Chemical composition and inhibitory effects of *Nectandra grandiflora* leaves essential oil against wood decay fungi. **Drewno: prace naukowe, doniesienia, komunikaty**, v. 59, n. 197, 2016. http://dx.doi.org/10.12841/wood.1644-3985.C29.10.

STANGERLIN, D. M.; COSTA, A. F. DA; GARLET, A.; PASTORE, T. C. M. Resistência natural da madeira de três espécies amazônicas submetidas ao ataque de fungos apodrecedores. **Revista Ciência da Madeira**, v. 4, n. 1, p. 15 - 32, 2013. http://dx.doi.org/10.15210/cmad.v4i1.4056.

TAHERPOUR, A.; MAROOFI, H.; CHANGIZI, M.; SHOUSHTARI, R. V.; LARIJANI, K.; KAZEMPOUR, A. Chemical compositions of the essential oil and calculation the biophysicochemical coefficients of the components of *Hymenocrater longiflorus* Benth. of Iran. **Natural Science**, v. 3, n. 2, p. 104 – 108, 2011. http://dx.doi.org/10.4236/ns.2011.32015.

TCHINDA, J. B. S.; NDIKONTAR, M. K.; BELINGA, A. D. F.; MOUNGUENGUI, S.; NJANKOUO, J. M.; DURMAÇAY, S.; GERARDIN, P. Inhibition of fungi with wood extractives and natural durability of five Cameroonian wood species. **Industrial Crops and Products**, v. 123, p. 183–191, 2018. https://doi.org/10.1016/j.indcrop.2018.06.078.

TORRES, A. M.; NDIKONTAR, M. K.; BELINGA, A. D. F.; MOUNGUENGUI, S.; NJANKOUO, J. M.; DURMAÇAY, S.; GERARDIN, P. *Nectandra megapotamica* (Spreng.) Mez.: phytochemical characterization and neutralizing effect on *Bothrops diporus* venom. **Journal of Essential Oil Research**, v. 26, n.3, p. 197 – 203, 2014. https://doi.org/10.1016/j.indcrop.2018.06.078.

XIE, Y.; HUANG, Q.; YANG, F.; LEI, C. Chemical variation in essential oil of *Cryptomeria fortunei* from various areas of China. **Industrial Crops and Products**, v. 36, n. 1, p. 308 – 312, 2012. https://doi.org/10.1016/j.indcrop.2011.10.023.

XIE, Y.; WANG, Z.; HUANG, Q.; ZHANG, D. Antifungal activity of several essential oils and major components against wood-rot fungi. **Industrial Crops and Products**, v. 108, p. 278 – 285, 2017. https://doi.org/10.1016/j.indcrop.2017.06.041.