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# Antifungal Activity of the Essential Oils of *Osteophloeum platyspermum* (Myristicaceae) against *Malassezia* spp. and *Candida albicans* Influenced by Seasonality and Climatic Factors

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** *Malassezia* spp. are involved in a wide range of mammalian skin diseases. The introduction of new drugs is a need. Natural products are known to be effective in the treatment of microbial pathogens. The present study analyzed the *O. platyspermum* leaf essential oils (EOs) antifungal activity.
**Study Design:** 18 terpenes from 13 *O. platyspermum* leaf EOs are related to seasonal and climatic variations occurring during the dry (DS) and rainy (RS) seasons in the Amazon Rain-Forest, verified by means of multivariate analyses.
**Place and Duration of the Study:** the study was conducted at the Center for Research in Biodiversity (Microbiology Laboratory and Cell Culture Laboratory), Paulista University, biological activity evaluations occurred between January/2019 and December 2019).

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**Methodology:** microdilution broth assay was used in the minimal bactericidal concentration (MBC). Multivariate analyses were used to access the relationship among MBC, seasonality and terpene composition of the EOs.
**Results:** *Malassezia pachydermatis* showed higher sensitivity to the EOs than *M. furfur* or *C. albicans*. The DS EOs were linked to the presence of limonene, myrcene, α-terpineol, linalool, terpinen-4-ol, cubenol-1-epi, influenced by insolation, temperature and evaporation, while β-elemene, γ-elemene, neo-intermedeol, elemol, α-cadinol, spathulenol, isospathulenol, viridiflorol, δ-amorphene and ledol were linked to the RS EOs, and were influenced by precipitation, relative humidity and wind velocity. DS EOs showed better antifungal activity against both *Malassezia* species, and the presence of the six discriminative terpenes was essential for the antifungal activity.
**Conclusions:** The DS EOs are a potential source of new leads to defeat animal dermatological microbes.

*Keywords: Volatile compounds; microdilution broth assay; principal component analysis; canonical correspondence analysis; seasonality.*

## 1. INTRODUCTION

*Malassezia furfur* and *M. pachydermatis* are usually found in the skin and in the mucosa microbiomes, both from animals and men [1]. In view of their commensal characteristics, they are frequently isolated from moist areas of the body that are rich in lipids and sebaceous glands, as the skin of healthy individuals [1]. *Malassezia* spp. represent 53 % to 80 % of the fungi existing in the human microbiome [2]. Although they live in commensalism, they can become pathogenic, depending on some predisposing factors as changes in the host defense mechanisms or in the skin microenvironment, which frequently cause superficial infections in both men and animals [3-5].

The treatment of veterinarian or human patients commited with *Malassezia* spp. fungal infection is based on the use of topical and / or systemic antifungal drugs [6,7] from a group known as azoles. Despite the range of effectiveness observed for these drugs, there are reports of *M. pachydermatis* resistance to fluconazole, itraconazole and ketoconazole [8], and to 5-fluorocytosine [9]. *Malassezia pachydermatis*, which frequently is associated with dog skin infections, can become epidemiologically significant in cases of transspecies infections from dogs to humans [10,11]. Also, *M. furfur*, a commensal microorganism to humans, can be the main associated pathogen present in infants under intensive care showing systemic infections, where the yeasts migrate from the hands of the patient, their parents or healthcare workers [12], and even associated to central catheters delivering parental nutrition [13,14], a condition that characterizes the importance of the pathogens to public health occurring in countries as the United States [15], India [16] or Brazil [17].

Studies made with essential oils as antimicrobial agents have been done for decades, and usually related to the traditional use of many medicinal plants, as *erva-baleeira* (*Cordia verbenacea* Jacq. – Boraginaceae [18]), or nutmeg (*Myristica fragrans* Hoult. – Myristicaceae [19]). The essential oil of *Lavanda* (*Lavandula pubescens*) contains carvacrol, a monoterpene that showed antifungal activity against *Candida albicans* [20], *Cordoncillo* (*Piper barbatum*) has hydroxylated sesquiterpenes in its essential oil that showed antifungal activity against two strains of *C. albicans* and against bacteria as *Staphylococcus aureus* and *Streptococcus mutans* [21].

*Malassezia furfur* and *M. pachydermatis* have shown high sensitivity to EOs, such as those obtained from *Zataria multiflora* and *Thymus kotschyanus* (both Lamiaceae), which inhibited the growth of *Malassezia* sp. isolated from infected animals [22]. Aerial organs of other lamiacs, as oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) [23], and mirtle (*Myrtus communis*) [24], showed activity against *M. furfur*, while the essential oil from the bark of *caneleira* (*Cinnamomum zeylanicum*) was effective against *M. pachydermatis* [25]. The use of both azole and essential oils to prospect for synergistic antifungal activity against *M. pachydermatis* clinical isolates is reported, and describes that the combination of Clotrimazole and the essential oils from *Melaleuca alternifolia*, *Origanum vulgare* or *Mentha piperita* showed the best results [26]. Nonetheless, few studies reporting the anti-*Malassezia* activity of essential oils obtained from Brazilian plants are available.

The essential oil obtained from the fruits of *Schinus terebinthifolius*, known in Brazil as *aroeira-vermelha* (rose pepper), showed activity against *M. furfur* [27]. The essential oil from the aerial organs of *Eugenia pyriformis*, known in Brazil as *uvaia*, has also shown activity against *M. furfur* [28].

*Osteophloeum platyspermum* (Myristicaceae) is a species widely distributed in the Amazon Rain Forest and part of Central America. Previous study has shown that 50 terpenes were identified in the 13 essential oils obtained from its leaves collected during a two-year period regarding November 2009 to October 2011 [29]. The present study reports the antifungal properties of the essential oils, as well as analyses how the seasonality and climatic factors have influenced the terpene composition of the essential oils and subsequently in their biological activity against *M. furfur*, *M. pachydermatis* and *C. albicans*.

## 2. MATERIALS AND METHODS

### 2.1. Sample Preparation for the Antifungal Assay

The leaves of the plant were collected in the Amazon Rain Forest, in a region near Manaus (, under Brazilian Ministry of Environment, license number 12A/2008/IBAMA/MMA/Brazil. The leaveswere collected from an individual tree [MBPaciencia, 846, (UNIP Herbarium)] and a voucher of the plant was deposited at Unip Herbarium under number UNIP5720. The essential oils were prepared at 100 %, 80 %, 60 %, 40 %, 20 % and 10 %. The oils were diluted with dimethyl sulfoxide (DMSO). The final test concentrations were 5.0 %, 4.0 %, 3.0 %, 2.0 %, 1.0 % and 0.5 %, due to the experimental methodology. DMSO did not show anti fungal activity at the final concentrations reached in the dilutions [29].

### 2.2 Antifungal Microdilution Broth Assay

Strains of *Malassezia furfur* CBS-1878, *Malassezia pachydermatis* CBS-1696 and *Candida albicans* ATCC10231 were used. The microdilution broth assay was adapted to *M. pachydermatis* and *M. furfur* from CLSI - *Clinical and Laboratory Standards Institute* [30]. Strains of *M. furfur* CBS-1878 and *M. pachydermatis* CBS-1696 were incubated in Dixon agar at 32 °C for five to seven days. Yeast suspensions were individually prepared from fresh colonies, in 1% tween 80 added to Sabouraud dextrose agar. A similar procedure was made for *C. albicans* ATCC10231, but regular Sabouraud dextrose agar (SDA) was used. The homogenized suspensions were prepared in saline solution (0.9 %) and suspensions were prepared at concentrations of $1.5x10^6$ CFU/mL for *M. pachydermatis* [31]. $1.5x10^5$ CFU/mL for *M. furfur* [32] and $1.5x10^5$ CFU/mL for *C. albicans* in Sabouraud dextrose broth medium (SDB) [33]. The assays were performed in U-bottom 96-well microplates, so 190 µL of the yeast SDB suspension were added to each well, then 10 µL of each oil were added to the corresponding wells, so as to give the final concentrations previously described. The tests were performed in duplicate. After that, microplates were incubated at 32 °C for 48 h to *Malassezia* spp. and at 36 °C to 24 h for *C. albicans*. In order to evaluate yeast growth inhibition, a subculture of 2 µL of each well were transferred to a 1.0 % tween 80 SDA Petri dish to *M. pachydermatis* and *M. furfur* and a regular SDA Petri dish to *C. albicans*. The dishes were taken to incubation under the conditions that were just described [34]. For those EOs that have inhibited yeast growth at the lower concentration of 0.5 %, the oils were half-fold diluted in order to be tested for the minimal fungicidal concentration.

### 2.3 Experimental Design

Fig. 1 represents the experimental design that was adopted in the present study. Collection, essential oil preparation and terpene identification were previously obtained [29], and biological and statistical analyses were performed in the present project.

### 2.4 Statistical Analysis

Statistical analyses were performed on the basis of independence, randomness and normality evaluation by the Shapiro-Wilk test. In order to compare the yields of the essential oils obtained from the leaves of *O. platyspermum* collected in DS and in the RS, a *t* test was conducted in order to evaluate the efficacy of the antifungal activity (based on the MFC) of the essential oils collected in the DS and in the RS, the Kruskal-Wallis followed by Dunn's post-test was conducted (GraphPad Prism® 7.0 statistical package). Principal component analysis (PCA) and canonical correspondence analyses (CCA; Multi Variate Statistical Package, version 3.22, Kovach Computing Services) were made to evaluate a possible ordination of the oils in relation to their terpene composition and amount,

and to verify possible correlations to climatic indices as temperature, insolation, evaporation, relative humidity, precipitation and wind velocity, by considering the 18 terpenes that commonly occurs in the oils, namely “factors”, and the 13 essential oils, namely “cases”. The three yeasts (*Malassezia furfur, M. pachydermatis* and *Candida albicans*) were used in the analyses as environmental factors to ordinate and to perform CCA [35]. For all the analyses the significance level was considered as $\alpha<0.05$.

## 3. RESULTS

### 3.1 Yields of the 13 Essential Oils of the Study and List of the 18 Commonly Occurring Terpenes

The yields of the EOs, expressed as % w/w, are described in Table 1, which also describes the season in which the leaves of the specimen were collected. Table 1 and Fig. 2 show that the yields of EOs obtained from the leaves collected during the DS are significantly smaller than the yields obtained from the leaves collected during the RS ($t=2.705$; $df=8$; $p=0.0269$).

Fig. 3 represents the 18 terpenes that commonly occurs in all 13 EOs from the leaves of *O. platyspermum*, and were considered relevant to the analysis.

### 3.2 Microdilution Broth Assay for the 13 Essential Oils

The 13 EOs obtained from the leaves of *O. platyspermum* were tested against *Malassezia furfur*, *M. pachydermatis* and *Candida albicans* in the broth microdilution assay. The MFCs obtained for each essential oil against micro-organisms can be seen in Table 2. Fig. 4 represents a statistical comparison of the antifungal activity of the EOs against the three yeasts. *M. pachydermatis* was significantly more sensitive to the EOs antifungal activity ($H\sim X^2_{(0.05, 3)} = 19.14$; $p<0.0001$). According to the results, *M. pachydermatis* showed more sensitivity to EOs than *M. furfur* ($p=0.0429$) and *C. albicans* ($p<0.0001$). Although the EOs have shown activity against *M. furfur*, only the EO11 was active at a very low concentration, whereas the oils were active only at the concentration of 1 % against *C. albicans*. Pure DMSO was tested in the broth assay (10 µL DMSO in 200 µL inoculated broth medium) and no fungal inhibition was observed.

### 3.3 Multivariate Analyses Study with the Essential Oils and Commonly Occurring Terpenes

Fig. 5A displays the principal component analysis (PCA) made with the 18 terpenes present in the essential oils obtained from the leaves of *O. platyspermum* collected in the dry (DS) and rainy (RS) seasons. The PCA in figure A resulted in a cumulative percentage of 84.140 in the first two axes. EOs obtained from leaves from plants collected in the DS are ordinated in accordance to a seasonal distribution.

Fig. 5B shows the ordination obtained from a PCA displaying the vectors that correspond to the terpenes. It is possible to observe that limonene, myrcene, and α-terpineol have influenced on the ordination of the EOs that were obtained from the leaves collected during the DS, while spathulenol, α-pinene and β-pinene have influenced on the ordination of the oils obtained from leaves collected in the RS.

Fig. 6A shows the ordination given by the canonical correspondence analysis (CCA) made with the 13 EOs and 18 terpenes. The relationship of the climatic factors with the terpene expression and with the essential oils are expressed. Daily data reported to the climatic factors were obtained from the *Instituto Nacional de Pesquisas Espaciais*, INPE (www.inpe.br). The cumulative percentage was 80.517 in the first axis. The essential oils, which are expressed in blue, were obtained from leaves collected during the DS, are seasonally ordinated according to the DS and related with the climatic factors insolation, temperature and evaporation, while the essential oils reported in green, obtained from leaves collected during the RS, are also seasonally ordinated and influenced by the relative humidity, precipitation and wind speed.

Fig. 6B shows how the terpenes spathulenol, isospathulenol and neo-intermediol are related to the precipitation factor, while α-pinene, β-pinene, and δ-amorphene are related to relative humidity. In the DS, it is observed that alpha-terpinene, terpinen-4-ol, linalool, myrcene, and limonene are related to average temperature and to the evaporation.

Fig. 7A reports the canonical correspondence analysis made with the EOs obtained from the leaves of *O. platyspermum* made with the 13 EOs and 18 terpenes, compared to the MFCs obtained from the yeasts *C. albicans*, *M.*

*pachydermatis* and *M. furfur*. As described in Table 1, all oils from the different collections showed some degree of yeast growth inhibition. Considering that the lower the value of the minimum fungicidal concentration of the EO the more effective is the antifungal power of the EO, the interpretation of the graph must be made by understanding that the best antifungal activity is observed for the essential oils located in opposition to the vector orientation. For *M. pachydermatis*, the oils that had the most significant antifungal activity were EO5, EO9 and EO10. For *M. furfur*, the oils with the best activity were EO7, EO11 and EO14, while for *C. albicans* the oil activity EO8, EO11 and EO14 were the most relevant.

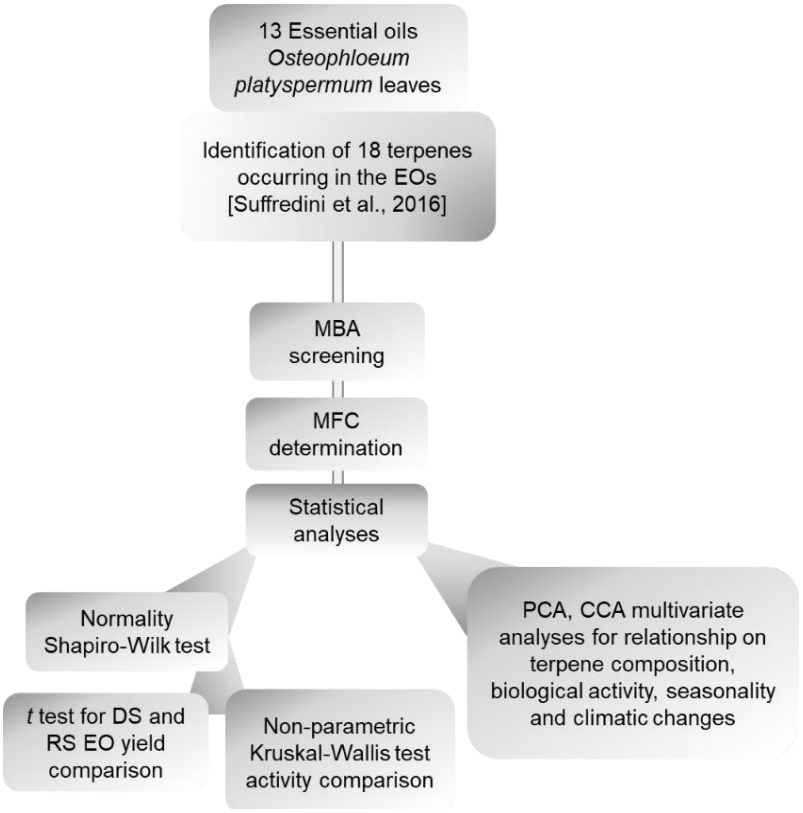


**Fig. 1. Experimental design adopted in the present analyses**

**Table 1. Season of leaves collection and yields of the essential oils prepared from it and statistical analysis comparing the yield mean and standard error, using Shapiro-Wilk (S-W) to seek for normality and *t*-test to compare means, for a significance level of α<0.05**

| Season | EO# | Yield (% w/w)) | Statistics | |
|---|---|---|---|---|
| DS | EO2 | 0.236 | Mean DS* | 0.3004 |
| RS | EO3 | 0.7346 | S.E. DS* | 0.04159 |
| RS | EO4 | 0.4202 | S-W DS | p=0.11527 |
| RS | EO5 | 0.5958 | | |
| RS | EO6 | 0.3025 | Mean RS* | 0.5481 |
| DS | EO7 | 0.4363 | S.E. RS* | 0.08158 |
| DS | EO8 | 0.3587 | S-W RS | p=0.59036 |
| RS | EO9 | 0.6873 | | |
| RS | EO10 | 0.2916 | t | 2.705 |
| DS | EO11 | 0.5706 | df | 8 |
| DS | EO12 | 0.2276 | p | 0.0269 |
| DS | EO13 | 0.2432 | Cohen d | 1.71097 |
| RS | EO14 | 0.2904 | Effect r | 0.6912 |

** = calculated after 1 outlier removal; S.E=standard error; DS=dry season; RS=rainy season*

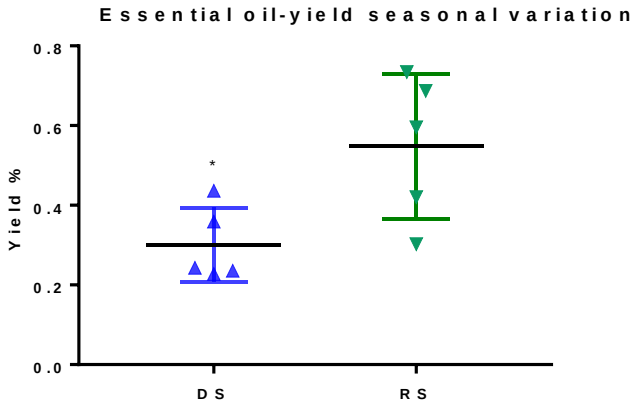


**Fig. 2. Comparison of the essential oil yields obtained from the extraction by hydrodistillation from the leaves of *Osteophloeum platyspermum* using a *t*-test, considering the significance as α<0.05. (*=p<0.05). DS=dry season; RS=rainy season.**

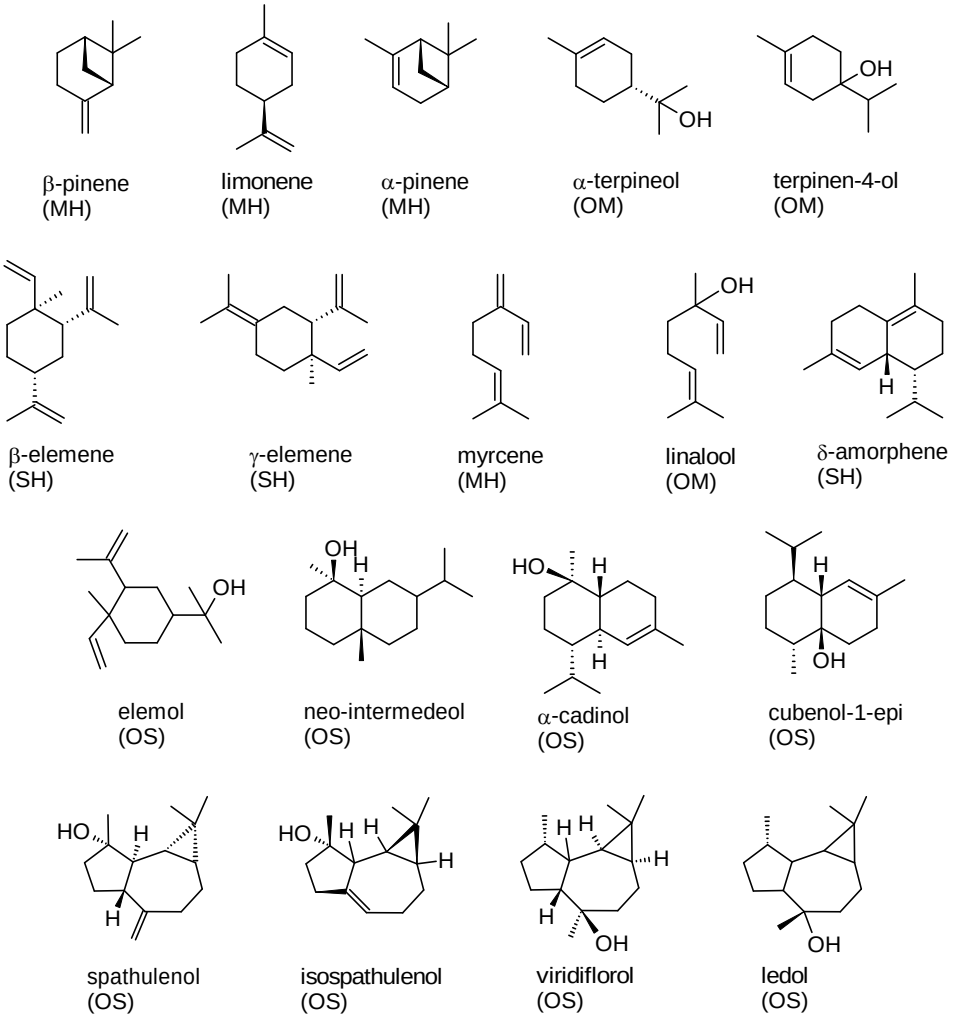


**Fig. 3. Chemical structure of the 18 essential oils obtained from the leaves of *Osteophloeum platyspermum*. MH=monoterpene hydrocarbon; OM=oxygenated monoterpene; SH= sesquiterpene hydrocarbon; OS=oxygenated sesquiterpene**

**Table 2. Minimal fungicidal concentration, given in percentage (%w/w), resulted from the microdilution broth assay made with essential oils obtained from the leaves of *Osteophloeum platyspermum* against *Malassezia pachydermatis, M. furfur* and *Candida albicans***

| Essential oil # | *Malassezia furfur* | *Malassezia pachydermatis* | *Candida albicans* |
|---|---|---|---|
| | Minimal fungicidal concentration (% w/w ) | | |
| EO2 | 1 | 1 | 1 |
| EO3 | 5 | 0.25 | 1 |
| EO4 | 1 | 0.125 | - |
| EO5 | 1 | 0.125 | 1 |
| EO6 | 0.25 | $6.25x10^{-2}$ | 1 |
| EO7 | 1 | $6.25x10^{-2}$ | 1 |
| EO8 | 0.25 | $6.25x10^{-2}$ | 1 |
| EO9 | 1 | 0.125 | 1 |
| EO10 | 0.25 | $6.25x10^{-2}$ | 1 |
| EO11 | $6.25x10^{-2}$ | $3.13x10^{-2}$ | 1 |
| EO12 | 0.25 | $6.25x10^{-2}$ | 1 |
| EO13 | 0.125 | 1 | 1 |
| EO14 | 1 | 0.125 | 1 |
| K-W(3,37)= 19.14 | p<0.0001 | | |
| S-W *Malassezia furfur* | p=0.0014 | | |
| S-W *Malassezia pachydermatis* | p<0.0001 | | |
| S-W *Candida albicans* | Not possible to calculate | | |
| (-) = not tested; S-W=Shapiro-Wilk normality test | | | |

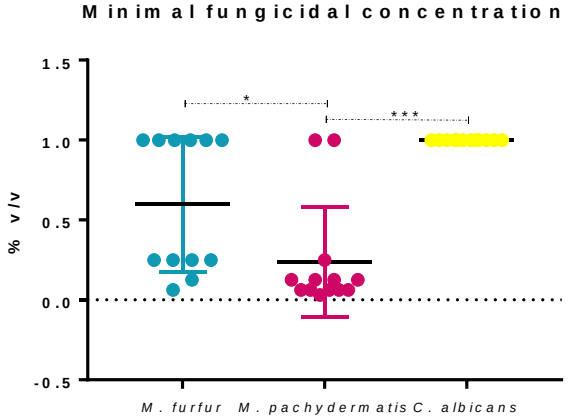


**Fig. 4. Comparison of the minimal fungicidal concentrations of the essential oils from the leaves of *Osteophloeum platyspermum* leaves against the three yeasts, *Malassezia furfur*, *M. pachydermatis* and *Candida albicans*. Kruskal-Wallis followed by Dunn's post-test was used, considering the significance level as α<0.05. *=p<0.05. ***=p<0.0001**

Fig. 7B shows which terpenes are most related to the yeast factor tested. Limonene and myrcene seemed to have more influence on *C. albicans* antifungal activity, whereas α-cadinol and cubenol-1-epi seemed to influence *M. furfur* activity. *M. pachydermatis* seemed to be influenced by the antifungal activity of EOs containing neo-intermediol. It seems that α-pinene, α-cadinol and β-pinene are important for antifungal activity for the three micro-organisms.

## 4. DISCUSSION

Previous works reported the antifungal activity of oregano, cinnamon, thyme, salvia and other essential oils against *Malassezia* spp. [34,36-40], but there is a lack of reports concerning the anti-yeast activity of Brazilian plants and their products, which the present report shall fill.

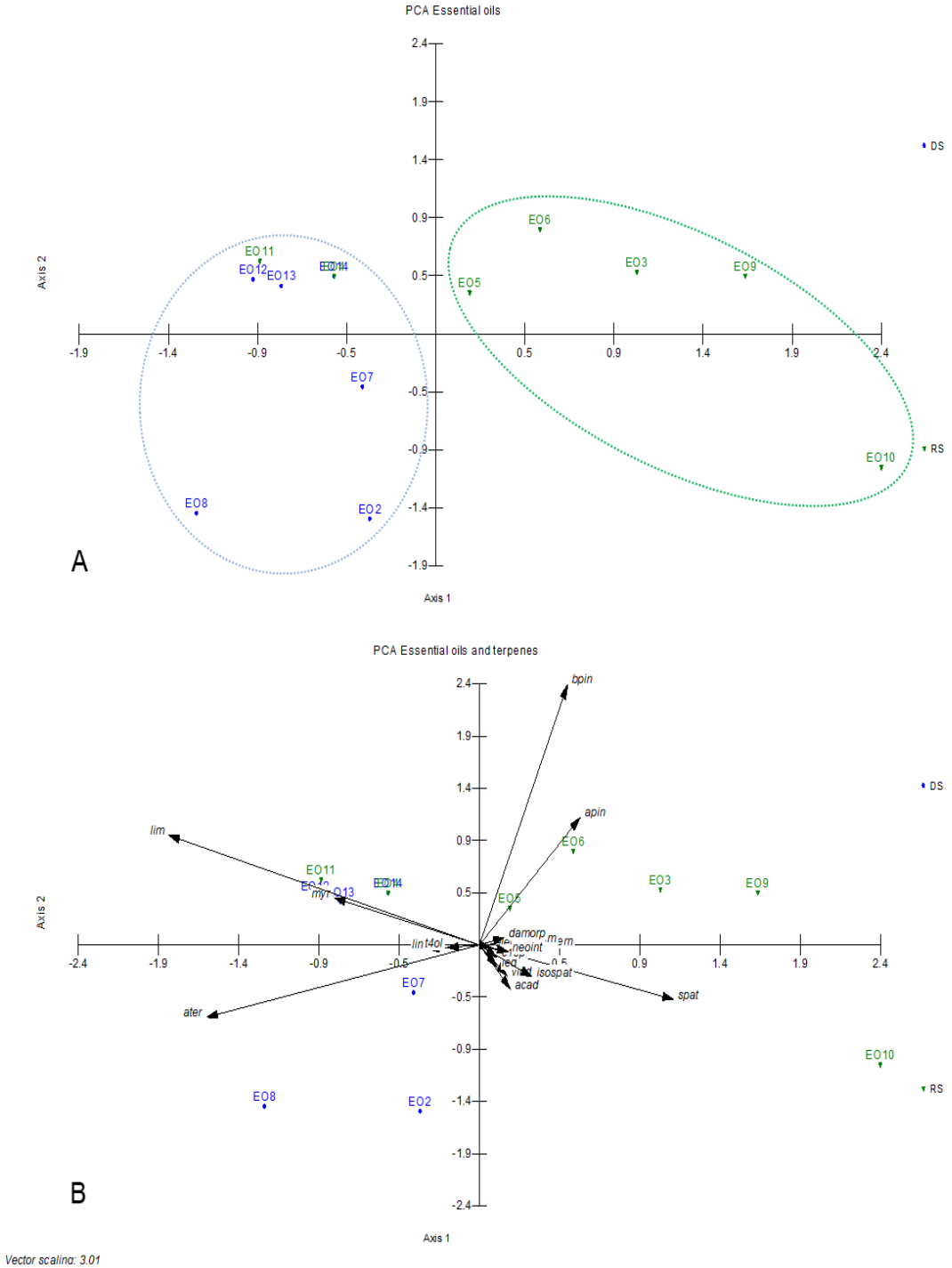


**Fig. 5. Principal component analysis (PCA) made with 13 EOs (cases) obtained from the leaves of *Osteophloeum platyspermum*, considering the 50 terpenes (factors) that were identified as the factors in the multivariate analysis. A. PCA with the 13 cases and 18 factors obtained from the EOs ordinating the cases according to the seasonal variation in dry season (DS) and rainy season (RS). B. The same PCA made before, but showing the vectors corresponding to the terpenes (factors)**

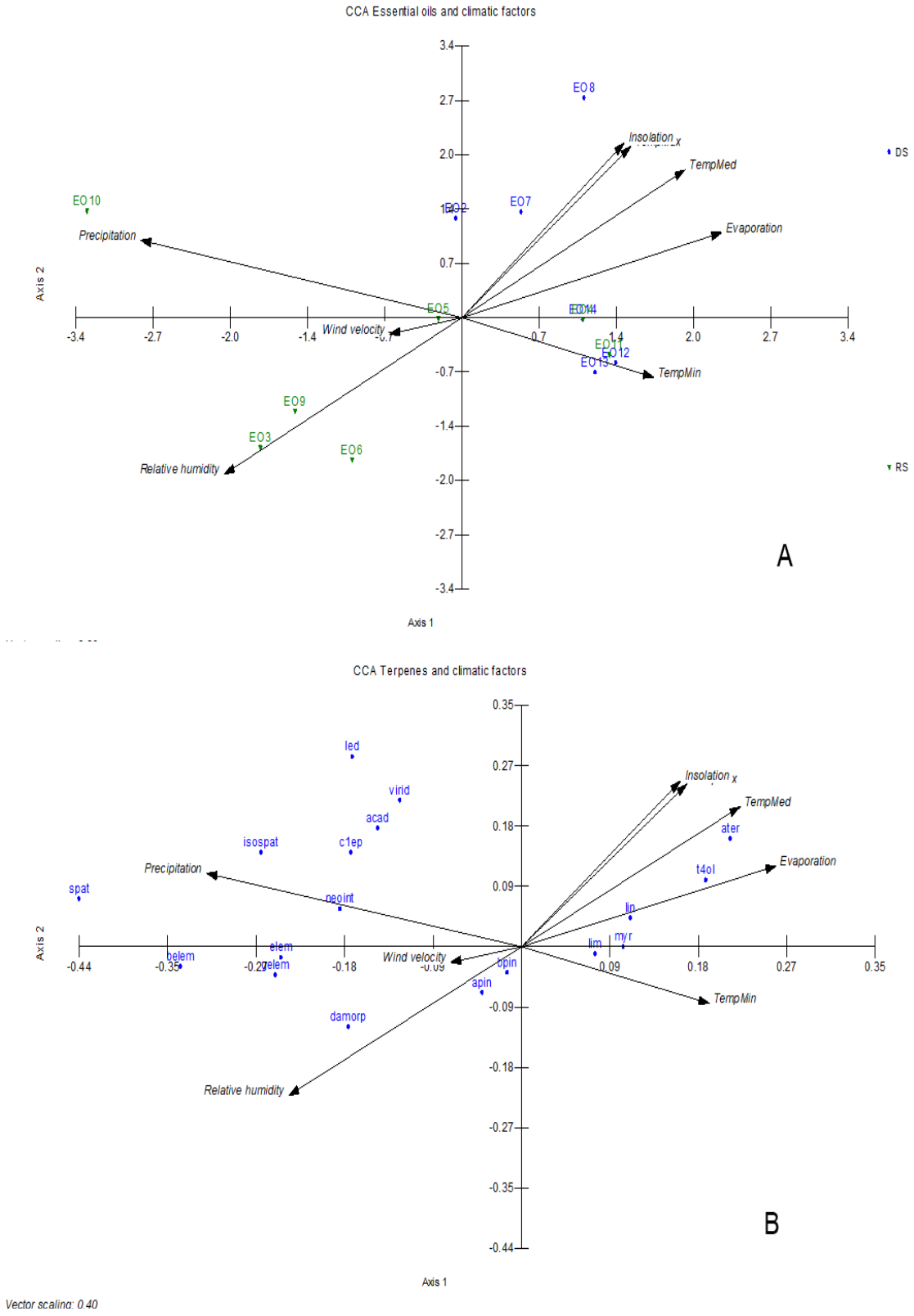


**Fig. 6. Canonical correspondence analysis made with the 13 essential oils (A) and 18 terpenes (B), considering the climatic factors as comparing variables. The oils were obtained from the leaves of *Osteophloeum platyspermum***

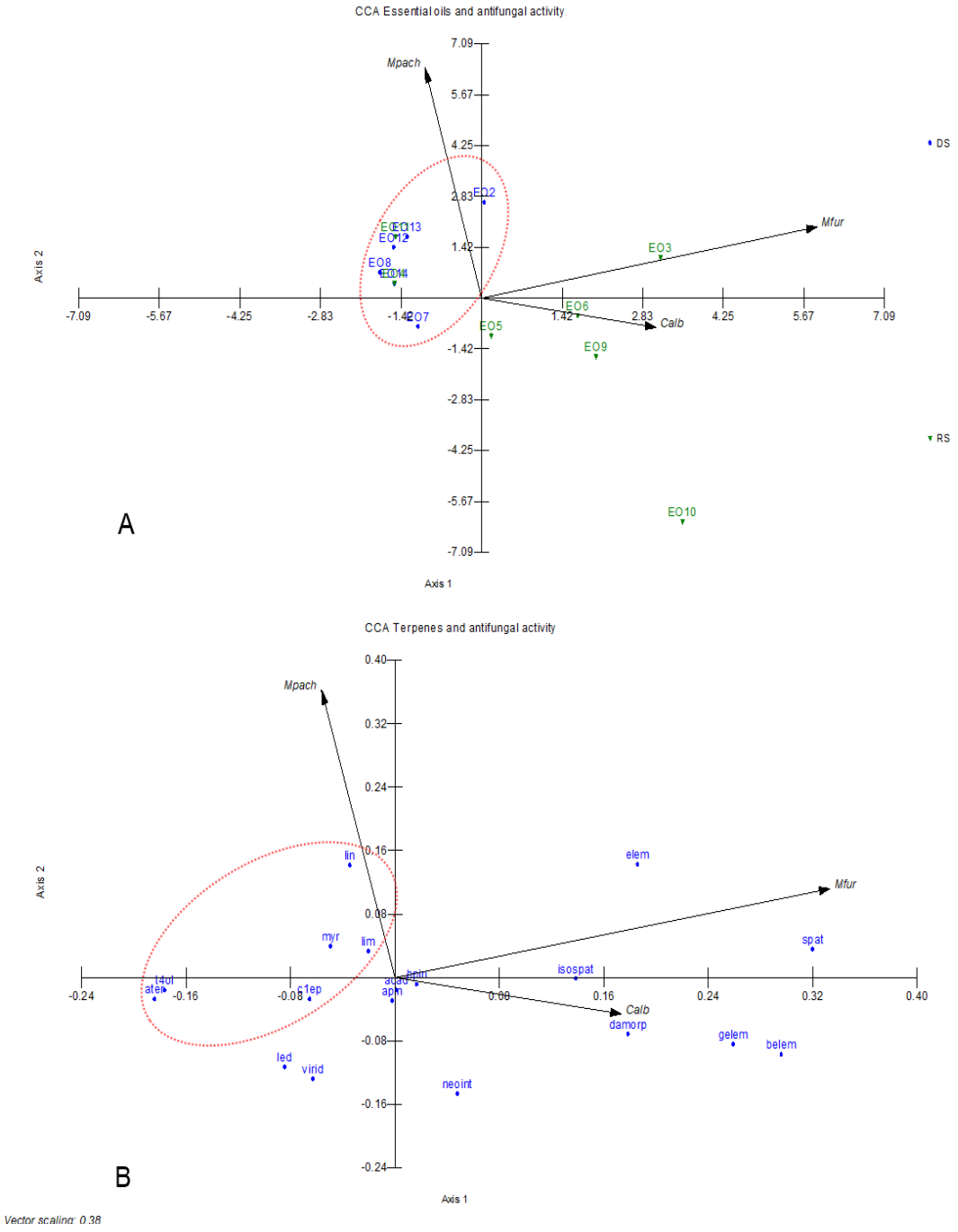


**Fig. 7. Canonical correspondence analysis made with the 13 EOs (cases; A) and 18 terpenes (factors; B) that were obtained from the leaves of *Osteophloeum platyspermum*. The comparison was made with the yeasts *Malassezia pachydermatis, M. furfur* and *Candida albicans***

In a previous work [29], the leaves of one individual tree, *Osteophloeum platyspermum* Warb. (Myristicaceae), popularly known as *ucuuba-chico-de-assis*, were collected 13 times (Brazil/MMA/IBAMA license no. 12A/08 and 30.622-1), spanning a two-year period comprising rainy (RS) and dry seasons (DS [41]). In the work, the authors report that they obtained the essential is by hydrodistillation, in a Clevenger apparatus, for 4 hours. Yields were calculated (% mass/mass), the compounds were identified [42], and were classified according to

their structure. Yields: EO2 (0.236%), EO3 (0.7346%), EO4 (0.4202 %), EO5 (0.5958 %), EO6 (0.3025%), EO7 (0.4363%), EO8 (0.35876%), EO9 (0.6873%), EO10 (0.2916%), EO11 (0.5706 %), EO12 (0.2276 %), EO13 (0.2432%); Compounds (mean ± standard error%): β-pinene (34.59±0.66 %), limonene (20.99±0.74%), α-pinene (9.8±0.44 %), myrcene (7.38±0.33%), α-terpineol (5.53±0.67%), spathulenol (2.75±0.45 %), α -cadinol (1.79±0.13%), linalool (1.29±0.14 %), isospathulenol (1.28±0.14%), terpinen-4-ol (1.03±0.08%), β-elemene (0.8±0.13%), γ-elemene (0.68±0.10%), ledol (0.66±0.08%), neo-intermedeol (0.64±0.08%), δ-amorphene (0.47±0.06%), elemol (0.32±0.04%), cubenol-1-epi (0.71±0.06%), and viridiflorol (2.75±0.45%). Structure: four hydrocarbon monoterpenes (β-pinene, limonene, α-pinene and myrcene), three oxidated sesquiterpenes (α-terpineol, terpinene-4-ol and linalool), three hydrocarbon sesquiterpenes (β-elemene, γ-elemene and δ-amorphene) and eight oxygenated sesquiterpenes (elemol, neo-intermedeol, α-cadinol, cubenol-1-epi, spathulenol, isospathulenol, viridiflorol and ledol [29]). This work supplied the analyses made in the present study by considering the EOs yields, and the terpene percentages used in the statistical analyses.

The yields of the essential oils obtained in the RS were significantly higher than those produced during the DS, possibly due to the higher water availability during RS, which may favor a greater volume of EO for this species. A similar observation was made in a study performed with the EO obtained from the leaves of *Myrocarpus frondosus*, a tree occurring in Southern Brazil [43], where the authors identified a higher oil yield during the winter. On the other hand, in a similar study carried out with *Lippia origanoides* (Verbenaceae), an herbaceous plant that grows in several Brazilian ecosystems, including the Amazon forest, the mean yields of the EOs collected in the DS was not statistically different from the mean yields of the EOs from the plant collected in the RS [44].

The evaluation of the antifungal activity of the 13 EOs has been done against *M. pachydermatis, M. furfur* and *C. albicans*, and all the EOs showed activity against the three yeasts, although variations in the antimicrobial activity were observed. *Candida albicans* showed to be more resistant to the EO activity than the *Malassezia* spp., and was used as reference strain [45-47]. Both *Malassezia* species that were used showed to be more sensitive to the EOs activity than that observed to *C. albicans*. EOs EO6, EO7, EO8, EO10, EO11 and EO12 were extremely active against *M. pachydermatis*, while only EO11 was active against *M. furfur*.

Despite a general variation in the terpene amounts in the EOs, the presence of the four major compounds β-pinene, limonene, α-pinene, and myrcene occurs in a constant pattern during seasonal change. On the other hand, a different situation occurs with *Iryanthera polyneura* Warb. (Myristicaceae), as there is a variation in spathulenol and τ-muurolol, the major compounds, according to a seasonal pattern: Spathulenol occurs in higher amounts during the RS while τ-muurolol occurs in higher amounts during the DS [48].

PCA has revealed that the EOs ordinated according to a seasonal variation, and discriminated those collected in the DS from those collected in the RS. Also, it showed that some of the terpenes have significantly contributed to the ordination, for example, limonene, myrcene, α-terpineol, linalool, and terpinen-4-ol have contributed to the ordination of EO EO2, EO7, EO8, EO12, EO13 and EO14 in the DS group, while α-pinene, β-pinene, spathulenol and the other terpenes contributed to the ordination of EO3, EO5, EO6, EO9 and EO10 in the RS group. The seasonal alterations in the EO composition have been previously reported by other groups. The EOs from the leaves of *Tretadenia riparia*, a plant that occurs in Southern Brazil, showed a significant alteration in the EO composition once 14-hydroxy-9-epi-caryophyllene was present in the EO from the spring collection and absent in the EO from the winter collection. The analyses were based on PCA [49], as was the present findings. Also, the EOs obtained from three species of *Nectandra* (Lauraceae) showed that according to the period the plant material was collected, there was a composition change, discriminated by a PCA, so higher concentrations of oxygenated sesquiterpenes were observed in *N. grandiflora* in the spring, while they were more concentrated in *N. lanceolata* that were collected in the autumn, but for *N. megapotamica* the group of terpenes that were more concentrated was the monoterpene hydrocarbons, in the plants collected in the autumn. All the EOs that were obtained from the three species showed α-pinene and β-pinene as the major compounds [50].

The CCA was used in the discrimination of essential oils and terpenes considering the climatic and the biological analysis factors, so as to prospect how the differences in the terpene expression varying according to a seasonal variation can influence the antimicrobial potential of the essential oils. In the first CCA analysis, two groups of essential oils were discriminated, as observed in the PCA analyses. The group composed by EOs EO2, EO7, EO8, EO12, EO13, and EO14 contains the oils made with leaves collected in the DS and were influenced by insolation, temperature and evaporation, which are climatic characteristics of the Amazon rain forest dry season. On the other hand, the group composed by EO3, EO5, EO6, EO9 and EO10 have been influenced by precipitation, wind velocity and relative humidity, which are climatic characteristics of the rainy season in the Amazon forest. Such results are in accordance with previous works [29]. Also, the ordination of the two groups of EOs was influenced by the terpene variation in each of the essential oil. It was observed that α-pinene, β-pinene, and α-cadinol had less influence on the CCA ordination than that observed for the other terpenes. Limonene, myrcene, linalool, terpinen-4-ol, and α-terpineol have influenced the discrimination of the DS set of EOs, while β-elemene, γ-elemene, neo-intermedeol, elemol, α-cadinol, cubenol-1-epi, spathulenol, isospathulenol, viridiflorol, δ-amorphene and ledol have influenced the discrimination of the RS set of EOs. Previous work also reports that the amounts of spathulenol are higher during the rainy season, as seen in the present findings [48].

The CCA made to analyze the ordination of the essential oils in relation to the antibacterial potential factor showed that the essential oils EO2, EO7, EO8, EO12, EO13 and EO14 were grouped in the DS, and were influenced by the amounts of myrcene, limonene, linalool, terpinen-4-ol, and α-terpineol, which were higher in the DS than in the RS. On the other hand, the essential oils EO3, EO5, EO6, EO9, and EO10 were grouped in the RS, and were influenced by a higher occurrence of spathulenol, elemol, β-elemene, γ-elemene, δ-amorphene, isospathulenol, and neo-intermedeol. The presence of α-pinene, β-pinene, and α-cadinol were significant to all the essential oils, independent of which season they were obtained. The CCA also helps in the elucubration on the relative importance the seasonality, as well as the significance of each terpene, in the general antifungal activity observed in the EOs. It is possible to state that the EOs from the DS are more active than those obtained from the RS against the yeasts, and consequently the occurrence of high amounts of myrcene, limonene, linalool, terpinen-4-ol, and α-terpineol, and the presence of cubenol-1-epi is important to the antifungal activity. Also, five out of six terpenes that are important for the DS discrimination are monoterpenes, and four out of six are oxygenated.

## 5. CONCLUSION

The present work reported how the antifungal activity observed for the essential oils has seasonally varied, and how the relative terpene amounts were significant to the antifungal activity. According to the observation, the essential oils obtained from leaves collected in the dry season, when temperatures are as higher as 40 $^{o}$C, the insolation and evaporation reaches elevated levels, as well as the presence of myrcene, limonene, linalool, terpinen-4-ol, α-terpineol, and cubenol-1-epi, showed to be essential to the antifungal activity. Those findings support the development of future remedies to be used against skin diseases caused by *Malassezia* spp., in animals.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Bond R, Guillot J, Cabañes FJ. *Malassezia* Yeasts in Animal Disease, In: Boekhout T, Guého E, Mayser P, Velegraki A. (Eds.), *Malassezia* and the skin. Science and clinical practice. Springer: Berlin. 2010;271–300.

2. Gao Z, Perez-Perez GI, Chen Y, Blaser MJ. Quantitation of major human cutaneous bacterial and fungal populations. J Clin Microbiol. 2010;48: 3575–3581.
3. Cafarchia C, Gallo S, Capelli G, Otranto D. Occurrence and population size of *Malassezia* spp. in the external ear canal of dogs and cats both healthy and with otitis. Mycopathologia. 2005; 160:143–149.
4. Cabañes FJ. *Malassezia* yeasts: How many species infect humans and animals? PLoS Pathogens;2014.
DOI: 10.1371/journal.ppat.1003892.
5. Velegraki A, Cafarchia C, Gaitanis G, Iatta R, Boekhout T. *Malassezia* infections in humans and animals: Pathophysiology, detection, and treatment. PLoS Pathogens;2015.
DOI: 10.1371/journal.ppat.1004523.
6. Lewis RE, Fothergill AW. Antifungal Agents. In: Hospenthal DR, Rinaldi MG, (Eds.), Diagnosis and Treatment of Human Mycoses. Humana Press Inc: Totowa, New Jersey. 2008;105–133.
7. Heeres J, Meerpoel L, Lewi P. Conazoles. Molecules. 2010;15:4129–4188.
8. Nijima M, Kano R, Nagata M, Hasegawa A, Kamata H. An azole-resistant isolate of *Malassezia pachydermatis*. Vet Microbiol. 2011;149:288–290.
9. Coutinho SD, Paula CR. Susceptibility to antifungal agents of *Malassezia pachydermatis* isolates from dogs. Pol J Vet Sci. 2001;4:77–81.
10. Čermáková Z, Wipler J, Hanzálek T, Horáková H, Buchta V. Pets (dogs/cats) as a possible source of opportunistic pathogenic fungi in humans. Klin Mikrobiol Infekc Lek. 2018;24:41-49.
11. Morris DO. *Malassezia pachydermatis* carriage in dog owners. Emerg Infect Dis. 2005;11:83-88.
12. Iatta R, Cafarchia C, Cuna T, Montagna O, Laforgia N, Gentile O, Rizzo A, Boekhout T, Otranto D, Montagna MT. Bloodstream infections by *Malassezia* and *Candida* species in critical care patients. Med Mycol. 2014;52:264-269.
13. Baker RM, Stegink RJ, Manaloor JJ, Schmitt BH, Stevens JC, Christenson JC. *Malassezia* Pneumonia: A rare complication of parenteral nutrition therapy. JPEN J Parenter Enteral Nutr. 2016;40:1194-1196.
14. Sizun J, Karangwa A, Giroux JD, Masure O, Simitzis AM, Alix D, De Parscau L. *Malassezia furfur*-related colonization and infection of central venous catheters. A prospective study in a pediatric intensive care unit. Intensive Care Med. 1994;20:496-499.
15. Chow NA, Chinn R, Pong A, Schultz K, Kim J, Gade L, Jackson BR, Beer KD, Litvintseva AP. Use of whole-genome sequencing to detect an outbreak of *Malassezia pachydermatis* infection and colonization in a neonatal intensive care unit-California, 2015-2016. Infect Control Hosp Epidemiol. 2020;41:851-853.
16. Gupta P, Chakrabarti A, Singhi S, Kumar P, Honnavar P, Rudramurthy SM. Skin Colonization by *Malassezia* spp. in hospitalized neonates and infants in a tertiary care centre in North India. Mycopathologia. 2014;178:267-272.
17. Pedrosa AF, Lisboa C, Rodrigues AG. *Malassezia* infections with systemic involvement: Figures and facts. J Dermatol. 2018;45:1278-1282.
18. Rodrigues FFG, Oliveira LGS, Rodrigues FFG, Saraiva ME, Almeida SCX, Cabral MES, Campos AR, Costa JGM. Chemical composition, antibacterial and antifungal activities of essential oil from *Cordia verbenacea* DC leaves. Pharmacognosy Res. 2012;4:161–166.
19. Das S, Kumar SV, Kumar DA., Kumar CA, Upadhyay NSA, Singh AKS, Ajay RC, Shaon P, Bhanu D, Nawal K. 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;2018.
DOI: 10.1080/14786419.2018.1519826.
20. Ali-Shtayeh MS, Abu-Zaitoun SY, Dudai N, Jamous RM. Downy Lavender oil: A promising source of antimicrobial, antiobesity, and anti-alzheimer's disease agents. Evid Based Complement Alternat Med;2020.
DOI: 10.1155/2020/5679408.
21. Noriega P, Ballesteros J, Cruz AD, Veloz T. Chemical composition and preliminary antimicrobial activity of the hydroxylated sesquiterpenes in the essential oil from *Piper barbatum* Kunth leaves. Plants;2020.
DOI: 10.3390/plants9020211.
22. Khosravi AR, Shokri H, Fahimirad S. Efficacy of medicinal essential oils against pathogenic *Malassezia* sp. isolates. J Mycol Med. 2016;26:28–34.
23. Vinciguerra V, Rojas F, Tedesco V,

Giusiano G, Angiolella L. Chemical characterization and antifungal activity of *Origanum vulgare*, *Thymus vulgaris* essential oils and carvacrol against *Malassezia furfur*. Nat Prod Res. 2019;33:3273–3277.

24. Barac A, Donadu M, Usai D, Tomic V, Mazzarello V, Zanetti S, Aleksic E, Stevanovic G, Nikolic N, Rubino S. Antifungal activity of *Myrtus communis* against *Malassezia* sp. isolated from the skin of patients with pityriasis versicolor. Infection. 2018;46:253–257.
25. Sim JXF, Khazandi M, Pi H, Venter H, Trott DJ, Deo P. Antimicrobial effects of cinnamon essential oil and cinnamaldehyde combined with EDTA against canine otitis externa pathogens. J Appl Microbiol. 2019;127:99–108.
26. Bohmova E, Conkova E, Harcarova M, Sihelska Z. Interactions between Clotrimazole and selected essential oils against *Malassezia pachydermatis* clinical isolates. Pol J Vet Sci. 2019; 22:173-175.
27. Oliveira MS, Gontijo SL, Teixeira MS, Teixeira KIR, Takahashi JA, Millan RDS, Segura MEC. Chemical composition and antifungal and anticancer activities of extracts and essential oils of *Schinus terebinthifolius* Raddi fruit. Revista Fitos. 2018;12:135-146.
28. Durazzini AMS, Machado CHM, Fernandes CC, Willrich GB, Crotti AEM, Candido ACBB, Magalhães LG, Squarisi IS, Ribeiro AB, Tavares DC, Martins CHG, Miranda MLD. *Eugenia pyriformis* Cambess: a species of the Myrtaceae family with bioactive essential oil. Nat Prod Res;2019.
DOI: 10.1080/14786419.2019.1669031.
29. Suffredini IB, Sousa SRN, Frana SA, Suffredini HB, Díaz IEC, Paciencia MLB. Multivariate analysis of the terpene composition of *Osteophloeum platyspermum* Warb. (Myristicaceae) and its relationship to seasonal variation over a two-year period. J Essent Oil-Bear Plants. 2016;19: 1380–1393.
30. CLSI. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard, 3rd ed., M27-A 3. Wayne, PA: Clinical and Laboratory Standards Institute;2008.
31. Cafarchia C, Iatta R, Immediato D, Puttilli MR, Otranto DMA. Azole susceptibility of *Malassezia pachydermatis* and *Malassezia furfur* and tentative epidemiological cut-off values. Med Mycol. 2015; 53: 743–748.
32. Rosana F, Davicino R, Anesini C. Antifungal activity of the aqueous extract of *Ilex paraguariensis* against *Malassezia furfur*. Phytother Res. 2010;24:715–719.
33. Liu W, Sun B, Yang M, Zhang Z, Zhang X, Pang T, Wang S. Antifungal activity of crude extract from the rhizome and root of *Smilacina japonica* A. Gray. Evid Based Complement Alternat Med;2019.
DOI: 10.1155/2019/5320203.
34. Santin R, Giordani C, Madrid IM, Matos CB, Freitag RA, Meireles MCA, Cleff MB, Mello JRB. Atividade antifúngica do óleo essencial de *Origanum vulgare* frente a *Malassezia pachydermatis*. Arq Bras Med Vet Zootec. 2014;66:367–373.
35. Martins ER, Díaz IEC, Paciencia MLB, Frana SA, Morais D, Eberlin MN, Silva JS, Silveira ER, Barros MP, Suffredini IB. Interference of seasonal variation on the antimicrobial and cytotoxic activities of the essential oils from the leaves of *Iryanthera polyneura* in the amazon rain forest. Chem Biodivers;2019.
DOI: 10.1002/cbdv.201900374.
36. Hammer KA, Carson CF, Riley TV. *In vitro* activities of ketoconazole, econazole, miconazole, and *Melaleuca alternifolia* (tea tree) oil against *Malassezia* species. Antimicrob Agents Chemother. 2000;44:467–469.
37. Rusenova N, Parvanov P. Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. Trakia J Sci. 2009;7:37–43.
38. Pistelli L, Mancianti F, Bertoli A, Cioni PL, Leonardi M, Pisseri F, Mugnaini L, Nardoni S. Antimycotic activity of some aromatic plants essential oils against canine isolates of *Malassezia pachydermatis*: an *in vivo* assay. The Open Mycol J. 2012;6:17–21.
39. Sreelatha GL, Babu UV, Kumar LMS, Soumya K, Sharmila T. Investigation on biochemical characterisation and *in vitro* antifulgal efficacy of plant extracts on *Malassezia furfur*. Int J Pharm Bio Sci. 2015;6:1027–1041.
40. Velegraki A, Graikou K, Kritikou S, Varsani M, Chinou I. Investigation of *in vitro* antifungal efficacy of selected extracts of Greek natural products on *Malassezia* spp. Planta Med. 2016.
DOI: 10.1055/s-0036-1596899.
41. Fisch G, Marengo J A, Nobre CA. Clima da Amazônia, at

http://climanalise.cptec.inpe.br/~rclimanl/boletim/cliesp10a/fish.html, assessed on 03/07/2016.
42. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4 Ed. Illinois: Allured Pub. Comp. Chicago, IL, USA. 2007;804.
43. Cabrera DC, Gomes GLS, Flach A, da Costa LAMA, Rosa GR, de Moura NF. Evaluation of climatic factors on the yield and chemical composition of the essential oil of *Myrocarpus frondosus.* Nat Prod Res. 2014;29:667–670.
44. Sarrazin SLF, Silva LA, Assunção APF, Oliveira RB, Calao VYP, Silva R, Stashenko EE, Maia JGS, Mourão RHV. Antimicrobial and seasonal evaluation of the carvacrol-chemotype oil from *Lippia origanoides* kunth. Molecules. 2015;20:1860–1871.
45. Berechet MD, Gaidau C, Miletic A, Pilic B, Râpă M, Stanca M, Ditu LM, Constantinescu R, Lazea-Stoyanova A. Bioactive properties of nano fibres based on concentrated collagen hydrolysate loaded with Thyme and Oregano essential oils. Materials;2020.
DOI: 10.3390/ma13071618.
46. Dai DN, Chung NT, Huong LT, Hung NH, Chau DTM, Yen NT, Setzer WN. Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils from five species of *Cinnamomum* growing wild in north central Vietnam. Molecules;2020.
DOI: 10.3390/molecules25061303.
47. Almeida ETC, Souza GT, Guedes JPS, Barbosa IM, Sousa CP, Castellano LRC, Magnani M, Souza EL. *Mentha piperita* L. essential oil inactivates spoilage yeasts in fruit juices through the perturbation of different physiological functions in yeast cells. Food Microbiol. 2019;82:20–29.
48. Martins ER, Díaz IEC, Paciencia MLB, Frana SA, Marques MOM, Morais DR, Eberlin M, Suffredini IB. Amazon climatic factors driving terpene composition of *Iryanthera polyneura* Ducke in *terra-firme* forest: A statistical approach. PLoS One; 2019.
DOI: 10.1371/journal.pone.0224406.
49. Gazim ZC, Amorim AC, Hovell AMC, Rezende CM, Nascimento IA, Ferreira GA, Cortez DAG. Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from leaves of *Tetradenia riparia* (Hochst.) Cdd in southern Brazil. Molecules. 2010;15:5509–5524.
50. Ferraz EO, Vieiram MAR, Ferreira MI, Junior AF, Marques MOM, Minatel IO, Albano M, Sambo P, Lima GPP. Seasonality effects on chemical composition, antibacterial activity and essential oil yield of three species of *Nectandra*. PLoS One;2018.
DOI: 10.1371/journal.pone.0204132.



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