MDGC-MS analysis of essential oils from *Protium heptaphyllum* (Aubl.) and their antifungal activity against *Candida* specie

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ABSTRACT: *Protium heptaphyllum* is found in the Amazon region, and in various Brazilian states and South American countries. Also Known as almecega, it produces an oil resin used in traditional medicine as analgesic, anti-inflammatory, cicatrizant and expectorant, it is rich in pentacyclic triterpenes and essential oil. The main objective of this study was to analyze the chemical composition of *P. heptaphyllum*resin (OEPh) over different extraction times and to evaluate their antifungal activity against Candida species, obtained from gardeners with onychomycosis, using the disk diffusion method. The OEPh was obtained by hydrodistillation and analyzed by Multidimensional Gas Chromatography coupled with Mass Spectrometry (MDGC / MS). Candida species were obtained from lesions on the nails of horticulturist from a community garden in the city of Teresina, Piauí, Brazil. The antifungal activity in concentrations of 1000 µg/L, 500 µg/L and 250 µg/L, PROTOCOL M44-A2 (CLSI 2009) OEPh was tested. The main constituents identified were: *l*-limonene, α-terpineol, p-cineol, o-cymene and α-phellandrene, however, its composition varies significantly with extraction time. All species, except *C. rugosa,* were inhibited with halo (≥ 14 mm) at 1000 µg / L. *C. krusei* is naturally resistant to the drug fluconazole, but when tested with OEPh the clinical species (case 9) demonstrated sensitivity in three dilutions (halo ≤ 10 ≥ 14) and the standard strain was inhibited at concentration of 1000 µg/Lg / L (halo 14mm). A similar situation also occurred with the standard strain of *C. parapsilosis* (halo ≥ 11mm). OEPh has considerable antifungal activity, which merits further investigation for alternative clinical applications, since this species is widely distributed in our community, and it presents good yields, and also has important therapeutic applications.

Key words: *Protiumheptaphyllum,* Análise Química, *Candida*, susceptibilidade, onicomicoses

RESUMO: Análise MDGC-MS de óleos essenciais de *Protium heptaphyllum* (Aubl.) e sua atividade antifúngica contra espécies de *Candida.* *Protium heptaphyllum* é encontrada na região amazônica, em vários estados do Brasil e países da América do Sul. Conhecida como almecega produz uma resina oleosa usada na medicina popular como analgésica, anti-inflamatória, cicatrizante e expectorante, é rica em triterpenos pentaciclicos e óleo essencial. O objetivo principal do presente trabalho foi analisar a composição química do óleo essencial da resina P. *heptaphyllum* (OEPh) em diferentes tempo de extração e avaliarsuaatividade antifúngica contra espécies de *Candida,* isoladas de horticultores com onicomicoses, por método de disco-difusão. O OEPh foi obtido por hidrodestilação, analisado por Cromatografia Gasosa Multidimensinal Acoplada a Espectrometria de Massas (MDGC/MS). As espécies de *Candida* foram obtidas de lesões nas unhas de horticultores de uma horta comunitária na cidade de Teresina, Piauí, Brasil. Testou-se a atividade antifúngica do OEPhnas concentrações de 1000 µg/L, 500 µg/L e 250 µg/L, protocolo M44-A2 (CLSI 2009). Os principais constituintes identificados foram *l*- limoneno, α-terpineol, *p*-cineol, *o*-cimeno e α-felandreno, entretanto, sua composição varia significativamente em decorrência do tempo de extração. Todas as espécies, exceto a *C. rugosa*, foram inibidas com halo ($\overline{X}$ ≥ 14 mm) na concentração de 1000 µg/L. *C. krusei* é naturalmente resistente ao fármaco fluconazol, mas quando testado com OEPh,a

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espécie clínico (caso 9) demonstrou sensibilidade nas três diluições (halo $\overline{X} \leq 10 \geq 14$) e a cepa padrão foi inibida na concentração de 1000 µg/L (halo $\overline{X}$ 14mm). Fato semelhante também ocorreu com a cepa padrão de *C. parapsilosis* (halo $\overline{X} \geq$ 11mm). O OEPh possui atividade antifúngica considerável, merecendo uma investigação mais aprofundada para aplicações clínicas alternativas, uma vez que esta espécie é amplamente distribuída em nossa comunidade, apresenta bom rendimento e, ainda, aplicações terapêuticas importantes.

Palavra-chaves: *Protium heptaphyllum,* Análise Química, *Candida*, Susceptibilidade, Onicomicoses

INTRODUCTION

Protium heptaphyllum (Aubl.) Marchand is a tree of the Burseraceae family, aromatic, evergreen or semi-deciduous, of humid sandy soil or dry and that reaches 10-20 m in height (Lorenzi & Matos, 2008). These species are widely spread throughout tropical and subtropical regions, mainly in the Amazon Rainforest.

P. heptaphyllum is found in the Amazon region and in some Northeastern states, such as Bahia, Ceara, Piaui, as well as in other countries of South America (Colombia, Paraguay, Suriname and Venezuela) (Citó et al. 2003; Lorenzi & Matos, 2008). It's recognized by the use of its leaves and bark as hemostatic, healing and anti-inflammatory, being used by the native people also as a nasal decongestant. Its stalk exudes an oily resin called almecega or breu-branco, which hardens on contact with air, of white-greenish color, pleasant aroma and rich in essential oils (Lorenzi & Matos, 2008). Contraceptive, antineoplastic, healing, expectorant and antimicrobial action were also related others authors (Oliveira et al. 2005, Rüdiger et al. 2007).

Phytochemical studies performed on gums and oil resin, obtained from *P. heptaphyllum* revealed the presence of binary mixtures of triterpenes, especially α- and β-amyrins (Susunaga et al. 2001, Oliveira et al. 2005). The chemical constituents of essential oil of this resin analyzed by gas chromatography-mass spectroscopy (GC/MS) are mainly monoterpenes (Bandeira et al. 2001, Citó et al. 2003), but, phenylpropanoids were also identified in the product (Siani et al. 1999a). In the essential oil of *P.heptaphyllum* was detected p-cymene (39.93 %) and α-tetradecane (13:38 %) as the main constituents (Marques et al. 2010).

Due to the high cost of antifungal agents, the side effect reactions observed and the occurrence of resistance of some species to antifungal agents, the use of essential oils from plants has been target of studies given its potential as natural antifungal agents. Onychomycosis is a fungal infection of nail which affects about 6 to 8% of the adult population worldwide (Amartya et al. 2013). The yeasts, including *Candida spp*. account for 2-10 % of fungal nail infections (Thomas et al. 2010).

In view of the popular knowledge of the antimicrobial activity of almecega and the absence of studies on the antifungal activity of this material in clinical isolates, this study was conducted to analyze the chemical composition of the essential oil of *Protium heptaphyllum* and evaluate their antifungal activity against yeast species, obtained from lesions of workers with onychomycosis in a community garden located in the neighborhood "Dirceu", in the City of Teresina, Piaui, Brazil.

MATERIAL AND METHOD

Plant Material

The resin was collected at a private property in the municipality of Timon, Maranhão, Brazil, in December 2012 and kept under refrigeration until the time of extraction of the oil. The specie was identified in the Herbarium Graziela Barroso of the Federal University of Piaui, Brazil, and a voucher specimen was registered under No. 28730.

Extraction

The resin was subjected to hydrodistillation in a Clevenger type system. Six successive fractions were collected at intervals of one hour. In another system was collected a fraction after 6 continuous hours (6h cont), this fraction was used for the antifungal tests, due its higher yield. The extracted oils were dried with sodium sulfate (Na_2SO_4), weighed and stored under refrigeration (5°C).

Analysis by gas chromatography-mass spectrometry

The analysis was realized by Gas Chromatography System Multidimensional, MDGC/GCMS-2010 SHIMADZU. In the chromatography of the components, an elite column Perkin Elmer mark with 30 m length and 0.25 mm in inner diameter and 0.25 µ film thickness was used in the first dimension (FID).Nitrogen was used as carrier gas with a flow rate of 1.0 mL/min, injector temperature 260 °C, the column was programmed with an initial temperature

of 50 °C (1 min) followed by an increase of 5 °C/min up to 180 °C (2 min), then 10 °C / min to reach 225 °C (5 min).

In the second dimension similar column and analysis methods were used, GC-MS. The conditions of MS were: ion detector quadrupole operating in electron impact and impact energy of 70 eV; scan speed 1,000; and the fragments found in the range of 45 to 650 Da.

The identification of the essential oil components was performed by comparing the retention indices, interpretation of the fragmentation pattern of the respective mass spectra, comparison with the database of the analysis system (being considered only the spectra with index of equal similarity or greater, at 95%) and with literature data. The pattern solution of n-alkanes (C_8-C_{20}) was used for the calculation of the index of Kolvats, and this value was compared to the one described in the literature (Adams 2007, Costa et al. 2010).

Antifungal activity

To evaluate the antifungal activity of the essential oil of *P. heptaphyllum* resin, we used disk diffusion tests that were performed in the Research Laboratory of the University Center UNINOVAFAPI, according to the protocol M44-A2 (CLSI 2009).

A suspension was prepared for each species of yeast with fresh culture of 24h of clinical isolates of: *Candida krusei, C. albicans, C. parapsilosis, C. metapsilosis, C. rugosa, C. guillermondii*. The identified agents came from lesions of onychomycosis of workers in a community garden located at Dirceu district, city of Teresina, State of Piaui, Brazil (after being approved by the research ethics committee 0207.0.043.000-10). 25 µL of each yeast suspension was inoculated on Petri dishes containing culture medium Mueller-Hinton (Difco™, USA), with glucose at 2% and methylene blue (MGB agar). Three oil concentrations were used, being 1000 µg/L, 500 µg/L and 250 µg/L. Withdrew 20 µL of each concentration, placed in sterile and standardized discs with a diameter of 6 mm, which were then deposited on the surface of the MGB medium. For quality control with strains of *C.parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258, and as negative control, we used solvent acetone/water at 15%. All tests were carriedout in triplicate.

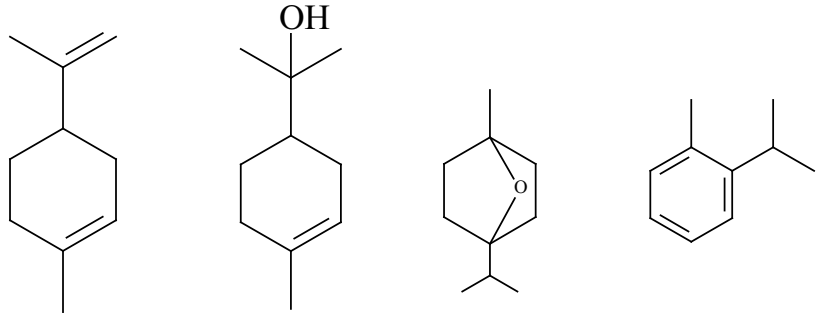
The descriptive analysis of the categorical variables was carried out by reading the absolute and relative frequencies (%), while in the numeric variables, the average position and standard deviation variability (SD) measurements were taken.

RESULTS AND DISCUSSION

Performance and Analysis of Chemical Constituents

The MDGC/GCMS-2010 is a MDGC system that offers excellent performance and highly reproducible separation with “multi-deans switching” technology, supporting the analysis of complex matrices, such as rocks extract samples, oils (petroleum), aromas and optical isomers. In this research work, we did not identified significant changes in the oil composition when using similar polarity column,however we focused especially on the separation and quantification power in the first dimension (MDGC) and identification in the second dimension (GCMS).The analysis of the essential oil of *P. heptaphyllum* resin by MDGC-MS allowed the identification of 26 constituents, predominantly monoterpenes (Table 1). The constituents present in greater abundance were: *l*-limonene, α-*terpineol, p-cineole* and *o-cymene* (Figure 1) in the first hour of extraction, with yield of 0.62 %.

It was possible to observed that as the extraction time is increased, the percentage of *l-limonene* (constituent in greater abundance in the first hour of extraction) decreases as the



l-Limonene α-Terpineol *p*-Cineole *o*-Cymene

FIGURE 1. Chemical structure of the main components of the essential oil of *P. heptaphyllum*.

concentration of α-*terpineol* increases, as well as other compounds that did not appear in the first hours begin to emerge. This fact is explained by the secondary reactions that may occur in the system due to the constant heating.

Research on antifungal effects of *DL-limonene* against various yeast species was presented by Ünal et al. (2012). More specifically, these authors demonstrated that *DL-limonene* at a concentration of 0.20% (w V-1) inhibited cell growth, ethanol formation, and sugar utilization by *S. cerevisiae*.

The α-*terpineol* is a major component of the essential oil of many plants and has been reported to enhance the permeability of skin to lipid- soluble compounds (Williams & Barry, 1991). Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria was evaluated by Park et al. (2012). The α-*terpineol* has also been described to have anti-inflammatory properties (Held et al. 2007), antibacterial (Kotan et al. 2007) and antifungal activities (Pitarokili et al. 2002). Hassan et al. (2010)showed that α-*terpineol* also has a potential anticancer agent.The p-cineole is responsible for several activities, amongst which,

TABLE 1. Extraction time influence on the chemical composition (%) of the essential oil of *P. heptaphyllum* resin.

		Extration Time						
Constituents	**IK***	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**6h cont**
α-Tujeno	924							0.40
α-Pinene	932	1.05	-	1.17	0.94	0.71	0.51	1.17
Sabenene	969	-	-	-	0.18	-	-	
β-Pinene	974	-	-	-	0.38	-	-	0.32
α-Phelandrene	1002	11.38	8.11	7.77	7.77	7.42	6.90	2.41
δ 3-Carene	1008	9.67	6.15	7.10	7.04	6.22	5.92	6.14
o-Cymene	1022	12.52	10.82	9.38	9.24	8.12	8.06	17.43
l-Limonene	1024	26.31	21.31	16.49	17.19	15.46	15.85	36.01
β-Phelandrene	1025	c/a	lc/a	lc/a	lc/a	lc/a	lc/a	1.49
p-Cineole	1026	18.98	14.34	8.73	9.96	8.59	8.06	18.68
γ-Terpinene	1054	-	-	-	-	0.35	-	
Cumicaldehyde	1077	-	-	-	-	0.30	-	
α-Terpinolene	1086	0.86	-	1.73	1.50	1.60	1.43	
β-Linalool	1095	-	-	1.05	0.83	-	0.70	
Cuminol	1104	-	-	-	0.47	0.3	-	
Mircene	1119							0.76
l-Camphor	1141	-	-	-	0.72	0.70	-	
4-Terpineol	1174	-	-	2.14	1.65	1.91	1.74	0.63
Cripton	1183							0.35
α-Terpineol	1186	19.22	32.60	30.05	31.14	30.91	32.57	6.60
Cis-Pinocarveol	1187	-	-	2.97	3.67	4.16	4.63	
Verbenone	1204	-	-	-	0.45	-	-	
Cis-Carveol	1226	-	-	-	-	0.52	0.53	0.31
Carvone	1239	-	-	0.86	-	0.77	0.76	1.06
Carvotanacetone	1244	-	-	-	-	1.69	1.33	
Piperitone	1249	-	-	2.51	2.16	-	-	0.85
Carvacrol	1298	-	-	1.21	1.55	2.25	2.87	
Methyleugenol	1403	-	-	-	0.66	1.13	1.22	
β-Sesquiphelandrene	1521	-	-	-	-	0.36	-	
Elemol	1537	-	-	-	-	0.50	-	
Yield (%)		0.62	0.25	0.17	0.17	0.11	0.06	1.38
Non- Identified		-	6.66	6.31	2.51	6.01	6.93	4.89

*Indice de Kolvats (Adams, 2007). lc/a = low concentration or absent.

it is used in the treatment of skin infections (Hammer et al. 2002). Recently, Santin et al. (2014) showed that the major compounds identified in the essential oil *Origanum vulgare*, are; thymol, 4-terpineol, α-terpinene which are responsible for antifungal activity against the yeast *M. pachydermatis.*

In the world market, much of the α-*terpineol* comes from synthetic route that uses pinene and turpentine as precursor substances (Baptistella et al. 2009) and other synthetic routes from *l-limonene* and *nerol* have been studied. The α-*terpinil trifluoroacetate* when derivative from *d-limonene*, through the addiction of Markvnikov using trifluoroacetic acid can be converted into d-α-terpineol by hydrolysis (Yuasa &Yuasa, 2006), as illustrated in (Figure 2).

The chemical composition of the oil from the resin of *Protium* varies according to time of year and the region where the material was collected. Previous studies performed with the same resin, but acquired from other sources showed that the chemical composition of the oil undergoes significant changes. Citó et al. (2003) found as major compounds β-*terpinilacetate* (23.2%), *limonene* (18.2%) and β-*ocimene* (11.2%), in resin from Teresina (Piaui , Brazil), while the oil obtained by Marques *et al*. (2010) in Cruzeiro do Sul, State of Acre (Brazil) showed *p-cymene* (39.93%), *n-tetradecane* (13.38%) and *dihydro-4-carene* (11.69%). The (Table 2) shows the major components identified in the essential oil of *P. heptaphyllum* collected in different regions.

The differences in the composition of essential oils extracts of *P. heptaphyllum* may occur due to other factors including in traspecific genetic differences in plants, seasonal and environmental influences such as temperature, humidity, soil, and time of collection. The time and method of storage of the resin is an important aspect to be considered, especially in view of the essential oils consisting mainly of *monoterpenes* and light compounds (volatiles, heat-unstable and photolabile) (Mattana et al. 2015; Rüdiger et al. 2007).

Antifungal Activity

So far there are no reports in the literature on the use of OEPh for the treatment of onychomycosis, which is considered a crucial disease in public health due to its high incidence and worldwide prevalence, representing more than 50 % of all onychopathies (Bokhari et al. 1999, Thomas et al. 2010, Zotti et al. 2011). Performance evaluation of disk diffusion method for determining the antifungal activity of OEPh, against yeasts agents of onychomycosis are shown in (Table3).

It was observed that all the species, except *C. rugosa,* were inhibited with halo ≥ 14 mm at 1000 µg/L. The *C. krusei*(case 9), *C. guilliermondii*(case 54) and *C. albicans* (cases 71, 89) were inhibited in all three concentrations (halo 12 ≤ $\overline{X}$ ≥ 14 mm). Fluconazole is an antifungal that has good clinical activity against most Candida spp. However,*C. krusei* is naturally resistant to this drug, but when tested with OEPh the clinical species showed sensitivity in three dilutions, and standard strain was inhibited at 1000 µg/L (halo ≥ 14mm). A similar situation also occurred with the standard strain of *C. parapsilosis,* which at the same concentration showed halo $\overline{X}$ ≥ 11mm. Comparing the performance of OEPh and fluconazole in relation to other Candida species, the drug Fluconazole is more reactive than the tested oil (halo 20 ≤ ≥35 mm).

Values of inhibition halo equal to or greater than 8 mm or 10 mm are indicative of the presence of inhibitory activity (Fenner et al. 2006, Lima et al. 2006, Aibinu et al. 2007, Dutta et al. 2007, Nascimento et al. 2007, Packer & Luz, 2007, Scorzoni et al. 2007, Pozzati et al. 2008, Menezes et al. 2009, Almeida et al. 2011, Cavalcanti et al. 2012). These values do not predict clinical response, but are associated to products with pharmacological potential in studies of drug screening (Cavalcanti et al. 2012).

Although the disk diffusion test is one of the most used and accepted for the search for new drugs, the results obtained in tests with this method are difficult to interpret. The substances used to

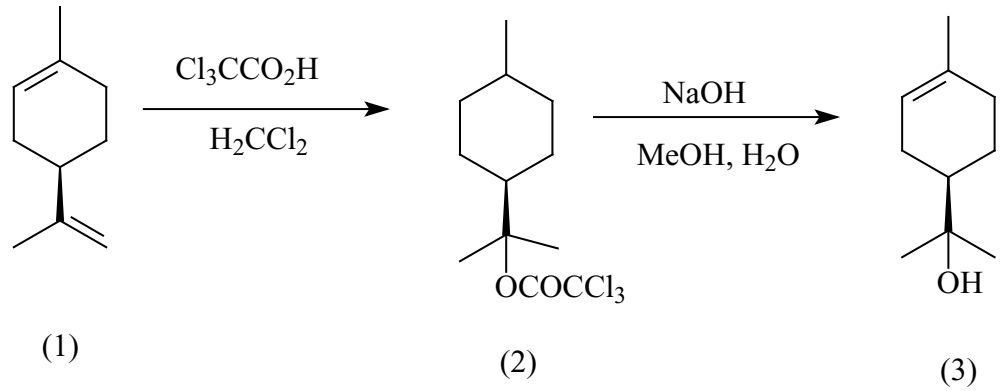


FIGURE 2. Synthesis of α-terpineol (3) from *d*-limonene (1) and α- *terpinyltrichloroacetate* (2)

TABLE 2. Literature review of major constituents of essential oil of *P. heptaphyllum*.

Constituents	Region	Material	Reference
p-Cimene (11.00%), limonene (4.20%), terpinolene (21.8%), dilapiol (16.1%).	Reserve of Campina, City of Manaus, State of of Amazonas Brazil	Resin	Siani et al., 1999a
terpinolene (28.5%), limonene (16.9%), α-felandrene (16.7%) e α-pinene (10.5%).	Crato (State of Ceará) Brazil	Resin	Bandeira et al. 2001
myrcene (18.6%) e β-caryophyllene (18.6%)	Crato, Brazil	Leaves	Bandeira et al. 2001
α-pinene (71.2 %).	Crato, Brazil	Fruits	Bandeira et al. 2001
α-terpinene (18.0%), *p*-cimene (36.0%), γ -terpinene (12,0%).	Reserve of Campina, Manaus - Amazonas, Brazil	FreshResin	Siani et al, 1999b
p-Cimene (11.0%), terpinolene,*p*-cimen-8-ol (11.0%), dilapiol (16.0%)	Reserve of Campina, Manaus - Amazonas, Brazil	AgedResin	Siani et al, 1999b
terpinilacetate (23.2%), limonene (18.2%) e β-ocimene(11.2%)	Obtained in the market in Teresina, State of Piaui, Brazil	Resin	Citó et al, 2003
9-epi (E)-cariophilene (21.4 %), trans-isolongifolanone (10.3 %) e 14-hidroxi-9-epi-β-caryophyllene (16.7%)	Biological Reserve of Guadalupe – State of Pernambuco, Brazil	Leaves	Pontes et al, 2007
α-terpinene (46.7%)	Biological Reserve of Guadalupe, Brazil.	Fruits	Pontes et al, 2007
Terpinolene (15.45%), β-elemene (22.09%), β-carifilene (11.11%).	390 km from Manaus, State of Amazonas (Brazil) road to Manaus-Caracarai (BR 174)	Leaves	Zoghbi et al, 1995
Terpinolene (40.28%)	390 km from Manaus, State of Amazonas (Brazil) road to Manaus-Caracarai (BR 174	Stalk	Zoghbi et al, 1995
p-cimene (39.93%), *n*-tetradecane (13.38%) e dihidro-4-carene (11.69%)	Cruzeiro do Sul, Stateof Acre, Brazil.	Resin	Marques et al, 2010

TABLE 3. Performance evaluation (halo inhibition, mm) of the disk diffusion method for determination of antifungal activity of the essential oil of *P. heptaphyllum* against yeast species

Cases	Species	Oil Concentration							
		1000 µg/**L**		**500** µg/**L**		**250** µg/**L**		**Fluconazol disc 25** µg	
		$\overline{X}$	DP	$\overline{X}$	DP	$\overline{X}$	DP	$\overline{X}$	DP
09	Candida krusei	14	-	10	10	10	-	-	-
11	C. parapsilosis	11.3	1.2	-	-	-	-	34	-
18	C. africana	13.3	1.2	9.3	9.3	9.3	1.2	30	-
30	C. parapsilosis	11.3	2.3	-	-	-	-	35	9.22
34	C. albicans	13.3	1.2	9.3	9.3	9.3	1.2	32	-
43	C. metapsilosis	12.7	1.2	-	-	-	-	26	-
44	C. albicans	11.3	2.3	-	-	-	-	20	-
54	C. guilliermondii	14	2.0	10	10	10	-	-	-
58	C. guilliermondii	10	-	-	-	-	-	20	-
63	C. parapsilosis	11.3	1.2	9.3	9.3	9.3	1.2	32	-
71	C. albicans	14.7	2.3	10	10	10	-	32	-
89	C. albicans	14	-	12	-	10	-	28	-
90	C. rugosa	-	-	-	-	-	-	20	-
104	C. metapsilosis	12.7	2.3	-	-	-	-	20	-
	C. parapsilosis ATCC 22019	11.3	2.3	8.0	8.0	8.0	-	30	-
	C. krusei ATCC 6258	14	2.0	-	-	-	-	-	-

$\overline{X}$, average; (-) null average due to the absence halo; standard deviation (SD) calculated for tests performed in triplicate.

dilute the compounds may constitute relevant factors affecting the result of inhibition of the isolates evaluated. For example, the dimethyl sulfoxide (DMSO), acetone and ethyl and methyl alcohols are organic solvents often cited in the specialized literature on plant products; however, the correct evaluation of the influence of the diluent on the result of inhibition obtained is required.

In this study we investigated solvents such as ethanol, methanol and acetone (data not shown) verifying that only the last of these diluents showed no inhibitory activity itself against clinical isolates and standard strains. Thus, the study was conducted by employing acetone in water (15%) as diluent. Moreover, the characteristics of the products evaluated, such as volatility, viscosity, hydrophobicity are factors of impact on the results. Thus, the different products have physico-chemical properties that interfere with diffusion in the agar.

Lima et al. (1993) evaluated the antifungal activity of the essential oil of various species of plants, against 16 species of *dermatophytes* (*Epidermophyton*, *Microsporum*, *Trichophyton*), and 80% of these plants showed antifungal activity, except the *P .heptaphyllum,* which presented β-terpinolene, *L*-phelkandrene e α-pinene as majority constituents. It is important to note that in this study, these constituents are in low relative abundance (*L*-phelkandrene -1.49%, α-pinene-1.17%) or absent (β-terpinolene), demonstrating the importance of the collected region in composition and also that these compounds can not be responsible for such activity.

The essential oil obtained from the resin of *Protium heptaphyllum* when analyzed by MDGC-MS and showed as major constituents *l-limonene*, α-*terpineol* and *p-cineol*. The analysis of the fractions collected at intervals of 1 hour showed that the abundance of the main components decrease, while the α-*terpineol* increases over time due to secondary reactions when subjected to heating. As compared to literature data, our study evidenced some differences in the chromatographic profile as well as in quantitative composition of essential oil *P. heptaphyllum*.

This study presented considerable antifungal activity of *Protium* oil resin, inhibiting species of *Candida*, by disk diffusion method, with diameter of halos ($\overline{X} \geq$ 14mm), which cause a broad spectrum of invasive superficial infections and nail infections that are public health problems.

Though the literature data reports the antifungal activity of some constituents identified in this study, there is still a need for more detailed studies of OEPh, including activity *in vivo* for possible alternative clinical applications, since this specie is widely distributed in our community, it presents good yield and also important therapeutic applications.

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REFERENCE

ADAMS, R.P. **Identification of essential oils components by gas chromatography/mass spectroscopy. 4. ed.** Carol Stream, USA: Copyrighted Material, 2007. 804p.

AIBINU, I. et al. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. African Journal of Traditional, Complementary and Alternative medicines, v.4, n.2, p185-190, 2007.

ALMEIDA, L.D.F.D.D. et al. Screening of antifungal activity of essential oils on *Candida albicans*. **Revista Brasileira de Ciências da Saúde** v.14, n.4, p.51-56, 2011.

AMARTYA DE, N. N.; TAHER, Abu. Onychomycosis and Its Treatment. **International Journal of advances in Pharmacy, Biology and Chemistry, v. 2, n.1, p.123-129,2013**

BANDEIRA, N.P. et al. Essential oil composition of leaves, fruits and resin of *Protium heptaphyllum* (Aubl.) March. Journal of Essential Oil Research v.13, n.1, p.33-34, 2001.

BAPTISTELLA, L.H.B. et al. Preparação do (+)-α-terpineol a partir do (+)-limoneno: monoterpenos de odor agradável em um projeto para química orgânica experimental. **Química Nova**, v.32, n.4, p.1069-1071, 2009.

BOKHARI, M.A. et al. Onychomycosis in Lahore, Pakistan. **International Journal of Dermatology**, v.38, p.591-595, 1999.

CAVALCANTI, Y.W. et al. Antifungal activity of brazilian plant extracts against *Candida* strains. **Revista Brasileira de Ciências da Saúde** v.16, n.1, p.43-48, 2012.

CITÓ, A.M.G.L. et al. Resina de *Protium heptaphyllum* march (Burseraceae): composição química do óleo essencial e avaliação citotóxica frente a *Artemia salina* Leach. **Anais da Associação Brasileira de Química**, v.52, n.2, p.71-73, 2003.

CLSI -Clinical and Laboratory Standards Institute-CLSI. **Method for antifungal disk diffusion susceptibility testing of yeasts**. 2ed. CLSI document M44-A2. Pennsykvania, USA, v.29, n.17, p.1-23, 2009.

COSTA, J.G.M.D. et al. Composição química e toxicidade de óleos essenciais de espécies de *Piper* frente a larvas de *Aedes aegypti* L. (Diptera: Culicidae). **Latin American Journal of Pharmacy**, v.29, n. 3, p.463-467, 2010.

DUTTA, B.K. et al. Anticandidial activity of some essential oils of a mega biodiversity hotspot in India. **Mycoses,** v.50, n.2, p.121-124, 2007.

FENNER, R. et al. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. **Revista Brasileira de Ciências Farmacêuticas,** v.42, n.3, p.369-393, 2006.

HASSAN, S. B. et al. Alpha terpineol: a potential anticancer agent which acts through suppressing NF-κB signalling. **Anticancer Research**, v.30, p.1911-20, 2010.

HAMMER, K.A. et al. In vitro activity of *Malaleuca alternifolia* (tea tree) oil against dermatophytes and other filamentous fungi. **Journal of Antimicrobial Chemotherapy**, v.50, n.2, p.195-199, 2002.

HELD, S. et al. Characterization α-terpineol as an anti-inflammatory component of orange juice by in vitro studies using oral buccal cells. **Journal of agricultural and food chemistry**, v.55, n.20, p.8040-8046, 2007.

KOTAN, R. et al. Screening of antibacterial activities of twenty-one oxygenated monoterpenes **Zeitschrift für Naturforschung C**, v.62, n.7-8, p. 507-513, 2007.

LIMA, E.O.; GOMPERTZ, O.; GIESBRECHT, A.; PAULO, M.Q. In vitro antifungal activity of essential oils obtained from officinal plants against dermatophytes. **Mycoses,** v.36, n.9-10, 1993, p.333-336.

LIMA, I.O. et al. Atividade antifúngica de óleos essenciais sobre espécies de *Candida*. **Brazilian Journal of Pharmacognosy**, v.16, n.2, p.197-201, 2006.

LORENZI, H.; MATOS, F.J.A. **Plantas medicinais no Brasil:** nativas e exóticas. 2.ed. Nova Odessa, SP: Plantarum, 2008. 544p.

MARQUES, D.D. et al. Chemical composition of the essential oils from two subespécies of *Protium heptaphyllum*. **Acta Amazonica**, v.40, n.1, p.227-230, 2010.

MATTANA, R.S. et al. Efeitos de diferentes tempos de extração no teor e composição química do óleo essencial de folhas de pariparoba [*Pothomorpheumbellata* (L.) Miq.]. **Revista Brasileira de Plantas Medicinais**, v.17, n.1, p.150-156, 2015.

MENEZES, T.O.A. et al. Avaliação in vitro da atividade antifúngica de óleos essenciais e extratos de plantas da região amazônica sobre cepa de *Candida albicans*. **Revista de Odontologia da UNESP**, v.38, n.3, p.184-91, 2009.

NASCIMENTO, P.F.C. et al. Atividade antimicrobiana dos óleos essenciais: uma abordagem multifatorial dos métodos. **Revista Brasileira de Farmacognosia,** v.17, n.1, p.108-113, 2007.

OLIVEIRA, F.A. et al. Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. **Journal Ethnopharmacology,** v.98, p.10-18, 2005.

PACKER, J.F.; LUZ, M.M.S. Método para avaliação e pesquisa da atividade antimicrobiana de produtos de origem natural. **Revista Brasileira de Farmacognosia,** v.17, n.1, p.102-107, 2007.

PARK, Soon-Nang et al. Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. **Anaerobe**, v.18, n.3, p.369-372, 2012.

PITAROKILI, D. et al. Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. **Journal of agricultural and food chemistry**, v.50, n.23, p. 6688-6691, 2002.

PONTES, W.J.T. et al. Chemical composition and acaricidal activity of the leaf and fruit essential oils of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). **Acta Amazonica**, v.37, n.1, p.103-109, 2007.

POZZATI, P. et al. In vitro activity of essential oils extracted from plants used as spices against fluconazole-resistant and fluconazole-susceptible *Candida spp*. **Canadian journal of microbiology**, v.54, n.11, p.950-956, 2008.

RÜDIGER, A.L. The chemistry and pharmacology of the South America genus *Protium Burm*. f. (Burseraceae). **Pharmacognosy reviews**, v.1, n.1, p.93-104, 2007.

SANTIN, R. et al. Atividade antifúngica do óleo essencial de Origanum vulgare frente a Malassezia pachydermatis. **Arquivo brasileiro de medicina veterinaria e zootecnia= Brazilian journal of veterinary and animal sciences**, v.66, n.2, p.367-373, 2014.

SCORZONI, L. et al. Comparative study of disk difusion and microdilution methods for evaluation of antifungal activity of natural compounds against medical yeasts *Candida* spp and *Cryptococcus* sp. **Revista de Ciências Farmacêuticas Básica e** Aplicada, v.28, n.1, p.25-34, 2007.

SIANI, A.C. et al. Volatile constituents from oleo resin of *Protium heptaphyllum* (Aubl.) March. **Journal of Essential Oil Research**, v.11, n.1, p.72-74, 1999.

SIANI, A.C. et al. Evaluation of anti-inflammatory-related activity of essential oils from leaves and resin of species of *Protium*. **Journal of Ethnopharmacology,** v.66, p.57-69, 1999.

SUSUNAGA, G. S. et al. Triterpenes from the resin of Protium heptaphyllum.**Fitoterapia**, v. 72, n. 6, p.709-711, 2001.

THOMAS J. et al. Toenail onychomycosis: an important global disease burden **Journal of Clinical Pharmacy and Therapeutics,** v.35, n.5, p.497- 519, 2010.

ÜNAL, M.Ü. et al. Research on antifungal and inhibitory effects of DL-limonene on some yeasts. **Turkish Journal of Agriculture and Forestry**, v.36, n.5, p.576-582, 2012.

WILLIAMS. A.C.; BARRY, B.W. Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. **Pharmaceutical research**, v.8, n.1, p.17-24, 1991.

YUASA, Y.; YUASA, Y. A pratical synthesis of d-α-terpineol via Markovnikov addition of d-limonene using trifluoroacetic acid. **Organic process research & development**, v.10, n.6, p.1231-1232, 2006.

ZOGHBI, M.G.B.; MAIA, J.G.S.; LUZ, A.I.R. Volatile Constituents from leaves and stems of *Protium heptaphyllum* (Aubl.) March. **Journal of Essential Oil Research**, v.7, n.5, p.541-543, 1995.

ZOTTI, M.; et al. Onychomycosis: First Case Due to *Aspergillus nomius*. **Acta dermato-venereologica**, v.91, n.5, p.591-592, 2011.