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Pharmacological prospection in-vitro of Lamiaceae species against human pathogenic fungi associated to invasive infections

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ABSTRACT

The medical importance of invasive fungal diseases (IFD) contrasts with the limited therapeutic arsenal available and the increasing resistance rate of pathogenic fungi. Thus, the search for news antifungal drugs is imperative. Many studies highlight the antimicrobial activity of plants from Lamiaceae family such as *Melissa officinalis, Mentha sp., Ocimum basilicum, Plectranthus barbatus* and *Rosmarinus officinalis*, but the antifungal potential these species remains still poorly known. The present study was to evaluate the effect of these Lamiaceae species against fungi involved in IFDs. Fresh plants were macerated with ethanol and the extract obtained was partitioned with different solvents. Phytochemical analyzes were conducted and the volatile compounds were investigated by gas chromatography/mass spectrometry (GC/MS). Subsequently, the standardized vegetal material was used to evaluate the antifungal activity through minimum inhibitory concentration (MIC) determination. Ethanol extract of *M. officinalis*, *Mentha sp.*, *O. basilicum*, and *R. officinalis* showed good to moderate activity against *Paracoccidioides* spp. (MIC 62.5-500 μg/mL). The spectrum of antifungal activity of *P. barbatus* ethanol extract includes, besides *P. brasiliensis* (MIC 125–500 μg/mL), several species of *Candida* spp. (MIC 31.25-500 μg/mL) and the yeasts *Cryptococcus gattii*, *Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa* (MIC 62.5 μg/mL). Flavonoids, steroids/triterpenes, and alkaloids were associated with antifungal activity of extracts and fractions, and the phytol was pointed as the main active compound of the extracts. Lamiaceae plants are a promising source of new antifungal prototypes and phytotherapeutic agents for the pharmaceutical industry, especially against *Paracoccidioides* spp.

1. Introduction:

Invasive fungal diseases (IFD) are among the main causes of morbidity and mortality in immunocompromised patients, especially in cases of organ transplant, burns, cancer and HIV/AIDS (Cesaro et al., 2017). Overall, it is estimated that 1 billion people are afflicted yearly with mycosis, and more than 1.5 million die due to complications of IFDs (Vos et al., 2016). A retrospective study that included 11,881 patients with IFDs showed that these infections carry a high mortality rate (next to 15%), and are associated with prolonged hospital stay (mean of 18.7 days) and high hospital costs (around $44,726 per hospitalization) (Menzin et al., 2009).

Yeast of the genus *Candida*, which includes *C. albicans* and *Candida* non-albicans (e.g. *C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*), are the most common agents in IFD (Andrade et al., 2018). According to the Centers for Disease Control and Prevention (CDC), about 46,000 new cases of nosocomial invasive candidiasis occur yearly in America (CDC, 2019); 40% of these cases lead to death (Horn et al., 2009). Moreover, *Cryptococcus* spp., dimorphic fungi (*Paracoccidioides* spp.) and molds (*Aspergillus* spp.) are other important causes of fatal IFD (Schmiedel and Zimmerli, 2016). In addition, rare yeasts like *Saccharomyces cerevisiae* (Smith et al., 2002) and *Rhodotorula mucilaginosa* (Kim et al., 2013) are also associated with IFD. The therapeutic arsenal available against IFDs includes only six approved antifungal compounds, and increased

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resistance to these drugs has led to uncertainties about the effectiveness of treatment in the future (Lima et al., 2019a). On the other hand, the pharmaceutical industry has neglected investment in new and effective antifungal agents. It has been estimated that, in period from 2000 to 2006, only three potential new antifungals were investigated by the largest pharmaceutical companies in the world (Talbot et al., 2006). In this adverse scenario, interest in alternative therapeutic measures has been growing, and the use of medicinal plants as adjuvants in antifungal therapy has been highlighted in recent years (Carvalho et al., 2018).

Lamiaceae (Labiateae) family, which is represented by about 236 genera and between 6,900 and 7,000 species distributed throughout the world, shows great pharmaceutical potential (Raja, 2012). In Brazil, the species lemon balm (*Melissa officinalis*), mint (*Mentha* sp.), basil (*Ocimum basilicum*), Brazilian boldo (*Plectranthus barbatus*) and rosemary (*Rosmarinus officinalis*) are traditionally used for their hypoglycemic, adaptogenic, antimicrobial, antimalarial, antiallergic, antifertility, antidiabetic, carminative, diaphoretic, antihypertensive, stimulant and analgesic properties (Abou El-Soud et al., 2015; Mimica-Dukic et al., 2004; Runyoro et al., 2006; Satyal et al., 2017; Tampieri et al., 2005). Many these pharmacological proprieties have been confirmed to be principally connected to the terpenoids present in the essential oils; however, flavonoids, alkaloids, iridoids and ursolic acid have also been identified as bioactive metabolites in these plants (Raja, 2012). Several studies has revealed the antifungal potential of Lamiaceae species, as highlighted in a recent review (Waller et al., 2017), but little is known about the activity of lemon balm, mint, basil, Brazilian boldo and rosemary. Thus, the present paper aimed to evaluate the antifungal potential of the ethanol extracts and fractions obtained from *Melissa officinalis*, *Mentha sp*., *Ocimum basilicum*, *Plectranthus barbatus* and *Rosmarinus officinalis* against frequent and rare pathogens involved in IFDs.

2. Material and methods:

2.1. Plant material and extraction:

The Lamiaceae species were collected in Carmópolis de Minas, Minas Gerais, Brazil, in April 2011. The plant materials were identified by Dr. Alexandre Salino, and voucher specimens (Table 1) were deposited at the Herbarium of the Institute of Biological Sciences of the Federal University of Minas Gerais in Belo Horizonte, Minas Gerais, Brazil.

The fresh plant materials were extracted by cold maceration in ethanol P.A. (Vetec, Brazil) for a period of 10 days at room temperature. Then, it was filtered and concentrated in a rotary evaporator at 40°C under reduced pressure and lyophilized to yield ethanol extracts (Araújo et al., 2014). Part of these extracts (1.0 g) were dissolved in 100 mL of EtOH/H_2O (7: 3) and then partitioned successively with 50 mL of hexane, dichloromethane, ethyl acetate and butanol (three times with each solvent), resulting in hexane (Hex), dichloromethane (DCM), ethyl acetate (EA), butanol (But) and hydroethanol (HE) fractions (Table 2). The volatile compounds from the ethanol extracts were subsequently investigated by gas chromatography/mass spectrometry (GC/MS) (Araújo et al., 2014).

Table 1
Summary of principal botanical information of the vegetal material employed.

Species	Parts	Voucher specimens
Melissa officinalis	Aerial parts	BHCB 147242
Mentha sp.	Aerial parts	BHCB 147244
Ocimum basilicum	Aerial parts	BHCB 147240
Plectranthus barbatus	Leaves	BHCB 147241
Rosmarinus officinalis	Aerial parts	BHCB 147245

Table 2
Masses obtained for the ethanol extract and fractions of Melissa officinalis, Mentha sp., Ocimum basilicum, Plectranthus barbatus and Rosmarinus officinalis.

Samples	Mass (g)					
	Et	Hex	DCM	EA	But	HE
Melissa officinalis	2.31	0.1946	0.1510	0.1186	0.1400	0.2323
Mentha sp.	10.17	0.1841	0.1783	0.2172	0.1500	0.1702
Ocimum basilicum	2.78	0.1729	0.1690	0.1054	0.2180	0.2053
Plectranthus barbatus	3.14	0.2870	0.2024	0.1914	0.1487	0.1308
Rosmarinus officinalis	6.30	0.1308	0.1071	0.1366	0.3201	0.1641

Et: ethanol extract; Hex: hexane fraction; DCM: dichloromethane fraction; EA: ethyl acetate fraction; But: butanol fraction; HE: hydroethanol fraction.

2.2. Antifungal activity

2.2.1. Microorganisms:

Yeasts employed in the study (except for the *P. brasiliensis* exemplars) originated from the American Type Culture Collection (ATCC) and were kindly provided by the Reference Microorganisms Laboratory of the Oswaldo Cruz Foundation (FIOCRUZ, Brazil). The species *Candida albicans* ATCC 18804, *C. glabrata* ATCC 2001, *C. krusei* ATCC 20298, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *Cryptococcus gattii* ATCC 24065, *C. neoformans* ATCC 24067, *Saccharomyces cerevisiae* ATCC 1562 and *Rhodothorula mucilaginosa* ATCC 32763 were included in this study. These fungal species were stored at −80 °C in glycerinated (25%) Sabouraud dextrose broth (SDB) (Acumedia®, Brazil) for later use. *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*, in turn, were obtained from of the collection the Microorganism-Host Interaction Laboratory, Biological Science Institute of Universidade Federal de Minas Gerais (UFMG). The exemplars of *P. brasiliensis* were represented by three phylogenetic species (PS1, PS2 and PS3) and were maintained in subcultures after 7 days of growth at 37°C in YPD (yeast, peptone and dextrose) medium. The mold *Aspergillus fumigatus* ATCC 1022 was obtained from the Laboratory of Mycology (UFMG) and maintained frozen in SBD with 30% glycerin at −80 °C. It was reactivated in YPD after 4 days of growth at 37 °C before use.

2.2.2. In vitro susceptibility tests:

2.2.2.1. Yeasts:. The antifungal activity of Lamiaceae species against pathogenic yeasts was performed in accordance with the guidelines in the CLSI document M27-A3 (Clinical and Laboratory Standards Institute, 2008a), with minor modifications. Isolated colonies were suspended in sterile saline and a working inoculum with a final concentration of 1×10^5 yeasts/mL was used. Next, microplates were prepared with 100 µL of inoculum in wells containing the extract and fractions (previously diluted in the range of 15.5–2000 µg/mL) in RPMI 1640 medium supplemented with L-glutamine and buffered to pH 7.0 with 0.165 M morpholine propanesulfonic acid (MOPS) (Sigma, St Louis, MO, USA). The minimum inhibitory concentration (MIC) was detected visually after incubation at 35 ± 2 °C for 72 h for *Cryptococcus* spp. or after 48 h for the other yeast species. Amphotericin B (range of 1-0.008 µg/mL) was included as a positive control and DMSO was used at ≤ 2% v/v as a vehicle control.

The determination of the antifungal effect against *Paracoccidioides* was performed according with method described by Johann et al. (2010). Initially, an inoculum of *Paracoccidioides* spp. was prepared by dilution of a suspension corresponding to 0.5 on the McFarland density scale in RPMI 1640 (1:10) to obtain a final inoculum with 1–5 × 10^5 yeasts/mL. The final suspension was inoculated in microplates containing serial dilutions of extracts and fractions in same range used for the other yeast species. Amphotericin B and sulfamethoxazole-trimethoprim (SMX-THT; 4.6-600 µg/mL) were

included as positive controls. The MIC was determined as the lowest concentrations that did not allow for the detection of visible fungal growth. All tests were performed in duplicate and in three independent experiments.

2.2.2.2. Mold:. The antifungal activity of mold *Aspergillus fumigatus* was determined according to CLSI document M38-A2 (Clinical and Laboratory Standards Institute, 2008b). Briefly, a conidial suspension of *A. fumigatus* was adjusted to a final concentration of 10^4 conidia/mL, and inoculated (100 µL) in wells containing RPMI and differ concentrations of the extracts and fractions. The plates were incubated at 35 °C for 48 h and the MIC was defined visually.

3. Results and discussion:

Natural compounds are already used as the main source of antifungal agents and possess great pharmacological potential (Carvalho et al., 2018; Lima et al., 2019b). The rapid and alarming spread of lineages resistant to conventional antifungal therapies, as well as the small number of agents currently in clinical use, has put pressure on research centers to support the development of new antifungal drugs (Perfect, 2017; Talbot et al., 2006). However, the lack of concern from pharmaceutical industries regarding investing in these agents has stimulated the search for alternative therapeutic entities (Talbot et al., 2006). Thus, products of vegetable origin, acting as a source of effective herbal medicines or of prototype compounds for the development of news antifungal agents, have assumed an important role in the therapeutic progress on fungal infections (Carvalho et al., 2018; Lima et al., 2019b). In this study, we evaluated the antifungal activity of the extracts from five Lamiaceae species whose pharmaceutical potential is still little explored. Plant extracts were tested against pathogens commonly involved in IFD belonging to the genera *Candida, Cryptococcus, Paracoccidioides* and *Aspergillus*. The activity against *S. cerevisiae* e *R. mucilaginosa*, yeasts that are rarely recovered in patients with IFD, was also evaluated. The minimum inhibitory concentrations (MICs) are summarized in Table 3 and Table S1 (Supplementary file). According to Holetz et al. (2002) antimicrobial activity of an extract is defined as good for MIC < 100 µg/mL, moderate when 100 µg/mL < MIC < 500 µg/mL and low when MIC > 500 µg/mL.

M. officinalis fractions were inactive (MIC > 2000 µg/mL) against all yeasts tested in the study (Table S1, supplementary file). However, the ethanol extract of this species had a potent antimicrobial effect against the dimorphic fungus *P. brasiliensis* (Table 3). The MIC against different *P. brasiliensis* and *P. lutzii* specimens ranged from 62.5 to 1000 µg/mL. The *P. brasiliensis* 9673 isolate showed high sensitivity (MIC of 62.5 µg/mL), and the extract of *M. officinalis* was five-fold more potent than the positive control TRI-STX (MIC 300 µg/mL) for this strain. Although other studies have shown the antifungal activity of *M. officinalis* against *C. albicans* (Abdellatif et al., 2014), *Bcinera mycelium*, *Rhizopus stolonifer*, *Penicillium expansum* (El Ouadi et al., 2017) and *Trichophyton* spp. (Mimica-Dukic et al., 2004), we have reported here for the first time the antifungal effect this Lamiaceae species against *P. brasiliensis* and *P. lutzii*. *Paracoccidioides* spp. is the etiologic agent of paracoccidioidomycosis (PCM), which is a fatal IFD when not correctly diagnosed and treated (de Pina et al., 2017). In Brazil, PCM is the first cause of death among IFDs, and problems with access, efficacy and toxicity regarding currently available antifungal treatments have stimulated the search for new therapeutic strategies in this case (Queiroz-Telles et al., 2017). In addition to the activity against *Paracoccidioides* spp., the ethanol extract of *M. officinalis* had a weak antifungal effect against the mold *A. fumigatus* (2000 µg/mL), corroborating a study that showed the effect this plant against *A. niger* (El Ouadi et al., 2017).

The profile of antifungal activity was similar for *Mentha* sp., *O. basilicum* and *R. officinalis* (Table 3). The antimicrobial activity of the ethanol extract of these Lamiaceae species was weak against the genera *Candida* spp. (MIC 1000–2000 µg/mL) and *Cryptococcus* spp. (MIC 500–2000 µg/mL). The antifungal effect was also weak against *A. fumigatus* (MIC, 2000 µg/mL). However, activity against *Paracoccidioides* spp. varied from good to moderate (MIC 62.5-1000 µg/mL). This study emphasizes the antifungal effect of the ethanol extract of *R. officinalis* aerial parts against the strain *P. brasiliensis* Pb-18, where the activity was five-fold higher that the positive control (MIC 62.5 µg/mL for the extract vs. 300 µg/mL for TRI-STX). The antifungal activity of the alcohol extract from *R. officinalis* have been shown against *Candida* spp., *Trichophyton* spp., *Microsporum gypseum* (Endo et al., 2015) and *Aspergillus* spp.(Centeno et al., 2010), but its antifungal effect against *Paracoccidioidis* spp. and *Cryptococcus* spp. has not yet been described.

Of the fractions, the hexane fraction of *Mentha* spp. showed moderate

Table 3
Minimal inhibitory concentration (MIC) of the ethanol extracts of Lamiaceae species against common and rare species involved in invasive fungal disease (IFDs).

Fungi species[a]	MIC (µg/mL)						
	Plants					Positive controls	
	M. officinalis	*Mentha sp.*	*O. basilicum*	*P. barbatus*	*R. officinalis*	Amphotericin B	STX-TRI
S. cerevisae ATCC 1562	–	–	–	62.5	–	1	NE
R. mucilaginosa ATCC 32763	–	–	–	62.5	–	1	NE
C. albicans ATCC 18804	–	2000	2000	500	2000	1	NE
C. glabrata ATCC 2001	–	2000	2000	31.2	2000	0.125	NE
C. krusei ATCC 20298	–	1000	2000	62.5	1000	0.5	NE
C. parapsilosis ATCC 22019	–	1000	1000	62.5	1000	0.5	NE
C. tropicalis ATCC 750	–	2000	2000	500	2000	0.25	NE
C. gattii ATCC 24065	–	500	2000	62.5	2000	1.2	NE
P. brasiliensis Pb18 (Pb7)	125	–	250	250	62.5	0.062	300
P. brasiliensis 1925 (Pb2)	250	125	–	125	62.5	0.031	150
P. brasiliensis MG5 (Pb11)	125	NE	62.5	125	62.5	0.031	150
P. brasiliensis B339 (Pb1)	250	125	62.5	–	62.5	0.062	75
P. brasiliensis 608 (Pb3)	500	125	–	500	125	0.015	300
P. brasiliensis 470 (Pb14)	250	250	–	–	62.5	0.125	75
P. brasiliensis 9673 (Pb8)	62.5	62.5	62.5	–	125	0.062	300
P. brasiliensis 1017 (Pb4)	500	250	–	500	125	0.062	150
P. lutzii 1578	500	125	62.5	–	125	0.062	75
P. lutzii 01	1000	500	1000	–	2000	0.125	300
P. lutzii ED01	250	125	62.5	NE	125	0.031	75
A. fumigatus ATCC 1022	2000	2000	2000	–	2000	4	NE

MIC: Minimal inhibitory concentration; STX-TRI: Sulfamethoxazole-trimethoprim; NE: Not evaluated; (–): No activity (MIC > 2000 µg/mL).

[a] Between parentheses are the names equivalent in [Morais, F.V., Barros, T.F., Fukada, M.K., Cisalpino, P.S., Puccia, R., 2000. Polymorphism in the gene coding for the immunodominant antigen gp43 from the pathogenic fungus *Paracoccidioides brasiliensis*. J. Clin. Microbiol. 38, 3960–3966].

activity against *C. gattii* (MIC 500 μg/mL), but the effect was not conserved for *C. neoformans*. Similarly, *C. neoformans* was not susceptible to the antifungal effects of *Mentha piperita* (Zheljazkov et al., 2010). The polar fractions of *R. officinalis* (hydroethanol and butanol), in turn, presented weak activity against *Candida* spp. and *C. neoformans* (MIC, 2000 μg/ml). However, the hexane fraction of *O. basilicum* showed good activity against *C. glabrata* (MIC 62.5 μg/mL). Considering the microbiological aspects of IFDs, *C. glabrata* represents a major clinical challenge due to its pattern of resistance to clinical antifungal agents. Resistance to echinocandins in *C. glabrata*, for example, doubled in only 4 years, from 4% of isolates in 2008 to 8% in 2014 (Vallabhaneni et al., 2015). In addition, increases in the MIC of amphotericin B have been reported among *C. glabrata* isolates (Scorzoni et al., 2017). Thus, the antifungal activity of the hexane fraction of *O. basilicum* against this species represents a promising pathway to the development of a new and effective therapy. In contrast to the findings of this study, the essential oils of *O. basilicum* were found to be inactive against fluconazole-sensitive and -resistant *C. glabrata* (Soares et al., 2015). However, corroborating our findings, basil has been shown to be active against the fungi *C. neoformans*, *C. gattii* (Cardoso et al., 2017), *C. albicans* (Tampieri et al., 2005) and *Aspergillus* spp. (Abou El-Soud et al., 2015).

Plectranthus barbatus leaves, in turn, showed the best spectrum of antifungal activity. The MIC against *Candida* spp. ranged from 31.25 μg/mL to 500 μg/mL. *C. glabrata* was the most susceptible species, as the MIC was two-fold lower than that observed for the hexane fraction of *O. basilicum* (31.25 μg/mL vs. 62.5 μg/mL). *Plectranthus barbatus* also exhibited good antifungal activity against *C. krusei* (62.5 μg/mL). In clinical medicine, the *C. krusei* is known to be intrinsically resistant to azoles, an important class of antifungal agents (Scorzoni et al., 2017). However, in contrast to our results, Tempone et al. (2008) have shown that *C. krusei* is not susceptible to the crude extract of *P. barbatus*. In addition to the activity against *Candida* ssp., the extract of *P. barbatus* also showed good activity against *C. gattii* (62.5 μg/mL). Cryptococcosis, a systemic disease caused by the fungus *Cryptococcus neoformans/Cryptococcus gattii*, is the most common IFD in immunocompromised individuals with HIV/AIDS (Chastain et al., 2017). The disease initially manifests as a pulmonary infection, but can affect the central nervous system and the skin by hematogenous spread, and is potentially fatal (Amaral et al., 2016). Worldwide, one million cases of cryptococcal meningitis are diagnosed with more than 600,000 deaths annually (Park et al., 2009). To the best of our knowledge, this is the first work that highlights the anti-cryptococcal activity of *P. barbatus*.

The ethanol extract of *P. barbatus* showed moderate antifungal activity against *P. brasiliensis* (MIC 125–500 μg/mL) and was not active against the tested *P. lutzii* isolates. In addition, this species had good antifungal activity against the yeasts *S. cerevisiae* and *R. mucilaginosa* (62.5 μg/mL for both). Although rarely involved in IFDs, these yeasts are related to poor prognosis and death rates that can exceed 80% in infected patients (Kim et al., 2013; Smith et al., 2002). Unlike the other Lamiaceae species, the antifungal potential of *P. barbatus* is still poorly studied. In this direction, only the effect of the essential oil of *P. barbatus*

Table 4
Results of preliminary phytochemical analysis of the ethanol extract and fractions of *Melissa officinalis, Mentha sp., Ocimum basilicum, Plectranthus barbatus* and *Rosmarinus officinalis*.

Samples	Phytochemical analysis					
M. officinalis	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Et	–	–	–	–	+	–
Hex	+	+	–	–	+	–
DCM	+	–	–	–	+	–
Ac	+	+	–	+	+	+
But	–	+	+	+	–	+
HE	+	+	+	+	–	+
Mentha sp.	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Et	+	+	–	–	+	+
Hex	–	+	–	–	+	–
DCM	–	+	–	–	+	–
Ac	–	–	+	–	+	+
But	–	+	–	–	+	+
HE	–	+	–	+	–	+
O. basilicum	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Et	+	+	–	–	+	+
Hex	+	+	–	–	+	–
DCM	+	–	–	–	+	–
Ac	–	+	+	+	–	+
But	–	+	–	+	–	+
HE	–	+	–	+	–	+
P. barbatus	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Et	+	–	–	–	+	–
Hex	+	+	–	–	+	–
DCM	–	+	–	–	+	+
Ac	–	+	–	+	+	+
But	–	+	+	–	+	–
HE	+	+	+	–	–	+
R. officinalis	Steroids/Triterpenes	Flavonoids	Saponins	Tannins	Alkaloids	Coumarins
Et	+	+	–	–	+	+
Hex	–	+	–	–	+	–
DCM	–	+	–	–	+	–
Ac	–	+	+	+	+	+
But	–	+	–	+	–	+
HE	–	–	+	–	–	+

Et: Ethanol extract; Hex: hexane fraction; DCM: dichloromethane fraction; EA: ethyl acetate fraction; But: butanol fraction; HE: hydroethanol fraction.

against *C. albicans* is actually known (Runyoro et al., 2006). Interestingly, the fractions of the extract of *P. barbatus* had considerably lower antifungal activity than that of the ethanol extract. Only the ethyl acetate and butanol fractions presented moderate effect against *C. krusei*. This activity profile suggests that the antifungal effect of the extract is more likely influenced by a synergistic interaction between components than by a specific phytocompound.

Phytochemicals such as steroids/triterpenes, flavonoids, coumarins, alkaloids, tannins and, saponins were detected by TLC analysis of ethanol extract and fractions from species employed in this study (Table 4). The good antifungal activity in the Lamiaceae species may be due to the presence of steroids/triterpenes, flavonoids, and alkaloids. In fact, the ethanol extract that showed the better antifungal activity profile (*O. basilicum, P. barbatus, Mentha* sp. and *R. officinalis*) were found to have basically these tree phytochemical classes (Table 4). Naturally occurring alkaloids in plant species are known for their antifungal effect, which is associated with the ability of these nitrogenous compounds to alter the membrane permeability, impair mitochondrial function, stimulate the generation of reactive oxygen species (ROS) and promote damage to cell wall (Khan et al., 2017). Flavonoids have the ability to inhibit RNA synthesis and form stable complexes with some proteins such as surface-exposed adhesins, cell wall polypeptides and, membrane bound enzymes, compromise so the structures of the surface cell as well as some components involved in the metabolism of the microorganism (Teixeira de Oliveira et al., 2018). In turn, steroids and triterpenes showed strong fungistatic/fungicidal activity *in vitro* apparently due to their ability to complex with ergosterol and disrupt membrane integrity (Smania et al., 2003).

The ethanol extracts from five species of Lamiaceae family employed in this study were subsequently investigated by gas chromatography/mass spectrometry (GC/MS) (Araújo et al., 2014). Among the major compounds identified, only phytol was present in all studied species (Araújo et al., 2014). This acyclic isoprenoid is the major constituent of the two most active species in antifungal trials (*P. barbatus* and *R. officinalis)*. Phytol (3,7,11,15-tetrametilhexadec-2-en-1-ol) is characterized as a long and branched chain acyclic alcohol, produced via chlorophyll metabolism (de Moraes et al., 2014). The antifungal activity of phytol has already been demonstrated against the yeast *C. albicans* (Ghaneian et al., 2015) and the molds *Aspergillus* spp., *Trichoderma* spp. and *Penicillium* spp. (Pejin et al., 2014), corroborating the results obtained for *P. barbatus* against *Candida* spp. and *R. officinalis* against *Aspergillus* spp. Furthermore, another major compounds identified, such as verbenone (Satyal et al., 2017), camphor (Sampietro et al., 2017; Satyal et al., 2017), cadinene (Sampietro et al., 2017), heptadecane (Chehregani et al., 2010) and 2(3H)-furanone (Teoh et al., 2012), also have a known antifungal effect. The presence of different substances with known antifungal activity in Lamiaceae extracts supports the possibility of synergistic interaction between these components. Thus, the greater activity of the ethanol extract in comparison to its fractions can be explained.

Melissa officinalis, Mentha sp., Ocimum basilicum and *Rosmarinus officinalis* showed good activity against the dimorphic fungus *Paracoccidiodes*. In some cases, the effect was greater than that of the positive control, which reveals the therapeutic potential of these species in the treatment of PCM. The ethanol extract of *Plectranthus barbatus*, in turn, exhibited a broad antifungal spectrum that ranged from the species commonly involved in IFDs to yeasts rarely associated with this infection. Molecules such as phytol, verbenone, camphor and cadinene were present in the studied extracts, and may interact synergistically to produce the observed antifungal effect. In summary, we conclude that botanical species of the Lamiaceae family show good antifungal activity and thus are a potential source for the development of new alternative treatments against IFDs. More studies should be undertaken in order to understand the efficacy and safety profile of these extracts in an *in vivo* model of IFD.

Conflicts of interest

None.

Contribution details

S.G.A. participated in the experimental studies, data acquisition, data analysis, and statistical analysis. W.G.L. was responsible for data analysis, manuscript preparation, manuscript editing and literature search. M.E.A.P. and M.I.M. participated in the optimization of the extracts and fractions employed, as well as the respective phytochemical analyzes of these materials. N.P.S., S.J., and C.A.R. designed, executed and assisted in the analysis of the results of biological activity (antifungal effect) and collaborated in the revision of the manuscript. L.A.R. S.L. was responsible for concepts, design, definition of intellectual content, manuscript editing and manuscript review.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101345.

References

Abdellatif, F., Boudjella, H., Zitouni, A., Hassani, A., 2014. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian Melissa officinalis L. EXCLI J 13, 772–781.

Abou El-Soud, N.H., Deabes, M., Abou El-Kassem, L., Khalil, M., 2015. Chemical composition and antifungal activity of Ocimum basilicum L. Essential oil. Open access maced. J. Med. Sci. 3, 374. https://doi.org/10.3889/oamjms.2015.082.

Amaral, D.M., Rocha, R.C., Carneiro, L.E., Vasconcelos, D.M., Abreu, M.A., 2016. Disseminated cryptococcosis manifested as a single tumor in an immunocompetent patient, similar to the cutaneous primary forms. An. Bras. Dermatol. 91, 29–31. https://doi.org/10.1590/abd1806-4841.20164582.

Andrade, J.T., Santos, F.R.S., Lima, W.G., Sousa, C.D.F., Oliveira, L.S.F.M., Ribeiro, R.I. M.A., Gomes, A.J.P.S., Araújo, M.G.F., Villar, J.A.F.P., Ferreira, J.M.S., 2018. Design, synthesis, biological activity and structure-activity relationship studies of chalcone derivatives as potential anti-Candida agents. J. Antibiot. (Tokyo) 71, 702–712. https://doi.org/10.1038/s41429-018-0048-9.

Araújo, S.G., Alves, L.F., Pinto, M.E.A., Oliveira, G.T., Siqueira, E.P., Ribeiro, R.I.M.A., Ferreira, J.M.S., Lima, L.A.R.S., 2014. Volatile compounds of Lamiaceae exhibit a synergistic antibacterial activity with streptomycin. Braz. J. Microbiol. 45, 1341–1347.

Cardoso, N.N.R., Alviano, C.S., Blank, A.F., Arrigoni-Blank, M. de F., Romanos, M.T.V., Cunha, M.M.L., da Silva, A.J.R., Alviano, D.S., 2017. Anti-cryptococcal activity of ethanol crude extract and hexane fraction from Ocimum basilicum var. Maria bonita: mechanisms of action and synergism with amphotericin B and Ocimum basilicum essential oil. Pharm. Biol. 55, 1380–1388. https://doi.org/10.1080/13880209.2017.1302483.

Carvalho, R.S., Carollo, C.A., de Magalhães, J.C., Palumbo, J.M.C., Boaretto, A.G., Nunes e Sá, I.C., Ferraz, A.C., Lima, W.G., de Siqueira, J.M., Ferreira, J.M.S., 2018. Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from Cochlospermum regium (mart. Et. Schr.) Pilger roots: mechanisms of action and synergism with tannin and gallic acid. South Afr. J. Bot. 114, 181–187. https://doi.org/10.1016/j.sajb.2017.11.010.

Statistics | invasive candidiasis | candidiasis | types of diseases | fungal diseases | CDC [WWW document]. n.d. URL. https://www.cdc.gov/fungal/diseases/candidiasis/invasive/statistics.html#one. accessed 1.21.19.

Centeno, S., Calvo, M.A., Adelantado, C., Figueroa, S., 2010. Antifungal activity of extracts of Rosmarinus officinalis and Thymus vulgaris against Aspergillus flavus and A. ochraceus. Pakistan J. Biol. Sci. PJBS 13, 452–455.

Cesaro, S., Tridello, G., Castagnola, E., Calore, E., Carraro, F., Mariotti, I., Colombini, A., Perruccio, K., Decembrino, N., Russo, G., Maximova, N., Baretta, V., Caselli, D., 2017. Retrospective study on the incidence and outcome of proven and probable invasive fungal infections in high-risk pediatric onco-hematological patients. Eur. J. Haematol. 99, 240–248. https://doi.org/10.1111/ejh.12910.

Chastain, D.B., Henao-Martínez, A.F., Franco-Paredes, C., 2017. Opportunistic invasive mycoses in AIDS: cryptococcosis, histoplasmosis, coccidiodomycosis, and talaromycosis. Curr. Infect. Dis. Rep. 19, 36. https://doi.org/10.1007/s11908-017-0592-7.

Chehregani, A., Mohsenzadeh, F., Mirazi, N., Hajisadeghian, S., Baghali, Z., 2010. Chemical composition and antibacterial activity of essential oils of Tripleurospermum disciforme in three developmental stages. Pharm. Biol. 48, 1280–1284. https://doi.org/10.3109/13880201003770143.

Clinical and Laboratory Standards Institute, 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, document M27-A3, third ed. Clinical and Laboratory Standards Institute. Wayne (PA).

Clinical and Laboratory Standards Institute, 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, document M38-A2, 2nd. Clinical and Laboratory Standards Institute, Wayne (PA).

de Moraes, J., de Oliveira, R.N., Costa, J.P., Junior, A.L.G., de Sousa, D.P., Freitas, R.M., Allegretti, S.M., Pinto, P.L.S., 2014. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease schistosomiasis mansoni. PLoS Neglected Trop. Dis. 8, e2617. https://doi.org/10.1371/journal.pntd.0002617.

de Pina, D.R., Alvarez, M., Giacomini, G., Pavan, A.L.M., Guedes, C.I.A., Cavalcante, R. de S., Mendes, R.P., Paniago, A.M.M., 2017. Paracoccidioidomycosis: level of pulmonary sequelae in high resolution computed tomography images from patients of two endemic regions of Brazil. Quant. Imaging Med. Surg. 7, 318–325. https://doi.org/10.21037/qims.2017.06.04.

El Ouadi, Y., Manssouri, M., Bouyanzer, A., Majidi, L., Bendaif, H., Elmsellem, H., Shariati, M.A., Melhaoui, A., Hammouti, B., 2017. Essential oil composition and antifungal activity of Melissa officinalis originating from north-Est Morocco, against postharvest phytopathogenic fungi in apples. Microb. Pathog. 107, 321–326. https://doi.org/10.1016/j.micpath.2017.04.004.

Endo, E.H., Costa, G.M., Nakamura, T.U., Nakamura, C.V., Dias Filho, B.P., 2015. Antidermatophytic activity of hydroalcoholic extracts from Rosmarinus officinalis and Tetradenia riparia. J. Mycol. Med. 25, 274–279. https://doi.org/10.1016/j.mycmed.2015.09.003.

Ghaneian, M.T., Ehrampoush, M.H., Jebali, A., Hekmatimoghaddam, S., Mahmoudi, M., 2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environ. Heal. Eng. Manag. J. 2, 13–16.

Holetz, F.B., Pessini, G.L., Sanches, N.R., Cortez, D.A.G., Nakamura, C.V., Dias Filho, B.P., 2002. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem. Inst. Oswaldo Cruz 97, 1027–1031. https://doi.org/10.1590/S0074-02762002000700017.

Horn, D.L., Neofytos, D., Anaissie, E.J., Fishman, J.A., Steinbach, W.J., Olyaei, A.J., Marr, K.A., Pfaller, M.A., Chang, C.-H., Webster, K.M., 2009. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin. Infect. Dis. 48, 1695–1703. https://doi.org/10.1086/599039.

Johann, S., Cisalpino, P.S., Watanabe, G.A., Cota, B.B., de Siqueira, E.P., Pizzolatti, M.G., Zani, C.L., de Resende, M.A., 2010. Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus Paracoccidioides brasiliensis. Pharm. Biol. 48, 388–396. https://doi.org/10.3109/13880200903150385.

Khan, H., Mubarak, M.S., Amin, S., 2017. Antifungal potential of alkaloids as an emerging therapeutic target. Curr. Drug Targets 18, 1825–1835. https://doi.org/10.2174/1389450117666160719095517.

Kim, H.A., Hyun, M., Ryu, S.-Y., 2013. Catheter-associated Rhodotorula mucilaginosa fungemia in an immunocompetent host. Infect. Chemother. 45, 339–342. https://doi.org/10.3947/ic.2013.45.3.339.

Lima, W.G., Alves-Nascimento, L.A., Andrade, J.T., Vieira, L., de Azambuja Ribeiro, R.I. M., Thomé, R.G., dos Santos, H.B., Ferreira, J.M.S., Soares, A.C., 2019. Are the Statins promising antifungal agents against invasive candidiasis? Biomed. Pharmacother. 111, 270–281. https://doi.org/10.1016/j.biopha.2018.12.076.

Lima, W.G., dos Santos, F.J., Cristina Soares, A., Macías, F.A., Molinillo, J.M.G., Maria Siqueira Ferreira, J., Máximo de Siqueira, J., 2019. Synthesis and antimicrobial activity of some benzoxazinoids derivatives of 2-nitrophenol and 3-hydroxy-2-nitropyridine. Synth. Commun. 1–11. https://doi.org/10.1080/00397911.2018.1554146.

Menzin, J., Meyers, J.L., Friedman, M., Perfect, J.R., Langston, A.A., Danna, R.P., Papadopoulos, G., 2009. Mortality, length of hospitalization, and costs associated with invasive fungal infections in high-risk patients. Am. J. Heal. Pharm. 66, 1711–1717. https://doi.org/10.2146/ajhp080325.

Mimica-Dukic, N., Bozin, B., Sokovic, M., Simin, N., 2004. Antimicrobial and antioxidant activities of Melissa officinalis L. (Lamiaceae) essential oil. J. Agric. Food Chem. 52, 2485–2489. https://doi.org/10.1021/jf030698a.

Park, B.J., Wannemuehler, K.A., Marston, B.J., Govender, N., Pappas, P.G., Chiller, T.M., 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23, 525–530. https://doi.org/10.1097/QAD.0b013e328322ffac.

Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., Radotic, K., Mojovic, M., 2014. Further in vitro evaluation of antiradical and antimicrobial activities of phytol. Nat. Prod. Res. 28, 372–376. https://doi.org/10.1080/14786419.2013.869692.

Perfect, J.R., 2017. The antifungal pipeline: a reality check. Nat. Rev. Drug Discov. 16, 603–616. https://doi.org/10.1038/nrd.2017.46.

Queiroz-Telles, F., Fahal, A.H., Falci, D.R., Caceres, D.H., Chiller, T., Pasqualotto, A.C., 2017. Neglected endemic mycoses. Lancet Infect. Dis. 17, e367–e377. https://doi.org/10.1016/S1473-3099(17)30306-7.

Raja, R.R., 2012. Medicinally potential plants of labiatae (Lamiaceae) family: an overview. Res. J. Med. Plant 6, 203–213. https://doi.org/10.3923/rjmp.2012.203.213.

Runyoro, D.K.B., Matee, M.I.N., Ngassapa, O.D., Joseph, C.C., Mbwambo, Z.H., 2006. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complement Altern. Med. 6, 11. https://doi.org/10.1186/1472-6882-6-11.

Sampietro, D.A., Gomez, A. de L.A., Jimenez, C.M., Lizarraga, E.F., Ibatayev, Z.A., Suleimen, Y.M., Catalán, C.A., 2017. Chemical composition and antifungal activity of essential oils from medicinal plants of Kazakhstan. Nat. Prod. Res. 31, 1464–1467. https://doi.org/10.1080/14786419.2016.1258560.

Satyal, P., Jones, T.H., Lopez, E.M., McFeeters, R.L., Ali, N.A.A., Mansi, I., Al-Kaf, A.G., Setzer, W.N., 2017. Chemotypic characterization and biological activity of Rosmarinus officinalis. Foods 6, 20. https://doi.org/10.3390/foods6030020.

Schmiedel, Y., Zimmerli, S., 2016. Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and Pneumocystis pneumonia. Swiss Med. Wkly. 146, w14281. https://doi.org/10.4414/smw.2016.14281.

Scorzoni, L., de Paula e Silva, A.C.A., Marcos, C.M., Assato, P.A., de Melo, W.C.M.A., de Oliveira, H.C., Costa-Orlandi, C.B., Mendes-Giannini, M.J.S., Fusco-Almeida, A.M., 2017. Antifungal therapy: new advances in the understanding and treatment of mycosis. Front. Microbiol. 08, 36. https://doi.org/10.3389/fmicb.2017.00036.

Smania, E.F.A., Delle Monache, F., Smania, A., Yunes, R.A., Cuneo, R.S., 2003. Antifungal activity of sterols and triterpenes isolated from Ganoderma annulare. Fitoterapia 74, 375–377.

Smith, D., Metzgar, D., Wills, C., Fierer, J., 2002. Fatal Saccharomyces cerevisiae aortic graft infection. J. Clin. Microbiol. 40, 2691–2692.

Soares, I.H., Loreto, É.S., Rossato, L., Mario, D.N., Venturini, T.P., Baldissera, F., Santurio, J.M., Alves, S.H., 2015. In vitro activity of essential oils extracted from condiments against fluconazole-resistant and -sensitive Candida glabrata. J. Mycol. Med. 25, 213–217. https://doi.org/10.1016/j.mycmed.2015.06.003.

Talbot, G.H., Bradley, J., Edwards, J.E., Gilbert, D., Scheld, M., Bartlett, J.G., Antimicrobial Availability Task Force of the Infectious Diseases Society of America, 2006. Bad bugs need drugs: an update on the development pipeline from the antimicrobial availability task force of the infectious diseases society of America. Clin. Infect. Dis. 42, 657–668. https://doi.org/10.1086/499819.

Tampieri, M.P., Galuppi, R., Macchioni, F., Carelle, M.S., Falcioni, L., Cioni, P.L., Morelli, I., 2005. The inhibition of Candida albicans by selected essential oils and their major components. Mycopathologia 159, 339–345. https://doi.org/10.1007/s11046-003-4790-5.

Teixeira de Oliveira, G., Siqueira Ferreira, J.M., Lima, W.G., Ferreira Alves, L., Duarte-Almeida, J.M., Alves Rodrigues dos Santos Lima, L., 2018. Phytochemical characterisation and bioprospection for antibacterial and antioxidant activities of Lippia alba Brown ex Britton & Wilson (Verbenaceae). Nat. Prod. Res. 32, 723–731. https://doi.org/10.1080/14786419.2017.1335727.

Tempone, A.G., Sartorelli, P., Teixeira, D., Prado, F.O., Calixto, I.A.R.L., Lorenzi, H., Melhem, M.S.C., 2008. Brazilian flora extracts as source of novel antileishmanial and antifungal compounds. Mem. Inst. Oswaldo Cruz 103, 443–449.

Teoh, Y.P., Don, M.M., Ujang, S., 2012. Nutrient improvement using statistical optimization for growth of Schizophyllum commune, and its antifungal activity against wood degrading fungi of rubberwood. Biotechnol. Prog. 28, 232–241. https://doi.org/10.1002/btpr.714.

Vallabhaneni, S., Cleveland, A.A., Farley, M.M., Harrison, L.H., Schaffner, W., Beldavs, Z. G., Derado, G., Pham, C.D., Lockhart, S.R., Smith, R.M., 2015. Epidemiology and risk factors for echinocandin nonsusceptible Candida glabrata bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008-2014. Open forum Infect. Dis. 2, ofv163. https://doi.org/10.1093/ofid/ofv163.

Vos, T., Allen, C., Arora, M., Barber, R.M., Bhutta, Z.A., Brown, A., Carter, A., Casey, D. C., Charlson, F.J., Chen, A.Z., Coggeshall, M., Cornaby, L., Dandona, L., Dicker, D.J., Dilegge, T., Erskine, H.E., Ferrari, A.J., Fitzmaurice, C., Fleming, T., Forouzanfar, M. H., Fullman, N., Gething, P.W., Goldberg, E.M., Graetz, N., Haagsma, J.A., Hay, S.I., Johnson, C.O., Kassebaum, N.J., Kawashima, T., Kemmer, L., Khalil, I.A., Kinfu, Y., Kyu, H.H., Leung, J., Liang, X., Lim, S.S., Lopez, A.D., Lozano, R., Marczak, L., Mensah, G.A., Mokdad, A.H., Naghavi, M., Nguyen, G., Nsoesie, E., Olsen, H., Pigott, D.M., Pinho, C., Rankin, Z., Reinig, N., Salomon, J.A., Sandar, L., Smith, A., Stanaway, J., Steiner, C., Teeple, S., Thomas, B.A., Troeger, C., Wagner, J.A., Wang, H., Wanga, V., Whiteford, H.A., Zoeckler, L., Abajobir, A.A., Abate, K.H., Abbafati, C., Abbas, K.M., Abd-Allah, F., Abraham, B., Abubakar, I., Abu-Raddad, L. J., Abu-Rmeileh, N.M.E., Ackerman, I.N., Adebiyi, A.O., Ademi, Z., Adou, A.K., Afanvi, K.A., Agardh, E.E., Agarwal, A., Kiadaliri, A.A., Ahmadieh, H., Ajala, O.N., Akinyemi, R.O., Akseer, N., Al-Aly, Z., Alam, K., Alam, N.K.M., Aldhahri, S.F., Alegretti, M.A., Alemu, Z.A., Alexander, L.T., Alhabib, S., Ali, R., Alkerwi, A., Alla, F., Allebeck, P., Al-Raddadi, R., Alsharif, U., Altirkawi, K.A., Alvis-Guzman, N., Amare, A.T., Amberbir, A., Amini, H., Ammar, W., Amrock, S.M., Andersen, H.H., Anderson, G.M., Anderson, B.O., Antonio, C.A.T., Aregay, A.F., Ärnlöv, J., Artaman, A., Asayesh, H., Assadi, R., Atique, S., Avokpaho, E.F.G.A., Awasthi, A., Quintanilla, B.P.A., Azzopardi, P., Bacha, U., Badawi, A., Balakrishnan, K., Banerjee, A., Barac, A., Barker-Collo, S.L., Bärnighausen, T., Barregard, L., Barrero, L.H., Basu, A., Bazargan-Hejazi, S., Beghi, E., Bell, B., Bell, M.L., Bennett, D. A., Bensenor, I.M., Benzian, H., Berhane, A., Bernabé, E., Betsu, B.D., Beyene, A.S., Bhala, N., Bhatt, S., Biadgilign, S., Bienhoff, K., Bikbov, B., Biryukov, S., Bisanzio, D., Bjertness, E., Blore, J., Borschmann, R., Boufous, S., Brainin, M., Brazinova, A., Breitborde, N.J.K., Brown, J., Buchbinder, R., Buckle, G.C., Butt, Z.A., Calabria, B., Campos-Nonato, I.R., Campuzano, J.C., Carabin, H., Cárdenas, R., Carpenter, D.O., Carrero, J.J., Castañeda-Orjuela, C.A., Rivas, J.C., Catalá-López, F., Chang, J.-C., Chiang, P.P.-C., Chibueze, C.E., Chisumpa, V.H., Choi, J.-Y.J., Chowdhury, R., Christensen, H., Christopher, D.J., Ciobanu, L.G., Cirillo, M., Coates, M.M., Colquhoun, S.M., Cooper, C., Cortinovis, M., Crump, J.A., Damtew, S.A., Dandona, R., Daoud, F., Dargan, P.I., das Neves, J., Davey, G., Davis, A.C., Leo, D. De, Degenhardt, L., Gobbo, L.C. Del, Dellavalle, R.P., Deribe, K., Deribew, A., Derrett, S., Jarlais, D.C. Des, Dharmaratne, S.D., Dhillon, P.K., Diaz-Torné, C., Ding, E.L., Driscoll, T.R., Duan, L., Dubey, M., Duncan, B.B., Ebrahimi, H., Ellenbogen, R.G., Elyazar, I., Endres, M., Endries, A.Y., Ermakov, S.P., Eshrati, B.,

Estep, K., Farid, T.A., Farinha, C.S. e S., Faro, A., Farvid, M.S., Farzadfar, F., Feigin, V.L., Felson, D.T., Fereshtehnejad, S.-M., Fernandes, J.G., Fernandes, J.C., Fischer, F., Fitchett, J.R.A., Foreman, K., Fowkes, F.G.R., Fox, J., Franklin, R.C., Friedman, J., Frostad, J., Fürst, T., Futran, N.D., Gabbe, B., Ganguly, P., Gankpé, F. G., Gebre, T., Gebrehiwot, T.T., Gebremedhin, A.T., Geleijnse, J.M., Gessner, B.D., Gibney, K.B., Ginawi, I.A.M., Giref, A.Z., Giroud, M., Gishu, M.D., Giussani, G., Glaser, E., Godwin, W.W., Gomez-Dantes, H., Gona, P., Goodridge, A., Gopalani, S. V., Gotay, C.C., Goto, A., Gouda, H.N., Grainger, R., Greaves, F., Guillemin, F., Guo, Y., Gupta, R., Gupta, R., Gupta, V., Gutiérrez, R.A., Haile, D., Hailu, A.D., Hailu, G.B., Halasa, Y.A., Hamadeh, R.R., Hamidi, S., Hammami, M., Hancock, J., Handal, A.J., Hankey, G.J., Hao, Y., Harb, H.L., Harikrishnan, S., Haro, J.M., Havmoeller, R., Hay, R.J., Heredia-Pi, I.B., Heydarpour, P., Hoek, H.W., Horino, M., Horita, N., Hosgood, H.D., Hoy, D.G., Htet, A.S., Huang, H., Huang, J.J., Huynh, C., Iannarone, M., Iburg, K.M., Innos, K., Inoue, M., Iyer, V.J., Jacobsen, K.H., Jahanmehr, N., Jakovljevic, M.B., Javanbakht, M., Jayaraman, S.P., Jayatilleke, A. U., Jee, S.H., Jeemon, P., Jensen, P.N., Jiang, Y., Jibat, T., Jimenez-Corona, A., Jin, Y., Jonas, J.B., Kabir, Z., Kalkonde, Y., Kamal, R., Kan, H., Karch, A., Karema, C. K., Karimkhani, C., Kasaeian, A., Kaul, A., Kawakami, N., Keiyoro, P.N., Kemp, A.H., Keren, A., Kesavachandran, C.N., Khader, Y.S., Khan, A.R., Khan, E.A., Khang, Y.-H., Khera, S., Khoja, T.A.M., Khubchandani, J., Kieling, C., Kim, P., Kim, C., Kim, D., Kim, Y.J., Kissoon, N., Knibbs, L.D., Knudsen, A.K., Kokubo, Y., Kolte, D., Kopec, J. A., Kosen, S., Kotsakis, G.A., Koul, P.A., Koyanagi, A., Kravchenko, M., Defo, B.K., Bicer, B.K., Kudom, A.A., Kuipers, E.J., Kumar, G.A., Kutz, M., Kwan, G.F., Lal, A., Lalloo, R., Lallukka, T., Lam, H., Lam, J.O., Langan, S.M., Larsson, A., Lavados, P.M., Leasher, J.L., Leigh, J., Leung, R., Levi, M., Li, Y., Li, Y., Liang, J., Liu, S., Liu, Y., Lloyd, B.K., Lo, W.D., Logroscino, G., Looker, K.J., Lotufo, P.A., Lunevicius, R., Lyons, R.A., Mackay, M.T., Magdy, M., Razek, A. El, Mahdavi, M., Majdan, M., Majeed, A., Malekzadeh, R., Marcenes, W., Margolis, D.J., Martinez-Raga, J., Masiye, F., Massano, J., McGarvey, S.T., McGrath, J.J., McKee, M., McMahon, B.J., Meaney, P.A., Mehari, A., Mejia-Rodriguez, F., Mekonnen, A.B., Melaku, Y.A., Memiah, P., Memish, Z.A., Mendoza, W., Meretoja, A., Meretoja, T.J., Mhimbira, F. A., Millear, A., Miller, T.R., Mills, E.J., Mirarefin, M., Mitchell, P.B., Mock, C.N., Mohammadi, A., Mohammed, S., Monasta, L., Hernandez, J.C.M., Montico, M., Mooney, M.D., Moradi-Lakeh, M., Morawska, L., Mueller, U.O., Mullany, E., Mumford, J.E., Murdoch, M.E., Nachega, J.B., Nagel, G., Naheed, A., Naldi, L., Nangia, V., Newton, J.N., Ng, M., Ngalesoni, F.N., Nguyen, Q. Le, Nisar, M.I., Pete, P. M.N., Nolla, J.M., Norheim, O.F., Norman, R.E., Norrving, B., Nunes, B.P., Ogbo, F. A., Oh, I.-H., Ohkubo, T., Olivares, P.R., Olusanya, B.O., Olusanya, J.O., Ortiz, A., Osman, M., Ota, E., PA, M., Park, E.-K., Parsaeian, M., de Azeredo Passos, V.M., Caicedo, A.J.P., Patten, S.B., Patton, G.C., Pereira, D.M., Perez-Padilla, R., Perico, N., Pesudovs, K., Petzold, M., Phillips, M.R., Piel, F.B., Pillay, J.D., Pishgar, F., Plass, D., Platts-Mills, J.A., Polinder, S., Pond, C.D., Popova, S., Poulton, R.G., Pourmalek, F., Prabhakaran, D., Prasad, N.M., Qorbani, M., Rabiee, R.H.S., Radfar, A., Rafay, A., Rahimi, K., Rahimi-Movaghar, V., Rahman, M., Rahman, M.H.U., Rahman, S.U., Rai, R.K., Rajsic, S., Ram, U., Rao, P., Refaat, A.H., Reitsma, M.B., Remuzzi, G., Resnikoff, S., Reynolds, A., Ribeiro, A.L., Blancas, M.J.R., Roba, H.S., Rojas-Rueda, D., Ronfani, L., Roshandel, G., Roth, G.A., Rothenbacher, D., Roy, A., Sagar, R., Sahathevan, R., Sanabria, J.R., Sanchez-Niño, M.D., Santos, I.S., Santos, J. V., Sarmiento-Suarez, R., Sartorius, B., Satpathy, M., Savic, M., Sawhney, M., Schaub, M.P., Schmidt, M.I., Schneider, I.J.C., Schöttker, B., Schwebel, D.C., Scott, J. G., Seedat, S., Sepanlou, S.G., Servan-Mori, E.E., Shackelford, K.A., Shaheen, A., Shaikh, M.A., Sharma, R., Sharma, U., Shen, J., Shepard, D.S., Sheth, K.N., Shibuya, K., Shin, M.-J., Shiri, R., Shiue, I., Shrime, M.G., Sigfusdottir, I.D., Silva, D. A.S., Silveira, D.G.A., Singh, A., Singh, J.A., Singh, O.P., Singh, P.K., Sivonda, A., Skirbekk, V., Skogen, J.C., Sligar, A., Sliwa, K., Soljak, M., Søreide, K., Sorensen, R.J. D., Soriano, J.B., Sposato, L.A., Sreeramareddy, C.T., Stathopoulou, V., Steel, N., Stein, D.J., Steiner, T.J., Steinke, S., Stovner, L., Stroumpoulis, K., Sunguya, B.F., Sur, P., Swaminathan, S., Sykes, B.L., Szoeke, C.E.I., Tabarés-Seisdedos, R., Takala, J. S., Tandon, N., Tanne, D., Tavakkoli, M., Taye, B., Taylor, H.R., Ao, B.J. Te, Tedla, B. A., Terkawi, A.S., Thomson, A.J., Thorne-Lyman, A.L., Thrift, A.G., Thurston, G.D., Tobe-Gai, R., Tonelli, M., Topor-Madry, R., Topouzis, F., Tran, B.X., Truelsen, T., Dimbuene, Z.T., Tsilimbaris, M., Tura, A.K., Tuzcu, E.M., Tyrovolas, S., Ukwaja, K. N., Undurraga, E.A., Uneke, C.J., Uthman, O.A., van Gool, C.H., Varakin, Y.Y., Vasankari, T., Venketasubramanian, N., Verma, R.K., Violante, F.S., Vladimirov, S. K., Vlassov, V.V., Vollset, S.E., Wagner, G.R., Waller, S.G., Wang, L., Watkins, D.A., Weichenthal, S., Weiderpass, E., Weintraub, R.G., Werdecker, A., Westerman, R., White, R.A., Williams, H.C., Wiysonge, C.S., Wolfe, C.D.A., Won, S., Woodbrook, R., Wubshet, M., Xavier, D., Xu, G., Yadav, A.K., Yan, L.L., Yano, Y., Yaseri, M., Ye, P., Yebyo, H.G., Yip, P., Yonemoto, N., Yoon, S.-J., Younis, M.Z., Yu, C., Zaidi, Z., Zaki, M.E.S., Zeeb, H., Zhou, M., Zodpey, S., Zuhlke, L.J., Murray, C.J.L., 2016. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388, 1545–1602. https://doi.org/10.1016/S0140-6736(16)31678-6.

Waller, S.B., Cleff, M.B., Serra, E.F., Silva, A.L., Gomes, A.D.R., de Mello, J.R.B., de Faria, R.O., Meireles, M.C.A., 2017. Plants from Lamiaceae family as source of antifungal molecules in humane and veterinary medicine. Microb. Pathog. 104, 232–237. https://doi.org/10.1016/j.micpath.2017.01.050.

Zheljazkov, V.D., Cantrell, C.L., Astatkie, T., Hristov, A., 2010. Yield, content, and composition of peppermint and spearmints as a function of harvesting time and drying. J. Agric. Food Chem. 58, 11400–11407. https://doi.org/10.1021/jf1022077.