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REGULAR ARTICLE

Traditional use, chemical composition and antimicrobial activity of *Pectis brevipedunculata* essential oil: A correlated lemongrass species in Brazil

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Abstract

For centuries, medicinal plants have been used as source of active principles for the treatment of many conditions. Ethnobotanical studies and bioassay guided isolation procedures have been successfully used in order to investigate and confirm their medicinal prescriptions. Traditionally used in Brazil, *Cymbopogon citratus* (lemongrass) is usually consumed as tea drink due to its calmative, anxyolitic as well as anti-hypertensive properties. Due to the similar lemongrass scent many species of *Pectis* genus have been used as infusion drinks for the same purposes as *C. citratus*. In Brazil, *Pectis brevipedunculata*, a sandy ornamental aromatic grass, is one of the "lemongrass odor" correlated species traditionally consumed. Chemical analysis of its essential oil was performed using GC-FID and GC-MS. Such essential oil was characterized by a high percentages of citral (81.8%: neral 35.6% and geranial 46.2%), followed by limonene (2.9%) and α-pinene (2.6%). Chemical and ethnobotanical investigations were performed involving one of the most commonly used *Pectis* species, known as lemongrass in order to confirm the medicinal indications compared to their chemical profile. The essential oil of *P. brevipedunculata* was tested against several clinical parasites. Our results were in agreement to the literature survey, suggesting the citral as the principal active constituent of the tested samples. Despite of the wide biological activities spectrum related to the major constituent presented in the essential oil of the most Pectis species, it is necessary to continue the phytochemical and pharmacological studies about the infusions constituents and validate the folk medicine.

Key words: *Pectis brevipedunculata*, Citral, Traditional medicine, Essential oil, Biological activities

Introduction

In the last decades, the natural products have increased their importance as alternative safety health against many synthetic drugs. Considering the low number and efficacy to the available drugs, as well as their side effects and the resistance developed by parasites, natural compounds may rise as an important source of new bioactive templates used in chemotherapies (Silva et al., 2008; Marques et al., 2010; Alviano et al., 2012). In this context, the information obtained with etnobotanical investigations associated to the phytochemical guided-isolation studies may be a useful tool in this new drugs discovery challenge. The scientific research about traditional medicines followed by discovery of new efficient phytotherapic drugs can provide more accessible and lower cost medicaments to the patients, specially to the patients of tropical neglected diseases (Alviano and Alviano, 2009). Several aromatic plants widely distributed in the tropics exhibit fungicidal activities and have traditionally been used as flavoring agents in native dishes, as incense, insect repellents and folk medicine since plant essential oils are a potentially useful source of antimicrobial compounds (Lewinsohn et al., 1998; Edris, 2007). Plant derivative products could be used as viable alternatives to microbial

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contaminants control in foods such as moulds, and be used in foods conservation systems, since many synthetic compounds are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (Yaouba et al., 2010). Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including foodborne pathogens (Garcia et al., 2008; Marques et al., 2011). It is known that the wide and indiscriminate use of synthetic antimicrobial medicines are leading to hypersensitivity reactions as well as developing resistance to many currently used medicines (Adinarayana et al., 2012). The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies (Rapp, 2004; Yaouba et al., 2010).

The Asteraceae, the largest family of flowering plants, comprises about 1,600 genera and more than 25,000 species. The genus *Pectis* is composed by roughly 76 species characterized by small herb plants of the daisy-like family with bright yellow flowers and dark green foliage (Albuquerque et al., 2007). In general, they are found as low branching, mostly heavily scented herbs, being founded in several countries of all Americas at altitudes varying from sea level to 4,000 feet (Keil, 2002). Some species of this genus have the citric scent due to the high concentration of citral in their volatile composition. These species are found specially in dry uplands, usually on sandy and calcareous soils and can grow in nutritionally poor soil places. Because of this pronounced citric fragrance some *Pectis* species were used by the Indians for flavoring their foods and by the Indian women as a perfume (Bradley and Haagen, 1949; Keil, 1984).

In Brazil, many plant species are known as lemongrass due to the citric fragrance from their volatile constituents. Widely known in Brazil, *Cymbopogon citratus* is the main traditionally used plant known as lemongrass. Its essential oil (EO) has a characteristic aroma and it is composed by a high content of citral (neral and geranial isomers, comprising 65–85% of the volatile compounds (Silva et al., 2008). Citral is a volatile essential oil component that is found in various aromatic plants with a stronger and sweeter aroma than lemon. This monoterpene aldehyde fraction is normally composed by a mixture of the two geometric *cis*- and *trans*-isomers: geranial and neral, commonly used as a taste enhancer, as an odorant in perfumes, and as an insect repellent. Geranial has a strong lemon odor while neral is less intense, but sweeter. Lemongrass oil exhibits a broad spectrum of fungi toxicity by inhibiting several fungal species at different concentrations (Belletti et al., 2008). It is known that its fungitoxic potency remains unaltered for 210 days of storage, after which it starts to decline (Mishra and Dubey, 1994; Tzortzakis and Economakis, 2007). There is therefore considerable interest in the application of lemongrass oil for human parasite affections as well as the preservation of stored food crops. For exemple, effects of citral in a broad spectrum of post-harvest and aflatoxigenic pathogens have been well documented, showing a strong fungistatic and fungicidal effect. Many biological activities were described to this aldehyde monoterpene fraction. Bacteriostatic and fungistatic properties have already been related to essential oils rich in citral (Shukla et al., 2009; Tyagi and Malik, 2010; Demuner et al., 2011; Kim and Park, 2012). Geraniol and citral isomers should probably account for the antifungal activity of lemongrass. De Billerbeck et al. (2001) showed that citral accounted for up to 70% of the antifungal activity of *C. citratus* essential oil. Kurita et al. (1981) have shown that citral acts as a fungicidal agent because it is able to form a charge transfer complex with an electron donor to fungal cells, which results in fungal death. In rats, cardiovascular effects as transient hypotension and bradycardia were induced by the citral-rich essential oil obtained from lemongrass species, *C. citratus* and *P. brevipedunculata* (Moreira et al., 2010; Pereira et al., 2013). Blanco et al. (2009) reported a study describing the effects of the citral-rich essential oil (EO) on the mouse central nervous system. No toxicant effect was observed in this oral treatment. The citral rich essential oil obtained from *C. citratus* was effective in increasing the sleeping time as well as decreasing the alert state of the mice. The essential oil effect was in agreement with the popular indication of drink infusions of this species usually used for nervous, stress, anxiety and insomnia in Brazil.

Pectis brevipedunculata, a Brazilian ornamental aromatic grass, is one of the "lemongrass odor" correlated species found in the country, (Figure 1). Ethnobotanical studies in Northeast region of Brazil described the traditional use of *Pectis elongata* Kunth and *Pectis oligocephala* var. *affinis* (Gardner) Baker as infusion drinks for stomach disorders and hypertension as well as cold, and flu. The genus is represented mostly by five species, including *Pectis apodocephala, Pectis oligocephala* and *Pectis linifolia* L. var. *linifolia* whose essential oils we have previously investigated (Albuquerque et al., 2003; Agra et al., 2007). Also in southeast region some *Pectis* species have traditional indication use as infusions or juice drink preparations for hypertension, stomach disorders and colds (Agra et al., 2007). Calmative and analgesic properties were also reported for some *Pectis* tea preparations (Soares et al., 2009; Oliveira et al., 2011). The leaves of these species are used as edible parts specially as condiment raw or cooked, being also used as flavouring due to the strong lemon scent. This plant is considered carminative and emetic while the crushed leaves are indicated in the treatment of stomachaches (Agra et al., 2007). In Mexican markets *P. papposa* is sold as limoncillo and used in moderation as a culinary spice to flavor meat (Moerman, 1998). In South America, the herb infusion of a particular ecotype of *P. elongata* occurring in French Guyane is utilized in tea and spices to replace lemongrass *C. citratus* as occurs in Brazil (Maia et al., 2005). Due to the similar citral content in the essential oil and characteristic strong lemon scent, many *Pectis* species have been used to replace *C. citratus* for infusions preparations in some regions of Brazil. Complementary phytochemical and toxicological studies could support the etnobotanical investigations assessing their traditional use of the folk preparations to confirm their carminative, antimicrobial, anxiolytic or sedative efficacy since the aqueous extract carry many of other secondary metabolites most of them probably different of *C. citratus*.

This work aims to record the etnobotanical values related to the traditional indication use of *P. brevipedunculata* and its chemical composition as well as to investigate the essential oil antimicrobial properties. Moreover we present this species as a potential source of citral rich essential oil.

Figure 1. *Pectis brevipedunculata* in natural habitat.

Materials and Methods

Plant Material and Essential Oil Extraction

Aerial parts (100 g) of *Pectis brevipedunculata* were collected in Rio de Janeiro, RJ in April 2011. The botanical vouchers were identified by Dr. Roberto L. Esteves and kept at the Herbarium (HB) of the Rio de Janeiro National Museum under number 1007 (R). The fresh aerial parts were submitted to hydrodistillation for 2h in a modified Clevenger-type apparatus. The obtained essential oil (EO) was dried over anhydrous sodium sulphate, yielding 0.4% w/w which was immediately stored in closed dark vials at 4°C until analysis.

GC-FID analysis

Quantitative analyses were carried out on a GC 2010 Shimadzu machine with a ZB-1MS fused silica capillary column (30m x 0.25mm x 0.25µm film thickness). The operating temperatures used were: injector 260°C, detector 290°C and column oven 60°C up to 240°C (3°C/min). Hydrogen at 1.0mL x min^{-1} was used as carrier gas. The percentages of the compounds were obtained by GC-FID analysis.

GC-MS analysis

Qualitative analyses were carried out on a GC-QP2010 PLUS Shimadzu machine with a ZB-5MS fused silica capillary column (30m x 0.25mm x 0.25µm film thickness). The operating temperatures used were: injector 270°C, detector 290°C and column oven 60°C up to 290°C (3°C/min). Helium at 1.0mL x min^{-1} was used as carrier gas for GC/MS. The essential oil components were identified by comparison of their retention indices and mass spectra with published data (Adams et al., 2001) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST 3.0) libraries provided with the computer controlling the GC–MS system. The retention indices were calculated for all volatile constituents using the retention data of linear n-alkanes C8–C24.

Evaluation of Antimicrobial Activity

Assays were performed at the Laboratory of Microorganisms Structural Surface I and II, Institute of Microbiology Professor Paulo de Goes, UFRJ, in collaboration with Prof. Dr. Daniela Sales Alviano and Prof. Dr. Celuta Sales Alviano. The extracts, partitions and crude citral rich essential oil of *Pectis brevipedunculata* had antimicrobial activity determined by Drop Test agar diffusion technique, described by Hili, 1997. The microorganisms used were the fungi *Candida albicans* serotype B ATCC 36802, *Cryptococcus neoformans* serotype A T1-444 (Federal University of São Paulo, SP-UNIFESP), *Trichophyton rubrum* T544, *Fonsecaea pedrosoi* 5VPL, *Microsporum canis*, *Microsporum gypseum* (collection of fungi Hospital Clementino Fraga Filho, UFRJ) and *Staphylococcus aureus* bacteria MRSA (BMB9393) (Hospital Clementino Fraga Filho, UFRJ), *Aspergillus niger, Escherichia coli* and *Staphylococcus epidermidis*. The samples tested were solubilized in dimethylsulfoxide (DMSO) to obtain a final concentration of 50mg/ml and after, were diluted to a concentration of 1:2 and 1:3 (V/V) in distilled water to eliminate the DMSO toxicity for microorganisms. Microorganisms (2 x 105 cells/ml) were spread on petri dishes containing BHI (Brain Heart Infusion of bovine) solid and, after 10 minutes, 10mL of each sample was placed in the center of each plate. All plates were incubated at 37°C or at room temperature, with incubation times varying from 1 to 7 days depending on the organism tested. After this period the diameter of inhibition zone was measured with a ruler in millimeters. The control was made with amphotericin B (1mg/ml) for fungi and vancomycin (1 mg/ml) for bacteria. The formation of inhibition zone where the sample was applied indicates the sensitivity of the microorganism to the same, being liable to occur mainly atypical results in tests with resistant microorganisms or mutants. In this case it is observed an inhibition zone, but with the presence of a few colonies in the region.

Statistical analyses

The percentage composition of the oils was computed by the normalization method from the GC-FID peak areas. The analyses were carried out in triplicate and the results are expressed as means ± SD. The biological testes (inhibition halo) data are expressed in millimeters as the means of average experiments performed in duplicate.

Results and Discussion

Chemistry and Ethnopharmacology

Popularly used in folk medicine, infusions prepared with fresh or dry leaves of *C. citratus* (lemongrass) are common in almost all the continents for a wide range of indications. In several states of Brazil, lemongrass is equally evidenced as popular medicinal plant with indication use for several conditions such as: restorative, digestive disorders, colds, analgesic, antihermetic, antithermic, anti-inflammatory, diuretic, antispasmodic, calmative and antiallergic (Negrelle and Gomes, 2007). Despite of all mentioned indications above, *C. citratus* is particularly used in the folk medicine for high blood pressure treatment. Many hypertensive patients drink daily tea of *C. citratus* due to its calmative, anxyolitic as well as anti-hypertensive properties. As occurs in lemongrass, the main components of the volatile fractions in the *Pectis brevipedunculata* essential oil (EO) are the isomers neral and geranial (citral > 80.0%) followed by nerol and geraniol as minoritary derivatives. Its essential oil chemical profile is quite similar to the *C. citratus*, being also characterized by the high percentage of citral ranging from 70-92% in the volatile composition, (Figure 2). In agreement with this chemical similarity, ethnopharmacological studies have reported the traditional use of *P. brevipedunculata* by some communities in the country as calmative tea drink as occurs with lemongrass (Oliveira et al., 2011). Other lemon scent *Pectis* species has also popular indicative use. In the State of Matogrosso, Brazil, infusions of *Pectis jangadensis* are also popularly used as calmative tea drink. In northeast of the country, it was found the popular use of *P. elongata* Kunth and *P. linifolia* L. var. *linifolia* in the folk medicine against hypotension and stomachic diseases while *P. oligocephala* (Gardner) Sch. Bip. infusions are usually indicated against grippes and colds (Agra et al., 2007; Maia et al., 2005).

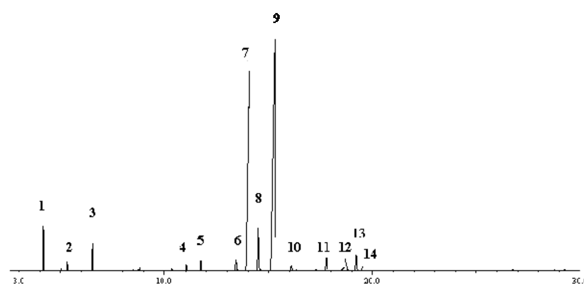

Figure 2. GC-FID chromatographic profile of *P. brevipedunculata* crude essential oil. Identified compound present in the essential oil: (1) α-pinene; (3) limonene; (4) α-pinene epoxide; (6) nerol; (7) neral; (8) geraniol; (9) geranial; (11) neryl acetate; (l2) 4-isopropylcyclohexanol; (14) geranyl acetate.

The main compounds of the oil of *P. apodocephala* are geranial (43 – 45%) and neral (32 – 34%) followed by α-pinene (10 – 11%) and limonene (6 – 7%) while the most prevalent compounds detected in the oils of *P. oligocephala* were *p*-cymene (50 – 71%) and thymol (24 – 45%). The essential oils exhibited significant activity and could be considered as potent natural nematicidal and larvicidal agents (Albuquerque et al., 2007). The chemiscal diversity found in the EO composition from different species is probably due to variations involving different biomes and climate conditions where they are under pressure. In fresh aerial parts of

North American species it is found that *P. Texana* presents 48% of content oil composed by thymol, while plants of *P. elongata* presented the essential oil comprising 60% citral. On the other hand, the percentage of cumaldehyde in *P. papposa* oil is approximately the same as in cumin oil 47%, followed by β-pinene 27% and carvone12% (Bradley and Haagen, 1949). Most of the traditional tea drink preparations are produced by decoction process. In this process warm water is able to extract considerable amounts of volatile compounds into the infusion drink. During the process, a small hydrophilic portion of the essential oil gets disproportionally partitioned into the water or hydrosol. The compositions of essential oils content present in the tea can be different from their field distilled primary water originally for commercial proposes, but still preserve the antimicrobial properties. In addition, recent studies have shown that even citral isomers oxides are also bioactive against natural parasites. The aldehyde epoxide mixture has shown to be more active than that of the original citral against several bacteria while the comparison between citral and crude lemongrass oil showed similar effect (Saddiq and Khayyat, 2010). Oliveira et al. (2011) investigated the influence of the drying-air temperature on content of citral present in the essential oil of *P. brevipedunculata*.

The maximum content of citral was observed in plant material dried at 40^{o}C while the minimum was found room temperature dried material. The chemical composition of *P. brevipedunculata* volatile fractions consists of monoterpene compounds, hydrocarbons, alcohols and aldehydes. The GC-FID and CG/MS methods were used to identify these compounds. The chemical analysis of the EO investigated is presented in Table 1. By the average analyses of tree EO independent extractions, it was characterized by a high percentage of citral (81.8%: neral 35.6% and geranial 46.2%), followed by limonene (2.9%) and α-pinene (2.6%). Alcohol *cis* and *trans* enantiomers derivatives were also detected: nerol and geraniol, corresponding to 1.1% and 6.6% in the whole oil, respectively. Citral is the major component (>80.0%) of *P. brevipedunculata* EO as occurs in lemongrass (*C. citratus*) where citral is usually found ranging from 65-85% in the oil (Silva et al., 2008). The fragrance similarity could explain the traditional use of this *Pectis* species for similar lemongrass proposes. The qualitative and quantitative analysis of the major compounds in the EO of lemongrass and *P. brevipedunculata* are quite similar, showing the citral as main compound in the mixture usually (citral> 70.0%) in both species. This fact could justify the same usage of these different species for the same proposes in some traditional medicines and the common nomenclature mistakes related to the citric lemon scent similarity. Despite the etnobotanical uses of them, few species of this genus has been the subject of scant phytochemical and biological studies, Table 2. Nevertheless, compounds extracted from *P. brevipedunculata* by warm water in tea preparations are different and unequal proportions of the essential oil could be presented in the infusions tea drinks consumed. In *C. citratus*, several secondary metabolites could interfere with absorption and distribution process since most of compounds were already characterized in aqueous drinks such as saponins, sesquiterpenes, lactones, alcacaloids, tanins, steroids, triterpenes and flavonoids (Vendruscolo et al., 2005; Omotade, 2009). Since no chemical data is found in the literature about *P. brevipedunculata*, in except volatile compounds, phytochemical studies should be taken in order to better known the chemical composition of these traditional drinks and confirm the possible bioactive compounds.

Table 1. Identified compounds in the aerial parts essential oil of *P. brevipedunculata*.

	Compounds	^{a} RI ^{Lit}	^{b}RI	FRESH-HD %	*Identification*
1	α-pinene	939	938	2.6 ± 0.7	RI, GCMS
2	limonene	1029	1032	2.9 ± 1.4	RI, GCMS
3	α-pinene epoxide	1089	1186	0.2 ± 0.8	RI, GCMS
4	nerol	1233	1233	1.1 ± 0.4	RI, GCMS
5	neral	1247	1248	35.6 ± 2.3	RI, GCMS
6	geraniol	1276	1260	6.6 ± 1.4	RI, GCMS
7	geranial	1277	1278	46.2 ± 3.5	RI, GCMS
8	neryl acetate	1362	1365	1.3 ± 0.3	RI, GCMS
9	4-isopropylcyclohexanol	-	-	1.3 ± 0.0	RI, GCMS
10	geranyl acetate	1381	1384	0.4 ± 0.5	RI, GCMS
Sum of identified peaks				98.1 ± 1.4	

aRI Lit: Literature Retention Indices; b RI: Experimental Retention Indices; H.D: Hydrodistillation; Data
are the means of three experiments performed in triplicate. The results are presented as average of three analyses ± SD (standard deviation).

Table 2. Volatile major constituent of *C. citratus* and literature described *Pectis* species with traditional indication.

Species	Key Marker	%	Traditional use	Use form	Reference
C. citratus	citral	65-85	cold, anxiety, hypertension	infusion	Silva et al., 2008
P. apodocephala	citral	75	hypertension, stomach disorders	infusion	Albuquerque et al., 2007
P. angustifolia	citral	na	flavoring, carminative	raw condiment	Moerman, 1998
P. brevipedunculata	citral	70-90	flavoring, calmative	infusion	Marques and Kaplan, 2013
P. ciliaris	citral	na	remedy for cold and fever	infusion	Beckwitb, 1927
P. elongata	citral	60	calmative, hypotension	infusion	Maia et al., 2005
P. jangadensis	citral	na	calmative	infusion	Soares et al., 2009
P. linifolia	citral	na	hypotension, stomach disorders	infusion	Machado et al., 2012
P. oligocephala	*p*-cymene	50-70	grippes and colds	infusion	Albuquerque et al., 2007
P. papposa	cumaldehyde	47	spice flavoring, carminative	raw condiment	Bradley and Haagen, 1949
P. texana	thymol	48	spice flavoring	raw condiment	Bradley and Haagen, 1949

na: no data available about volatile composition (citral was ascribed as the principal component according to the strong lemon scent described by the literature source and traditional indications).

Table 3. Antimicrobial activities of *P. brevipedunculata* crude extracts and sub-fractions.

Microorganisms		[a]Fungi		[b]Bacterias		
	Samples	*Inhibition zone (mm)*		Inhibition zone (mm)		
	EXTRACTS	*C. albicans*	C. neoformans	*S. aureus*	*E. coli*	*T. rubrum*
1	Hex	9	9	10	very small	8
2	MeOH	---	---	---	---	---
	SUB-FRACTIONS					
2a	Hex	---	---	---	---	very small
2b	$CHCH_2$	---	---	---	---	very small
2c	AcoEt	---	---	---	---	---
2d	BuOH	---	---	---	---	---
	control	10	22	15	ND	20

a: room temperature; b: incubated at 37oC for 24h. Microorganisms: *A. niger: Aspergillus* niger*; C. albicans*: *Candida albicans; C. neoformans: Chryptococcus neoformans; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; T. Rubrum: trichophyton rubrum.* The inhibition halo is measured in mm. ND: not determined. Data are the average means of experiments performed in duplicate.

Table 4. Antimicrobial activity of *P. brevipedunculata* citral rich essential oil.

	Microorganisms	Inhibition zone (mm)		
	Concentration	1:3	1:2	Control
	Fungi			
1	*A. niger*	---	30	ND
2	*C. albicans*	---	23	10
3	*C. neoformans*	---	12	22
4	F. pedrosoi	---	25	18
5	*M. canis*	5	+++	19
6	*M. gypseum*	6	+++	17
	Bacteria			
1	E. coli	---	10	ND
2	*S. aureus*	8	22	15
3	*S. epidermidis*	---	14	11
4	*T. rubrum*	15	+++	20

a: room temperature; b: incubated at 37oC for 24h. Microorganisms: *A. niger: Aspergillus* niger*; C. albicans*: *Candida albicans; C. neoformans: Chryptococcus neoformans; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; T. Rubrum: trichophyton rubrum.* (+++): very strong toxic effect; (---): no inhibition observed. ND: not determined. Data are the average means of experiments performed in duplicate.

Citral rich essential oil biological activities

Bioactive phytocompounds present in the crude extracts and essential oils of medicinal plants are components of an important strategy linked to the discovery of new medicines (Alviano et al., 2012). Many plant essential oils have been described as a potentially useful source of antimicrobial compounds. Numerous essential oils have been tested for *in vivo* and *in vitro* antimycotic activity and some demonstrated to be potential antifungal agents (Silva et al., 2012). In fact, they are characterized by a wide range of volatile compounds, some of which are important flavor quality factors (Belleti et al., 2008; Wannissorn et al., 2009). Because of its characteristic lemon aroma, citral is also an interesting raw material used in the pharmaceutical, perfumery and cosmetics industries. Several *Cymbopogon* citral-rich species supply essential oil that is applied in the industrial products as well as for medicinal proposes, being this use of reasonable economic importance (Negrelle and Gomes, 2007; Silva et al., 2008). Independently of the place origin the predominant compound of the lemongrass essential oil is the aldehyde isomers mixture. As a natural acyclic monoterpenes, citral was found also in a wide variety of plant and was shown to act effectively in chemoprevention and chemotherapy of different cancers diseases in animal models, at cellular level, and in human clinical trials (Saddiq and Khayyat, 2010). Over the last decade the increased incidence of fungal infections poses a great challenge to healthcare professionals. The increasing number of fungal infections is related to the great number of immunocompromised individuals due to use of extensive chemotherapy and other immunosuppressive drugs or related diseases (Kumar et al., 2012). Concerning about the affections caused by common parasites it is well known that bacteria and fungi are the causative organisms for several infectious diseases posing a great threat not only to humans, but also to plant health (Yaouba et al., 2010). In recent years, there has been a considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods (Garcia et al., 2008). Plants and natural products can represent a source of natural antimicrobials to improve the shelf life and the safety of food. For exemple, citron essential oil is characterized by a broad spectrum antimicrobial activity (Friedman et al., 2002; Belleti et al. 2004, 2008, 2010). The relevant antimicrobial activity of citron (*Citrus medica*) and lemon myrtle (*Backhousia citriodora*) essential oils was ascribed to their high citral content (Belletti 2004). Effects of citral in a broad spectrum of post-harvest and aflatoxigenic pathogens have shown a strong fungistatic and fungicidal effect against many fungi such as: *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*. Also, in apples (*Penicillium expansum*) was strongly inhibited by this natural compound. The results obtained with a citron essential oil were particularly interesting because it is commonly used as flavouring agent in soft drinks. The antimicrobial activity of this oil was confirmed in vitro and was related to the high concentration of citral (Belleti et al., 2008; Belleti et al., 2010; Cardoso and Soares, 2010).

The extracts, semi-purified fractions and crude citral rich essential oil of *P. brevipedunculata* were tested for their antimicrobial activity against Gram positive bacteria *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* and Gram-negative *Escherichia coli* as well as in fungi *Aspergillus niger*, *Candida albicans, Cryptococcus neoformans, Fonsecaea pedrosoi*, *Microsporum canis*, *Microsporum gypseum* and *Trichophyton rubrum*. The hexane (Hex) and methanol (MeOH) extracts of aerial parts, were preliminary tested. The hexane extract showed better antimicrobial activity promoting some growth inhibition of all fungi and bacteria tested. The results are summarized in Tables 3 and 4. The best result was found against *C. albicans*. Phytochemical studies with nonpolar fractions suggested the aldehyde citral as the responsible for this antimicrobial effect since this Hex extract is 12% composed by citral. It is reasonable this indication since citral has many antimicrobial activities described in the literature. The sub-fractions obtained from MeOH extract were not effective in the tested parasites. This data were in agreement to the fact there was no essential oil present in that sub-fractions once hexane was previous used to remove the great part of nonpolar as terpenoid compounds from the plant material before the maceration with MeOH. As is shown in the Table 3, a very small inhibition halo was found with Hex (2a) and CH_2Cl_2 (2b) fractions provenient from MeOH extract against *T. rubrum* while the crude Hex extract was active against all tested microorganisms. It is found that citral (25-200 μg/mL) is able to inhibit the mycelial growth of *C. albicans*, suggesting the potential value of citral rich oils for the treatment of cutaneous candidiasis. Lemongrass oil is highly effective also in vapour phase against *C. albicans*, leading to deleterious morphological changes in cellular structures and cell surface alterations (Tyagi and Malik, 2010). Additionally, pre-clinical studies conducted with ointments containing essential oils, including lemongrass oil used in animals infected with dermatophyte fungi (*T. rubrum* and *M. gypseum*) showed the efficacy of these preparations. To obtain more data concerning antimicrobial activities of *P. brevipedunculata* essential oils against clinical parasites, the inhibitory assay was also performed using the bacteria *S. aureus*, *S. epidermidis* and *E. coli* and fungi *A. niger*, *C. albicans*, *C. neoformans*, *F. pedrosoi*, *M. canis*, *M. gypseum* and *Trichophyton rubrum*.

The results showed that the citral rich essential oil possessed inhibitory activity against these bacteria and fungi. Inhibition zone diameters against fungi and bacteria tested are represented in Table 4. It was observed that diameters inhibition zones produced by the crude EO varied from 5 to 15mm when in dilution ratio of 1:3 and from 10 to 30mm when tested in dilution ratio of 1:2.

Differences in the inhibition growth among microorganisms may be explained on the basis particular characteristics of prokaryotic and eukaryotic parasites once the sensitivity of prokaryotic is different than that of eukaryotic because the changes in the cell wall and plasma membrane and also the nuclear substances moreover (Saddiq and Khayyat, 2012). The results are in agreement to the data related to the citral antimicrobial potential since most of the tested parasites were susceptible in front of the aldehyde isomers. In fact, in most of the cases the inhibition halo was superior to the observed in the control when analyzed in ration 1:2. The inhibition activity of the EO was very strong for *C. albicans, F. pedrosoi* and *S. aureus*. Strong for *S. epidermidis* and moderate for *C. neoformans* and *E. coli*. In three cases it was observed a very toxic effect of the EO inhibiting the complete growing development of the parasites in the agar plate. The EO activity was just inferior to the control when compared to the *C. neoformans*. Once again our results are in agreement with literature data.

The infusions preparations could also display a noteworthy antimicrobial effect once Adinarayana et al. (2012) has found that the water-soluble oil recovered by redistilling the hydrosol containing lemongrass oil still showed strong activity against *Staphylococcus aureus*, showing a strong activity with zones of inhibition of 16 - 22 mm for this parasite. The same effective inhibition growing was found to *Aspergillus niger*. For essential oil samples, the inhibition halos will depend on the ability of oil to diffuse uniformly through an agar medium and the effect on the parasites of oil vapors that may be released. The biological activity may also vary depending of the composition of the EO. The presence of two or more active components may interact antagonistically, additively, or synergistically at low concentrations (Friedman et al., 2002). Thus, despite of the documented toxic effect of citral to several parasites, the inhibition growth evaluation involving these oils may display some variation range effect when compared to different oils and experiments. Because of high volatility and lipophilicity of the essential oils, they are readily attached to penetrate into the cell membrane to exert their biological effect (Machado et al., 2012). Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration. These actions can occur in an isolate or concomitant way and culminate with mycelium germination inhibition (Silva et al., 2008; Kumar et al., 2012). Lipophilic agents may be able to execute their action at membrane integrity level, affecting embedded enzymes and fatty acid composition, being citral and α-pinene the most referred of these hydrocarbons (Garcia et al., 2008). This fact is relevant data since in both species *C. citratus* and *P. brevipedunculata* the EO is also composed by α- and β-pinene followed by limonene. Hydrophobic molecules can easily diffuse across cell membranes and consequently gain advantage in what concerns to interactions with intracellular targets, being a valuable research option also for the search of new anti-Leishmania drug templates (Edris, 2007; Machado et al., 2012). Out of the 1114 strains belonging 105 sensible and resistant species of microbes (molds, yeasts and bacteria) have been already investigated front of citral rich essential oil, 38.2% were sensitive to lemongrass oil discs containing 50 μg oil/disc. Citral rich essential oil was able to instantly kill *C. albicans* and *Escherichia coli,* and *S. aureus* in 10 min at 1 mg/ml concentration, indicating of its wide spectrum potency antimicrobial activity at easily achievable concentrations (Singh et al., 2011). Based on the several biological benefits involving the essentials oils containing citral as main constituent, is reasonable to believe that *P. brevipedunculata* EO is also endowed of these noteworthy properties.

Conclusions

According to these facts, citral is related as active natural agent presented in many of these plant species. Although this aldehyde fraction display many biological activities it is suggested that the concentration found in the small quantity of plant material usually used in a cup of tea (2-10g) could not be sufficiently enough to be effective as responsible for some described activities in human beings. However, the availability of citral was found to be considerable sufficient to display the reported activities involving this compound. Further studies should be important to investigate how much these aldehydes isomers are present in the *Pectis* infusions in order to estimate the curative/preventive oral effect of these folk drinks, its water vapour effect on the microorganisms as well as the antihypertensive activity related also to these species. Thus, further investigations should be carried out in order to improve the knowledge of its efficacy and safety in validate these traditional use.

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