

Essential oils found in the smoke of “tira-capeta”, a cigarette used by some quilombolas living in pantanal wetlands of Brazil

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RESUMO: “Óleos essenciais encontrados na fumaça do “tira-capeta”, um cigarro utilizado pelos quilombolas que vivem no pantanal do Brasil”. Pesquisa etnofarmacológica realizada entre os quilombolas que vivem na Sesmaria Mata-Cavalos, no Estado de Mato Grosso, Brasil, um cigarro conhecido como “tira-capeta”, foi citado por melhorar a memória e cognição, além de possuir outras indicações terapêuticas, tais como, “contra a sinusite”, “para evitar resfriado”, e “para aliviar problemas de insônia”. No presente estudo, foi feita a caracterização dos óleos essenciais liberados durante o aquecimento das plantas utilizadas na confecção do cigarro, usando um método simples, rápido e livre de solvente baseado em cromatografia gasosa acoplada a espectrometria de massas usando “headspace” e microextração em fase sólida. Os principais constituintes encontrados foram 1,8-cineol, cânfora e α -pineno. Na tentativa de correlacionar a atividade biológica desses constituintes com as indicações terapêuticas relatadas pelos quilombolas, encontramos vários trabalhos realizados por diversos autores que mostraram que estes óleos possuem atividades similares às indicações terapêuticas relatadas pelos quilombolas.

Unitermos: Etnofarmacologia, óleos essenciais, medicina popular, sistema nervoso central, quilombolas, memória.

ABSTRACT: An ethnopharmacological survey developed among quilombolas living in Sesmaria Mata-Cavalos, in the State of Mato Grosso, Brazil; a cigarette known as “tira-capeta” (removing-the-devil), had been cited mainly “to improve memory and cognition”, and also showed other therapeutical indications, such as: “against sinusitis”, “to avoid cold”, “to relieve sleep problems”. The purpose of the present study was carried out a screening of essential oils delivered in the heating of plants used to produce the “tira-capeta” cigarette, using a simple, rapid and solvent-free method based on gas chromatography–mass spectrometry and headspace solid-phase microextraction. The principal essential oils found were 1,8 cineole, camphor and α -pinene. In the tentative to correlate these constituents with therapeutical indications reported by the quilombolas, were found some works carried out by many authors that corroborated the therapeutical indications reported by the quilombolas.

Keywords: Ethnopharmacology, essential oils, traditional use, central nervous system, quilombolas, memory.

INTRODUCTION

Few ethnopharmacological surveys among *Quilombolas* (descendants of Afro-Brazilian runaway slaves) have been conducted in Brazil, despite the existence of 178 *Quilombola*'s communities in its territory (Fundação Cultural Palmares, 2008). Some exceptions are the studies carried out by Albuquerque (2001) and by Rodrigues & Carlini (2004).

The use of a great number of plants in a single prescription is very common among *Quilombola*'s therapeutic practice; being also observed among the river dwellers from Brazilian Amazon (Rodrigues, 2006) also in Ayurveda and Chinese therapeutics (Patwardhan

et al., 2005). This practice may produce synergistic or antagonistic effects (Gilbert & Alves, 2003; Rodrigues & Carlini, 2004).

Plants belonging to *tonics for the brain's* category are utilized to improve memory and/or cognition; nine out of thirteen plants cited in this category are used as a cigarette known as “tira-capeta” (removing-the-devil). This cigarette is recommended for persons suffering from nervous breakdown due to overwork, being also used by teenagers and children to improve their performance in learning abilities (Rodrigues et al., 2008).

Although the main use of the “tira-capeta” cigarette is as tonic for the brain, it also has other indications, such as: against sinusitis, to gastrointestinal disorders, to avoid

cold, to relieve sleep problems and to diminish the use of cannabis. In a previous work (Rodrigues et al., 2008), an hydroalcoholic extract (ETC) of “tira-capeta” cigarette was submitted to pharmacological and phytochemical investigations. The phytochemical screening indicated the presence of tannins, phenolic acids, flavonoids, saponins and alkaloids; tannins and phenolic acids being the main components. The pharmacological tests showed that ETC induced a biphasic effect, with intense initial stimulation of CNS, followed by a general depressor state; decreased the latency for sleeping and increased the total sleeping time, without causing prejudice in motor coordination. The purpose of the present study was carried out a screening of essential oils delivered in the heating of plants used to produce “tira-capeta” cigarette, since the route of administration utilized by *Quilombolas* is the inhalation of the smoke produced by the burning of a cigarette. Gas chromatography-mass spectrometry and headspace solid-phase microextraction had been successfully applied for the analysis of essential oils in various medicine plants. Below it will be presented the plants used in the preparation of this cigarette.

Petiveria alliacea L., Phytolaccaceae, grows in all regions of Brazil where it is known by many vernacular names; among them: “erva-guiné”, “guiné”, “pipi” and “tipi” and “macuracaá”, by the Guarani Indians. It is popularly used in folk medicine for treating a wide variety of disorders. In the State of Minas Gerais the roots of this plant are used to treat rheumatism (Gomes et al., 2005) and in other Brazilian regions, both the roots and the leaves are popularly used as stimulant. Phytochemical analysis revealed the presence of coumarins, triterpenes and flavonoids (Rocha & Da Silva, 1969) amino acids and trisulphide (Kim et al., 2006). So far, there is not information about essential oils.

Eucalyptus globulus Labill., Myrtaceae, is a native dicotyledonous tree of Southeast Australia States, that was introduced in Latin America. *E. globulus* is one of the most interesting species among the more than 600 species comprising the genus *Eucalyptus* (Hasegawa et al., 2008). In Brazil this genus also has a great therapeutical importance and several species of *Eucalyptus* are used in traditional medicine as antiseptics, and against infections of the upper respiratory tract, such as the common cold, influenza and sinus congestion; besides this exhibit antibacterial, analgesic and anti-inflammatory activities (Silva et al., 2003). Essential oil from these species has a therapeutic application in treatment of pulmonary infections by inhalation (Ramezani et al., 2002). Phytochemical analysis showed that the profile of the terpenoids varies among the *Eucalyptus* species, with potential variations in medicinal properties. The major components identified were aromadendrene, followed by α -phellandrene, 1,8-cineole, ledene and globulol (Pereira et al., 2005). In general, all the oils of *Eucalyptus* species contain α -pinene, β -pinene, β -caryophyllene and 1, 8-cineole as major components

(Sacchetti et al., 2005).

In Brazil, several plants known as “catuaba” are recognized for their aphrodisiac and stimulant properties. One of the species available in Brazilian commerce, traded as “catuaba”, was identified as *Anemopaegma arvense* (Vell.), Bignoniaceae. In the ethyl acetate extract of the stem bark of this plant were found catuabin A and known flavan-3-ol type phenylpropanoids, which possess antioxidant activities (Tabanca et al., 2007). So far, there is not information about essential oils of this plant.

Siparuna guianensis Aubl., Monimiaceae, is a genus of small trees or shrubs that occurs in the neotropics from Vera Cruz, Mexico, until tropical South America. This plant was known as “capitiú” or “negramina” and had been utilized as anxiolytic by South American Indians and “caboclos” river-dwellers (Rodrigues, 2006; Rodrigues et al., 2008). Alkaloids, steroids (Braz Filho et al., 1976), flavonoids (Leitão et al., 2005) and essential oils are found in this plant. Essential oils epi- α -cadinol as the major component, except for the fruit and stem bark oils that contained 2-undecanone and terpinolene as main constituents, respectively (Viana et al., 2002). Curzerenone and its derivative were also found in essential oil from leaves (Antonio et al., 1984). The fruit oil consisted mainly of 2-undecanone, β -pinene and limonene (Fischer et al., 2005).

Ruta graveolens L., Rutaceae, have different established effects like antimicrobial, cytotoxic (Ivanova et al., 2005), fungicide (Meepagala et al., 2005), anti-inflammatory (Raghav et al., 2006) and hypotensive properties. It is one of the most ancient and effective contraceptive plants (Maurya et al., 2004; de Freitas et al., 2005). Ketones and alcohols, especially 2-nonanone, 2-undecanone and nonan-2-ol are common in this specie (Fredj et al., 2007, Ivanova et al., 2004). This species possess α -pinene, limonene and 1,8-cineole as the main monoterpene constituents. Among other compounds, were detected considerable amounts of valeric acid, octanoic acid and methyl salicylate (De Feo et al., 2002).

Syzygium aromaticum (L.) Merr., Myrtaceae, also known as clove tree, is a perennial tropical plant used as a source for obtaining an essential oil, extracted from the dried flower buds. Clove bud oils showed antibacterial, antifungal, and antioxidant properties, and had been used traditionally as flavoring agent and antimicrobial material in food (Velluti et al., 2003). The main constituents of its essential oil are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde (Chaieb et al., 2007), followed by β -caryophyllene, α -humulene, and eugenyl acetate (Park & Shin, 2005; Jirovetz et al., 2006).

Allium sativum L., Liliaceae, garlic had been used worldwide as a food and medicinal plant since ancient times, and their biological effects have been attributed to its characteristic organosulfur compounds. Garlic is also known as an anticancer, antiseptic, tonic, bactericide, expectorant, stomachic, and antihypertensive.

Garlic essential oils are reported to consist primarily of diallyl, dimethyl, and allylmethyl mono-, di-, trisulfides (Park & Shin, 2005; Chung et al., 2007). Allicin (diallyl thiosulfinate) is the main biologically active compound of fresh garlic juice (Camargo et al., 2007).

Plants of *Hyptis* spp. are recommended in folk medicine for various conditions, among them nasal congestion, skin diseases, gastric disorders, fever, and pain. Bicyclogermacrene, 1,8-cineole, α -pinene and β -caryophyllene are the major components found in *Hyptis fruticosa* essential oil (Menezes et al., 2007). So far, there is not information about the essential oils of *Hyptis cana* Pohl ex Benth (Lamiaceae).

Several *Dorstenia* species "carapiá" are used in folk medicine, mainly against skin diseases, because of the presence of biologically active compounds. These plants are distributed in the subtropical regions of the world, and they are known by their ability to synthesize furocoumarins. The genus *Dorstenia* is used in the medicinal plant therapy in many countries as antiophidic and anti-rheumatic, and against infections and skin diseases (Cardoso et al., 2002). So far, there is not information about the essential oils of *Dorstenia asaroides* Hook (Moraceae).

MATERIAIS AND METHODS

Fieldwork

Ethnopharmacological study was approved by the Ethics Committee on Research of Federal University of São Paulo (n. 056/00) and residents of Sesmaria signed a written consent form to access *Quilombola* knowledge and botanical material. Samples of each plant mentioned by the interviewees were collected in compliance with methods recommended by Lipp (1989) and the plants were identified at the Botanical Institute of São Paulo (IB), where the vouchers were deposited. The phytochemical study was also approved by Ethics Committee on Research (n. 0147/07).

Botanical material

Parts of the nine plants used to prepare "tira-capeta" cigarette were collected in Sesmaria Mata-Cavalos by one of the researchers (ER) accompanied by one of the interviewee, the spiritual and political leader, Mr. Cezário. The leaves of the following plants [their respective vouchers] were collected: "guiné" - *Petiveria alliacea* L., Phytolaccaceae [E.Rodrigues 823], "eucalipto" - *Eucalyptus globulus* Labill., Myrtaceae [E.Rodrigues 525], "alecrim-do-norte" - *Anemopaegma arvense* (Vell.) Stellfeld ex de Souza, Bignoniaceae [E.Rodrigues 519], "negramina" - *Siparuna guianensis* Aubl., Monimiaceae [E.Rodrigues 531], "arruda" - *Ruta graveolens* L., Rutaceae, and "hortelã-da-várzea" - *Hyptis cana* Pohl ex Benth, Lamiaceae [E.Rodrigues 530]. Also,

the rhizomes of "caia-piá" - *Dorstenia asaroides* Hook., Moraceae [E.Rodrigues 878]), the flowers of "cravo-da-india" - *Syzygium aromaticum* (L.) Merr. & L.M. Perry, Myrtaceae, and skin of one bulb of garlic - *Allium sativum* L., Liliaceae. After collection, the respective parts were cut in small pieces, mixed and exposed to the sun.

Analytical procedure

The procedure was based on methodology published by Polzin et al. (2007) with modifications. Aliquot of 4 g (approximately 10 mL) of powder plants (ground in a grinding Mill Mesh 20) was introduced in the 20 mL headspace-vials (glass vials for headspace analysis crimped by 20 mm Teflon/silicone septa), sampling by automated headspace solid-phase microextraction (SPME), closed hermetically and placed into the oven. In the optimized conditions, the sample was heated at 150 °C for 30 min. The headspace sample was thermally desorbed from the SPME fiber upon insertion in the GC. The needle (heated at 150 °C) of the autosampler syringe, once entered the vial and filled the syringe, injected 0.8 mL of headspace sample directly into the injector liner, which was heated at 200 °C, and led it directly into the ionisation chamber of the mass spectrometer, using nitrogen as cleaning inert gas. Then, a helium stream carried the volatiles through the transfer line, heated at 250 °C, to the mass detector Shimadzu GCMS-QP505A gas chromatograph coupled to a quadrupole mass selective spectrometer and with split of 2. The direct interface between the injector and the detector is ensured by a transfer line, which consists of a 15 cm long empty retention gap placed in an oven with a temperature controller. Helium was the carrier gas, at a linear velocity of 1.8 mL/min. The separation of compounds was performed on DB-5 (Shimadzu, Br) capillary column (30 m \times 0.25 mm internal diameter and 0.25 μ m film thickness). The initial oven temperature was 40 °C, held for 4 min, ramped at 4 °C min⁻¹ to 200 °C, held at 200 °C for 1 min, and ramped at 4 °C min⁻¹ to 250 °C and held for 2 min. The quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV of electron energy, a source temperature of 200 °C, quadrupole temperature of 200 °C, and the transfer line was 250 °C, with a continuous scan from m/z 40 to 500. The total GC run time was 19.15 min. Constituents were identified by comparing their mass spectra with those reported in the GC/MS computer database (Wiley 275, Wiley 229 and NIST 21) and reference compounds. The relative amounts of the individual components were obtained from GC analysis, based on peak areas without Flame Ionization Detector factor correction.

RESULTS AND DISCUSSION

Chemical composition of essential oils found in the plants used in "tira-capeta" cigarette.

A simple, rapid and solvent-free method based on gas chromatography-mass spectrometry (GC-MS) using headspace solid-phase microextraction was developed for the analysis of the essential oils. Compared with conventional extraction techniques e.g. steam distillation and solvent extraction, SPME is a simple, sensitive and solvent-free method for the analysis of essential oils. The principal essential oils obtained from mixture of plants that produce a "tira-capeta" cigarette were 1,8 cineol (52.0%), camphor (21.7%) and α -pinene (11.5%), with low amounts of 3-methylbutanal (4.9%), camphene (3.6%), *trans*-caryophyllene (3.7%), (E,Z)-3,3-dimethyl-1,4-hexadiene (1.8%) and β -elemene (0.5%). According to Pereira et al. (2005), *E. globulus* contain 60-90% of eucalyptol (1,8-cineol), 2.8-24% of α -pinene and 0.0-5.5% of β -caryophyllene. 1,8-Cineole was also found in *R. graveolens* (De Feo et al., 2002). α -Pinene was also found in *R. graveolens* (De Feo et al., 2002) and *S. guianensis* (Fischer et al., 2005). β -caryophyllene was also found in *S. aromaticum*. A comprehensive profiling of *P. alliacea*, *A. arvense*, *H. cana* and *D. asaroides* essential oils has not been reported so far and the source of the others essential oils found in the present study, mainly camphor, is unknown. However, 1,8-cineole, α -pinene, and β -caryophyllene were the major compounds detected in the essential oil of *Hyptis fruticosa* (Menezes et al., 2007).

As shown in Table 1, these essential oils are known and were identified by their respective mass spectrum, which was compared with literature data and reference compounds. The mass spectrum of 1,8 cineol showed molecular ion at m/z 154 (M^+ , $C_{10}OH_{18}$, 70), and fragments at m/z 139 (M^+-CH_3) (70), 111 [$M^+-CH(CH_3)_2$] (75), 108 (85), 93 (80), 81 (100), while the mass spectrum of α -pinene showed molecular ion at m/z 136 (M^+ , $C_{10}H_{16}$, 20), and fragments at m/z 121 (M^+-CH_3) (30), 105 (M^+-OCH_3) (30), 93 [$M^+-CH(CH_3)_2$] (100), 77 (50).

For camphor, the mass spectrum showed the molecular ion at m/z 152 (M^+ , $C_{10}OH_{16}$, 40), and fragments at m/z 137 (M^+-CH_3) (5), 108 (137 - COH) (45), 95 (100), 81 (75). Camphene showed molecular ion at m/z 136 (M^+ , $C_{10}H_{16}$) (10), and fragments at m/z 121 ($M^+ - CH_3$) (65), 107 (M^+-COH) (40), 93 [$M^+-CH(CH_3)_2$] (100), 79 (50), 67 (45). β -Caryophyllene showed molecular ion at m/z 204 (M^+ , $C_{15}H_{24}$, 1), and fragments at m/z 164 (10), 147 (5), 133 (80), 120 (40), 105 (70), 93 (90), 91 (100), 79 (90). β -Elemene showed molecular ion at m/z 204 (M^+ , $C_{15}H_{24}$, 1), and fragments at m/z 161 [$M^+-CH(CH_3)_2$] (20), 133 (20), 119 (20), 105 (50), 93 (60) 91 (100), 79 (50). For 3-methylbutanal, the mass spectrum showed molecular ion at m/z 86 (M^+ , C_5OH_{10}) (1) and a fragment at m/z 57 (M^+-COH) (60). For (E,Z)-3,3-dimethyl-1,4-hexadiene, the mass spectrum showed molecular ion at m/z 110 (M^+ , C_8H_{14}) (25) and a fragment at m/z 95 (M^+-CH_3) (100).

Biological activity of essential oils found in this analysis.

In the tentative to correlate these constituents with therapeutical indications reported by the *Quilombolas*, some works were found carried out by many authors, which showed biological activities for these essential oils similar to the therapeutical indications reported by the *Quilombolas*. Thus, below will be presented the biological activities, described in scientific literature, to the essential oils found in the plants used for produce the "tira-capeta" cigarette, where some correlations tentatively were carried out between these activities and the therapeutical uses reported by the *Quilombolas*.

Perry et al. (2000) verified that α -pinene, 1,8-cineole and camphor have uncompetitive and reversible acetylcholinesterase inhibitory activity. It is known that compounds with anticholinesterase properties improved memory and attention in people (Scholey et al., 2008). This pharmacological evidence could be tentatively to explain the main therapeutical indication made to this cigarette: *tonic for the brain*.

Besides, other uses described by the *Quilombolas* to "tira-capeta" could be explained using studies already carried out by others authors. The Quilombola's indication "to avoid cold" could be explained by the presence of monoterpenoid components of the oils of *Eucalyptus globulus*, which are commercially available for the treatment of the common cold and other symptoms of respiratory infections (Cockcroft, 1992). Also, the use "against sinusitis" could have some relation to the presence of 1,8-cineol. According to Juergens et al., (2004) the role of 1,8-cineol to control airway mucus hypersecretion by cytokine inhibition, suggested that long-term treatment with this compound reduce exacerbations in asthma and sinusitis.

Anxiety is widespread and individuals aged between 10 and 25 years are at highest risk for developing an anxiety condition (. Ligands that bind to the benzodiazepine binding site on the gamma-aminobutyric acid receptor type A (GABAA) receptor can attenuate cognition. Anxiolytics act together with the gabaergic receptors facilitating coupling to GABAA, which is the main neurotransmitter inhibitor to the central nervous system (Harris et al., 2008). Aoshima & Hamamoto (1999) verified that pinene potentiate the response in the presence of GABA at low concentrations, possibly because they bound to the potentiation-site in GABA(A) receptors and increase the affinity of GABA to the receptors. Also, Deramciclane, a camphor derivative, is an anxiolytic agent with a unique mechanism of action (Naukarinen et al., 2005; Monostory et al., 2005). 1,8-Cineol showed anticonvulsant and hypnotic activities (Ismail, 2006). Since *Quilombolas* declared that one of the uses of "tira-capeta" is "to relieve sleep problems", the pharmacological data above could corroborate this use.

Besides the biological activities cited above, that corroborated the use of "tira-capeta" cigarette by *Quilombolas*, some other biological activities, such as,

anticonvulsant, antinociceptive and psychoactive were reported for these essential oils.

At anticonvulsant doses, cineol, α -pinene and β -pinene produced sedation and motor impairment (Sayyah et al., 2004). Intraperitoneal administration of 1,8-cineol and caryophyllene significantly increased ambulatory activity in mice, suggesting that these chemicals possess pharmacological actions on behavior (Umezue et al., 2001). Cineole exhibited an antinociceptive activity comparable to that of morphine. Pinene exerted supraspinal antinociceptive actions (Liapi et al., 2007).

β -Caryophyllene exhibited antinociceptive effect (Menezes et al., 2007) and anti-inflammatory activity in several experiments (Guan et al., 2007; Fu et al., 2007).

Thus, the results obtained in this study showed that the main essential oils found in the plants used in "tira-capeta" cigarette were 1,8-cineol, α -pinene and camphor. In the tentative to carry out a correlation among constituents and biological activity of this essential oil using articles published by some authors, was verified that they produce the majority of the effects described by *Quilombolas*, "to improve memory and/or cognition", "against sinusitis", "to avoid cold" and "to relieve sleep problems".

Table 1. Essential oils found in the smoke of "tira-capeta" cigarette.

Rt (min)	Content (%)	Molecular weight	GC/EIMS fragments (m/z, %)	Proposed structure
2.2	4.9	C ₅ H ₁₀ - 86	86 (M ⁺ , 1), 57 (M ⁺ -COH) (60) 136 (M ⁺ , 20), 121 (M ⁺ -CH ₃) (30),	3-methylbutanal
9.3	11.5	C ₁₀ H ₁₆ - 136	105 (M ⁺ - OCH ₃) (30), 93 [M ⁺ -CH(CH ₃) ₂] (100), 77 (50) 136 (M ⁺ , 10), 121 (M ⁺ -CH ₃) (65),	α -pinene
9.8	3.6	C ₁₀ H ₁₆ - 136	107 (M ⁺ -COH) (40), 93 [M ⁺ -CH(CH ₃) ₂] (100), 79 (50), 67 (45) 154 (M ⁺ , 70), 139 (M ⁺ -CH ₃) (70),	camphene
13.4	51.9	C ₁₀ H ₁₈ - 154	111 [M ⁺ -CH(CH ₃) ₂] (75), 108 (85), 93 (80), 81 (100) 152 (M ⁺ , 40), 137 (M ⁺ -CH ₃) (5),	1,8-cineol (eucapyptol)
17.9	21.7	C ₁₀ H ₁₆ - 152	108 (M ⁺ -CH ₃ COH) (45), 95 (100), 81 (75)	camphor
19.0	1.8	C ₈ H ₁₄ - 110	110 (M ⁺ , 25), 95 (M ⁺ -CH ₃) (100) 204 (M ⁺ , 1), 164 (10), 147 (5), 133	(E,Z)-3,3-dimethyl-1,4-hexadiene
27.8	3.7	C ₁₅ H ₂₄ - 204	(80), 120 (40), 105 (70), 93 (90), 91 (100), 79 (90) 204 (M ⁺ , 1), 161 (M ⁺ -CH(CH ₃) ₂)	β -caryophyllene
28.5	0.5	C ₁₅ H ₂₄ - 204	(20), 133 (20), 119 (20), 105 (50), 93 (60) 91 (100), 79 (50)	β -elemene

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