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Chemical composition of the essential oils from two subspecies of *Protium heptaphyllum*

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ABSTRACT

Qualitative and quantitative analyses of the volatile constituents from resin of *Protium heptaphyllum* (Aubl.) Marchand subsp. *ulei* (Swat) Daly (PHU), and *Protium heptaphyllum* (Aubl.) Marchand subsp. *heptaphyllum* (PHH), Burseraceae were performed using GC-MS and GC-FID. The resins were collected around the city of Cruzeiro do Sul, state of Acre, Brazil. Essential oils from the two subspecies were extracted by hydrodistillation with a yield of 8.6% (PHU) and 11.3% (PHH); the main components were terpinolene (42.31%) and *p*-cymene (39.93%) for subspecies *ulei* (PHU) and *heptaphyllum* (PHH), respectively.

KEYWORDS: *Protium heptaphyllum*, essential oils, Burseraceae, terpinolene, *p*-cymene.

Composição química do óleo essencial de duas subespécies do *Protium heptaphyllum*

RESUMO

As análises qualitativa e quantitativa dos óleos essenciais obtidos das resinas das espécies *Protium heptaphyllum* (Aubl.) Marchand subespécie *ulei* (Swat) Daly (PHU) e *Protium heptaphyllum* (Aubl.) Marchand subespécie *heptaphyllum* (PHH), Burseraceae, foram realizadas utilizando cromatografia em fase gasosa acoplado a um espectrômetro de massa (CG-EM) e cromatografia a gás com detector de chama (CG-DIC). As resinas foram coletadas no Município de Cruzeiro do Sul, Acre, Brasil. O óleo essencial das oleoresinas foi extraído por hidrodestilação fornecendo rendimento 8,6% para PHU e 11,3% para PHH. Os monoterpenos terpinoleno (42.31%) e p-cimeno (39.93%) foram os constituintes principais para PHU e PHH, respectivamente.

PALAVRAS-CHAVE: *Protium heptaphyllum*, óleo essencial, Burseraceae, terpinoleno, p-cimeno.

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INTRODUCTION

Many species of vegetal kingdom are characterized by having a large production of essential oil with possibilities of economic exploration, as occurs with the species of *Citrus* and *Eucalyptus*. Brazilian exportation of essential oils, plant extracts and oleoresin is around US$ 50.8 million dollars per year, with orange oil having the largest share of this amount (Sousa, 2005). The species of Burseraceae are known to exude resins that are rich in essential oils (Siani *et al.*, 2004), triterpenes and other constituents (Rudiger *et al.*, 2007). Some species of this family produce a great amount of essential oil that has potential possibilities of application in the perfumery industry (Siani *et al.*, 2004) and the diversification of the plant species provides large economic viability (Gottelib & Kaplan, 1990).

The Burseraceae family comprises 18 genera with 700 species divided in three tribes: *Protieae* (three genera), *Canarieae* (eigth genera) and *Bursereae* (seven genera). The *Protium* genus (Tribe *Protieae*) is the main family member with 150 species. All tribes are represented in the America, Africa and Indo-Asian tropics, with the highest diversity found in Southern Hemisphere (Weeks *et al.*, 2005). These species are widely spread all over Brazil, mainly in the Amazon Region, where the genus *Protium* makes up 80% of the Burseraceae (Siani *et al.*, 2004). Alto Juruá Region, in the state of Acre, Brazil, *P. heptaphyllum* (Aubl.) March. subsp. *ueli* (Swat) Daly (PHU) and *P. heptaphyllum* (Aubl.) March. subsp. *heptaphyllum* (PHH) are widely found, and they are known as an excellent source of oleoresin. Its popular use in medicine is very common, being used as anti-inflammatory, analgesic, expectorant and insect repellant. The resin is also used in the manufacture of varnishes, as impermeable agent of woody boats and as incense in religious rituals (Bandeira *et al.*, 2001). This paper reports by first time the chemical composition of the oleoresin from PHU and PHH collected in state of Acre.

MATERIAL E MÉTODOS

PLANT MATERIAL AND ESSENTIAL OILS EXTRATION METHOD

The resins of *P. heptaphyllum* (Aubl.) Marchand. Subsp. *ulei* (Swat) Daly (PHU) and *P. heptaphyllum* (Aubl.) Marchand subsp. *heptaphyllum* (PHH) were collected in March 2006 the city of Cruzeiro do Sul, western area of Acre state, North of Brazil in BR 364 highway, (07º 35' 45.5" S/72º 46' 36.4" W) and in BR 307 highway (07º 29' 23.4" S/72º 54' 19.8" W), respectively. All species were classified by Dr. Douglas C. Daly and voucher specimens (PHU No. 18.379 and PHH No. 18.381) were deposited in the Herbarium of the Zoobotanic Park of the Universidade Federal do Acre (UFAC), Brazil. The resins of PHU (136g) and PHH (101g) were manually triturated and submitted to hydrodistillation for 8 hs. The oils were obtained in a modified Clevenger-type apparatus. After separation from water, the oils were dried with Na_2SO_4, stored in sealed glass tubes and maintained under refrigeration. The yields (w/v) to PHU (8.6%) and PHH (11.3%) were calculated based on the weight of fresh resin and the volume of oil extracted.

GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Gas chromatography(GC-FID): The quantitative analysis of the oil were performed in an apparatus gas chromatography (GC) model Trace GC Ultra, equipped with a flame ionization detector (FID) using a dimethylpolysiloxane OV-5 fused silica capillary column (30m x 0.25mm, id x 0.25 µm film thickness) using the following conditions: helium was used as the carrier gas at a flow rate of 1 mL/min and 40 psi inlet pressure; column inlet split ratio 1:48; temperature program: 40-180ºC at 4ºC/min, then heated at a rate of 20ºC/min to 280ºC and held isothermal for 7 min; injector temperature 250ºC; detector with temperature of 280ºC.

Gas chromatography-mass spectrometry (GC-MS): The oil samples were analyzed in a gas chromatography Shimadzu model KP 5050A coupled to a mass detector using a OV-5 capillary column (30 m x 0.25 mm, i.d. x 0.25 µm film thickness) in the following: carrier gas hydrogen flow rate 1 mL/min and with split mode at ratio 1:48, ion source temperature 280ºC, ionizations voltage 70 eV, electron impact detection. The thermal conditions of the column were from 40ºC to 180ºC at 4ºC/min and then 180ºC to 280ºC at 20ºC/min, and held isothermal for 7 min.

The essential oils were analyzed by GC-FID an GC-MS, and individual components was identified comparing the mass spectrum obtained with the Wiley library, simulation of the Kovat index (Alencar *et al.*, 1990), as well as visual comparison with the standard of fragmentation of spectra of mass described in the literature (Adams, 2007) and the database provided with the mass spectra (SciFinder).

RESULTS AND DISCUSSION

The composition of the oil from resin of two species of *Protium* named PHU and PHH is summarized in Table 1. A total of 32 compounds in the two oil samples were identified and they are arranged in the order of elution sequence in column OV-5. The analysis of oil from the resin of PHU and PHH showed predominantly constituted of monoterpenes.

Sixteen constituents (95.74%) were identified in the sample PHU representing fifteen monoterpenes (94.98%). The major constituent was terpinolene (42.31%) followed by *p*-cymen-8-ol (13.62%), limonene (11.87%) and *p*-cymene (4.75%). In the oil PHH were identified twenty-

Table 1 - Chemical composition of essential oil from resin of the *P. heptaphyllum* (Aubl.) March. subsp. *ulei* (Swat) *Daly* (PHU) and *P. heptaphyllum* (Aubl.) March. subsp. *heptaphyllum* (PHH).

Compounds	RI	PHU (%)	PHH (%)
thujene	928	-	1.88
α-pinene	936	3.96	-
β-pinene	972	3.42	0.60
cis-pinane	980	-	1.17
p-menth-3-ene	991	-	3.17
α-phellandrene	997	2.02	7.41
α-terpinene	1010	1.49	1.68
p-cymene	1017	4.75	39.93
pseudocumene	1020	3.06	
dihydro-4-carene	1024		11.69
1,8-cineole	1025	3.07	
limonene	1029	11.87	
γ-terpinene	1058	2.62	
p-mentha-2,4(8)-diene	1082		0.85
terpinolene	1097	42.31	4.22
p-mentha-1,3,8-triene	1114	0.68	
trans-p-menthan-8-ol	1144		2.46
p-cymen-8-ol	1186	13.62	
α-terpineol	1190	1.00	
verbenone	1197	0.39	
chrysanthenyl acetate	1242	0.72	
n-tridecane	1307		1.75
methyl eugenol	1401	0.76	
n-tetradecane	1405		13.38
β-(E)-caryophyllene	1429		1.16
α-humulene	1460		0.43
γ-gurjunene	1480		0.45
n-pentadecane	1502		4.49
β-bisabolene	1509		0.44
α-bisabolene	1515		0.25
elemicin	1545		0.27
n-hexadecane	1594		0.85
Total (%)		95.74	98.53

RI. Retention indices

one constituents (98.53%). The *p*-cymene (39.93%) was the main constituent followed by n-tetradecane (13.38%), dihydro-4-carene (11.69%) and α-phellandrene (7.41%). The presence of the 5 sesquiterpenes [β-(*E*)-caryophyllene, α-humulene, γ-gurjunene, β-bisabolene, α-bisabolene] and four hydrocarbons (n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane) in the oil from PHH was a difference between the two analyzed subspecies, besides the main constituent.

The phytochemistry investigation of the essential oil from resin from the *P. heptaphyllum* (Aubl.) Marchand collected in the state of Ceará - Brazil, has enabled identification of 86.4% of monoterpenes, being the terpinolene (28.5%) the main constituent, followed by α-pinene (10.5%) and α-phellandrene (16.7%) (Bandeira *et al.*, 2001). Other specimens have been collected in Amazonas - Brazil (Zoghbi *et al.*, 1995) and in Pernambuco - Brazil (Pontes *et al.*, 2007) where they performed investigations of the essential oil from the fruits and leaves.

The comparison of the chemical profile of the essential oil from the resin of *P. heptaphyllum* (Bandeira *et al.*; 2001) with the oil extracted *P. heptaphyllum* subsp. *ulei* (PHU) has provided some likenesses. The two species of *P. heptaphyllum* and PHU, presented a lot of monoterpenes 86.4% and 91.93%, respectively, and both had the terpinolene as the main component. However, the essential oil from the resin of *P. heptaphyllum* subsp. *heptaphyllum* (PHH) shows relevant differences among the three investigated species of *Protium*. The (PHH) specie was the only that showed four linear hydrocarbons with yield of 20.47%, being n-tretadecano (13.38%) the second most concentrated component.

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