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Chemical Composition and Antimicrobial Activity of the Essential Oil from Oleoresin of *Protium neglectum* S.

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Abstract: The hydrodistilled oil from the oleoresin of *Protium neglectum* S., was analyzed by GC-MS. The yield of the resin oil was 1.0 % (w/w), in which twenty-eight compounds were identified, accounting for 99.3 % of the total oil. The most abundant compounds were the oxygenated monoterpenes (78.4%), with piperitenone (25.4%) as the major constituent, followed by thymol (17.5%), durenol (15.6%), methyl eugenol (9.2%), α-terpineol (6.9%) and p-cymene (5.2%). The antimicrobial activity using the disk susceptibility test as well as the minimum inhibitory concentration (MIC) on four pathogenic and non pathogenic Gram (+) and Gram (-) was screened. It was found that the essential oil from the resin exhibited antimicrobial activity on the Gram (+) bacteria, especially against *Bacillus subtilis*.

Key Word Index: *Protium neglectum*, Burseraceae, essential oil composition, piperitenone, thymol, durenol, antibacterial activity.

Introduction: Plants of the genus *Protium* (Burseraceae) are well-known in the traditional medicine of many countries around the world. Members of the genus such as *Protium heptafyllum*[1-3], *Protium icicariba*[4], *Protium kleinii*[5], *Protium unifoliolatum*[6], *Protium tenuifolium*[7] have been frequently used to treat skin diseases, inflammations, as an analgesic inhalant and to treat microbial infections. Plants of the Burseraceae are characterized by the presence of aromatic exudates and resins rich in volatile substances[8-9]. These resins are used as food additives, as fragrances as anti-inflammatory agent[10], and for the treatment of many other diseases[11-12]. *Protium neglectum* Swartz locally called currucay and tacamahaca, has been used in Venezuela as traditional remedy for inflammations, as an

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inhalant to clear the respiratory and bronchial passages and for hound healing. In view of the ethnobotanical uses of this plant, and as part of pharmacological-phytochemical integrated studies of medicinal plants from Venezuela, we investigated the chemical composition of this plant, and here we report the composition of the oil obtained from the oleoresin of *P. neglectum* collected in the north-east region of Venezuela and the results of the antimicrobial activity of this oil against Gram (+) and Gram (–) bacteria. The effect of *P. neglectum* oil was also compared with standard concentration of the antibiotic ampicillin. To the best of our knowledge, there is no previous work on the essential oil of *Protium neglectum* from Venezuela.

Experimental

Plant Material: The resin of *Protium neglectum* was collected in March 2003 from trees growing in Maturin, Monagas state of Venezuela. The botanical identification was achieved by Dr. Stephen Tillett, voucher specimens were deposited in the Herbarium Victor Manuel Ovalles of the Facultad de Farmacia of the Universidad Central de Venezuela.

Extraction of essential oil: Fresh oleoresin of *P. neglectum* (19.8 g) was subjected to hydrodistillation for 4 h using a Clevenger–type apparatus. The oil obtained was dried over anhydrous sodium sulphate and subsequently filtered and stored at 5°C until the analysis was conducted.

Gas Chromatography-Mass Spectrometry: The GC-MS analysis of the essential oil was performed on a Varian Saturn 2000 Spectrometer instrument using an ionic trap detector. Fused silica capillary columns with two different stationary phases were used; CP-Sil 5CB (100% dimethylpolysiloxane) and CP-Sil 8CB (5% phenyl and 95% of dimethylpolysiloxane), length 30 mts and 0.25 mm of diameter. The analytical conditions were: carrier gas; helium; injector temperature, 250°C; split rate; 20:1; temperature program, 5 min at 60°C rising to 290°C at a rate of 3°C/min; ionization mode: electron impact (EI) at 70 eV.

The essential oil components were identified by calculating their Kovats indices in relation to homologous series of n-alkanes (C_6 - C_{24}) under the same conditions, and by comparing mass spectra with those reported in the literature[13] and mass spectra provided by Wiley [14]and Nist [15] libraries in the data system.

Antimicrobial activity: Test microorganisms used in this study were the Gram-positive bacteria *Staphylococus aureus* (ATCC 25963), *Bacillus subtilis* (ATCC 25962); the Gram-negative bacteria *Pseudomona aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 35218). The microbial strains were from the American Type Culture Collection (ATCC), obtained from the collections of the Institute of Biology Experimental (IBE) of the Universidad Central de Venezuela.

The activity was measured by the standard disc agar diffusion method[16-17]. The bacteria were grown on the Mueller-Hinton agar medium (pH 7.2-7.4). Microbial suspensions were then made from the agar plates using relevant broths. The agar medium were poured

into the plates to a uniform depth of 5 mm and allowed to solidify. The microbial suspensions were then streaked over the media surface using a sterile cotton swab to ensure confluent growth of the organism. Aliquots of the pure oil and the oil diluted in a mixture of DMSO/ EtOH/H2O 60:20:20 (C = 1000 and 100 ppm), were impregnated on Whatman N°. 1 filter paper discs of 5mm size. The discs were then aseptically applied to the surface of agar plates at well-spaced intervals. The plates with *P. aeruginosa*, and *B. subtilis* were incubated at 30°C for 24 h, while *E. coli* and *S. aureus* were incubated at 37°C during the same time, and the observed zones of inhibition were measured. Control discs impregnated with 10 ìL of solvent mixture, were used as negative control and the standard antibiotic ampicillin as positive control with a concentration of 1mg/ml.

Results and Discussion: The essential oil obtained from the oleoresin of *P. neglectum* was pale yellow with a very pleasant and characteristic odor, gave a yield of 1.0 % (w/w). The chemical components with their retention indices and percentage composition are shown in Table I. Relative percentage amounts of the separated compounds were calculated automatically from peaks areas of the total ion chromatogram without using corrections factors. Twenty-eight components were identified in the *P. neglectum* essential oil, amounting to 99.3% of the total content.

The oil was found to be characterized by oxigenated monoterpenoids in which the major compounds were: piperitenone (25.4%), thymol (17.5%), durenol (15.7%), p-cymene (5.2%), α-terpineol (6.9%), and piperitone (2.4%). Others identified constituents includc: methyl eugenol (9.2 %), α-phellandrene (2.4 %), 6-camphenol (2.0 %), 1,8 cineol (1.6 %), and α-copaene (1.21 %). Based on the literature data[18-21], the oil composition of *P. neglectum* seems to be significantly different of other species of the same genus, specially from Brazil, which are characterized mostly by the predominance of terpenes as p-cymene, α-pinene, β-phellandrene and limonene, while piperitenone and piperitone two of the major components founds by us in *P. neglectum,* are only reported in traces. The results obtained in this investigation showed that the chemo-geographical variations are very important and may affect the chemical composition of the essential oil in the *Protium* genus.

The results of antimicrobial activity of the essential oil obtained from the oleoresin of *P. neglectum* are presented in Table II. The oil exhibited a significant antibacterial activity against Gram (+) bacteria, an interesting effect on *Bacillus subtilis,* moderate activity against *Staphylococus aureus*, and was completely inactive against the Gram-negative pathogens assessed.

Higher sample concentration exhibited higher activity against the microorganisms. The reference antibiotic (ampicillin) showed the highest antimicrobial activity against all tested microorganisms. From our results, it may be concluded that the antibacterial activity of the oil could be exerted by synergistic effect of the all compounds in the oil or, may in part be associated with the high concentration of oxygenated monoterpenes: piperitone and thymol, which have been reported with interesting antibacterial activity[22].

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Table I. Volatile compounds identified in *Protium neglectum* oleoresin

Compound	KI Value	% Composition
Monoterpenes		
6-Camphenol	770	2.0
p-Cymene	1027	5.2
1,8-Cineole	1030	1.6
Linalool	1092	0.3
2-Cyclopentenone	1115	0.3
Durenol	1183	15.6
α-Terpineol	1195	6.9
Piperitenone	1207	25.4
O-Cumenol	1217	1.1
Piperitone	1252	2.4
Thymol	1293	17.5
Phenylpropanoids		
Methyl eugenol	1370	9.2
iso-eugenol	1407	1.0

table 1. (continued)

Compound	KI Value	% Composition
3,4-Dimethoxybenzaldehyde	1606	0.3
Elemicin	1546	0.5
Apiole	1692	0.6
α-Phellandrene	1829	2.4
Sesquiterpenes		
α-Copaene	1377	1.2
β-Elemene	1388	1.2
α-Gurjene	1415	0.3
Acoradiene	1484	0.1
α-Cubebene	1505	0.4
δ-Cadinene	1525	1.3
Calamenene	1531	1.2
Spathulenol	1580	0.2
Viridiflorol	1604	0.4
Torreyol	1650	0.4
τ-Cadinol	1666	0.2

Table II. Antimicrobial activity of the essential oil from the oleoresin of. *P. neglectum*

Microrganisms Species	Inhibition zone (mm)		Ampicillin (1mg/ml)
	1000 ppm	100 ppm	
B. subtilis	25	15	32
S. aureus	15	8	29
P. aeruginosa	0	0	31
E. coli	0	0	33