African Journal of Biotechnology Vol. 7 (14), pp. 2462-2464, 18 July, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Chemical composition and antibacterial activity of the essential oil of *Pinus caribaea* from Nigeria

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Accepted 18 June, 2008

The chemical composition of the essential oil obtained from the needles of *Pinus caribaea* by hydrodistillation was analyzed by Gas Chromatography-Mass Spectrometry. A total of twenty nine compounds, representing 93.8% of the oil were identified. The major constituents of the essential oil were β-phellandrene (67.9%), β-caryophyllene (10.2%) and α-pinene (5.4%). High concentration of β-phellandrene in the oil suggests its usefulness as fragrance. The antibacterial activity of the essential oil was evaluated using disc diffusion method. The essential oil exhibited moderate activity against *Pseudomonas aeruginosa* at minimum inhibitory concentration (MIC) of 1000 ug/ml and no activity against *Candida albican*, *Bacillus subtilis*, *Staphylococcus typhi*, *Bacillus aureus* and *Proteus mirabilis*. The antibacterial activity of the oil against *P. aeruginosa* suggests its potential use as a remedy for food-borne diseases.

Key words: *Pinus caribbea*, Pinaceae, essential oil, β-phellandrene, β-caryophyllene, antibacterial.

INTRODUCTION

The genus *Pinus* belongs to the family Pinaceae and comprises about 250 species. It is the largest genus of conifers occurring naturally in the Northern Hemisphere and has been planted in the temperate regions of the Southern Hemisphere. They are evergreen and resinous trees growing to 3 – 80 m tall with needle-like gray-green leaves that grow in pairs. Essential oils from *Pinus* species have been reported to have various therapeutic properties (Fuentes et al., 2006; Kozan et al., 2006). The chemical compositions of essential oil of many *Pinus* species from different part of the world have been reported by many workers (Ekundayo, 1978; Ekundayo, 1988; Dagne et al., 1999; Macchioni et al., 2003; Stevanoic et al., 2004; Dob et al., 2005).

The aim of this work was to investigate the chemical composition and antibacterial activity of the essential oil of *Pinus caribaea* from Nigeria.

MATERIALS AND METHODS

Plant material

Fresh needles of *Pinus caribaea* were collected from the University of Ibadan campus, Nigeria. The plant was authenticated by Mr. Shasanya Olufemi of the Forest Herbarium, Forest Research Institute of Nigeria (FRIN), where voucher specimen was deposited (FHI 107916).

Essential oil isolation

Air-dried needles (500 g) were subjected to hydrodistillation in all–glass Clevenger type apparatus for 4 h. The oil was collected and dried over anhydrous sodium sulphate and stored at 4°C until analysed.

Gas Chromatography (GC)

The oil was analyzed on an Agilent Model 6890 Gas Chromatography equipped with a DB-5 fused silica capillary column (30 x 0.25 mm, film thickness 0.25 µm). Analytical conditions were: Oven temperature: 60°C, with 2 min initial hold, and then to 250°C at 4°C/min, with final hold time of 20 mins; helium as carrier gas at a flow rate of 1 ml/min. Retention indices were determined with reference to a homologous series of normal alkanes (C_8-C_{26}) analyzed under the same conditions. Percentage composition of each constituent was calculated by integration of the GC peak areas.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent Model 6890 GC with split/splitless injector interfaced to an Agilent 5973 Mass Selective Detector. The temperature program used for the GC was

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Table 1. Chemical composition of essential oil of *Pinus caribbea* from Nigeria.

Constituents	RI[a]	Percentage (%)
α-Thujene	925	0.1
α -Pinene	932	5.4
Camphene	947	0.3
Sabinene	971	0.1
β-Pinene	976	0.5
α -Phellandrene	1006	2.3
α -Terpinene	1016	0.3
β -Phellandrene	1027	67.9
δ-Terpinene	1056	0.2
(E)-Menth-2-en-1-ol	1064	0.1
Terpinolene	1087	0.3
(Z)- Menth-2-en-1-ol	1096	0.1
Linalool	1098	0.1
Terpinen-4-ol	1166	0.2
Thymol methyl ether	1233	0.1
Isobornyl acetate	1284	0.6
α -Cubebene	1349	0.1
β -Elemene	1391	0.1
Longifolene	1402	0.6
β -Caryophyllene	1418	10.2
α - Bergamotene	1435	0.3
γ - Muurolene	1475	0.2
D-Germacrene	1480	2.4
B- Germacrene	1494	0.1
Bicyclogermacrene	1496	0.1
α - Muurolene	1498	0.2
γ - Cadinene	1513	0.1
δ - Cadinene	1524	0.6
Caryophyllene oxide	1580	0.2
Monoterpenehydrocarbon		77.4
Oxygenatedmonoterpene		15.0
Sesquiterpenehydrocarbon		1.2
Oxygenated sesquiterpene		0.2

[a]Retention indices relative to *n*-alkanes on DB-5 capillary column.

the same as described above. The MS was operated in EI mode with ionization voltage 70eV and ion source temperature, 230°C.

Components identification

The components of the essential oil were identified on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra (Adams, 1989) and those of reference compounds from the Library of National Institute of Standard and Technology (NIST) database.

Antimicrobial screening

The essential oil was screened for antimicrobial activities against 6 standard strains of bacteria representing both Gram +ve and Gram –ve (*Candida albican* MTTC 227, *Bacillus subtilis* ATTC 33923, *Staphylococcus typhi* ATTC 2785, *Pseudomonas aeruginosa* ATTC 27856, *Bacillus aureus* ATTC 14579 and *Proteus mirabilis* ATTC 21784). Disc diffusion method was used to determine minimum inhibitory concentration (MIC). The bacteria were grown on nutrient agar (Mueller Hinton) which was prepared by dissolving agar (28 g) in distilled water (1000 ml). The mixture was heated to dissolve and autoclaved at 121°C for 15 mins. The nutrient agar was poured into sterile petri dishes at uniform depth of 5 mm and allowed to solidify. The microbial suspensions were streaked over the surface of the agar media using a sterile cotton swab to ensure uniform inoculation. Different concentrations of the oil (10, 100, 1000, 10000 ppm) were prepared in dimethyl sulphoxide (DMSO). Different concentrations (0.01 ml) were impregnated on Whatman filter paper No 2 disc. The discs were then aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37°C for 24 h. The zones of inhibition as well as the minimum inhibitory concentration (MIC) were measured. Disc impregnated with gentamicin (0.01 ml) dissolved in DMSO (5 µg/ml) was used as control.

RESULTS AND DISCUSSION

The oil yield obtained from hydrodistillation of the *Pinus caribaea* was 0.02% (v/w). Table 1 shows the constituents identified in the essential oil with their percentage composition and retention indices. Twenty-nine compounds, representing 93.8% of the essential oil were identified. Like the essential oil from other *Pinus* species (Ekundayo, 1978; Ekundayo, 1988; Macchioni et al., 2003; Stevanoic et al., 2004), the chemical composition of our *P. caribaea* is dominated by monoterpenes hydrocarbons (77.4%). The sesquiterpenes hydrocarbons and oxygenated compounds constitute 15.0 and 1.4% respectively. The major constituents of the monoterpene hydrocarbons were β-phellandrene (67.9%), α-pinene (5.4%) and α-phellandrene (2.3%). Sesquiterpenes hydrocarbons were dominated by β-caryophyllene (10.2%) and D-germacrene (2.4%). β-phellandrene which is present in high percentage in the oil is an important constituent in fragrance because of its pleasing aroma. The monoterpenes hydrocarbon constituents of our studied species differ from those reported by Ekundayo (1978) and Dagne et al. (1999) for the same specie, in which α-pinene (63.2 – 87.1%) was reported as the major constituent. The difference in the oil composition may be due to factors such as time of collection, genetic, geographic and climatic conditions. Table 2 summarizes the results of the antibacterial test of the essential oil from *Pinus caribaea* on the six standard strains of bacteria. The essential oil shows moderate activity against *P. aeruginosa* at minimum inhibitory concentration (MIC) and zone of inhibition values of 10000 ppm and 25 mm, respectively. The oil shows no activity against the remaining 5 bacteria strains (*C. albican*, *B. subtilis*, *S. typhi, B. aureus* and *P. mirabilis*) at 10000 ppm. It has been reported that components with phenolic structures such as carvacrol, eugenol and thymol, were highly active against microorganism (Lambert et al., 2001; Knowles et al., 2005; Valero and Frances, 2006; Bouhdid et al., 2008).

Table 2. Antibacterial activity of the essential oil from *Pinus caribaea* from Nigeria.

Microorganism	Minimum inhibitory concentration (ppm)	Zone of inhibition (mm)
Pseudomonas aeruginosa	10000	25
Proteus mirabilis	10000	NA
Bacillus subtilis	10000	NA
Candida albican	10000	NA
Staphylococcus typhi	10000	NA
Bacillus aureus	10000	NA

NA = No activity.

None of these phenolic compounds was found in the essential oil of the *Pinus* species studied. This observation may be responsible for the non-activity of the oil against most of the tested bacterial. However, moderate activity of the oil against *P. aeruginosa* may suggest its potential use as a remedy for food-borne diseases.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. I. Oladosu and Mrs. S. Aboaba for their assistance during the analyses.

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