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Topical anti-inflammatory activity and chemical composition of essential oil of *Sabina virginiana* L. Antoine (Cupressaceae)

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Abstract

Essential oil of from the foliage leaves of Sabina virginiana L. Antoine (Cupressaceae) was obtained by hydrodistillation and analyzed for their constituents by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Thirty-one compounds were identified with monoterpenes accounting for 68.3% of the oil content. The major constituents were limonene (32.9%), safrole (23.0%), asarone (15.9%) and α-pinene (5.2%). The topical anti-inflammatory assay of the whole essential oil was also investigated in mice ear edema induced by tetradecanoylphorobol-13-acetate. The anti-inflammatory activity of 66.7% at 5.0mg dose level may be an indication of the therapeutic application of the oil.

Keywords: *Sabina virginiana*, essential oil composition, monoterpene, limonene, safrole, asarone, anti-inflammatory

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INTRODUCTION

Sabina virginiana (syn. *Juniperus virginiana*) commonly referred to as 'eastern red-cedar' is a perennial tree up to 65 inches tall. It belongs to the family Cupressaceae. The distribution of the plant extends from Atlantic to the Pacific coasts and from Southern Canada to Texas and New Mexico [1]. The plant has been introduced to other parts of the world including Nigeria. The plant is a source of lignans [2]. Essential oils from the wood of *S. virginiana* are indicated for hemorrhoids, for lining of clothes to deter moths and repel vermin in the storage of valuables. In addition, the leaves are found to exert effects on emmenagogue, stimulant and as diaphoretic in rheumatism [3]. Chemosystematic studies of the volatile oils from the foliage of *Juniperus horizontalis, J. scopulorum* and *J. virginiana* have been reported [4]. The study represented oils

from 10 trees from 6 different locations. Elemol acetates (35-44.5%), limonene (17-29.5%), sabinene (6.0-28.0%) and α-pinene (0.5-6.0%) were the most abundant compounds.

The present work provides information on the topical anti-inflammatory activity and chemical constituents of the foliage leaves of *S. virginiana*, because this activity may justify the use of the plant as phytotherapeutics for external use. This is part of our extensive research aimed at the characterization of the chemical constituents and biological activities of Nigerian medicinal plants and herbs as they are made available [5].

MATERIALS AND METHODS

Plant Material

Mature foliage leaves of *S. virginiana* were collected from already identified and well labeled trees growing beside Faculty of Agriculture, Block IV, Kyushu University, Hakozaki Campus, Fukuoka, Japan, in November 2006. Further taxonomic identification was achieved by curators at the Herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimen was deposited. The air-dried plant samples were chopped and hydrodistilled for 3 h using a Clevenger-type apparatus.

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis was accomplished with a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/splitless injector. The column used was a DB-5 (Optima-5, 30m x 0.25mm, 0.25 μm film thickness) coated with 5% diphenyl-95% polydimethylsiloxane. The operating conditions were oven temperature 50 ^{o}C, held for 1 min, rising at 3 ^{o}C /min to 250 ^{o}C, held for 5 min, rising at 2 ^{o}C /min to 280 ^{o}C, held for 3 min. Injection and detector temperatures were 250 ^{o}C and 280 ^{o}C respectively, while 1.0μL of the oil was used for the analysis at a split ratio of 30:1. The carrier gas was nitrogen at 30cm/s linear velocity and inlet pressure 99.8Kpa; hydrogen flow rate, 50mL/min; air flow rate 400 mL/min; make up (H_2/air) flow rate 50 mL/min. Data were acquired by means of GC software (Shimadzu). The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factor.

GC-MS analysis was performed with an Agilent 6890N GC interfaced with a VG Analytical 70-250s double focusing Mass Spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 ^{o}C. The GC was fitted with a 30m x 0.32 mm fused capillary silica column coated with DB-5 (0.25 μm film thickness). The GC operating parameters were identical with those of the GC analysis.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear indices relative to the series of n-hydrocarbons, and by matching their fragmentation pattern in mass spectra against commercially available spectral [6-9].

Animal Experiment

The animal experiment were approved by the University of Ibadan (UI) Animal care and Use Committee and conducted according to standard guidelines. Male Swiss Webster mice (UI

breed) 21 days old, weighing 22-25g were housed in groups of six in an NIH-approved facility. All groups were fed with standard rodent diet (TestDiet®570B, Purina Mills, St. Louis, MO) *ad libitum* with free access to water. Animals were in the fed condition throughout the experiment. The lights in the facility were turned off between 1900 and 0700 h, with the environmental temperature recorded at 25 ^{o}C ± 1 ^{o}C.

All experimental procedures were conformed to the National Institute of Health, Public Health Service and Animal Welfare Act guidelines for the ethical treatment of laboratory animals.

Topical anti-inflammatory assay

The topical anti-inflammatory activity was evaluated as inhibition of the tetradecanoylphorobol-13-acetate induced ear edema in mice. Edema was induced in ears of each mouse by the topical application of 2µg of tetradecanoylphorobol-13-acetate dissolved in 20 µL of acetone to both the inner and outer surfaces of the right ear (surface area: 1 cm^2). Thirty minutes after the application of tetradecanoylphorobol-13-acetate, the inner and outer surface of each ear was treated (10 µL to each side) with;

(i) 50% ethanolic solutions of the test essential oil (eo) in doses of 0.075, 1.25, 2.5 and 5.0 mg eo/ear (n=6 at each dosage)
(ii) 50% ethanol (vehicle control)
(iii) indomethacin (0.25 mg/ear dissolved in 50% ethanol as an anti-inflammatory drug standard).

The thickness of each ear was measured using a micrometer (Mitutoyo Series IP65, Mitutoyo America Aurora, IL) before and at 4 h and 24 h after TPA administration. The micrometer was applied near the top of the ear distal to the cartilaginous ridges. At 24 h each animal was sacrificed and a plug biopsies (6 mm diameter hole punch) were removed from both the treated (right) and the untreated (left) ears immediately, weighed, frozen and stored at -80 ^{o}C. The edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percentage of the edema reduction in treated mice compared to the control mice. The pharmacological data were analysed by the student's t-test, and a probability level lower than 0.05 was considered as significant.

RESULTS AND DISCUSSION

The hydrodistillation of the semi air-dried foliage leaves of *S. virginiana* yielded colourless oil in 1.5% (w/w). The identities of the 31 compounds representing 97.9% of the total oil contents are summarized in Table 1. The oil was dominated by monoterpenes (68.3%) and sesquiterpenes (26.0%). The major constituents were limonene (32.9%), safrole (23.0%), asarone (15.9%) and *α*-pinene (5.2%). Other quantitatively significant constituents of the oil include guaiol (4.4%) and caryophyllene (2.1%). Although limonene has been described as an abundant constituent of studied *Juniperus* species [4], other compounds such as elemol acetate and sabinene that are characteristic of the previous study were not identified in the present oil sample. In addition, safrole and asarone which are present in higher quantities in this study have not been mentioned to be of importance in previous study.

The essential oil was tested at different concentrations for its anti-inflammatory assay evaluated as inhibition of tetradecanoylphorobol-13-acetate induced ear edema in mice. Ear edema was observed in all tetradecanoylphorobol-13-acetate treated animals after 4 and 24 h. The results on the topical *in vivo* anti-inflammatory activity of the oil and indomethaccin are reported in Table 2. All experimental groups had significantly reduced ear edema compared with non-oil treated control.

Table 1: Compounds identified from *Sabina virginiana*

LRI	Compound	Percentage
941	α-Pinene	5.2
952	Camphene	0.5
990	Myrcene	0.6
1000	Δ-2-Carene	0.2
1010	Δ-3-Carene	1.5
1020	α-Terpinene	0.4
1030	α-Phellandrene	0.8
1031	**Limonene**	**32.9**
1096	β-Ocimene	0.4
1119	2-Pinanol	0.4
1120	5-Caranol	0.8
1146	2-Thujanol	0.2
1287	**Safrole**	**23.0**
1289	Anethole	0.4
1351	α-Cubebene	0.5
1376	α-Copaene	0.2
1380	Patchoulene	0.3
1391	β-Elemene	0.5
1408	Methyl eugenol	0.7
1423	Caryophyllene	2.1
1595	Guaiol	4.4
1620	**Asarone**	**15.9**
1640	Hinesol	0.6
1641	Spathulenol	0.3
1710	Pentadecanal	0.3
1713	Geranic acid	0.4
1733	β-Santalol	0.5
1922	Eicosene	0.4
1986	Manoyl oxide	0.6
1999	Retinol	0.3
2105	Menthol	0.3
Total		**97.9%**

The average initial ear thickness of the experimental animals was 0.3 ± 0.02 mm (mean ±SEM). At the end of 24 h, the ear thickness had increased to 0.44 ± 0.05 mm after treatment. The oil at 5.0 and 2.5 mg/ear dose levels exhibited moderate anti-inflammatory activity with percentage edema reduction of 66.7 and 51.3 respectively. The oil at 5.0 mg/ear dose level was significantly more effective than indomethacin at 0.25 mg/ear dose level in reducing edema. This action may be attributed to the compounds identified so far in the oil. Further study is in progress aimed at the isolation and characterization of the main compound (among the identified compounds) responsible for the anti-inflammatory activity of the oil.

Table 2. Anti-inflammatory activity of the oil of *Sabina virginiana*

Group	**Dose (mg)**	**# of animals**	**Change in ear weight (Mean ± SEM) mg**	**% of edema reduction**
Control	-	6	7.8 ± 0.3	-
Indomethacin	0.25	6	3.3 ± 0.1	57.7
S. virginiana	5.0	6	2.6 ± 0.3	66.7
	2.5	6	3.8 ± 0.5	51.3
	1.25	6	7.0 ± 0.5	10.3
	0.075	6	7.6 ± 0.3	2.6

CONCLUSION

It could be seen that the essential oil of *s. virginiana* may be employed for both medicinal and industrial purpose because of the different chemical compounds it contains.

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