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Chemical Composition and Anti-inflammatory Activity of the Essential Oil of the Aerial Part of *Mezoneuron benthamianum* Baill. (Caesalpinoideae)

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Abstract: The chemical composition of the essential oil from the aerial part of *Mezoneuron benthamianum* Baill. (Caesalpinoideae) was studied using GCand GC-MS. The oil contains fifteen compounds which constituted about 93.4% of the oil and this was dominated by monoterpenes (36.5%), sesquiterpenes (20.4%) and sesquiterpenoids (19.6%). A non-ubiquitous apocarotenoid $C_{20}H_{30}O$ with similar fragmentation pattern to that of trans-phytofluene was isolated and found to be the most abundant compound (16.7%) in the oil. Five other major compounds were 3-carene (14.2%), α-trans-nerolidol (13.3%), α-pinene (11.7%), α-farnesene (11.6%) and α-thujene (6.7 %). The topical anti-inflammatory effect of the oil was assayed as inhibition of the 12-O-tetradecanoylphorobol-13 acetate (TPA) induced ear edema in mice. The oil at 5.0 and 2.5 mg dose levels exhibited more effect than indomethacin (0.25 mg) in reducing edema. The results demonstrated the oil of *M. benthamianum* has an anti-inflammatory agent, supporting the use of this plant in folk medicine. Aside these, nine other compounds were reported along with the anti-inflammatory activity and these seemed to receive no mention in any previous literature known to us and hence novel.

Key words:

INTRODUCTION

Mezoneuron benthamianum Baill. (Caesalpinoideae) is a woody climber with recurred thorn on its black stem. It is also referred to as ‘tiger’s claw’. The leaves and roots are important medicinal parts in folk medicine [1]. The leaves are bright red when young and dark green when old. They are native to West Africa. The plant is known locally as “Jenifiran” in western part of Nigeria [2]. The plant is used locally for the treatment of dermal infection, healing of refractory sore, blood disorders; laxatives; stomach troubles, venereal diseases, eye treatments, genital stimulants/depressants, hemorrhoids, pain-killers, pulmonary troubles and as chewing stick [3-5].

Literature survey revealed that very little phytochemical works have been carried out on the chemotype and activity of *Mezoneuron benthamianum* and there seems to be no report on the essential oil content and constituents so far.

As part of our continuing investigation on the anti-inflammatory activities of essential oils from lesser-known plants in western part of Nigeria, we report here the volatile constituents as well as anti-inflammatory activity of the aerial part of this plant species grown in Nigeria. Our results have been useful in understanding the benefits of traditional uses of the fresh plant as a fragrant topical healing for refractory sore and pulmonary inflammation by the Yoruba’s in Nigeria.

MATERIALS AND METHODS

Plant Materials: Aerial parts of *M. benthamianum* were harvested in an open forest in Isara town, Ogun-State. Nigeria in April, 2008. It was botanically identified by Mr. Esimeldunai Donatus of the Department of Biological Sciences, University of Ibadan, Ibadan, Nigeria. The plant sample was authenticated at Department of Biological

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Sciences, University of Ibadan, Ibadan, Nigeria, Ibadan by Prof Egunyomi. Voucher specimen was deposited at the Institute's Herbarium (UIH 22321).

Isolation of Essential Oils: The oil was obtained by hydrodistillation of fresh crushed aerial part (520.2 g) using a Clevenger-type apparatus for 3 h according to the British Pharmacopoeia specifications [6]. The oil was dried over anhydrous sodium sulphate and stored in vial at low temperature until analysis.

Gas Chromatography: Quantitative and qualitative data were determined by GC and GC-MS respectively. *M. benthamianum* oil was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/ splitless injector. The column used was an DB-5 (Optima-5), 30 m . 0.25 mm i.d., 0.25 µm *d*f, coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following oven temperature programme: 50°C, held for 1 min, rising at 3°C/min to 250°C, held for 5 min, rising at 2°C/min to 280°C, held for 3 min; injection temperature and volume, 250°C and 1.0 µl, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280°C; hydrogen, flow rate, 50 ml/min; air flow rate, 400 ml/min; make-up (H_2/air), flow rate, 50 ml/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu).

Gas Chromatography-mass Spectrometry Analyses: Agilent 6890N GC was 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°C. The GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of the GC analysis.

The percentage compositions of the oil were computed in each case from GC peak areas and are shown in Table 1. Retention indices for all the compounds were determined according to the Kovats method relative to the *n*-alkanes series. The identification of the compounds was done by comparison of retention indices and by matching their fragmentation patterns in mass spectra with those of published mass spectra data [7-9]. In a few cases, identification of components was carried out by means of commercial libraries (Wiley, NIST05 and Hochmuth) [10].

Table 1: Chemical composition of the aerial part essential oils of *M. benthamianum*

Compound[a]	Ri[b]	%
α-thujene	933	6. 7
α-pinene	941	11.8
α-sabinene	974	0.1
3-carene	1010	14.2
α-ocimene	1038	3.7
Isocaryophyllene	1411	3.2
α-caryophyllene	1423	2.1
α-bergamotene	1435	0.2
α-elemene	1477	3.3
α-farnesene150411.6		
γ-trans-nerolidol	1564	13.3
9-octadecenoic acid	2144	0.2
cetiol	2187	3.0
farnesol	2419	3.3
unknown apocarotenoid[c]	2658	16.7
Total		93.4

[a] The compounds were identified by the combination of both the mass spectra and retention indices on DB-5 capillary coated column except where stated. Values (%) represent percentage composition; [b]retention index relative to *n*-alkanes on DB-5 capillary coated column; [c]Identified probably for the first time in *M. benthamianum* oil.

Animals: The animal experiments were approved by the University of Ibadan Animal Care and Use Committee and conducted according to standard guidelines. Male Swiss Webster mice (UI breed) 21 d old, weighing 22-25 g were housed in groups of 8 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 maintained at 25±1°C. All experimental procedures were conformed to the National Institutes of Health, Public Health Service and Animal Welfare Act guidelines for the ethical treatment of laboratory animals.

Topical Anti-inflammatory Assay: A modification of the method of Young *et al*., [11] was used. The topical anti-inflammatory activity was evaluated as inhibition of the 12-O-tetradecanoylphorobol-13 acetate (TPA) induced ear edema in mice following standard procedure [11-13]. Edema was induced in ears of each mouse by the topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer surfaces of the right ear (surface: about 1 cm^2). Thirty minutes after the application of TPA, the inner and outer surface of each ear was treated (10 µL to each side) with:

Table 2: The results of anti-inflamatory activity of the aerial part essential oils of *M. benthamianum*

Group	Dose mg	No of animals in the group	Change in ear weight (Mean ±SEM) mg	Percentage of edema reduction
Control	-	8	7.8±0.3	-
indomethacin	0.25	8	3.3±0.1	57.7
Essential oil	5.0	8	0.6±0.3	92.3
	2.5	8	1.8±0.5	76.9
	1.25	8	4.0±0.5	48.7
	0.075	8	6.6±0.3	15.4

- 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 = 8 at each dosage).
- 50% ethanol (vehicle control)
- 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 tetradecanoylphorobol-13 acetate 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°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 analyzed by the student's t-test and a probability level lower than 0.05 was considered as significant.

RESULT AND DISCUSSION

The constituents of the aerial volatile oil of *M. benthamianum* are presented in Table 1. The aerial parts of *M. benthamianum* (520 g) produced 1.12 g light yellow oil (0.22% w/w). The essential oil has its own characteristic strongly pungent odour, due to the constituents it contained. The fifteen compounds identified for *M. benthamianum* oil constituted 93.4% of the total constituents based on GC-MS analysis (Table 1). The oil is dominated by monoterpenes (36.5%). Other major classes of compounds identified are sesquiterpenes (20.4%) and sesquiterpenoids (19.6%). The six most abundant components identified accounted for 74.2% of the oil. Noteworthy is the presence of the non-ubiquitous apocarotenoid $C_{20}H_{30}O$, which has very similar pattern of fragmentation [286 (5%), 243 (10%), 227 (8%), 201 (3%), 175 (2%), 161 (5%), 147 (7%), 135 (6%), 119 (15%), 107 (30%), 93 (45%), 81 (60%), 69 (100%), 55 (32%), 41 (54%), 29 (15%)] to that of trans-phytofluene. It is the most abundant compound in the oil (16.7%) and may likely be responsible for the special biological activity displayed by the oil. Five other major compounds are 3-carene (14.2%), γ-trans-nerolidol (13.3%), α-pinene (11.7%), α-farnesene (11.6%) and α-thujene (6.7 %). The other nine compounds present in the oil are listed in Table 1.

The isolated aerial part essential oil was tested at different concentrations for its anti-inflammatory activity evaluated as inhibition of tetradecanoylphorobol-13-acetate induced ear edema in mice. Ear edema was observed in all tetradecanoylphorobol-13-acetate treated animals by 4 and 24 h after treatment. The results on the topical anti-inflammatory activity *in vivo* of the oil and indomethacin are reported in Table 2. All experimental groups had significantly reduced ear edema compared with no-oil treated control. The average initial ear thickness of the experimental animals equaled 0.3±0.02 mm (mean±SEM). At the end of 24 h, the ear thickness has increased to 0.44±0.05 mm after treatment. The oil at 5.0 and 2.5 mg dose levels exhibited a significant anti-inflammatory activity with percentage edema reduction of 92.3 and 76.9 respectively. The oil at these concentrations was significantly more effective than indomethacin in reducing edema. The results obtained justified the use of this plant as a remedy for inflammation of eye, hemorrhoids and related diseases.

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