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Volatile oil constituents and anti-inflammatory potential of the essential oil of the leaves and roots of *flabellaria paniculata* car (malpighiaceae)

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ABSTRACT

The chemical composition of the essential oil from the leaves and the roots of *Flabellaria paniculata* Cav. (Malpighiaceae) were studied using GC and GC-MS. The leaves oil contains twenty-four compounds which constituted about 91.2% of the oil. This was dominated by sesquiterpenoids (69.9%). The roots oil contains fourteen compounds which constituted about 96.2% of the oil. The roots oil was also dominated by diterpenes (84.1%). The topical anti-inflammatory effects of the two oils were assayed as inhibition of the 12-O-tetradecanoylphorobol-13 acetate (TPA) induced ear edema in mice. The oils at 5.0 and 2.5 mg dose levels exhibited more effect than indomethacin (0.25 mg) in reducing edema. The results demonstrated the leaves oil of *Flabellaria paniculata* has an anti-inflammatory agent, supporting the use of this plant in folk medicine. Aside these, 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.

Introduction

Flabellaria paniculata Cav (Malpighiaceae) is a herb with slivery leaves and white pale pink flowers. Its flower is known as "Ajidere" in Western Nigeria (Yorubaland). The plant is common in tropical Western Africa. The plant found uses in the treatment of wounds, skin diseases, dysentery, sore, snake bite, amonorrehoea, and ancebolic[1-3]. *Flabellaria paniculata* has been implicated locally as having antibacterial, antifungal, and antiinfective properties [4-5].

Literature survey revealed that very little phytochemical works have been carried out on *Flabellaria paniculata* and there is 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 leaves and roots of the *F. paniculata* 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.

Material and methods

Plant Materials

The leaves and the roots of *Flabellaria paniculata* were harvested from a farm settlement in Ago-Iwoye, 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 Sciences, University of Ibadan, Ibadan, Nigeria, Ibadan by Prof Egunyomi. Voucher specimen was deposited at the Institute's Herbarium.

Isolation of Essential Oils

The oils were obtained by separate hydrodistillation of fresh crushed leaves (300 g) and roots (520 g) of *Flabellaria paniculata* using a Clevenger-type apparatus for 3 h according to the British Pharmacopoeia specifications (1980) [5]. The oils were dried over anhydrous sodium sulphate separately and stored in vials at low temperature until analysis.

Gas Chromatography

Quantitative and qualitative data were determined by GC and GC-MS respectively. *Flabellaria paniculata* oils were injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/ splitless injector separately. The column used was an DB-5 (Optima-5), 30 m . 0.25 mm i.d., 0.25 µm *df*, 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 ^{0}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.

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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].

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.* (1984)[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:

- 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 leaves and roots volatile oils of *Flabellaria paniculata* are presented in Table 1. The leaves of *Flabellaria paniculata* (350 g) produced 1.10 g light yellow oil (0.31% w/w). The essential oil has strongly pungent odour, due to the constituents it contained. The twenty-four compounds identified for *Flabellaria paniculata* leaves oil constituted 91.2% 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 seven most abundant components identified accounted for 85.6% of the oil. Noteworthy is the presence of the sesquiterpenoids γ-trans-nerolidol and α-farnesene. They are the most abundant compounds in the leaves oil (58.6%) and are likely be responsible for the special biological activity displayed by the leaves oil. The five other major compounds are α-sabinene (6.0%), α-ocimene (8.1%), β-elemene (4.2%), α-caryophyllene (4.1%) and farnesol (4.6 %). The other seventeen compounds present in the leaves oil are listed in table 1.

The roots of *Flabellaria paniculata* (520 g) produced 2.85 g yellow oil (0.55% w/w). The essential oil has light aromatic odour, due to the constituents it contained. The fourteen compounds identified for *Flabellaria paniculata* roots oil constituted 96.2% 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 four most abundant components identified accounted for 92.1% of the roots oil. Noteworthy is the presence of the canthaxanthin and acoratien. They are the most abundant compounds in the roots oil (80.1%) and are likely be responsible for the special biological activity displayed by the leaves oil. The four other major compounds are hexadecanoic acid (8.0%), ergst-5-en-3-ol (4.0%), molurolene (0.8%) and γ-trans-nerolidol (0.9%). The other compounds present in the roots oil are listed in table 1.

A total of 28 compounds were identified from separate GC and GC-MS analyses of the leaves and roots of *Flabellaria paniculata*. We identified twenty-four compounds representing 91.2% of the leaves oil and fourteen compounds representing 96.2% of the roots oil. The two essential oils are highly terpenic with (--%, --%) monoterpene, (--%, --%) sesquiterpene, (--%, --%) monoterpenoids and (--%, --%) sesquiterpenoids respectively in leaves and roots. Ten of the twenty-eight are found in both leaves and roots essential oils (Table 1). The four most abundant compounds in each of the leaves and roots essential oils are [leaves: (%);(%);(%);(%)] and [roots: (%);(%);(%);(%)]. Three of the four most abundant compounds in the roots essential oil are non-ubiquitous to essential oil constituents. Canthaxanthin is a merely reported compound in phytochemistry.

The isolated essential oils from leaves and the roots were tested at different concentrations for its anti-inflammatory activities evaluated as inhibition of tetradecanoylphorobol-13-acetate induced ear edema in mice separately. 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 oils 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 leaves oil at 5.0 and 2.5 mg dose levels exhibited 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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Table I: Chemical composition of the aerial part essential oils of *Flabellaria paniculata*

Compound	RI	leaves	roots
α-pinene	941	0.2	0.3
α-sabinene	974	6.0	-
1,8-cineol	1033	0.2	-
α-ocimene	1038	8.1	-
2-methylisoborneol	1182	0.5	-
α-patcholene	1380	0.2	0.2
β-elemene	1391	4.2	-
α-cedrene	1410	0.2	0.3
α-caryophyllene	1423	4.1	0.5
α-bergamotene	1435	0.4	-
β-farnesene	1454	0.6	0.2
trans-psi-ionone	1485	0.5	-
zunurolene	1499	0.2	0.8
α-farnesene	1504	26.3	-
γ-trans-nerolidol	1564	32.3	0.9
spathulenol	1576	0.3	-
α-cadinol	1652	0.4	-
β-santalol	1713	0.7	-
hexanoic acid	1982	-	8.0
manool	2060	-	4.0
9-octadecenoic acid	2144	0.2	0.2
thymol	2166	0.3	-
octadecanoic acid	2200	0.2	0.5
phytol acetate	2223	0.2	-
tricosane	2300	0.3	-
farnesol	2419	4.6	0.2
geranyl linalool	2450	-	20.1
unknown retinol derivative	2467	-	60
Total		91.2	96.2

[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 *Flabellaria paniculata* oils.

Table 2: The results of anti-inflammatory activity of the leaves and roots essential oils of *Flabellaria paniculata*

Group	Dose mg	No of animals in the group leaves roots	Change in ear weight (Mean ±SEM) mg leaves roots	Percentage of edema reduction leaves roots
Control	-	8 8	7.8 ± 0.3 7.8 ± 0.3	- -
indomethacin	0.25	8 8	3.3 ± 0.1 3.3 ± 0.1	57.7 57.7
Essential oil	5.0	8 8	5.8 ± 0.4 0.6 ± 0.3	25.8 92.5
	2.5	8 8	6.9 ± 0.4 1.8 ± 0.5	11.6 77.1
	1.25	8 8	7.6 ± 0.1 4.0 ± 0.5	02.5 48.9
	0.075	8 8	7.6 ± 0.2 6.6 ± 0.3	02.5 15.4

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