CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF THE TANZANIAN PLANT *OCIMUM SUAVE*

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ABSTRACT.—The essential oil of *Ocimum suave* Wild contains eugenol (71.5%) and six identified terpenoid substances. The oil is an excellent mosquito repellent and has moderate antimicrobial activity. Oleanolic acid, β-sitosterol, stigmasterol, phytol, and common fatty acids were identified in leaf extracts. Some indigenous uses of the plant are explained.

The tropical shrub *Ocimum suave* Wild (1) of the family *Labiatae* is a native of Africa and India. It grows extensively in Tanzania (2) and has a variety of indigenous uses including the flavoring of tobacco and snuff and as a body perfume (3). It is frequently used as a mosquito repellent (3); branches are burned or placed on the roofs and walls of huts. The leaves are also reputed to act as an insecticide towards mosquitos, flies, and other insects. Claims have been made for various medicinal activities, and extracts of the plant are used for treating coughs, eye and ear complaints, and abdominal pains (4).

This investigation was designed to explore the chemical basis of the above claims and also to obtain accurate data on the biological activity of the plant. Previous work on this plant has been confined to the essential oil, with only the principal constituents having been identified. Eugenol was found in amounts between 2.8–62% in oil from various sources, (1,5) while methyl eugenol was the main constituent (56%) in Kenya oil (6). Linalool and linalyl acetate have also been identified in some East African samples (5).

DISCUSSION

The chemical composition of *O. suave* essential oil is shown in table 1 and is based on glc on two separate columns, glc/ms analysis and separation of components by preparative glc. Table 1 indicates the methods used to identify the components. This oil contains the highest reported concentration of eugenol (71.5%) for this species and, also, a significant amount of cis-β-ocimene (13.5%). Apart from eugenol and linalool the other compounds in table 1 are reported for the first time in *O. suave* oil.

The oil had powerful mosquito repellent properties (table 2) and was superior in activity to citronella oil (7,8), previously considered to be one of the most efficient natural repellents. This activity is almost entirely due to the eugenol, since removal of this component rendered it inactive. Moreover pure eugenol, tested at the same time, had a marginally higher effect than *O. suave* Wild oil. In view of the pleasant odor and non-irritant nature of the oil, the above results indicate that it has a potential for commercial exploitation and use as a mosquito repellent.

On the other hand the oil was found to have no significant insecticidal properties when tested against *Musca domestica* and was inactive as a larvicide for day-old

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TABLE 1. Constituents of *Ocimum suave* Wild oil.

Constituent	% Total	Identification method
1. β-Pinene	0.3	ms, pe[a]
2. Cis-β-ocimene	13.5	ms, ir, nmr
3. Linalool	0.5	ms, pe
4. Eugenol	71.5	ms, ir, pe
5. Unidentified sesquiterpene	1.4	ms
6. β-Caryophyllene	2.8	ms, pe, ir
7. β-Cubebene	5.1	ms, ir
8. β-Bisabolene	4.9	ms, ir

[a]pe=peak enrichment (enriched peaks obtained by co-injection of authentic material on two columns).

larva of *Aedes aegypti* mosquitos. Antimicrobial activity was demonstrated for the oil (table 3), but once again it appears that this is due to the eugenol, which showed a similar but slightly higher pattern of activity. Confirmation of this was obtained by testing a specimen of the oil from which eugenol had been removed; this sample gave negative results at the levels used.

The leaf extract of the plant yielded oleanolic acid, β-sitosterol, stigmasterol, and phytol, which are all common plant constituents. Palmitic, linoleic, and

TABLE 2. Comparative repellency results (*Aedes Aegypti* mosquitos).

Repellent	Mean protection time in minutes[a]
O. suave Wild oil	100
O. suave Wild oil with eugenol removed	0
Eugenol	105
Citronella oil	75

[a]Mean of six tests.

linolenic acids were also identified, together with their ethyl esters. The latter are considered to be artifacts formed during ethanol extraction. No alkaloids were detected in the extract.

The results of this investigation show that *O. suave* oil is a good mosquito repellent and anti-microbial agent, with eugenol as the active component. This gives credibility to the indigenous use of the plant as an insect repellent and may explain its use for the treatment of skin, eye, ear, and stomach complaints.

TABLE 3. Antimicrobial assays of *Ocimum suave* Wild oil.

Material	*E. coli*	*M. luteus*	*S. aureus*	*S. cerevisiae*
O. suave Wild oil	900[a]	700[a]	800[a]	500[a]
Eugenol	600	600	700	400
O. suave Wild oil without eugenol	>1000	>1000	>1000	>1000

[a]Figures are minimum inhibitory concentrations of μg/ml.

EXPERIMENTAL[2]

PLANT MATERIALS.—Plant material was collected at Arusha, Tanzania, in the month of January. Plants were identified by the herberia of the Tropical Pesticide Research Institute at Arusha and the University of Dar es Salaam, and the Lushoto herberium.

PLANT DISTILLATION.—The essential oil was obtained by steam distillation of the freshly picked leaves and terminal branchlets in a conventional apparatus. The oil obtained in 0.4% yield, was a pleasant smelling pale yellow liquid with the following constants: $d_{20}=0.996$; $n^{20}D=1.5260$; $[\alpha]^{20}D-15.7°$; solubility, 1:1 in 80% ethanol.

PLANT EXTRACTION.—Leaf components were extracted from fresh plant material with 95% ethanol. The crude filtered extract was concentrated to low bulk (rotary under reduced pressure) and re-extracted into diethyl ether. This extract was separated into neutral, acidic and basic components by partition between the solvent and aqueous acid (10% HCl) and base (10% NaOH).

CHROMATOGRAPHY OF ACIDIC FRACTION.—The acidic fraction (15 g from about 50 g crude extract) was separated on a column (6.5 cm I.D.) of Merck type 60 silica gel (200 g) packed as a slurry in light petroleum ether (40–60°). Gradient elution with light petrol containing 2.5, 5, 10, 25 and 30% ethyl acetate was carried out and fractions (200 ml) were collected.

Fractions 1–6 (6.9 g). The brown oily liquid obtained was identified as eugenol by comparison of ir, nmr and mass spectral data with the spectra of authentic material. This identification was confirmed by glc analysis on the two columns.

Fractions 7–34 (3.4 g). This material was obtained in the form of a waxy oil. The ir spectrum had peaks at 1725 and 2500–3300 cm^{-1}. A portion, 20 mg, was methylated by refluxing in methanol (5 ml) with *p*-toluene sulphonic acid (1 mg) as catalyst. An aliquot (2 μl) was injected into the gas chromatograph fitted with a diethylene glycol succinate column (20% on chromasorb W) at 180°. Peaks were obtained with the same retention times as ethyl palmitate, ethyl linoleate and ethyl linolenate.

Fractions 35–69 (2.2 g). White needles were obtained which were recrystallized from hot ethanol, mp 289–290°, $[\alpha]^{20}D+77.2°$. Oleanolic acid was reported (9) to have mp 310 and $[\alpha]^{20}D+83.3°$. Found C, 78.9; H, 10.5% calculated for $C_{30}H_{48}O_3$: C, 78.9; H, 10.6%. Mass spectrum M^+ 456. The mixed mp of a specimen and authentic material was not depressed, and nmr spectra of the two samples were identical. Methyl oleanate was prepared from the acid by treatment with dimethyl sulfate/potassium carbonate and was obtained in the form of colorless plates, mp 199–200° (lit (9) 201°), M^+ 470.

CHROMATOGRAPHY OF NEUTRAL FRACTION.—The neutral fraction (10 g from 70 g of crude extract) was similarly chromatographed on a column of 200 g silica gel. Gradient elution with light petroleum ether containing 0, 1, 2.5, 10, and 20% ethyl acetate was carried out; 200 ml fractions were collected.

Fractions (1–3) (0.6 g). This was a pale yellow oil, shown to consist of β-caryophyllene and β-bisabolene by glc analysis.

Fractions 4–10 (2.5 g) were shown to be a mixture of ethyl palmitate, ethyl linoleate and ethyl linolenate by glc/ms analysis. Authentic ethyl esters were prepared and used as reference compounds.

Fractions 19–28 (1.9 g). A brown oil was obtained which was shown to be phytol by ir, nmr and mass spectrometry. All spectra were identical to those of authentic phytol.

Fractions 29–50 (1.8 g). Colorless crystals were obtained, mp 137–138°, which were not resolved on tlc plates. High resolution mass spectra showed peaks at 414.3872 and 412.3684. β-Sitosterol (10) ($C_{29}H_{50}O$) has M^+ 414.3863, and stigmasterol (11) ($C_{29}H_{48}O$) has M^+ 412.3705. Glc of the trimethylsilyl ethers of the mixture gave peaks on two columns identical to retention times of the trimethyl silyl derivatives of authentic β-sitasterol and stigmasterol.

Fractions 71–105 (2.1 g). Colorless crystals, mp 290–295°, were obtained with all spectral properties identical to those of oleanolic acid [lit mp 310° (9)].

CHROMATOGRAPHY OF THE BASIC FRACTION.—The basic fraction (0.1 g from 15 g crude extract) was examined by tlc analysis (silica gel plates, methanol-ammonia, 100:1). The plates

[2]Glc analysis was carried out on a Packard model 417 instrument with glass columns (6 ft x ¼") packed with either SE30(10%) or FFAP(5%) on Chromosorb W (80–100 mesh). Nitrogen was the carrier gas (60 ml/min), and the columns were programmed from 80–200° at 8°/min. Glc/ms analysis was performed on a Shimadzu GC 6 AMP instrument fitted with an OV–17 Scot column (40 M) coupled to an AEI MS12 mass spectrometer. Helium (2 mls/min) was the carrier gas, the column was programmed for 55–200° at 5°/min, and the mass spectrometer was run at 70 eV. Preparative glc was performed on a Varian 712 fitted with a column (6 ft x ⅜") packed with SE30 (20%) on Chromosorb W. Nitrogen flowed at 100 ml/min, and the column temperature was programmed from 90–200° at 2°/min. Ir spectra were measured on a Pye Unicam SP1000 and nmr spectra on Jeol NM 4H 100 spectrometer.

were sprayed with iodoplatinate, Marquis, and Dragendorf reagents, however, no alkaloid positive spots were identified.

REPELLENCY TESTS (12, 13).—*Aedes aegypti* mosquitos, bred in the Zoology Department of the University of New South Wales were used. Test materials (100 mg) in acetone (150 ml) were applied to a cotton arm band by dipping. Eugenol free essential oil was prepared by extraction with excess 10% sodium hydroxide solution. Absence of eugenol was confirmed by glc analysis. Rose bengol dye in the solution was used to ensure even penetration. After the drying of the acetone, the arm band was applied to the fore-arm of a volunteer who placed his arm into a cage containing 40 female mosquitos (3–4 days old) for 2 min at 10 min intervals until the first bite was recorded. The arm band dipped in acetone containing rose bengol only was used for controls. Results are the mean of at least six volunteer results.

INSECTICIDAL TESTS.—The essential oil and the neutral and acidic portions of the leaf extract (1 μg) in acetone, with an Arnold hand applicator was applied to anaesthesized (diethy ether) house flies. Test insects were kept in a conditioned chamber (25°, 60% R.H.), and mortality counts were made after 24 and 48 hrs.

LARVICIDAL TESTS.—Day-old larvae of *Aedes aegypti* were placed in a 100-ml aqueous solution of test materials (essential oil, neutral and acidic leaf extracts—1 ppm concentration). Mortality counts were made after 12 hrs.

ANTI-MICROBIAL TESTS.—The organisms used were *Escherichia coli* UNSWO24101, *Micrococcus luteus* ATCC 9341, *Staphyloccoccus aureus* ATCC 6538 and *Saccharomyces cerevisiae* UNSA Y-40. Bacteria were grown in nutrient broth (oxoid) at 35°; yeast was grown in Sabourad Dextrose broth at 31°. Assays were carried out by the addition to the appropriate medium of a known quantity of test material solution or emulsion and a standard quantity of microorganism culture. Assay tubes were cultured for 48 hrs at the appropriate temperature on an orbital shaking water bath. Minimum inhibitory concentrations were measured by visual inspection and by colony counts.

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