Oleanolic Acid, a Bioactive Component of the Leaves of Ocimum Gratissimum (Lamiaceae)

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Abstract:

Bioactivity-guided fractionation of the leaves of *Ocimum gratissimum* L. (Lamiaceae), using the brine shrimp lethality test, led to the isolation of oleanolic acid. Oleanolic acid showed bioactivity against a panel of six human solid tumor cell lines, the nematode *Caenorhabditis elegans* and yellow fever mosquito larvae *Aedes aegypti*. Details of the isolation and bioactivities are described.

Keywords: Aedes aegypti | brine shrimp test | Caenorhabditis elegans | cytotoxicity | Ocimum gratissimum | oleanolic acid | yellow fever mosquito

Article:

INTRODUCTION

A decoction of the leaves of *Ocimum gratissimum* L. (Lamiaceae) is used as a diaphoretic, febrifuge and anthelmintic (Oliver, 1960; El-Said *et al.*, 1969). Various essential oil components have been detected from the leaves, the major ones being thymol and eugenol (Sofowora, 1970; Ntezurubanza *et al.*, 1987). Lipidsoluble principles in the leaves have been shown to exhibit smooth muscle contracting activity (Onajobi, 1986). Awuah (1994) indicated that the crude steam distillate of the leaves had some inhibitory effect on *Phytophthora palmivora* known to cause blackpod disease of cocoa. The present work was directed towards finding the bioactive principles in the leaves, apart from the volatile oil or lipid fractions, which may be responsible for its claimed anthelmintic and other biological activities.

MATERIALS AND METHODS

Plant Material

The leaves of *O. gratissimum* were collected from the Nsukka area in Enugu, Nigeria, in May 1995. The plant material was identified as *O. gratissimum* L. by Mr. A.O. Ozioko of the Botany Department, University of Nigeria, Nsukka, and a voucher sample has been deposited there for purposes of reference.

Extraction and Separation

Air-dried leaves of *O. gratissimum* (710 g) were ground into powder and extracted with 95% ethanol at room temperature. The extract was evaporated under rotary vacuum at 35°C to yield 115 g of the ethanol residue (F001). This was partitioned between H2O and CH2Cl2 to give the H2O residue (F002, 74 g) and CH2Cl2 residue (F003, 41 g) on evaporation. F003 was partitioned between hexane and 90% methanol to give the methanol residue (F005, 33 g) and the hexane residue (F006, 7.87 g) on evaporation. Based on the brine shrimp lethality bioassay (BST) (Table 1), F005 was identified as the most bioactive fraction. F005 was further chromatographed over silica gel columns (60–200 mesh) eluted with gradients of hexane/ethyl acetate/methanol. Further purification of the resulting most bioactive fractions was carried out by hplc over silica gel eluted with 10% THF in methanol/hexane gradients (5–10%) and acetonitrile and water (10–20%) over C-18 silica gel to yield 17.6 mg of bioactive compound. The TLC, proton and carbon NMR spectra, and mass spectra of this compound were identical to an authentic sample of oleanolic acid (He, 1995).

Brine Shrimp Test (BST)

The BST was performed according to standard protocols (Meyer *et al.*, 1982; McLaughlin, 1991). The LC50 values in μg/ml (ppm) were determined for the partitioned fractions, the pooled chromatographic fractions, and the isolated compound (Table 1) using the Finney probit analysis.

Table 1. Brine Shrimp lethality of partitioned fractions.

Fraction	**F001**	**F002**	**F003**	**F005**	**F006**
LC_{50} (μg/ml)	1807.6	> 2000	118.89	76.6	> 2000

Cytotoxicity Assays

The isolated oleanolic acid was tested for cytotoxicity in a panel of six human solid tumor cell lines at the Purdue Cancer Center by using a 7-day MTT assay and standard protocols for A-549 (lung carcinoma) (Fogh and Trempe, 1975), A-498 (kidney carcinoma) (Giard *et al.*, 1973), MCF-7 (breast carcinoma) (Soule *et al.*, 1973), HT-29 (colon adenocarcinoma) (Fogh and Trempe, 1975), PC-3 (prostate adenocarcinoma) (Kaighn *et al.*, 1979) and PACA-2 (pancreatic carcinoma) (Yunis *et al.*, 1977). AdriamycinR served as a positive control.

Caenorhabditis elegans Assays

Using a standard method (Perret and Whifield, 1995) a lethality assay was carried out using both larvae and adult *C. elegans*. The nematodes were raised on nematode growth agar previously

seeded with *Escherichia coli* (OP50 strain) and were obtained from the *Caenorhabditis* Genetics Center, 1445 Gortner Ave., St. Paul Minnesota, 55108-1095. They were added to in-well concentrations of oleanolic acid at 8, 4, and 1 ppm in water. IvomecR (ivermectin) and febendazole were used as positive controls. Mortalities were counted 8 h after the addition of compounds.

Yellow Fever Mosquito Larvae Assay (YFM)

A standard microtitre plate was used to evaluate the pesticidal activity of oleanolic acid. Dr. Steven Sackett (New Orleans Mosquito Control Board, New Orleans, Louisiana 70126) kindly provided the eggs of *Aedes aegypti* used for the assay. Overnight hatching of the eggs in 300 ml of distilled water was initiated. The emerging larvae were transferred into a 400 ml beaker containing bovine liver powder (ICN Biochemicals), at a concentration of 2–4 mg/ml, and were allowed to develop for 4 days. Harvested larvae were then transferred into a 20 ml scintillation vial filled with 10 ml of 5 mM MES (2-[N-morpholino] ethanesulfonic acid, C6H13NO4S.H2O) in buffer (pH 6.5). With the larvae ready for the experiment, wells in a 96-well flat bottom assay plate were filled with 240 µl of 5 mM MES per well using a Vaccupette/96 with the aid of a model edp microlitre repipettor (Rainin Instrument). Single larva were introduced per well, and 5 µl aliquots of oleanolic acid in methanol were added to each well. Rotenone served as a positive control, while a 2% methanol solution served as a negative control in each experiment. With a 1:10 dilution of test compound in methanol, 500, 50, and 5 ppm in-well concentrations were obtained. There were 8 replications of each dose. After adding the test compounds, the plates were covered and incubated in the dark at room temperature for 4 days. Dead and live larvae were then counted. The LC50 values and 95% confidence intervals in µg/ml (ppm) were calculated using a Finney probit analysis computer program (McLaughlin, 1991).

RESULTS

The toxic effects against *C. elegans* are presented graphically (Fig. 1). Brine shrimp lethalities and cytoxicities are summarized in Tables 1 and 2, and mortalities to the yellow fever mosquito larva are presented in Table 3.

Table 2. Brine Shrimp lethality and cytotoxicities against human solid tumor cell lines.

	BST[a]	Cytotoxicity (ED_{50}, µg/mL)					
Compd	**LC_{50} (µg/ml)**	**A-549[b]**	**MCF-7[c]**	**HT-29[d]**	**A-548[b]**	**PC[e]**	**PACA-2[g]**
F005	76.6	28.24	43.99	33.86	39.34	16.46	27.83
Oleanolic acid	0.07	3.16	2.46	3.12	3.13	2.58	3.47
Adriamycin[h]	2.27×10^{-1}	0.019	0.349	0.0372	0.0439	0.0335	0.00755

[a] Brine shrimp lethality test. [b] Human lung carcinoma. [c] Human breast carcinoma. [d] Human colon adenocarcinoma. [e] Human renal carcinoma. [f] Human prostate adenocarcinoma. [g] Human pancreatic carcinoma. [h] Positive control.

Table 3. Yellow fever mosquito assay (YFM).

Sample	**LC_{50} (µg/ml)**
F005	60.3
Oleanolic acid	4.4
Rotenonea	0.12

[a] Positive control.

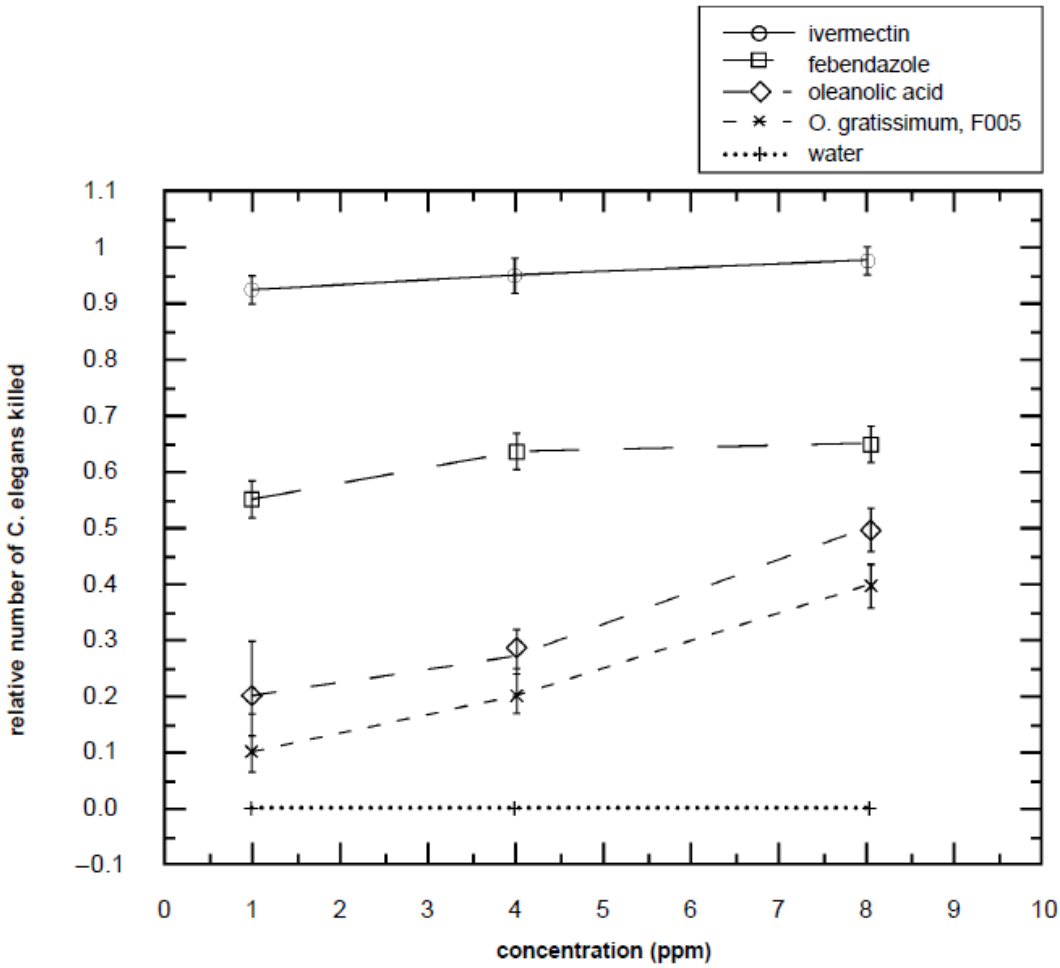

Fig. 1. The *y*-axis represents the number of *C. elegans* killed in the assay, while the *x*-axis represents the concentration in µg/ml (ppm). For example, ivermectin killed over 90% of the nematodes at all doses while water, as a control, did not kill any. Relative to each other the *O. gratissimum* extract and oleanolic acid responded similarly, with the latter being slightly more active than the crude extract.

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DISCUSSION

Considering the regular use of *O. gratissimum* leaves to relieve various ailments, in particular the deworming of dogs with ascarid worms in Nigeria, it appears that oleanolic acid contributes to the observed beneficial activity. Most of the previous work carried out on *O. gratissimum* has been on its essential oil or lipidsoluble fractions. Though oleanolic acid is a widespread triterpene in plants, apart from its possible protection to the plants, it may also provide beneficial effects to humans and animals. It was generally cytotoxic but appeared to have slightly more

effect on breast cancer cells compared to the other cell types tested. Oleanoic acid may be useful as an inexpensive anthelmintic, and its insecticidal action is significant.

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REFERENCES

Awuah, R.T. (1994) *In vivo* use of extracts from *Ocimum gratissimum* and *Cymbopogon citratus* against *Phytophthora palmivora* causing blackpod disease of cocoa. *Ann. Appl. Biol.* **24:** 173–178.

El-Said, F., Sofowora, E.A., Malcolm, S.A. and Hofer A. (1969) An investigation into the efficacy of *Ocimum gratissimum* as used in Nigeria native medicine. *Planta Med.* **17:** 194–200.

Fogh, J. and Trempe, G. (1975) New Human Tumor Cell Lines, in *Human Tumor Cells in vitro* edited by Fogh, J., 115 pp., Plenum Press, New York.

Giard, D.J., Aaronson, S.A., Todaro, G.J., Arnstein, P, Kersey, H.J., Dosik, H. and Parks, W.P. (1973) *In vitro* cultivation of human tumors; establishment of cell lines derived from a series of solid tumors. *J. Nat. Cancer Inst.* **51:** 1417–1423.

He, K. (1995) Phytochemical investigations of medicinal plants from Chile and the Fiji Islands. pp. 90–92, University of Arizona, Tucson

Kaighn, M.E., Narayan, K.S., Ohnuki, Y., Lechner, J.F. and Jones, L.W. (1979) Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Investigative Urol.* **7:** 16–23.

McLaughlin, J.L. (1991) Crown Gall tumors in potato discs and brine shrimp lethalities: two simple bioassays for higher plant screening and fractionation, in *Methods in Plant Biochemistry vol 6* edited by Hostettmann, K., pp. 1–33, Academic Press, London.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobson, L.B. *et al.* (1982) Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med.* **45:** 31–34.

Ntezurubanza, L. Scheffer, J.J.C. and Svendsen, B.A. (1987) Composition of the essential oil of *Ocimum gratissimum* grown in Rwanda. *Planta Med.* **53:** 421–423.

Oliver, B. (1960) Medicinal plants in Nigeria, Nigeria College of Arts, Science and Technology, Ibadan, Nigeria, 42 pp.

Onajobi, D.F. (1986) Smooth muscle contracting lipid-soluble principles in chromatographic fractions of *Ocimum gratissimum*. *J. Ethnopharmacol.* **8:** 3–11.

Perrett, S. and Whifield, P.J. (1995) Anthelmintic and pesticidal activity of *Acorus gramineus* (Araceae) is associated with phenylpropanoid asarones. *Phytotherapy Res.* **9:** 405–409.

Sofowora, E.A. (1970) A study of the variations in essential oil of cultivated *Ocimum gratissimum*. *Planta Med.* **18:** 173–176.

Soule, H.D., Vazquez, J., Long, A., Albert, S. and Brennan, M.J.A. (1973) Human cell lines from a pleural effusion derived from a breast carcinoma. *J. Nat. Cancer Inst.* **51:** 1409–1416.

Yunis, A.A., Arimura, G.K., Russin, D.J. (1977) Human pancreatic carcinoma (MIA PaCa-2) in continuous culture. Sensitivity to asparaginase. *Int. J. Cancer* **19:** 128–135.