

## Poster Papers

### Antioxidant, Anticarcinogenic and Termiticidal Activities of Vetiver Oil

Feng Chen<sup>1</sup>, Xi Wang<sup>2</sup>, and Hyun-jin Kim<sup>1</sup>

<sup>1</sup>*Department of Food Science and Human Nutrition, Clemson University, SC 29634*

<sup>2</sup>*Department of Biochemistry and Genetics, Clemson University, SC 29634*

**Abstract:** Vetiver oil (VO) was assayed for its antioxidant, anticarcinogenic and termiticidal properties. At the concentration of 10 µl/ml, the DPPH radical scavenging effect of vetiver oil (93%) in antioxidant activity test was higher than that of 1mM BHT (73%) and equivalent to 1mM α-tocopherol (93%). At 100ppm in cancer cell lines, vetiver oil inhibited the growth up to 89% of SiHa cervical cells, 88% of CaSki cervical cells and 89% of MCF-7 breast cancer cells. Besides, vetiver oil was found to possess strong termiticidal activity against the Formosan subterranean termite *Coptotermes formosanus*. Vetiver oil decreased termite tunneling at concentration as low as 5 g/g sand, and entirely inhibited termites' tunneling and paper consumption at concentrations higher than 25 g/g sand.

**Contact:** Feng Chen <[fchen@clemson.edu](mailto:fchen@clemson.edu)>

#### 1 INTRODUCTION

Vetiver oil is an important plant-derived product that contains hundreds of terpenes, terpenoids, phenols, etc. It currently has three primary commercial applications: as aromas in perfumes, as food flavoring additives, and as natural insecticide.

In recent years, biological activities, e.g. antioxidant, antimicrobial, insecticidal, anticancer effects, etc., of various essential oils and natural plant extracts have been widely and deeply studied. However, except of few reports of the chemical composition of vetiver oil, few studies have been conducted on the biological activities of vetiver oil (Zhu et al., 2001). Therefore, the objective of this study was to evaluate the antioxidant, anticarcinogenic and termiticidal activities of the VO to explore its more practical applications.

#### 2 METHODS AND MATERIALS

Vetiver (*Vetiveria zizanoides*, Haiti) oil was purchased from the Good Scents Company (TGSC, Oak Creek, WI). The authentic standard of nootkatone (crystalline, 97%) was purchased from the Lancaster Synthesis Inc. (Windham, New Hampshire). Other standards were purchased from Sigma-Aldrich, Inc. USA.

##### 2.1 Silica Gel Column Chromatography

The column (3 x 2.5 cm) packed with silica gel (70-230 mesh, 60Å) was equilibrated with hexane and eluted with the mixture solvents of hexane, methylene chloride (DCM) and methanol. Five ml of VO was loaded and fractionated in size of 1ml. The first two fractions were isolated with pure hexane,

other fractions were separated with DCM and methanol (70:30,V/V).

## 2.2 HPLC Separation

A Pinnacle II C18 column (150 X 4.6mm, 5 $\mu$ m; Restek, PA, USA) was connected with Shimadzu LC-10AT HPLC system (Kyoto, Japan). 50  $\mu$ l of vetiver oil partially purified by silica gel was injected and eluted with methanol for further antioxidant test and chemical identification by GC-MS. The flow rate was 1ml/min and the eluant was detected by Shimadzu SPD-M10V PAD.

## 2.3 GC-MS Identification

Chemical profile of VO was performed on Shimadzu GC-17A coupled with QP5050 MSD. Chemicals were separated by DB-5 capillary column (60m X 0.25mm X 0.25 $\mu$ m; J&W Scientific, Folsom, CA). Oven temperature was set from 60C to 240C at the increase rate of 3C/min, and held at 240C for 10 min. Identification of compounds were based on comparison of mass spectra and retention indices with authentic standards or references.

# 3 BIOASSAY OF VO

Formosan subterranean termites (Figures 3 and 4) were collected in a fallen oak tree from New Orleans, Louisiana, USA

## 3.1 Repellency Assay

The testing procedure was modified from Lewis et al. (1978). A Petri dish (5x1 cm) was used for testing the repellency and toxicity of the VO against workers of the termite. The VO was dissolved in ethyl alcohol and prepared in a series of dilutions for testing. One ml of hot agar solution (1.5%) was spread evenly in the bottom of each dish, and covered with blasting sand (fine, #4). In each dish, one half of the sand was chemically treated and covered with a piece of treated filter paper (Whatman #1, 0.5x1cm). The other half part was covered with sand and paper that were not chemically treated. Four concentrations (5, 10, 25, 50 $\mu$ g/g sand) of the VO was prepared, and three replicates at each concentration and control were tested. This experiment was repeated twice. Ten worker termites were added into each Petri dish. Termite activities were recorded every 24 hours for 4 weeks. If more than 23 out of 30 termites were observed on the untreated sand, then the oil was considered repellent to termite. If the termites behaved sluggishly or was found moribund and dead, the oil was considered toxic.

## 3.2 Tunneling, Paper Consumption and Mortality Assay

The experimental method of this bioassay is the same as that published (Zhu et al., 2001). Tunneling response of termite to chemicals were recorded by a scanner for measurement for total tunnel length. Paper consumption was calculated as the difference between the weight of filter paper before and after the test. ANOVA test was used for the analysis of tunneling response, paper consumption and mortality rate.

## 3.3 DPPH Assay for the Determination of Antioxidative Capacity

DPPH method of Yamahuchi *et al.* (2002) was modified to determine the radical scavenging activity of VO. Butylated hydroxytoluene (BHT) and  $\alpha$ -tocopherol was used as comparable standard antioxidants. The reactant mixed with 0.4ml of 0.5mM DPPH solution, 0.2ml of ethanol, and 0.2ml of sample was incubated in the darkness at room temperature for 1 hr. Antioxidative activity was measured spectrophotometrically at 517nm and calculated.

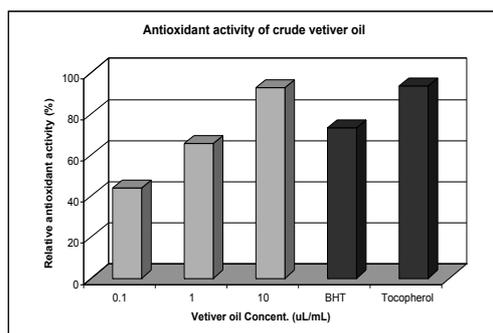
### 3.4 Anticancer Bioassays

VO was tested against cultures of two aggressive cervical cancer cell lines (CaSki & SiHa) and one breast cancer cell line (MCF-7). The anticancer activity was assessed with the MTS viability assay (CellTiter 96 AQueous One Solution kit, Promega). 15,000 cells of each cancer cell line per well were added into 96-microtiter plate, mixed with appropriate concentrations of the vetiver oil. The cells then were incubated for 24 hrs at 37C. After that 20  $\mu$ l of MTS solution was added and incubated for 4 hours, then the absorbance was read at 490nm in an ELISA plate reader. Dead cells% =  $(1 - \text{Abs.}@490\text{nm of sample} / \text{Abs.}@490\text{nm of control}) \times 100\%$ .

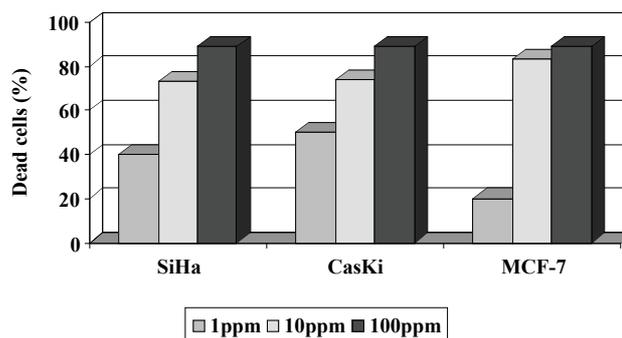
## 4 RESULTS AND DISCUSSION

Fig. 1 shows the DPPH radical scavenging effect of vetiver oil at different concentrations compared to 1mM BHT and  $\alpha$ -tocopherol. VO has the values at 44%, 66%, and 93% at the concentration of 0.1  $\mu$ l/ml, 1  $\mu$ l/ml, and 10  $\mu$ l/ml, respectively, compared to that of 73% for 1mM BHT and 93% 1 mM  $\alpha$ -tocopherol. This result indicated that VO is an excellent alternative natural antioxidant. However, because of the complicated chemical composition of the VO, further instigation is underway to identify the components responsible for the strong antioxidant effect.

**Fig. 1 DPPH radical scavenging effect of VO compared with BHT and tocopherol**



**Fig. 2 Inhibition of cell reproduction by vetiver oil**



**Fig. 3 Termite Damage**



**Fig. 4 *Coptotermes formosanus***



Fig. 2 shows the values representing the anticancer activity against three cancer cell lines at concentrations of VO at 1ppm, 10ppm, and 100ppm. VO strongly inhibited the growth of all three cancer cell lines approximately up to 89% at the 100ppm level. Even at the 1ppm level, the cervical cancer cells, i.e. SiHa and Caski cells, were kill up to 40% and 50%, respectively, and the dead percentage MCF-7 breast cancer cells was in 20%.

VO also demonstrated strong repellency and antifeedant activity against the Formosan subterranean termites, *Coptotermes formosanus* (Table 1). At the concentration of 5\_g/g sand, VO significantly decreased the termite tunneling activity and paper consumption at concentration as low as 10\_g/g sand ( $F=39, 40, df=4, 15; P<0.0001$ ). When the concentration of VO increased to 25ug/g sand and above, the tunneling activity and paper consumption of termites entirely stopped. Further investigation found that nootkatone was one of the major chemicals in VO responsible for the termiticidal activity, for which patents have been filed.

**Table 1 Mean ( $\pm$ SD) of paper consumption, percent mortality and tunneling length of Formosan subterranean termite after a 14-day exposure**

Concentration of vetiver oil ( $\square$ g/g sand)	Weight loss of filter paper (mg)	% Termite mortality	Tunneling length (cm)
0	64.05 $\pm$ 8.85 A	13.50 $\pm$ 4.03 A	39.75 $\pm$ 3.18 A
5	15.80 $\pm$ 8.35 B	21.33 $\pm$ 2.08 A	27.87 $\pm$ 2.86 AB
10	8.65 $\pm$ 5.05 BC	13.00 $\pm$ 4.66 A	19.87 $\pm$ 4.52 B
25	0.00 $\pm$ 0.00 C	13.20 $\pm$ 3.78 A	0.00 $\pm$ 0.00 C
50	0.00 $\pm$ 0.00 C	15.50 $\pm$ 9.32 A	0.00 $\pm$ 0.00 C

From the above results, it can be concluded that vetiver oil possesses multi-functional biological activities. VO is not only an excellent natural antioxidant, also a potential alternative termiticide. Considering its anticarcinogenic ability, VO also might be used in aromatherapy and provide clues for novel drugs against some specific cancers.

## References

- Lewis DL, Michaels GE, Hays DB, *et al.* 1978. Evaluation of the antitermitic activity of hydroxyquinoline and naphthol derivative formulations using *Reticulitermes* in laboratory and field experiments. *J. Econ. Entomol.*, 71: 818-821
- Wedge DE, Meepagala KM, Magee JB, *et al.* 2001. Short communication. Anticarcinogenic activity of strawberry, blueberry, and raspberry extracts to breast and cervical cancer cells. *Journal of Medicinal Food.*, 4(1): 49-51
- Yamaguchi T, Takamura H, Matoba T, *et al.* 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.*, 6: 1200-1204
- Zhu BCR, Herderson G, Chen *et al.* 2001. Nootkatone is a repellent for Formosan subterranean termite (*Coptotermes formosanus*). *J. Chem. Ecol.*, 27: 523-531