*Philippine Journal of Crop Science December 2004, 29(3): 41-43*

*Released 11 April 2005*

# ESSENTIAL OILS AS ANTI-PROTOZOAL AGENTS

ELMER-RICO E MOJICA[1], CUSTER C DEOCARIS[2,3] & MARLA A ENDRIGA[4]

[1]Assistant Professor, Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna (rico@chem.uplb.edu.ph ). [2] Graduate Researcher, Gene Function Research Center, 1-1-1 Higashi, AIST Central 4, Tsukuba 305-8562, Japan. [3] PhD student, Department of Chemistry and Biotechnology, School of Engineering, University of Tokyo, Hongo, Tokyo 113-8656, Japan. [4] Instructor, Institute of Biology, University of the Philippines Diliman, Quezon City

*The anti-protozoal effects of essential oils were examined using microplate-based in-vitro bioassays on axenically cultured trophozoites of Acanthamoeba sp. (a keratitis-causing amoeba) and Tetrahymena pyriformis (a test organism for cytotoxicity). Essential oils were extracted by steam distillation from leaves, fruits and other parts of 10 plant species: Citrus sinensis (orange), Citrofortunella mitis (syn. Citrus microcarpa, calamansi), Citrus poonensis (ponkan), Citrus reticulata (dalandan), Eucalyptus citriodora (eucalyptus), Zingiber officinale (ginger), Cananga odorata (ilang-ilang), Cymbopogon winterianus (citronella), Pandanus* sp. *(pandan) and Mentha piperita (peppermint). Of the 10 species, Citronella, dalandan and ponkan showed 100% anti-protozoal activity. Interestingly, extracts from plants of the citrus family showed homogeneity in toxicity profiles for both protozoans while ginger and pandan showed differential reactivities.*

*Acanthamoeba* sp., anti-protozoal activities, aromatherapy, Citronella, citrus family, dalandan, essential oils, ponkan, *Tetrahymena pyriformis*

## INTRODUCTION

Essential oils are complex mixtures of compounds that are produced in specialized glands within various aromatic plants. These oils can be harbored in leaves, stems, seeds, wood, barks, resins, fruits, roots and needles of vascular and non-vascular plants. These oils contain the chemical components responsible for the distinguishing odor of some flora.

Essential oils are used in industry for the essence and flavoring of all kinds of finished consumer products. They are also popular in aromatherapy. Buchbauer & Jirovetz (1994) provide a comprehensive survey on the uses of essential oils as medicaments. They describe various studies on the physiological effects of essential oils on humans. These include brain stimulation, anxiety-relieving sedation and antidepressant properties as well as increasing cerebral blood flow. They also describe the effects of odors on cognition, memory and mood.

Essential oils are also rich sources of defensive chemicals which can counter insect, fungal and viral attacks (Rosenthal 1986). Studies on some of these oils have shown them to be antimicrobial and insecticidal in nature. Deans & Ritchie (1987) have examined 50 plant essential oils for their antibacterial properties against 25 genera of bacteria using an agar diffusion technique. Some essential oils are active against insects (Rejesus et al 1990), dermatophytes (Dubey et al 1998) and viruses (Hayashi et al 1995). Activity against a cancer cell line has also been reported (Hailat et al 1995).

This paper presents a preliminary screening of the toxicity of some essential oils against *Acanthamoeba* sp. and *Tetrahymena pyriformis* for possible utilization as anti-protozoal agents. *Acantamoeba* sp. is an amoeba that causes keratitis, which is a serious and potentially devastating corneal infection generally seen in soft contact lens wearers (Perrine et al 2003), while the ciliated *T. pyriformis* is a well-known organism that has been used to assess the cytotoxicity of different compounds and therapeutic agents (Nilsson 1989).

## MATERIALS & METHODS

### *Extraction of essential oils*

Ten plant species available locally were used as material sources of crude extracts of the essential oils (Table 1). The citrus fruits and ginger were purchased from the local market and grocery stores in Los Baños, Laguna. Other plant specimens were obtained from different parts of the University of the Philippines Los Baños campus. Eucalyptus leaves were collected from the College of Forestry campus; citronella, pandan and peppermint were obtained from the Department of Horticulture area; and ilang-ilang flowers were obtained from the Physical Science premises.

The fresh plant materials were cut into small pieces and mixed with water. Isolation of essential oils was done by steam distillation.

### *Bioassay*

The extracted essential oils were then tested for their anti-protozoal properties against *Acanthamoeba* sp. and *Tetrahymena pyriformis* using microplate-based in vitro bioassays. This was done by mixing cultured cells of the test organisms (100 μL) with the essential oils (50 μL) in microtiter plate wells. A small amount of dimethyl sulfoxide (DMSO) (6.67% final concentration) was used to facilitate the mixing of the immiscible essential oils and cultured cells. Mortality of the cultured cells was examined microscopically upon addition of essential oils and after 24 hours. For the control treatment, a 100 mg/L concentration of cytosine β-o-arabinofuranoside (Sigma Chemical Co) or Ara C was used. All treatments were done in triplicates.

**Table 1. Sources of essential oils evaluated for their anti-protozoal property**

| Common/Local Name | Scientific Name | Parts Used |
|---|---|---|
| Calamansi | *Citrofortunella mitis,* syn. *Citrus microcarpa* | fruit rind |
| Dalandan | *Citrus reticulata* | fruit rind |
| Orange | *Citrus sinensis* | fruit rind |
| Ponkan | *Citrus poonensis* | fruit rind |
| Citronella | *Cymbopogon winterianus* | leaves |
| Eucalyptus | *Eucalyptus citriodora* | leaves |
| Pandan | *Pandanus* sp. | leaves |
| Peppermint | *Mentha piperata* | leaves |
| Ginger | *Zingiber officinale* | rhizomes |
| Ilang-ilang | *Cananga odorata* | flowers |

## RESULTS & DISCUSSION

Screening of the 10 essential oil extracts against *Acanthamoeba* sp. and T*etrahymena pyriformis* showed varying results (Table 2). Citronella, dalandan and ponkan resulted in 100% mortality of the organisms upon addition of the essential oils, while pandan and ginger essential oils showed no lethal effects against *Acanthamoeba* sp.

Several active compounds might have a synergistic effect in certain plant species but not in others. It has also been reported that the time of harvest influences the composition of the oil and consequently the potency of its biological activity (Deans & Svoboda 1988, Marotti et al 1994). This could be the reason why dalandan and ginger were observed to be inactive against *Acanthamoeba* sp. The extracts from other citrus fruits had a lethal effect on *Acanthamoeba* sp. and the time of harvest may have a bearing on the composition of the essential oils and thus, their biological activity.

**Table 2. Percent mortality of essential oils against *Acanthamoeba* sp. and *Tetrahymena pyriformis* at different observation periods**

| Samples / Microorganism | *Acantamoeba* sp. | | *Tetrahymena pyriformis* | |
|---|---|---|---|---|
| | Initial | After 24 hours | Initial | After 24 hours |
| Calamansi | 0 | 50 | 100 | 100 |
| Citronella | 100 | 100 | 100 | 100 |
| Dalandan | 0 | 33 | 0 | 100 |
| Eucalyptus | 0 | 100 | 100 | 100 |
| Ginger | 0 | 0 | 0 | 100 |
| Ilang-ilang | 0 | 100 | 100 | 100 |
| Orange | 100 | 100 | 100 | 100 |
| Pandan | 0 | 0 | 0 | 100 |
| Peppermint | 0 | 100 | 100 | 100 |
| Ponkan | 100 | 100 | 0 | 100 |
| Ara C (100 ppm)* | 0 | 100 | 0 | 100 |

**positive control*

Other factors such as genotype, chemotype, geographical origin and environmental and agronomic conditions can influence the composition of the final natural product (Svoboda et al 1995). Ravid et al (1994) found that the enantiomeric composition of various monoterpenes in different species can further complicate the biological activity of a given essential oil.

It is possible that the mechanism of action of some components of essential oils like terpenes involve membrane disruption by the lipophilic compounds. This is supported by the finding of Mendoza et al (1997) that the addition of a methyl group to kavrene diterpenoids reduced antimicrobial activity.

Other essential oils, particularly those that are biologically active against microorganisms, may also exhibit activity against protozoans and amoeba that are harmful to humans.

These preliminary results show the feasibility and potential of utilizing essential oils as anti-protozoal agents. The availability of the plants from which they were derived indicates quick access to affordable sources of possible alternative medicines against diseases caused by protozoans and amoeba.

At present, other essential oils are being assayed for their anti-protozoal activity. It is recommended that those that have potential as anti-protozoal agents be tested at different dosage levels. It is also suggested that these active essential oils be tested against other harmful protozoans and amoeba.

## Literature Cited


Buchbauer G & L Jirovetz. 1994. Aromatherapy – Use of fragrances and essential oils as medicaments. *Flavour and Fragrance Journal* 9: 217-222

Deans SG & G Ritchie. 1987. Antibacterial properties of plant essential oils. *International Journal of Food Microbiology* 5: 165-180

Deans SG & KP Svoboda. 1988. Antibacterial activity of French tarragon (*Artemisia dracunculus* L.) essential oil and its constituents during ontogeny. *Journal of Horticultural Science* 63: 503-508

Dubey NK, P Yadav & VK Joshi. 1998. Screening of some essential oils against dermatophytes. *Philippine Journal of Science* 127(2): 139-147

Hailat N, Z Batinheh, S Lafi, E Raweily, M Aqel, M Al-Katib & S Hanash. 1995. Effect of *Nigella sativa* volatile oil on Jurkat T cell leukemia polypeptides. *International Journal of Pharmacognosy 33(1): 16-20*

Hayashi K, M Kamiya & T Hayashi. 1995. Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus and HIV. *Planta Medica* 61(3): 237-241

Marotti M, R Piccaglia, E Giovanelli, SG Deans & E Eagkesham. 1994. Effects of planting time and mineral fertilization on peppermint (*Mentha piperita* L.) essential oil composition and its biological activity. *Flavour and Fragrance Journal* 9: 125-129

Mendoza L, M Wilkens & A Urzua. 1997. Antimicrobial study of resinous exudates and of diterpenoids and flavonoids isolated from some Chilean *Pseudographalium* (Asteraceae). *Journal of Ethnopharmacology* 58: 85-88

Ravid U, E Putievsky & I Katzir. 1994. Determination of the enantiomorphic composition of the $\alpha$-terpineol in essential oils. *Flavour and Fragrance Journal* 10: 281-284

Rejesus BM, HA Maini & K Ohsawa. 1990. Insecticidal action of several plants to *Callosobruchus chinensis*. In **Bruchids And Legumes Economics Ecology And Coevolution**, AMR Fujik-Gatehense, CD Johnson, R Michel & T Yoshida (ed), Kluwer Academic Publisher, pp 91-100

Rosenthal GA. 1986. The chemical differences of higher plants. *Scientific American* 254: 76-81

Svoboda KP, J Gough, J Hampson & B Galambosi. 1995. Analysis of the essential oils of some *Agastache* species grown in Scotland from various seed sources. *Flavour and Fragrance Journal* 10: 139-145