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**Research Article**

# Chemical constituents and cytotoxic effects of the essential oil from the root of *Cyperus kyllingia* Endl.

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**Abstract** - The chemical constituents of the essential oil from the root of *Cyperus kyllingia* Endl. were obtained from hydrodistillation and subsequently analyzed by a gas chromatography/mass spectrometry (GC-MS) technique. Twenty-eight compounds were identified, accounting for 89.62% of the total oil that consisted mainly of oxygenated sesquiterpenes (50.21%) and sesquiterpene hydrocarbons (38.08%). α-Cadinol (18.62%), caryophyllene oxide (12.18%), α-muurolol (11.56%), Cyperene (10.15%), and α-Cyperone (5.72%) were identified as the major components. The essential oil showed significantl cytotoxicity against NCI-H187, and MCF-7 cell lines with $IC_{50}$ values of 6.7 and 13.3 μg/mL, and was non-cytotoxic against Vero cells. Furthermore, the oil was active against *Mycobacterium tuberculosis* with a MIC value of 25.0 μg/ mL.

**Keywords:** *Cyperus kyllingia* Endl., essential oil, anticancer activity, cytotoxicity, anti-TB activities

## 1. Introduction

Continued searching for new and more effective anticancer drugs is urgently needed due to the high worldwide mortality rates and the expected continuing increase in numbers of new cancer cases (Siegal *et al*., 2018). Essential oils provide great molecular diversity and biological functionality, which are indispensable for novel drug discovery. Essential oils have demonstrated numerous biological activities, such as antibacterial, antioxidant, anti-inflammatory and anticancer activities (Bakkali *et al*., 2008; Dhifi Wissal *et al*., 2016).

*Cyperus kyllingia* Endl. belongs to the family Cyperaceae, *Cyperus* genus. It is commonly found in tropical regions of Asia and well distributed over all parts of the world (Seo *et al*., 2001). This species is widely used in traditional medicine for fever, diuretic, emollient, stimulant, analgesic, stomachic, anthelmintic and expectorant activities (Feizbakhsh and Naeemy, 2011). Pharmacological and biological activities including antidiarrhoeal, antidiabetic (Somasundaram *et al*., 2010) antibacterial, anticancer and antimalarial activities (Khamsan *et al*., 2011) have been reported for this plant.

The previous phytochemical studies on *Cyperus* species revealed the presence of alkaloids (Jeong *et al*., 2000), flavonoids (Harborne *et al*., 1982), tannins, triterpenoids, sesquiterpenoids (Xu *et al*., 2008, Thebtaranonth *et al*., 1995) and glycosides (Sayed *et al*., 2007). The chemical composition of essential oil from this species has been studied by several authors and the major compounds were α-cyperone, cyperene, cyperotundone and β-selinene, along with other components including α-copaebe, caryophyllene oxide, valernal, pachoulenyl acetate and sugeonyl acetate (Lawal and Oyedeji, 2009).

The screening of biological activity of essential oils from Thai medicinal plants as therapeutic agents for treatment of cancer is the focus of our ongoing research program. The aims of this present work are to investigate the essential oil compositions and its biological activities.

## 2. Materials and methods

### 2.1 Plant material

The plant material was identified in 2008 by J. F. Maxwell. A voucher specimen was deposited at the Herbarium of Biology department, Chiang Mai University, Chiang Mai, Thailand (Number S. Khamsan 2). The fresh root parts of *C. kyllingia* were collected from Phayao province, Thailand.

The fresh roots (500 g) were washed with distilled water, chopped into small pieces and subjected to hydrodistillation for 6 hours in a modified Clevenger-type apparatus. The oil was collected, dried over anhydrous $Na_2SO_4$ and stored at 4°C for further analysis.

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**2.2 Analysis of the Essential Oil**

The essential oil was analyzed on a Hewlett-Packard (Agilent Tecnology GC 7890A) gas chromatograph equipped with HP-5 (HP 19091J-433E) fused silica capillary column 30 m X 0.25 mm, 0.25 $\mu$m film thickness (composed of 5% phenyl methyl polysiloxane) for the volatile components separation, temperature programmed as follows: 80°C held for 4 min, then to 260°C at 8°C /min for 20 min. High purity helium was use as the carrier gas with a constant flow rate at 1.00 mL/min; injector port and detector temperatures were 250 and 280°C, respectively. Samples were injected by splitting and the split ratio was 100:1. GC/MS analysis was performed on Hewlett-Packard 6850GC coupled with a Hewlett-Packard 5973N mass selective detector under the same conditions as for GC. Significant quadrupole MS operating parameters: interface temperature 240°C; electron impact ionization at 70 eV with scan mass range of 35-550 m/z at a sampling rate of 1.0 scan/s. The volatile components were identified by comparison of their retention indices (determined relatively to *n*-alkane series with those given in the literature and their mass spectra authentic samples, which were compared with those libraries of mass spectral data (Wiely7n.1, NIST mass spectral Database (2008) and W8NO8 library).

**2.3 Determination of Anticancer Activity**

The anticancer activities of the essential oil against the cancerous human-cell lines. - MCF-7 cell line (breast adenocarcinoma, ATCC HTB-22) and NCI-H 187 cell line (small cell lung carcinoma, ATCC CRL-5804) were assayed employing the Resazurin microplate assay (REMA) as described by Brien *et al*. (2000), with suitable modification. In brief, cells at a logarithmic growth phase were harvested and diluted to 9x104 cells/mL, in fresh medium. Successively, 5 $\mu$L of the essential oil diluted in 5% DMSO, and 45 $\mu$L of cell suspension were added to 384-well plates, incubated at 37ºC in 5% $CO_2$ incubator. After the incubation period (5 days), 12.5 $\mu$L of 62.5 $\mu$g/mL resazurin solution was added to each well, and the plates were then incubated at 37ºC for 4 hours. Fluorescence signal was measured using SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 and 590 nm, respectively. Percent inhibition of cell growth was calculated by the following equation.

$$\%\text{Inhibition} = [1-(\text{FUT}/\text{FUC})]*100 \quad (1)$$

Whereas FUT and FUC are the mean fluorescent unit from treated and untreated conditions, respectively. Dose response curves were plotted from 6 concentrations of 2-fold serially diluted test compounds and the sample concentrations that inhibit cell growth by 50% ($IC_{50}$) can be derived using the SOFTMax Pro software (Molecular Devices, USA). Ellipticine and Doxorubicine were used as positive controls.

**2.4 Determination of Cytotoxicity Assay against Vero cell**

The cytotoxicity against primate cell line (Vero) of the extract was assayed by using Green fluorescent protein (GFP) detection described by Hunt *et al*. (1999), with suitable modification. In brief, the GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line (Vero, ATCC CCL-81), with pEGFP-N-1 plasmid (Clontech). The cell line was maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate and 0.8 mg/mL geneticin, at 37 ºC in a humidified incubator with 5% $CO_2$. The assay was carried out by adding 45 $\mu$L of cell suspension at $3.3x10^4$ cells/mL to each well of 384-well plates containing 5 $\mu$L of test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days in 37ºC incubator with 5% $CO_2$. Fluorescence signals were measured by using SpectralMax M5 microplate reader (Molecular Devices, USA) in the bottom reading mode with excitation and emission wavelengths of 485 and 535 nm, respectively. Fluorescence signal at day 4 was subtracted with background fluorescence at day 0. The percentage of cytotoxicity was calculated by the following equation (Equation 2), where FUT and FUC represent the fluorescence units of cells treated with test compound and untreated cell, respectively.

$$\%\text{ cytotoxicity} = [1-(\text{FUT}/\text{FUC})] \times 100 \quad (2)$$

The $IC_{50}$ values were derived from dose-response curves, using 6 concentrations of 2-fold serially diluted samples, by the SOFTMax Pro software (Molecular device). 0.5% DMSO was used as a negative control. Ellipticine and doxorubicine were used as positive controls.

**2.5 Antimycobacterial assay**

Antituberculosis activity of the essential oil was evaluated against *M. tuberculosis* $H_{37}$Ra using the green fluorescent protein microplate assay (GFPMA) as described previously (Colins *et al*., 1998). The standard drugs, rifampicin, streptomycin, isoniazid and ofloxacin were used as the standard reference compounds.

**3. Results and Discussion**

The study aimed to investigate the volatile components of the essential oil obtained from hydrodistillation of the root parts of *C. kyllingia* with a yield of 0.11% (w/w). The composition of this oil was analyzed by GC (FID) and GC-MS. Table 1 shows the results of the qualitative and quantitative essential oil analyses listed in order of elution in the HP-5, a nonpolar type phase column. The components identified from the essential oil, their retention indices and percentage composition are summarized in Table 1. In total, 28 compounds were identified, corresponding to 89.62% of the oil that consisted mainly of oxygenated sesquiterpenes (50.21%), and sesquiterpene hydrocarbons (38.08%). The most representative compounds were α-cadinol (18.62%), caryophyllene oxide (12.18%), α-muurolol (11.56%), cyperene (10.15%), and α-cyperone (5.72%) (Fig. 1).

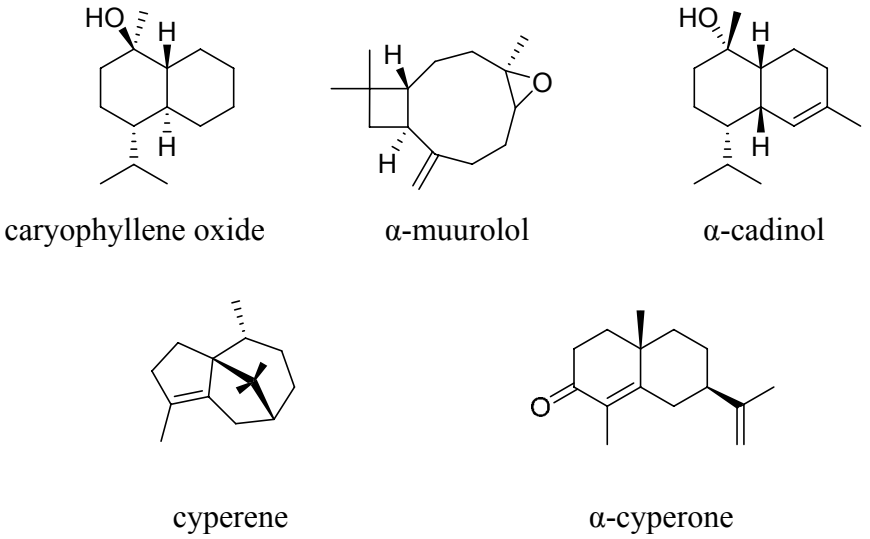


**Figure 1.** Major constituents of essential oil from the root parts of *C. kyllingia*.

The chemical compositions of essential oil of other *Cyperus* species have been studied. The oils of the underground parts of *C. brevifolius* and *C. kyllingia* f. Humulis are rich in terpenes including α-cyperone, β-selinene and α-humulene (Komai and Tang, 1989). The volatiles of *C. giganteus* were investigated and analyzed by GC and GC/MS and cyperotundone and cyperene were identified as the major components (Zoghbi *et al*., 2006). Cyperene (25.9%) and caryophyllene oxide (10.4%) were the major constituents of *C. compressus* root oil (Rameshkumar *et al*., 2011). More recently, Khamsan *et al*. (2011) analyzed the essential oil of aerial parts of *C. kyllingia* finding that the most representative compounds were α-cadinol (19.3%), caryophyllene oxide (12.2%), α -muurolol (11.6%), α-humulene (9.9%), and α-atlantone (6.1%) (Khamsan *et al*., 2011).

The essential oil showed significant anticancer activity against NCI-H187 and MCF-7 cell lines with $IC_{50}$ values of 6.7 and 13.3 $\mu$g/mL respectively and was non-cytotoxic against Vero cells (Table 2). The chemical compositions indicated that high sesquiterpene contents in the essential oil were responsible for its anticancer activity. The anticancer activity of some sesquiterpenes in the essential oil has been reported in the literature that α-cadinol is the highest representative compound in *C. kyllingia*. Essential oil shows selective toxicity against human colon adenocarcinoma cell lines HT-29 (He *et al*., 1997). Furthermore, α-humulene is active against A-549 and DLD-1 cell lines with the $IC_{50}$ value of 62±2 and 71±2 $\mu$M, respectively (Legault *et al*., 2003) and β-caryophyllene exhibited against cancer cell lines (Sylvestre *et al*., 2006). Therefore, the essential oil of *C. kyllingia* might be a possible new anticancer drug. The anti-tuberculosis activity of the essential oil was also evaluated against *Mycobacterium tuberculosis* $H_{37}$Ra. The essential oil showed activity against *M. tuberculosis* $H_{37}$Ra strain with MIC value of 25.0 $\mu$g/mL.

**Table 1.** Chemical constituents from the root of *C. kyllingia* essential oil.

| Identified Compounds | RI[a] | percentage[b] | Identification[c] |
|---|---|---|---|
| α-Pinene | 935 | 0.86 | RI, MS |
| β-Pinene | 979 | 0.43 | RI, MS |
| β-Myrcene | 990 | 3.65 | RI, MS |
| α-Cubebene | 1353 | 1.51 | RI, MS |
| α-Copaene | 1370 | 0.28 | RI, MS |
| β-Elemene | 1387 | 0.15 | RI, MS |
| Cyperene | 1397 | 10.15 | RI, MS |
| β-Caryophyllene | 1415 | 0.81 | RI, MS |
| α-Gurjunene | 1431 | 5.63 | RI, MS |
| α-Humulene | 1464 | 3.27 | RI, MS |
| γ-Muurolene | 1477 | 2.13 | RI, MS |
| Rotundene | 1490 | 3.26 | RI, MS |
| β-Selinene | 1485 | 0.15 | RI, MS |
| α-Selinene | 1491 | 0.21 | RI, MS |
| α-Muurolene | 1498 | 0.25 | RI, MS |
| γ-Cadinene | 1500 | 0.11 | RI, MS |
| (Z)-Calamenene | 1509 | 0.02 | RI, MS |
| β-Calamenene | 1514 | 0.78 | RI, MS |
| -Cadinene | 1528 | 4.43 | RI, MS |
| Germacrene B | 1546 | 0.45 | RI, MS |
| Caryophyllene oxide | 1572 | 12.18 | RI, MS |
| Humulene-1,2-epoxide | 1609 | 1.12 | RI, MS |
| α-Muurolol | 1627 | 11.56 | RI, MS |
| α-Cadinol | 1639 | 18.62 | RI, MS |
| α-Atlantone | 1679 | 0.56 | RI, MS |
| α-Cyperone | 1771 | 5.72 | RI, MS |
| Methyl hexadecanoate | 1929 | 0.92 | RI, MS |
| Palmitic acid | 1940 | 0.41 | RI, MS |
| Sesquiterpene hydrocarbons | | 38.08 | |
| Oxygenated sesquiterpenes | | 50.21 | |
| Ester | | 0.92 | |
| Carboxylic acid | | 0.41 | |
| **Total** | | 89.62 | |

[a]RI retention indices; relative to n-alkane series (C7-C30)
[b]Results obtained by peak area normalization
[c]Methods of identification: MS, comparison of the mass spectrum with MS libraries; RI of literature

**Table 2.** Cytotoxicity of *C. kyllingia* essential oil on human cancer cell lines.

| | $IC_{50}$[a] (µg/mL) | | |
|---|---|---|---|
| **Sample** | **Vero cells** | **MCF-7** | **NCI-H187** |
| essential oil | Inactive[b] | 13.3 | 6.7 |
| Ellipticine[c] | 1.3 | - | 1.2 |
| Doxorubicine[d] | - | 1.2 | 0.06 |

[a]Concentration that killed 50% of cell lines
[b]Inactive at 50 $\mu$g/ mL
[c,d]Anticancer drugs used as positive controls

## 4. Conclusions

In conclusion, the chemical constituents of the essential oil of *C. kyllingia* Endl. were analyzed by GC, GC-MS and contained both oxygenated sesquiterpenes (50.21%) and sesquiterpene hydrocarbons (38.08%). The essential oil of *C. kyllingia* Endl. demonstrated promising selective cytotoxic activity against human cell lines and was non-cytotoxic against Vero cells. Furthermore, the essential oil showed activity against the *M. tuberculosis* $H_{37}$Ra strain.

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