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Antimalarial, Anticancer, Antimicrobial Activities and Chemical Constituents of Essential Oil from the Aerial Parts of *Cyperus kyllingia* Endl.

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Abstract: The chemical constituents of the essential oil from *Cyperus kyllingia* Endl. were analyzed by a GC, GC-MS. Twenty-three compounds were identified, accounting for 93.75% of the total oil that consisted mainly of oxygenated sesquiterpenes (53.52%), particularly sesquiterpene hydrocarbons (38.97%), and carboxylic acid (1.26%). The most representative compounds were α-cadinol (19.32 %), caryophyllene oxide (12.17%), α-muurolol (11.58 %), α-humulene (9.85%), and α-atlantone (6.07%). The oil showed significant activities against *Plasmodium falcipalum* (K1, multi drug resistant strain) and NCI-H187 (Small Cell Lung Cancer) with the IC_{50} values of 7.52 and 7.72 µg/mL, respectively. The oil exhibited highly active against *Staphylococcus aureus* ATCC25923 and moderately active against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27553, *Aspergillus flavus* and *Candida albicans*.

Keywords: *Cyperus kyllingia* Endl.; essential oil; antimalarial activity; anticancer activity; antimicrobial activity

1. Plant Source

Cyperus kyllingia Endl. (Cyperaceae) is commonly found in tropical regions of the world. According to ethnomedical investigation this plant was found to have numerous biological activities, such as antidiarrhoeal, diuretic, stomachic, anthelmintic and expectorant activities. It is also used in fever, hepatopathy, splenopathy, diabetes and tumors [1].

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The aerial parts of *C. kyllingia* Endl. were collected in July 2008 from Chiang Mai University, Thailand. The plant material was identified by J. F. Maxwell. A voucher specimen was deposited at the Herbarium of Chiang Mai University, Chiang Mai, Thailand (No. S. Khamsan 2).

2. Previous Studies

The oils of *C. brevifolius* and *C. kyllingia* f. Humulis underground parts contain terpenes including α-cyperone, β-selinene and α-humulene [2]. More recently, the phytochemical investigation of the plant was found that the ethanolic extract of the rhizomes possesses flavonoids, triterpenoids and glycosides. The petroleum ether extract was found to possess triterpenoids and glycosides [1].

There is no report on chemical investigation and biological activities of the essential oil from *C. kyllingia* Endl.. This is the first report on chemical constituents and its biological activity.

3. Present Study

The fresh aerial parts (500 g) were subjected to hydrodistillation in a modified Clevenger-type apparatus for 4 h. The oil was collected, dried over anhydrous Na_2SO_4 and stored at 4°C for further analysis.

The oil was analyzed on a Hewlett-Packard GC-6850 gas chromatograph (FID) equipped with a HP-1 (HP 19091S-933E) fused silica capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, programming from 80°C (4 min) to 260°C at 8°C/min, 20 min hold, ending at 260°C (15 min); carrier gas He, constant flow rate of 20.0 mL/min; injector port and detector temperature were 250°C and 280°C, respectively. Samples were injected by splitting mode (1:20).

The 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; carrier gas He, constant flow rate of 1.0 mL/min and the split ratio was 1:20.

The components of the oil were identified by comparing of their retention indices (RI) relative to n-alkane indices on HP-1 column and by a comparison of mass spectra from libraries (Wiely7n.1 and NIST) with literature data [5-9].

Antimalarial activity against *P. falcipalum* (K1) was determined by microculture radioisotope techniques (Desjardins *et al.*) [3]. The anticancer activity against the NCI-H187 was performed using the method described by Brien *et al.* [4]. The preliminary antimicrobial activities were also evaluated using the agar diffusion method. The microorganisms used were: *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27553, *Candida albican, Aspergillus flavus* and *Trichophyton mentagrophyte*.

The qualitative and quantitative analysis of the essential oil were listed in order of elution in the HP-1 column (Table 1) with a yielded of 0.26% (w/w). In total, 23 compounds were identified, corresponding to 93.75% of the total oil that consisted mainly of oxygenated sesquiterpenes (53.52%) particularly sesquiterpene hydrocarbons (38.97%), and carboxylic acid (1.26%) including α-cadinol (19.32%), caryophyllene oxide (12.17%), α-muurolol (11.58%), α-humulene (9.85%), and α-atlantone (6.07%) as the major components.

The oil showed significant activities against *P. falcipalum* (K1) and NCI-H187 (Small Cell Lung Cancer) with the IC_{50} values of 7.52 and 7.72 μg/mL, respectively (Tabel 2). The potent activities of the oil might be attributable to its high sesquiterpene content (92.49%). The bioactivity of some sesquiterpenes present in this essential oil has been reported that α- cadinol, α-humulene and β–caryophyllene exhibited anticancer activity [10].

Table 1 Chemical constituents of *C. kyllingia* Endl. essential oil

Compound	HP1[a]	DB5[b]	%	Identification[c]	References
Sesquiterpene hydrocarbons					
α–Cubebene	1353	1351	1.11	RI, MS	[5] Fraternale *et al.*, *Fitoterapia*. (2007) **78**, 443–335.
α–Copaene	1370	1377	1.01	RI, MS	[6] Vian *et al.*, *J. Chromatogr. A.* (2008) **1190**, 14–17.
β–Bourbonene	1379	1388	0.74	RI, MS	[6]
β–Elemene	1387	1391	3.78	RI, MS	[6]
β–Caryophyllene	1415	1419	1.43	RI, MS	[6]
α–Humulene	1464	1455	9.85	RI, MS	[5]
γ–Muurolene	1474	1477	0.86	RI, MS	[5]
Germacrene D	1493	1480	5.51	RI, MS	[5]
β–Selinene	1496	1485	2.26	RI, MS	[5]
α–Valencene	1490	1496	3.09	RI, MS	[5]
α–Muurolene	1498	1500	2.26	RI, MS	[7] Bendimerad *et al.*, *J. Agric. Food Chem.* (2005) **53**, 2947–2952.
γ-Cadinene	1500	1511	0.63	RI, MS	[7]
(Z)-Calamenene	1509	1512	0.76	RI, MS	[8]
δ–Amorphene	1519	1518	4.20	RI, MS	[8]
δ–Cadinene	1528	1523	1.48	RI, MS	[5]
Oxygenated sesquiterpenes					
Spathulenol	1567	1578	0.81	RI, MS	[6]
Caryophyllene oxide	1572	1581	12.17	RI, MS	[6]
T–Muurolol	1627	1608	1.8	RI, MS	[8] Boyom *et al.*, *Phytochemistry.* (2003) **64**, 1269–1279.
α– Muurolol	1628	1645	11.58	RI, MS	[8]
α– Cadinol	1639	1653	19.32	RI, MS	[8]
α–Atlantone	1679	1717	6.07	RI, MS	[8]
Farnesol	1691	1746	1.77	RI, MS	[8]
Carboxylic acid					
Palmitic acid	1940	1963	1.26	RI, MS	[7]
Sesquiterpene hydrocarbons			38.97		
Oxygenated sesquiterpenes			53.52		
Carboxylic acid			1.26		
Total			93.75		

[a]RI retention indices on HP1 column; relative to n-alkane, [b]RI retention indices on DB5 column [9]: Adams (2001), [c] Methods of identification: MS, comparison of the mass spectrum with MS libraries; RI of literature.

Table 2 Antimalarial and anticancer activity of *C. kyllingia* Endl. essential oil

Sample	IC_{50}[a] (µg/mL)	
	P. falciparum (K1)	NCI-H187
C. kyllingia Endl. oil	7.52	7.72
Dihydroartemisinin[b]	0.0012	-
Ellipticine[c]	-	1.18
Doxorubicine[d]	-	0.058

[a]Concentration that killed 50% of cell lines, [b]Antimalarial drug used as positive control
[c,d] Anticancer drug used as positive control

The oil possessed strong antimicrobial activity against *S. aureus* and showed moderately active against all bacteria and fungi strains with their respective diameter of the inhibition zones (Table 3), but did not inhibit the growth of *T. mentagrophyte*.

According to this result the essential oil of *C. kyllingia* Endl. exhibited significant antimalarial, anticancer and antimicrobial activites. Thus, essential oil from this plant might be another potential source for the discovery of new drugs to treat infectious diseases.

Table 3 Antimicrobial activity of *C. kyllingia* Endl. essential oil

Sample test	Concentration	Zone of inhibition (mm)[a]					
		Bacteria strains			Fungi strains		
		E. coli	*S. aureus*	*P. aeruginosa*	*A. flavus*	*C. calbican*	*T. mentagrophyte*
Oil	10 mg/mL	15	25	15	12	16	-
10% DMSO[b]	-	-	-	-	-	-	-
Gentamicin[c]	75 µg/mL	27	35	27	-	-	-
Ketoconazole[d]	250 µg/mL	-	-	-	25	37	16

[a] Diameter of inhibition zones (mm); [b]Negative control; [c,d]Antibiotic used as positive control

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