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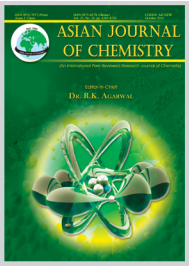

Chemical and Biological Studies of *Cyperus alternifolius* Flowers Essential Oil

AMAL H. AHMED

Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Corresponding author: E-mail: a-elmerigy@hotmail.com

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The hydro-distilled volatile oil of the flowers of *Cyperus alternifolius* (Family: Cyperaceae) was analyzed using GC and GC/MS for the first time. Forty compounds representing *ca.* 98 % of the oil were characterized. Sesquiterpenes constituted the bulk of the oil (*ca.* 62 %); the major components of the oil were α-cyperone 19.6 %, β-selinene 9.8 %, caryophyllene oxide 7.2 % and cyperene 5.2 %. Antioxidant activity of the volatile oil was tested using a DPPH free radical assay and found to exhibit a significant antioxidant activity.

Key Words: *Cyperus alternifolius*, Cyperaceae, Essential oil, α-Cyperone, β-Selinene, Caryophyllene oxide, Cyperene, Antioxidant activity.

INTRODUCTION

Cyperus is a large genus of about 600 species of sedges, distributed throughout all continents in both tropical and temperate regions[1,2]. They are annual or perennial plants, mostly aquatic and growing in still or slow-moving water up to 0.5 m deep[1-4]. The species vary greatly in size, with small species only 5 cm tall, while others can reach 5 m in height[4-8].

Cyperus alternifolius (umbrella papyrus, umbrella sedge or umbrella palm) is a grass-like plant in the very large genus *Cyperus* of the sedge family, *Cyperaceae*[6-8]. It is native to Madagascar[9,10], but is frequently cultivated worldwide as an ornamental or pot plants, it can grow to 3-5 feet if given plenty of water[5,6,11], the flowers are a brownish spadix produced in the centre of the leaves[11-13]. Traditionally, *C. alternifolius* leaves, stem & roots are used as aphrodisiac and stimulating[11,14]. Study of root and leaf extracts of *Cyperus alternifolius* showed moderate activity against yeast, while the root extract showed a clear antioxidant activity. It showed strong activity against both sensitive and resistant *S. aureus* strains and against *E. faecalis*[15,16]. The aim of this work is to investigate the chemical composition of volatile oil of the flowers of *Cyperus alternifolius* as well as its antioxidant activity.

EXPERIMENTAL

Fresh flowers of *Cyperus alternifolius* were collected at September 2009 from El-Orman garden, Giza, Egypt. The plant was kindly authenticated by Eng. Teresa (Agriculture Engineer at El-Orman botanical garden).

Isolation of volatile components: Fresh flowers (100 g) were subjected to hydro-distillation; the yield (v/w) of volatile oil was 0.2 %. The volatile oil was dried over anhydrous sodium sulfate and stored at 4 ºC for analysis.

Gas chromatography: Gas chromatography analysis were performed on an Orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with CP-Sil 5 and CP-Sil 19 (fused silica, 25 m × 0.25 mm, 0.15 µm film thickness) and flame ionization detector (FID). The volume injected was 0.2 µL and the split ratio was 1:30. Oven temperature was programmed from 50-230 ºC at 3 ºC/min using hydrogen as carrier gas. Injection and detector temperatures were maintained at 200 and 250 ºC, respectively. Qualitative data were obtained by electronic integration of FID area percents without the use of correction factors.

Gas chromatography/mass spectrometry: A Hewlett-Packard HP5890A GC, interfaced with a VG Analytical 70-250s double focusing mass spectrometer was used. Helium was the carrier gas at 1.2 mL/min. The MS operating conditions were: ionization voltage 70 eV, ion source 230 ºC. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15 µm. The GC operating conditions were identical with those of GC analyses. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the oils were computed in each case from GC peak areas. The identification of the components was based on comparison of retention indices (determined relative to the retention times of series of *n*-alkanes) and mass

spectra with those of authentic samples and with data from literature[17-19].

Determination of antioxidant activity

Scavenging activity of DPPH free radical by TLC: To measure the antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay by TLC was used[20]. Briefly, 10 µL of each extract (1 mg mL^{-1} in ethanol) was applied to a chromatographic plate. Chromatography was conducted using ethyl acetate:acetic acid:formic acid:water (100:11:11:27) as eluent. The plate was developed using a DPPH solution (2 mg mL^{-1} in ethanol); after 0.5 h, the yellow spots from reduced DPPH were clearly observed against a purple background.

Scavenging activity of DPPH free radical by spectrophotometry[21]: 1 mL of 0.135 mM DPPH prepared in ethanol was mixed with 1 mL of aqueous extract ranging from 0.2-0.8 mg/mL. The reaction mixture was vortexed and left in dark at room temperature for 0.5 h. The absorbance was measured spectrophotometrically at 517 nm. Quercetin was used as a positive control. The capacity to scavenge the DPPH radical was calculated as follows:

$$\text{Radical scavenging activity (\%)} = \frac{(A - B)}{A} \times 100$$

where, A is the absorbance of the negative control (DPPH plus ethanol) and B is the absorbance of the sample (DPPH, ethanol plus sample). The correlation between each concentration and its percentage of scavenging was plotted and its percentage of scavenging was plotted and the EC_{50} was calculated by interpolation. The activity was expressed as EC_{50} (the effective concentration that scavenges 50 % of DPPH radicals).

RESULTS AND DISCUSSION

The volatile oil of the flowers of *Cyperus alternifolius* was obtained by hydro-distillation and was analyzed by GC and GC/MS. The compounds were identified by comparison retention times with those reported in literature[17-19], wherever possible, by co-injection with authentic sample and by matching their fragmentation patterns in mass spectra with published mass spectra[22].

Table-1 shows the retention indices, relative percentages and identities of the constituents of the oil. A total of 40 compounds representing about 98 % of the flower oil were identified from their mass spectra. Quantitatively the oil was characterized by the abundance of sesquiterpenes (*ca.* 62 %) than monoterpenes (*ca.* 32 %); the major sesquiterpenes were found to be α-cyperone 19.6 %, β-selinene 9.8 %, caryophyllene oxide 7.2 %, cyperene 5.2 %. Other sesquiterpene compounds that existed in appreciable proportions of the oil included aristolone 2.3 %, caryophylla-3,8(13)-diene-5-β-ol 2.4 %, oplopenone 2.4 %, aristolone 2.3 % humulene epoxide 2 %, patchoulenyl acetate 2 %, oxo-α-ylangene 1 %, α-gurjuene 0.2 %, spathulenol 0.3 % and globulol 0.6 %, major monterpenes were β-pinene 8.2 %, geraniol 5.3 %, α-pinene 3.5 % and myrtenol 3.4 %.

To test antioxidant activity, volatile oil was analyzed by a DPPH free radical assay using TLC (thin layer chromatography). Oil displayed a strong antioxidant activity on the chromatographic plate. The oil was further tested for its scavenging activity using a DPPH spectrophotometric assay. The percentage reduction of DPPH radical exhibited by the different concentrations of volatile oil and quercetin as a positive control was calculated (Table-2). The oil showed appreciable free radical scavenging activities at the highest concentrations of 0.8 mg/mL on DPPH. All activities followed a concentration dependent manner.

TABLE-1
CHEMICAL COMPOSITION (%) OF THE FLOWER OIL OF *Cyperus alternifolius*

Compound	RI	Composition (%)
α-Thujene	925	0.6
α-Pinene	935	3.5
Camphene	952	Traces
β-Pinene	978	8.2
Myrcene	991	0.5
Sabinene	971	1
α-Phellandrene	1001	Traces
P-Cymene	1026	1
1,8-Cineole	1029	0.8
Terpinolene	1082	0.6
Perillene	1096	0.1
Camphene hydrate	1147	0.2
Borneol	1167	0.2
Terpinen-4-ol	1178	0.9
Myrtenol	1202	3.4
Verbenone	1209	0.5
Geraniol	1268	5.3
Eugenol	1354	1.2
α-Copane	1375	0.5
β-Elemene	1391	0.4
Cyperene	1397	5.2
β-Caryophyllene	1418	0.9
α-Gurjuene	1431	0.2
Germacrene D	1479	1.2
β-Selinene	1484	9.8
α-Selinene	1491	3.7
Germacrene B	1546	1.1
Acetyl eugenol	1553	0.4
Spathulenol	1572	0.3
Caryophyllene oxide	1584	7.2
Humulene epoxide	1601	2
Oplopenone	1608	2.4
Globulol	1623	0.6
Patchenol	1628	2.1
Caryophylla-3,8(13)-diene-5-β-ol	1642	2.4
Caryophyllenol11	1661	4.8
Aristolone	1752	2.3
α-Cyperone	1771	19.6
Patchoulenyl acetate	1775	2
Oxo-α-ylangene	1779	1

TABLE-2
SCAVENGING EFFECTS OF *Cyperus alternifolius* ESSENTIAL OIL AND QUERCETIN (POSITIVE CONTROL) ON DPPH AT DIFFERENT CONCENTRATIONS

Oil (mg/mL)	CE_{50} (µg/mL)	Quercetin (mg/mL)	CE_{50} (µg/mL)
0.2	8.4 ± 1.0	0.2	4.9 ± 1.0
0.4	7.9 ± 1.3	0.4	3.8 ± 1.3
0.6	6.5 ± 2.0	0.6	3.2 ± 1.0
0.8	5.6 ± 2.9	0.8	3.0 ± 1.3

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