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CHEMICAL CONSTITUENTS AND ANTI-INFLAMMATORY ACTIVITY OF LEAF ESSENTIAL OIL OF NIGERIAN GROWN *Chenopodium album* L.

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ABSTRACT

Hydrodistilled leaves of *Chenopodium album* yielded 0.64 % v/w of essential oil. GC and GC/MS analyses of the oil revealed that the bulk of the oil was constituted by aromatic compounds (60.1 %). The abundant constituents of the oil were: p- cymene (40.9 %), ascaridole (15.5 %), pinane-2-ol (9.9 %), α-pinene (7.0 %), β-pinene (6.2 %) and α-terpineol (6.2 %). The oil displayed strong anti-inflammatory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA) – induced ear edema in mice.

Keywords: *Chenopodium album*; chenopodiaceae; p-cymene; ascaridole; pinane-2-ol; α-terpineol; anti-inflammatory

INTRODUCTION

Chenopodium album L. (family Chenopodiaceae) is an annual shrub widely grown in Europe, North America, Asia, and Africa. It is commonly known as pigweed, fat hen or lamb-quarters' (Bailey, 1977; GRIN Database, 2005). The plant is used in folk medicine in different parts of the world. As therapeutic agents, it is used as laxative, antihelmintic against round and hook worms and blood purifier. Its use for the treatments of hepatic disorders, spleen enlargement, intestinal ulcers and burns has also been documented (Sarma et al., 2008).

Various bioactivities such as antifungal (Tahara et al., 1994 Maruta et al., 1995), antipruritic, antinociceptive (Dai et al., 2002) and hypotensive (Gohar and Elmazar, 1997) properties of crude and isolated compounds from the plant justified its uses in traditional medicine. Phytochemical analyses revealed the presence of alkaloids (Horio et al., 1993; Cutillo et al., 2004), apocarotenoids (DellaGreca et al., 2004), flavonoids (Gohar and Elmazar, 1997), phytoecdysteroids (Dinan, 1992; Dinan et al., 1998; DellaGreca et al., 2005a) and an unusual xyloside (DellaGreca et al., 2005b) in the plant. Earlier work on the leaf essential oil of East Mediterranean grown *C. album* revealed the abundance of Limonene (23.2 %), α-terpinyl acetate (13.7 %), α-terpinene (12.3 %) and cis-ascaridole (12.2 %) in the oil (Dembitsky et al., 2008).

It has been established that geographical and agroclimatic conditions could affect the composition pattern of essential oil of a par-

ticular plant and therefore its biological activities (Lahlou, 2004). Based on these factors, we investigate the leaf essential oil of Nigerian grown *C. album* and its anti-inflammatory activity.

EXPERIMENTAL

Plant materials

The fresh leaves of *Chenopodium album* were obtained in Ilorin, Kwara State, North Central, Nigera. Identification was carried out at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, where voucher specimens were deposited.

Oil isolation

Pulverized leaves of *Chenopodium album* were hydrodistilled for 3 h in a Clevenger-type apparatus according to the British Pharmacopoeia (1980) specification. The resulting oil was collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas chromatography

GC 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° C, 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 (HP 5890A) 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 temperature 230° C. 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 analysis. The MS data were acquired and processed by online desktop computer equipped with disk memory. The percentage compositions of the oil were computed in each case from GC peak areas. The identification of the components was based on the 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 (Jennings and Shibamito, 1980; Adams 1995; Joulain and Koenig, 1998).

Anti-inflammatory activity of leaf essential oil of C. album

The animal experiment were approved by the Department of Biochemistry, University of Ilorin, Animal Care and Use Committee and conducted according to standard guidelines. Sixty-four, 21-day old male swiss Webster mice, weighing between 22 and 25 g were housed in groups of 8 in an NIH – approved facility. All groups were fed with standard rodent diet (Test diet 570B, Purina Mills, St. Louis, MO) and supplied water *ad libitum*, throughout the experimental period. The light in the facility were turned off between 1900 and 0700 h, with the environmental temperature maintained at 24 ± 1° C. All experimental procedures were conformed to the National Institute of Health, Public Health Service and Animal Welfare Act guidelines for the ethical treatment of laboratory animals.

Topical anti-inflammatory assay

A modification of the method of Young et al. (1984) was used. The topical anti-inflammatory activity was evaluated as inhibition of the 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ear edema in mice by the oils following standard procedure (Young et al., 1984; Griffiths et al., 1988; Bralley et al., 2008). Edema was induced in ears of each mouse by the topical application of 2 µg TPA dissolved in 20 µl of acetone to both the inner and outer sur-

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faces of the right ear (surface: about 1 cm^2). Thirty minutes after the application of TPA, the inner and outer surfaces of each ear were treated (10 ul to each side) with:

1. 50 % ethanolic solutions of the test essential oil (EO) in doses of 0.625 (CA4), 1.25 (CA3), 2.5 (CA2) and 5.0 (CA1) mg eo/ear (n = 8 at each dosage)
2. 50 % ethanol (vehicle control)
3. Indomethacin (0.25 mg/ear dissolved in 50 % ethanol (as a standard anti-inflammatory drug).

The thickness of each ear was measured using a micrometer (Mitutoyo Series IP65, Mitutoyo America, Aurora, IL) before and at 4 h and 24 h after tetradecanoylphorobol-13-acetate administration. The micrometer was applied near the top of the ear distal to the cartilaginous ridges. At 24 h, each animal was sacrificed and a plug biopsies (6 mm diameter hole punch) were removed from both the treated (right) and the untreated (left) ears immediately, weighed, frozen and stored at 8° C. The edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percentage of the edema reduction in treated mice compared to the control mice. The pharmacological data were analyzed by the Student's t-test, and a probability level lower than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Pulverized leaves of C*henopodium album* afforded oil in the yield of 0.64 % v/w. Table 1 shows the retention indices, mass spectra data, identities and relative percentage of the constituents of the oil. A total of 37 compounds that represent 95.6 % of the oil were identified from their mass spectra. Hydrocarbon and oxygenated monoterpenes constituted 17.4 and 18.1 % of the oil. The percentage composition of aromatic compounds in the oil was 60.9 %.

The oil was characterized by the abundance of aromatic compounds. Bulk of the aromatic compounds was constituted by p-cymene (40.9 %). Other notable aromatic compounds in the oil were ascaridole (15.5 %) and ethyl cinnamate (3.7 %).

α-pinene was the most abundant hydrocarbon monoterpene in the oil. Other hydrocarbon monoterpenes that were found in significant proportions were β-pinene (6.2 %), limonene (4.2 %). The most abundant oxygenated monoterpene in the oil was pinane-2-ol (9.9 %). α-terpineol (6.2 %) and linalyl acetate (2.0 %) also occurred in appreciable quantities.

Qualitative and quantitative compositions of the oil were found to be different from the leaf essential oil of East Mediterranean grown *C. album* (Dembitsky et al., 2008). For instance, the principal constituents in the leaf oil of the Nigerian grown species, α-terpineol, pinane-2-ol, linalyl acetate, p-cymene and ethyl cinnamate, were not identified in the oil of East Mediterranean *C. album*. The most abundant constituent of the oil was p-cymene; hence, the oil was of p-cymene chemotype. On the other hand, terpinenyl acetate and terpinen-1-ol that were found in appreciable amounts in the oil of East Mediterranean grown *C. album* were not identified in the oil of the Nigerian grown *C. album*. Meanwhile, α-pinene, β-pinene, limonene and ascaridole were identified in both oils. These constituents were of greater abundance in the oil of the Nigerian grown *C. album* except limonene that predominate the leaf oil of East Mediterranean grown *C. album*. Variations in composition patterns of the oils may be attributed to agroclimatic and geographical conditions.

It has been established that anti-inflammatory activities of essential oils are attributable to the presence of substituent such as; limonene, linalool, linalyl acetate and α-pinene (Peana et al., 2002; Özbek and Sever, 2005; Karaca et al., 2007).

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Table 1: Chemical composition (%) of leaf oil of *Chenopodium album*

Compound[a]	RI[b]	Percentage composition	Mass spectra data
Tricyclene	922	Tr	136, 121, 105, 93
α-thujene	926	Tr	136, 121, 115, 105
α-pinene	933	7.0	136, 121, 105, 93
Camphene	946	Tr	136, 121, 107, 93
Sabinene	971	Tr	136, 121, 107, 93
β-pinene	976	6.2	136, 121, 107, 93
Myrecene	990	Tr	136, 121, 115, 107
p-cymene	1022	40.9	134, 119, 103, 91
Limonene	1027	4.2	136, 121, 107, 93
Benzyl alcohol	1028	Tr	108, 91, 89, 79
1,8-cineole	1029	Tr	154, 139, 125, 108
Cis-ocimene	1035	Tr	136, 121, 105, 93
γ-terpinene	1057	Tr	136, 121, 105, 93
Linalool	1098	Tr	139, 121, 109, 97
Pinane-2-ol	1136	9.9	139, 111, 93, 69
Allo ocimene	1142	Tr	136, 121, 105, 93
Citronellal	1150	Tr	154, 136, 121, 111
Borneol	1162	Tr	139, 121, 110, 95
Terpinen-4-ol	1175	Tr	154, 136, 71, 43
α-terpineol	1188	6.2	136, 121, 105, 93
Citronellol	1226	Tr	138, 123, 109, 99
Ascaridole	1237	15.5	139, 125, 69, 41
Neral	1238	Tr	135, 119, 109, 99
Linalyl acetate	1255	2.0	136, 121, 105, 93
Geranial	1268	Tr	152, 137, 123, 109
Borneol acetate	1284	Tr	196, 154, 136, 108
Thymol	1290	Tr	150, 135, 121, 107
Carvacrol	1299	Tr	150, 135, 121, 107
Ethyl cinnamate	1460	3.7	176, 158, 147, 131
Acetyl eugenol	1523	Tr	207, 164, 149, 121
Elemicin	1553	Tr	208, 193, 177, 150
Benzyl benzoate	1759	Tr	152, 105, 91, 51
Total		95.6	

[a] Compounds are listed in order of elution from Silica Capillary Column coated on CP-Sil 5
[b] Retention indices on fused Silica Capillary Column coated with CP-Sil 5=trace (<0.1 %)

The result of anti-inflammatory activity of leaf essential oil of *Chenopodium album* is presented in Table 2.The result revealed that the anti-inflammatory action of the oil is concentration dependent. Hence, the percentage reduction in the ear edema increases with increase in concentration of the oil. Furthermore, the oil caused significant reduction ($p < 0.05$) in the ear edema except at 0.625 mg concentration. The reduction may be due to the presence of α-pinene, linalool and linaly acetate in the oil. Hence, the oil can be use as anti–inflammatory agent.

Table 2: Anti-inflammatory activity of leaf essential oil of *Chenopodium album*

Group	Dose mg	No of animals in the group	Change in ear weight (Mean ±SEM) mg	Percentage of edema reduction
Control	-	8	7.20 ± 0.02	-
Indomethacin	0.25	8	3.10 ± 0.01	57.00
CA1	5.00	8	0.50 ± 0.02	91.80
CA2	2.50	8	0.70 ± 0.05	89.40
CA3	1.25	8	1.90 ± 0.04	74.40
CA4	0.625	8	4.60 ± 0.01	41.00

P < 0.05, Student's t-test

CA = *Chenopodium album*

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