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Chemical composition and biological activity of essential oil of *Chenopodium ambrosioides* from Yemen

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Abstract
The chemical composition of the hydrodistilled leaf essential oil from *Chenopodium ambrosioides* L. growing wild in Yemen was determined by GC-MS analysis, and its cytotoxic, and general antioxidant potential were evaluated. Major compounds of *C. ambrosioides* oil were ascaridole (54.2%), isoascaridole (27.7%) and *p*-cymene (8.1%). At concentrations of 50 and 25 µg/mL, the essential oil showed cytotoxic activity against HT29 (human colon adenocarcinoma cells), with growth inhibition of 100 and 56% (± 3). The free radical scavenging ability of the oil was assessed by the DPPH assay to show antiradical activity with IC_{50} of 10.4 µg/mL. TLC-bioautographic assay was used to identify the acetylcholinesterase inhibitory effect, and ascaridole was isolated and characterized (ESIMS, ^{1}H NMR, ^{13}C NMR and HMBC) as the responsible constituent for anticholinesterase activity.

Keywords: *C.ambrosioides*; GC-MS, ascaridole, antioxidant, cytotoxic.

1. Introduction
The genus *Chenopodium* (Chenopodiaceae) includes varieties of weedy herbs (more than 200 species) native to much of Europe, Asia including India and China, and both North and South America [1] and eight of which are found in Yemen [2].
C. ambrosioides L.is widely used in popular medicine as a vermifuge, emmenagogue and abortifacient [3]. *C. ambrosioides* acts as a diuretic and an anthelmintic, and it is used to treat wounds, respiratory problems, inflammatory and painful processes, bronchitis, tuberculosis, and rheumatism [4]. Externally, it has been used as a wash for hemorrhoids, as a poultice to detoxify snake bites and other poisons and is thought to have wound-healing properties [5]. The essential oil of *C. ambrosioides* (EOCA) is known to inhibit the growth of dermatophytes [6] and other filamentous fungi such as *Aspergillus, Fusarium,* and *Colletotrichum* [7]. EOCA possessed antiaflatoxigenic, antimalarial and antioxidant properties [4] as well as antihelmintica worm expelling activities [8].
There have been several reports on the essential oil composition of *C. ambrosioides*; EOCA has high levels of monoterpenes, mainly ascaridole, α-terpinene, *p*-cymene, and isoascaridole [7, 9-14]. At least seven different chemotypes have been identified: (1) ascaridole, (2) α-terpinene, (3) α-pinene, (4) *p*-cymene, (5) carvacrol, (6) α-terpinyl acetate, and (7) limonene [11]. To our knowledge, there are no published reports on the chemical composition, cytotoxic and antioxidant activity of *C. ambrosioides* oil from Yemen. For this reason, the chemical composition of EOCA was analyzed and its antiproliferative activity on a human colon adenocarcinoma cell line was investigated. The antioxidant potential of the oil was also evaluated by the chemical DPPH assay. A bioautographic assay was used to identify the acetylcholinesterase inhibitory activity. The active compound was isolated and characterized using ESIMS, LC-MS, ^{1}H NMR, ^{13}C NMR and HMBC, and identified as ascaridole.

2. Materials and Methods
2.1 Plant Material
The leaves of *C. ambrosioides* were collected in the early morning from the Alselw district, Taiz province, Yemen, on 13th August 2012. The plant was identified by Dr. Hassan M. Ibrahim of the Botany Department, Faculty of Sciences, Sana'a University. A voucher specimen (YMP-chen-14) has been deposited at the Pharmacognosy Department, Sana'a University, Yemen.

2.2 Essential Oil Extraction

Dried leaves from *C. ambrosioides* were hydrodistilled for 3 h in a Clevenger type apparatus according to the European Pharmacopoeia [15]. The obtained oil was subsequently dried over anhydrous Na_2SO_4 and kept at 4 °C until analysis.

2.3 Gas Chromatography – Mass Spectral Analysis

The essential oil of *C. Ambrosioides* was analyzed by GC-MS according to method described in [16]. The essential oil composition of *C. ambrosioides* is summarized in Table 1.

2.4 Antioxidant Activity Screening

The antiradical activity of the oil was studied using DPPH-assay as previously reported [16].

2.5 Cytotoxicity Screening

The cytotoxic activity of oil was studied against HT29 tumor cells (human colonic adenocarcinoma cells) as previously reported [16].

2.6 Modified TLC Bioautographic Assay for Acetylcholinesterase Inhibition

The test was carried out according to reference [17]. PTLC layers were developed with toluene: ethylacetate (93:7, v/v). The positive band was scratched out, eluted with methanol and purified by HPL Cusing an RP-C_{18}cartidge and subjected to ESI and LC-MS measurements.

3. Results and Discussions

EOCA was obtained by hydrodistillation with a yield of 0.52%, v/w, on a dry-weight basis. Eleven compounds, representing 94.1% of the oil, were identified. As can be seen in Table 1, the main constituents of the essential oil were oxygenated monoterpenoids and monoterpene hydrocarbons at concentrations of 85.3% and 8.8%, respectively. Ascaridole (54.2%), isoascaridole (27.7%) and *p*-cymene (8.1%) were the main compounds in the oil. Yemeni oil composition therefore is somewhat similar to the compositions described in previous reports on *C. ambrosioides* oil from Madagascar with ascaridole (41.8%), isoascaridole (18.1%), *p*-cymene (16.2%) [9], Brazil with ascaridole (61.4%), isoascaridole (18.6%) [7], China with ascaridole (29.7%), isoascaridole (13.0%), *p*-cymene (12.7%) [18], cultivated *C. ambrosioides* from Uttarakhand, India with ascaridole (45.0%), isoascaridole (2.9%), *p*-cymene (27.1%), α-terpinene (8.3%) [19], and Cuba with ascaridole (30.5-47.1%), *p*-cymene (20.2-21.1%) α-terpinene (17.0-20.7%) [10]; but differed from those described in Nigeria by Owolabi *et al.* [11] [with α-terpinene (63.1%), *p*-cymene (26.4%) and ascaridole (3.9%)], in wild *C. ambrosioides* from Himalayan India by Lohani *et al.* [19] [with α-terpinene (44.7%), *p*-cymene (21.3%) and ascaridole (17.9%)], and in southern India by Gupta *et al*. [12] [with α-terpinene (63.6%) and *p*-cymene (19.5%)].The composition of EOCA from Yemen places this plant in the ascaridole chemotype [11].

Table I: Essential oil composition of *Chenopodium ambrosioides* from Yemen.

RI	Compound	%
1017	α-Terpinene	0.7
1025	*p*-Cymene	8.1
1125	Unidentified	4.8
1185	*p*-Cymen-8-ol	tr
1224	Unidentified	0.8
1238	Ascaridole	54.2
1251	*cis*-Piperitone epoxide	0.4
1257	*trans*-Piperitone epoxide	1.2
1268	*trans*-Ascaridole glycol	tr
1288	*cis*-Ascaridole glycol	1.8
1295	Carvacrol	tr
1303	Isoascaridole	27.7
1316	4-Hydroxycryptone	tr
	Oxygenated monoterpenoids	85.3
	Monoterpene hydrocarbons	8.8
	Total Identified (11)	94.1

EOCA shows a potent radical scavenging activity at concentrations ranging from 20 to 5 µg/mL. Its chemical antiradical activity with an IC_{50} of 9.7µg/mL, thus is better than that of ascorbic acid (IC_{50} 12.7 µg/mL). EOCA is markedly rich in oxygenated monoterpenoids (85.3%) which may act as the radical-scavenging agents. It seems to be a general trend that the essential oils that contain oxygenated monoterpenoids have greater antioxidative properties than non-oxygenated ones [20].

The potential anticancer activity of EOCA was assessed against HT29 (human colon adenocarcinoma) cells. At concentrations of 50 and 25 µg/mL, EOCA showed good cytotoxic activity, with growth inhibition of 100% and 56% (± 3.45), respectively. The potent antineoplastic properties of EOCA may be attributed to the main component of the oil, ascaridole which is known to exhibit activity against different tumor cell lines *in vitro* [21, 22].

EOs and their components with marked anticholinesterase activity can find practical applications, for example, as natural insecticides, anti-dementia agents, and for treating head lice [23, 24]. The inhibitory effect of EOCA on AChE was detected by bioautographic TLC assay. The active band was isolated, purified and characterized by ESIMS, LC-MS, ^{1}H NMR, ^{13}C NMR and HMBC as ascaridole.

4. Conclusions

In conclusion, EOCA is characterized by high content of oxygenated monoterpenes, mainly ascaridole and isoascaridole. The results clearly show that the oil possesses a significant cytotoxic activity against human tumor HT29 cell line. The cytotoxic activity can be explained partly by the presence of ascaridole. Our results show a good correlation between antioxidant and cytotoxic activity of EOCA. A further study is needed to determine the anticholinesterase activity of ascaridole.

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