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CYTOTOXIC AND ANTIPHYTOFUNGAL ACTIVITY OF THE ESSENTIAL OILS FROM TWO ARTEMISIA SPECIES

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ABSTRACT

Hydrodistilled essential oils from aerial parts of *Artemisia abyssinica* Sch.Bip. ex A. Rich, and *Artemisia arborescens* L**.** growing in Yemen were screened for their cytotoxic and antiphytofungal properties as well as their chemical compositions. Twenty-seven components were identified in the essential oils and the main components of these species were found to be davanone (42.34%), camphor (22.88%), nerolidol (8.96%), and chamazulene (4.46%), from *A. abyssinica* oil and artemisia ketone (51.05%), camphor (14.09%), α-bisabolol (12.56%) and α-phellandrene (8.69%) from *A. arborescens*. At concentration of 50 and 25 µg/mL, *A. arborescens* oil showed a strong cytotoxic activity with growth inhibition of 95%(±1.6) and 74%(±3.8) (IC_{50} of 16.91 µg/mL) against HT29 tumor cells (Human colonic adenocarcinoma cells), while *A. abyssinica* oil exhibited at concentration of 100 and 50 µg/mL growth inhibition of 71.0% (±12.5) and 27.3%(±14.4) (IC_{50} of 75.42 µg/mL) respectively. Bioautographic assay was used to evaluate the antiphytofungal activity of the oils against *Cladosporium cucumerinum*.

KEY WORDS: *Artemisia abyssinica*; *Artemisia arborescens*; cytotoxic activity *Cladosporium cucumerinum*; essential oil.

INTRODUCTION

Many natural compounds extracted from plants exhibit important biological activities. Among these diverse natural compounds, essential oils extracted from aromatic plants are attracting special attention [1]. Essential oils exhibit a very interesting chemotherapeutic potential; several essential oil constituents have been described as anticancer agents [2]. Moreover essential oils, obtained from the aromatic plants, have been traditionally used to protect the grains and vegetables during the storage[3]. Artemisia is included in the tribe Anthemideae of Asteraceae (Compositae), and comprises over 500 species, which are mainly found in Asia, Europe and North America. *Artemisia abyssinica* is used in folk medicine as an anthelmintic, antispasmodic, antirheumatic and antibacterial agent[4]. Antioxidant, antileishmanial, antitrypanosomal activity was recorded for *A. abyssinica* oil [4,5]. *Artemisia arborescens* is used traditionally as an anti-inflammatory remedy. The oil showed antimicrobial and antiviral activity[4] In this study, we assessed for the first time the cytotoxic and fungicidal activity of the essential oils obtained from *A. abyssinica* (EOAab), and *A. arborescens* (EOAar) against HT29 tumor cells (Human colonic adenocarcinoma cells) and against the phytopathogenic *Cladosporium cucumerinum* using semeiquantative bioautographic assay

MATERIALS AND METHODS

Plant material

The aerial parts of *A. abyssinica* and *A. arborescens* were collected from Sana'a (Sana'a city), at April-2009, were identified by Dr. Hassan Ibrahim, Department of Biology, Faculty of Science, Sana'a University. Voucher specimens (comp. art.-1 and comp. art-2) were deposited in the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University

Oil extraction

Hydrodistillation of the aerial parts of *A. abyssinica* and *A. arborescens* using Clevenger-type apparatus was carried out according to the procedure described in[6] and yielded 3% v/w and 1.2% v/w of the both oils respectively.

Table 1. Main Components of EOs from *A. abyssinica*.(1) and A *arborescens* (2)

compound	RI	Adams	% (1)	% (2)
Sabinene	978	975	----	1.54
β -Pinene	986	979	1.54	
Yomogi alcohol	989	999	----	0.96
α-Phellandrene	1007	1002	0.49	----

α-Terpinene	1017	1017	0.40	8.70
Artemisia ketone	1038	1062	-----	51.1
Artemesia alcohol	1055	1083	-----	0.77
Terpinolene	1061	1088	-----	1.60
Linalool	1073	1096	2.45	
Camphor	1140	1146	22.89	14.09
cis-Chrysanthenol	1160	1164		0.68
Borneol	1164	1169	0.82	0.65
Terpinen-4-ol	1176	1177	2.23	
Bornyl acetate	1280	1288	2.68	
Ethyl (E)-Cinnamate	1460	1467	1.09	
(E)-Nerolidol	1562	1563	8.96	
Davanone	1581	1587	42.34	
α-Bisabolol	1685	1685	------	12.56
Chamazulene	1723	1731	4.47	
Palmitic acid	2012	1960	1.63	2.36
Linoleic acid	2141	2133	0.67	1.41
monoterpene hydrocarbons			2.43	11.84
Oxygenated monoterpenes			31.07	68.25
Sesquiterpene hydrocarbons			4.47	
Oxygenated sesquiterpenes			51.30	12.56
phenylpropanoid			1.09	
Fatty acids			2.30	3.77
Total			92.66	96.42

Adams RP. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry, 4th Ed.Allured Publishing, Carol Stream, Illinois, 2007

GC-MS analysis

(EOAab) & (EOAar) were analyzed by GC-MS as described previously in [7].

Cytotoxicity test

The cytotoxic activity of (EOAab) & (EOAar) was studied against HT29 tumor cells (human colonic adenocarcinoma cells) as previously reported[6].

Antiphytofungal assay

This semiquantitative test allows a relative estimation of the activity of compounds and extracts with similar diffusion characteristics. The phytopathogenic fungus *Cladosporium*

cucumerinum Ell. et Arth. was used as test organism. Initial tests of fungicidal activity were carried out by the method described previously in [7] .

RESULTS AND DISCUSSIONS

EOAab and EOAar were screened for their cytotoxic, antiphytofungal properties as well as their chemical compositions. In EOAab, 14 compounds were identified, representing **92.7**% of the total oil. The most abundant components were oxygenated sesquiterpenes (**51.3%**) and oxygenated monoterpenes (**28.4**%). davanone (42.3%), camphor (22.9%), nerolidol (9.0%), chamazulene (4.5%), bornyl acetate (2.7%), linalool (2.4%), and terpinen-4-ol (2.2%) were the major compounds of EOAab (Table 1).

In EOAar, 13 compounds were identified, representing (96.4%) of total oil content. In contrast to *A. abyssinica* oil, EOAar was characterized by a high content of monoterpene fraction (**80.1**%), with artemisiaketone (51.1%), camphor (14.1%), α-phellandrene (8.7%), α-pinene (2.2%), terpinolene (1.59%) and sabinene (1.5%) as the main constituents.

The cytotoxic properties of EOAab and EOAar were assessed against HT29 tumor cells (Human colonic adenocarcinoma cells). The results showed that EOAar exhibited at 50 and 25µg/mL stronger inhibitory effect (95% ±1.6 and 74% ±3.8 (IC_{50} of 16.91 µg/mL) on the human cancer cells than EOAab (27.3% ±14.4) (IC_{50} of 75.42 µg/mL). Studies showed that monoterpenes exert antitumor activities and suggest that these components are a good source of cancer chemopreventive agents[8].The cytotoxic activity of EOAar could be also attributed to the presence of sesquiterpenes such α-bisabolol[9]. Harada et al. reported the strong cytotoxic effect of palmitic acid on cancer cells[10]. The synergistic effects of these active chemicals with other constituents of EOAar should be taken into account

The antiphytofungal activity of the oils was assessed against the phytopathogenic fungus C. *cucumerinum* using a standardized bioautographic technique[7] At the application of 400 µg, EOAar exhibited more potent antifungal activity than EOAab with inhibition zones of 14 (± 1.6) and 10 (± 2.1) respectively. There are few reports on the antiphytofungal activity of EOs and their components against C. *cucumerinum*[11] From our results, it could be concluded that the oils isolated from Yemeni *A. abyssinica* and *A. arborescens* are characterized by a high content of davanone and Artemisia ketone, respectively. EOAar is more as cytotoxic agent on HT29 tumor cells than EOAab. This is the description of a potential cytotoxic activity for this oil, suggesting the presence of active components.

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