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Isolation of compounds from the roots of *Ambrosia artemisiifolia* and their effects on human cancer cell lines

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Abstract: Common ragweed (*Ambrosia artemisiifolia* L.) is an invasive plant in Europe with spreading use in the contemporary folk medicine. The chemical composition of the above-ground parts is extensively studied, however, the metabolites of the roots are less discovered. By multiple chromatographic purification of the root extracts, we isolated thiophene A (**1**), *n*-dodecene (**2**), taraxerol-3-*O*-acetate (**3**), *α*-linoleic acid (**4**), (+)-pinoresinol (**5**), and thiophene E (7,10-epithio-7,9-tridecadiene-3,5,11-triyne-1,2-diol) (**6**). The ^{1}H NMR data published earlier for **1** were supplemented together with the assignment of ^{13}C NMR data. Thiophene E (**6**), which is reported for the first time from this species, exerted cytotoxic and antiproliferative effects on A-431 epidermoid skin cancer cells, whereas taraxerol-3-*O*-acetate (**3**) and *α*-linoleic acid (**4**) had slight antiproliferative effect on gynecological cancer cell lines. Thiophene E (**6**) and taraxerol-3-*O*-acetate (**3**) displayed antiproliferative and cytotoxic effects on MRC-5 fibroblast cells. Thiophene E (**6**) exerted weak antibacterial activity (MIC 25 μg/mL) on MRSA ATCC 43300, on *Staphylococcus aureus* ATCC 25923, *Escherichia coli* AG100 and *E. coli* ATCC 25922 both thiophenes were inactive. Although the isolated compounds exerted no remarkable cytotoxic or antiproliferative activities, the effects on MRC-5 fibroblast cells highlight the necessity of further studies to support the safety of ragweed root.

Keywords: *Ambrosia artemisiifolia*; lignan; ragweed; sterol; thiophene.

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1 Introduction

Common ragweed (*Ambrosia artemisiifolia* L., Asteraceae) is a naturally occurring species in North America, with a center of origin and diversity in the Sonoran Desert [1]. Several *Ambrosia* species have spread to Eurasia. Of these, common ragweed, is the most successful and widespread and has been recorded almost throughout Europe as an invasive plant [2]. Because of it purported allelopathic effect and since it does not have any natural pests in Europe, it can colonize territories of a high level of disturbance (crop fields, building sites, vacant lots, etc.) quickly. The fast spreading is also triggered by the climate change [3]. Ragweed is classified as one of the top hundred worst invasive plants, therefore the European Food Safety Authority promotes its eradication [4].

Common ragweed does not belong to the important medicinal plants in North America, the ethnobotanical reports focus mainly on ethnographic aspects without discussing its folk medicinal use [5]. Unexpectedly, oral ragweed herb

preparations are widely utilized as highly valued panaceas in Hungary, despite the fact that their use lacks both the scientific and ethnomedicinal foundation [5]. In a previous study assessing the repeated-dose toxicity of ragweed puree on rats, toxic effects on liver and kidneys and controversial effect on brain tissue were reported, therefore the safety of its long-term human consumption is questionable [6].

Nevertheless, the chemical composition of common ragweed had been intensively investigated for its special metabolites, focusing mainly on sesquiterpene lactones [7–14], characteristic ingredients of the plant, as potential antiproliferative, antimicrobial or antiparasitic agents [6]. Recently, two guaianolide sesquiterpenes together with a pseudogaianolide and eudesmane glycosides with neuroprotective effects were described from the aerial plant parts as new natural compounds [15, 16].

However, few studies reported the chemical composition of ragweed roots. Its main constituents include the red-colored thiarubrin A, accompanied by its photoproduct thiophene A, isolated first by Bohlmann et al. from the plant [17]. Further acetylenes, thiophene D and the acetylenic hydrocarbon pentayneene, methyl caffeate, *β*-sitosterol, stigmasterol, *β*-bisabolene, and taraxerol acetate were also isolated from the roots [18, 19].

The antiproliferative and cytotoxic activities of *Ambrosia* sp. extracts have been reported in several trials [20]. However, these activities have been observed with the extracts of (or sesquiterpenes lactones isolated from) aerial parts. Cytotoxic and antiproliferative activities are characteristic of sesquiterpene lactones. Cytotoxic sesquiterpenes typically contain a lactone function, which is α,β-unsaturated and an exocyclic α-methylenic linkage. The presence of a C-11 or C-13 exocyclic double bound conjugated to the γ-lactone is also important for cytotoxicity. These compounds alkylate the nucleophilic groups of enzymes thereby controlling cell division [20].

The sesquiterpene content or cytotoxic/antiproliferative properties of root samples have only been the subject of a few studies so far. Moreover, there are some reports on the lack of sesquiterpene lactones in the roots of certain species [14]. In case of *Ambrosia artemisifolia*, there are no data on the sesquiterpene lactone content or antiproliferative/cytotoxic activities of the roots except one earlier study, where *in vitro* antitumor effects of an *n*-hexane extract of ragweed root against human skin epidermoid carcinoma cells (A-431) we detected [21]. In our present work we aimed to characterize the chemical profile of ragweed root in order to identify metabolites of therapeutic interest and also to contribute to the bioactivity profile of this invasive plant by evaluating antiproliferative and antibacterial effects of the isolated compounds.

2 Experimental

2.1 Chemicals and chromatographic materials

Solvents for extraction were obtained from Molar Chemicals Kft. (Halásztelek, Hungary). Analytical-grade solvents were purchased from Chem-Lab NV (Zedelgem, Belgium) and VWR Chemicals International S. A. S. (Fontenay-sous-Bois, France).

For vacuum liquid chromatography (VLC), SiO_2 (silica gel 60 GF254, 15 μm, Merck 11677; VLC 1) and reversed-phase SiO_2 (LiChroprep RP-18, 25–40 μm, Merck 9303; VLC 2) were applied. Rotation planar chromatography (RPC) was performed by a Chromatotron instrument (model 8924, Harrison Research) on manually coated SiO_2 (silica gel 60 GF254, Merck 7730) plates of 1 (RPC 1–5 and RPC 7) or 2 mm (RPC 6) thickness, at a flow rate of 3 or 7 mL/min, respectively. Preparative layer chromatography (PLC) was carried out on aluminum sheets coated with SiO_2 (silica gel 60 F254, Merck 5554; PLC 1 and PLC 4–5) or reversed-phase SiO_2 (silica gel 60 RP-18 F254s, Merck 5559; PLC 3 and PLC 6), or on glass plates coated with SiO_2 (HPTLC silica gel 60 RP-18 F254s with concentrating zone, Merck 115498; PLC 2). Medium pressure liquid chromatography (MPLC) were performed on a Büchi apparatus (Büchi Labortechnik AG) equipped with a 2 × 150 mm C-18 RP-MPLC column (40–63 μm, Büchi), at a flow rate of 6 mL/min.

2.2 Plant material

The roots of *A. artemisiifolia* L. were collected in August 2017 before full flowering in the Southern Great Plain (Hungary) near Szeged (46°13′50.6″ N 19°53′52.3″E) and authenticated by Dóra Rédei PhD (Department of Pharmacognosy, University of Szeged, Hungary). A voucher specimen (No. 815) has been deposited at the Department of Pharmacognosy, University of Szeged.

2.3 Extraction and isolation

The air-dried and ground roots of the plant (1.35 kg) were extracted once with MeOH (8 L) in ultrasonic bath at room temperature. The concentrated extract (50 mL) was diluted in 150 mL H_2O and extracted with EtOAc (18 × 150 mL). After evaporation, the EtOAc-soluble phase was subjected to VLC (vacuum liquid chromatography) with gradient elution (VLC I; 6.5 × 19 cm), using mixtures of *n*-hexane, $CHCl_3$ and MeOH with increasing polarity. A total of 60 fraction with a volume of 50 mL were collected and combined according to the TLC results, yielding 16 main fractions (I–XVII).

Fraction II (26.5 mg; eluted with *n*-hexane–$CHCl_3$ 75:25) was chromatographed by rotation planar chromatography (RPC I) in two steps, first with a solvent system of cyclohexane–CH_2Cl_2 95:5, and then with cyclohexane, which resulted in the isolation of compound **1** (11.5 mg, yellow oil). Fraction III (31.3 mg; eluted with *n*-hexane–$CHCl_3$ 75:25) was also processed by means of RPC (RPC II), using a solvent system of petroleum ether–CH_2Cl_2 95:5. For the final purification step, reversed-phase PLC was applied (PLC-1) using EtOAc–MeOH 3:7 as developing system, affording compound **2** (7.3 mg, colorless oil). From fraction IX (31.2 mg; eluted with *n*-hexane–$CHCl_3$ 6:4), after an RPC purification process (RPC III) with an eluent system of cyclohexane–EtOAc 95:5 compound **3** was crystallized in pure form (3.8 mg, white crystals). For the separation of fraction X (54.0 mg; eluted with *n*-hexane–$CHCl_3$ 6:4) a

subsequent RPC (RPC IV) was achieved. A solvent system of petroleum ether–CH_2Cl_2–MeOH with increasing polarity was used as eluent. From the subfractions eluted with petroleum ether–CH_2Cl_2 55:45, a small amount of compound **3** was crystallized (1.2 mg). Subfractions eluted with MeOH were purified by reversed-phase PLC (PLC-2), using acetonitrile–H_2O 9:1 as developing system, to yield compound **4** (3.5 mg, colorless oil). Fraction XII (1.035 g; eluted with *n*-hexane–$CHCl_3$ 4:6 and *n*-hexane–$CHCl_3$–MeOH 8:12:1) was separated by means of RP-VLC (VLC II; 2.7 × 10 cm), using an eluent system of $CHCl_3$–MeOH–H_2O with decreasing polarity. From the subfractions eluted with $CHCl_3$–MeOH 3:7 a white substance was crystallized and further purified by NP-RPC (RPC V), applying cyclohexane–CH_2Cl_2–MeOH 7:13:1 as eluent, which afforded a mixture of *β*-sitosterol and stigmasterol (2.0 mg, white crystals). Fraction XV (625.2 mg; eluted with *n*-hexane–$CHCl_3$–MeOH 4:16:1 and 1:8:1) was subjected to RP-MPLC, applying a gradient system of MeOH and H_2O, with decreasing polarity. Subfractions eluted with the above system at 4:6 and 1:1 were separated via RPC (RPC VI), using cyclohexane–CH_2Cl_2–isopropanol 10:10:1, and purified by PLC in two steps. The first one (PLC-3) was carried out on RP-silica, using MeOH–H_2O 6:4 as eluent, while the following one (PLC-4) was achieved on NP-silica with an eluent of cyclohexane–CH_2Cl_2–isopropanol–MeOH 13:10:1:1, to furnish compound **5** (1.4 mg, white powder). Subfractions eluted with MeOH–H_2O 9:1 from MPLC were re-chromatographed by PLC (PLC-5), using cyclohexane–CH_2Cl_2–isopropanol–MeOH 13:10:1:1 as eluent. The final purification was carried out by RP-PLC (PLC-6), applying an eluent system of acetonitrile – H_2O 7:3, which led to the isolation of compound **6** (1.8 mg, yellow amorphous powder).

2.4 Structure elucidation

NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) or 125 MHz (^{13}C) in $CDCl_3$ and CD_3OD. The residual peaks of the deuterated solvents were taken as reference points. The NMR data were acquired and processed with MestReNova v12.0.0–20080 software. Mass spectrometric measurements were performed on an API 2000 LC-MS/MS (AB Sciex) system.

2.5 Assay for cytotoxic effect

The cytotoxic effect of **1**, **3**, and **6** was tested against A-431 epidermoid carcinoma cells (ATCC CRL-1555, from LGC Standards, Teddington, UK) and MRC-5 non-cancerous human embryonic lung fibroblast cells (ATCC CCL-171, from LGC Standards, Teddington, UK) by MTT assay. Before the assay, a limited number of cells (1×10^4/well) were seeded in Dulbecco's Modified Eagle's Medium (DMEM; Gibco Life Technologies Co., UK), or Eagle's Minimal Essential Medium (EMEM, Sigma-Aldrich, St Louis, MO, USA), respectively, in flat-bottomed 96-well microtiter plates overnight. The culture plates with the isolated compounds and the clinically used anticancer reference agent cisplatin were incubated at 37 °C for 24 h, as it was described earlier [22]. Cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA).

In the cytotoxicity and antiproliferative assays, pure cell culture medium (MEM) was utilized as a negative control. The tested substances were dissolved in dimethylsulfoxide (DMSO) to prepare stock solutions of 10 mM. The highest DMSO concentration in the medium during the assay was 0.3%. According to our previous experiments, the growth of the used adherent cell lines is not changed significantly by DSMO up to 1%.

2.6 Assay for antiproliferative effect

The antiproliferative effects of **1**, **3**, and **6** against A-431 and MRC-5 lines were measured with MTT assay. Before the assay, a limited number of cells (5×10^3/well) were seeded in DMEM or EMEM medium, respectively, in 96-well microplates. MTT assay was performed after an incubation of 72 h of the culture plates. Absorbance values of the samples were measured at 545 nm using a microplate reader (Stat Fax-2100, Awareness Technologies Inc., Palm City, FL, USA), and untreated cells were used as a control. Other details of the assay have been described earlier [22].

Further MTT assays (**3** and **4**) were performed against a panel of human adherent cancer cell lines of gynecological origin (breast cancer: MCF-7 (ATCC HTB-22), T-47-D (ATCC HTB-133) and MDA-MB-231 (ATCC HTB-26), and cervical cancer: HeLa (ATCC CLL-2)). All the cell lines were purchased from European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK). The cells were incubated with the tested substances for 72 h, and cisplatin (Ebewe Pharma GmbH, Unterach, Austria) was included as a reference agent. All details of these assays have been described earlier [23]. Cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA).

2.7 Determination of minimum inhibitory concentrations (MICs) by microdilution method

As Gram-positive strains, *Staphylococcus aureus* ATCC 25923 was used as methicillin-susceptible reference strain; furthermore, the methicillin resistant *S. aureus* MRSA ATCC 43300 strain were investigated in the study. As Gram-negative strains, wild-type *Escherichia coli* K-12 AG100 strain and *E. coli* ATCC 25922 reference strain were used in the experiment.

MICs of compounds were determined according to the Clinical and Laboratory Standard Institute guidelines (CLSI) [24]. MIC values of the compounds were determined by visual inspection. The solvent was also assayed to ensure there was no antibacterial effect and the concentration (1 v/v %) applied in the assays had no antibacterial activity. DMSO was used at subinhibitory concentration (1 v/v %) in the assays.

3 Results

Altogether 6 compounds were isolated from the roots of *A. artemisiifolia* by means of multiple chromatographic methods. Compound **1** was identified as thiophene A based on the comparison of ^{1}H and ^{13}C NMR with those reported in the literature [25] (Figure 1). The ^{1}H NMR data published earlier were supplemented [5.83 (1H, dd, *J* = 17.5, 2.3 Hz, H-1a), 5.67 (1H, dd *J* = 10.9, 2.3 Hz, H-1b), 5.91 (1H, dd, *J* = 17.5, 10.9 Hz, H-2), 7.12 (1H, d, *J* = 3.8 Hz, H-8), 6.94 (1H, d, *J* = 3.8 Hz, H-9), 2.07

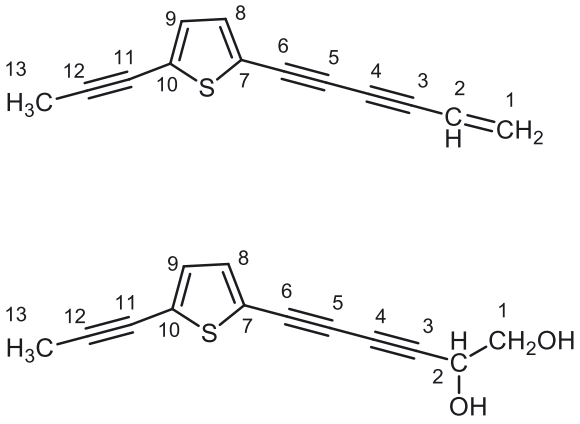

Figure 1: Thiophenes (**1** and **6**) isolated from *A. artemisiifolia*.

Table 1: NMR spectral data of compounds **1** and **6** [125 MHz (^{13}C), $CDCl_3$, δ (ppm)].

Position	Compound 1	Compound 6
1	130.8	66.3
2	116.2	64.0
3	82.9	82.6
4	74.4	70.8
5	73.1	77.3
6	74.3	71.9
7	121.9	121.2
8	134.2	134.6
9	130.9	130.9
10	127.3	127.7
11	72.7	72.6
12	92.2	92.4
13	4.9	4.9

(3H, s, H-13)] together with the assignment of ^{13}C NMR data (Table 1). The mass spectrometric experiments reassured that the molecular composition of **1** is $C_{13}H_{10}SO_2$. In the APCI spectrum, $[M\text{-}H_2O + H]^+$ is the major peak at 213 (212.99) (Figure S17). The structure of compound **6** was determined as 7,10-epithio-7,9-tridecadiene-3,5,11-triyne-1,2-diol (thiophene E) based on the accordance of the measured and literature NMR and MS data [25]. Compound **3** was identified as taraxerol-3-*O*-acetate [26], compound **5** as (+)-pinoresinol [27], compound **4** as *α*-linoleic acid [28], and compound **2** as *n*-dodecene [29] by comparing their NMR spectral data with already reported data. The presence of *β*-sitosterol and stigmasterol was also demonstrated [30].

Compounds **1**, **3**, **4**, and **6** isolated from the roots were tested for their antiproliferative and cytotoxic activities on various cancer cell lines and on non-cancerous human fetal lung fibroblast (MRC-5) cells. The antiproliferative and cytotoxic properties of the compounds were determined similarly by MTT test. Instead of measuring the cell growth inhibitory activity, the direct killing effect can be assessed by cytotoxicity testing, which requires a higher cell population and a shorter exposure time.

First, to investigate the role of these compounds in the previously reported antiproliferative activity of the root extract against human skin epidermoid carcinoma cells (A-431), the compounds were assayed on this cell line. Only taraxerol-3-*O*-acetate (**3**) and thiophene E (**6**) were cytotoxic as well as having antiproliferative properties (Table 2). To further elucidate the antiproliferative profiles of these compounds, they were tested on a set of gynecological cancer cell lines (human cervix adenocarcinoma cell line HeLa, human breast cancer cell lines MCF-7, T-47-D, and human breast adenocarcinoma cell line MDA-MB-231). Here, only taraxerol-3-*O*-acetate (**3**) and *α*-linoleic acid (**4**) were active and since these activities were lower than those on A-431, we assessed these activities in three different concentrations. The compounds did not elicit higher than 50% inhibition even at the maximal concentration (30 μM), therefore, instead of calculating IC_{50} values, we present the activities at the tested 3 concentrations (Table 3). These effects were concentration-dependent and substantially less pronounced than those of the positive control cisplatin. Moreover, in some cases at lower concentrations no activity could be observed. The dose-response curves of taraxerol-3-*O*-acetate (**3**) and *α*-linoleic acid (**4**) are published as supplementary material (Figure S18). *α*-Linoleic acid (**4**) was not toxic to MRC-5 cells, which is not surprising, considering that this is a ubiquitous compound of many plants that are considered to be safe, including food crops.

Some thiophenes possess antimicrobial activities [31]. To assess the potential activities of the compounds isolated by us, the antimicrobial activities of the thiophenes **1** and **6** were assayed on the following bacterial strains: *S. aureus* ATCC 25923, MRSA ATCC 43300, *E. coli* AG100 and *E. coli* ATCC 25922. Weak antibacterial activity (MIC 25 μg/mL) was observed only in case of thiophene E (**6**) on MRSA ATCC 43300, in other cases the MIC was >100 μg/mL.

Table 2: Antiproliferative and cytotoxic activities of the isolated compounds against human cancer (A-431) and normal (MRC-5) cell lines.

Compound	Antiproliferative activities IC_{50} (μM) ± SD		Cytotoxic activities IC_{50} (μM) ± SD	
	A-431	MRC-5	A-431	MRC-5
Taraxerol-3-*O*-acetate	27.15 ± 19.30	6.35 ± 1.43	58.34 ± 17.76	44.63 ± 1.33
Thiophene A	>100	>100	>100	>100
Thiophene E	31.04 ± 4.51	22.66 ± 1.50	53.55 ± 9.88	40.55 ± 10.83
Cisplatin	6.23	3.46	24.77	>100

Table 3: Antiproliferative properties of the tested compounds on gynecological cell lines.

Compound	Concentration (μM)	Growth inhibition (%) ± SD			
		MCF-7	T-47-D	MBA-MB-231	HeLa
Taraxerol-3-*O*-acetate	3	20.80 ± 1.56	–[a]	–	16.06 ± 2.66
	10	31.91 ± 0.81	–	12.48 ± 0.93	30.02 ± 0.88
	30	47.76 ± 1.46	33.83 ± 1.12	23.36 ± 0.99	45.18 ± 1.03
α-Linoleic acid	3	11.29 ± 1.13	–	–	–
	10	27.44 ± 1.06	–	–	12.23 ± 2.23
	30	47.63 ± 1.06	18.86 ± 2.15	19.03 ± 2.65	26.77 ± 2.49
Cisplatin	3	16.36 ± 1.90	21.10 ± 1.69	27.07 ± 1.84	20.09 ± 1.81
	10	54.06 ± 1.17	40.76 ± 1.81	42.72 ± 2.68	32.23 ± 1.16
	30	95.45 ± 0.28	59.96 ± 0.66	86.44 ± 0.42	93.70 ± 0.83

[a]Cancer cell growth inhibition values less than 10% were considered negligible and are not given numerically.

4 Discussion

Polyynes are widely distributed in the Asteraceae family. A subgroup of these compounds, sulphur-containing polyynes are characteristic to Helianthae, and reported from several *Ambrosia* species [32]. Dithiacyclohexadiene polyynes, also called as thiarubrines, are usually found in the roots. The red thiarubrines are chemically unstable and easily converted into colourless thiophenes via ring contraction and sulphur extrusion on sunlight at ambient conditions. The first identified thiarubrine was thiarubrine A, the metabolite of which is thiophene A (**1**) [32]. **1** was first identified in *Calocephalus citreus* (Asteraceae) by Sørensen et al. in 1964 [33] and later reported from the *A. artemisiifolia* as well. The only reported thiarubrine of this species was thiarubrine A [34]. Thiophene E (**6**) was reported by us for the first time from this species (previously, it was isolated from *Ambrosia chamissonis*) [25]. For **6**, full ^{13}C signal assignment in $CDCl_3$ is reported for the first time (Table 1).

There are significant differences in the cytotoxic and antiproliferative activities of the tested compounds, demonstrating their real cell proliferation inhibitory activity rather than direct killing effects. Several mono-, di- or tri-substituted thiophene derivatives have been reported to have antitumor effects and raloxifene, the representative of this group is marketed as an anticancer medicine [35]. The antitumor effect of thiophene A (**1**) has not been reported previously and our results point out that this compound is not involved in the antiproliferative effect of the root extract. However, thiophene E (**6**) might have a role in the effect of the herbal extract. Interestingly, **3** and **6** were more toxic to normal cells than to cancer cells. Previously we demonstrated the antiproliferative effects of *β*-sitosterol and stigmasterol on A-431 and MRC-5 cell lines, hence these compounds may also have role in the overall effect of the root extracts [36].

Although the bioactivity of taraxerol-3-*O*-acetate (**3**) was not very remarkable, its anticancer effect may only be partly relied on its antiproliferative activity. This compound was reported to exert anticancer effects in U-87 glioblastoma cells in *in vitro* and *in vivo* experiments, which effects were mediated via antiproliferative effect, the induction of autophagy, apoptosis, cell cycle arrest and inhibition of cell migration [37].

However, the remarkable antiproliferative and moderate cytotoxic effects of taraxerol-3-*O*-acetate (**3**) and thiophene E (**6**) on normal cells raises safety issues. Although common ragweed is not a widely used traditional medicinal plant, its application in contemporary folk medicine is spreading [5]. This species is categorized as unapproved novel food in the European Union, since neither its traditional food use nor its safety as food have been confirmed [38]. In a toxicity study, the repeated use of ragweed resulted in nephro- and hepatotoxic effects in rats, and these results question the safe human consumption of common ragweed [6].

5 Conclusions

Thiophene E is reported for the first time from *A. artemisiifolia*. Thiophene A and E did not exert significant antimicrobial activity; however, this is the first report on the assessment of their antibacterial activities. These compounds also lack remarkable antitumor effects; however, thiophene E exerts cytotoxic and antiproliferative effects on normal MRC-5 cells. Experiments on gynecological cancer cell lines supplemented the antitumor profile of taraxerol-3-*O*-acetate. The use of common ragweed as a medicinal plant may also be constrained by its potentially harmful effects, which may be partly attributed to the cytotoxic and antiproliferative actions of its metabolites on normal cells. Although the compounds isolated from the root extract have only moderate effects on cell proliferation, they could still be unsafe. Preparations containing root extract may increase the deleterious effects of cytotoxic

medicines when used in conjunction with cancer therapy because the investigated compounds' actions are not selective towards cancer cells. Therefore, a thorough evaluation of the toxicity profiles of plant metabolites and chemically well-characterized extracts should precede the medicinal use of this plant.

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