Cytotoxic Effect of *Conyza canadensis* (L.) Cronquist on Human Lung Cancer Cell Lines

*Conyza canadensis* (L.) Cronquist’ın İnsan Akciğer Kanser Hücre Hatları Üzerine Sitotoksik Aktivitesi

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ABSTRACT

Asteraceae family plants are receiving great attention because they have potential anticancer activity. Therefore, in this study, the cytotoxic effect of *Conyza canadensis* (L.) Cronquist (Asteraceae) were tested against human lung adenocarcinoma cell lines (A549 and H1299) for the first time. Cytotoxic effect of the *n*-hexane, chloroform, *n*-butanol and remaining water (R-H₂O) extracts fractioned from the methanol extracts of the aerial parts and roots of *C. canadensis* was investigated using Sulforhodamine B (SRB) assay and percent (%) viability was measured. The results indicate that the extracts of *C. canadensis* have cytotoxic activities on these cells in a dose-dependent manner. The root extracts exhibited relatively higher cytotoxic effects than the aerial parts of the plant. The most active extract was found to be *n*-hexane extract of the roots with IC₅₀ values 94.73 and 84.85 µg/mL on A549 and H1299 cell lines, respectively. These results suggest that *C. canadensis* exhibits moderate cytotoxic effect in lung cancer cells. This might be taken into account in its use for therapeutic purposes.

Key words: Asteraceae, *Conyza canadensis*, Cytotoxic activity, Lung cancer, Sulforhodamine B assay

INTRODUCTION

Cancer is a major group of diseases and is still among the leading of death in the world (1). Natural products are important sources of anticancer lead molecules. Many success plant-derived anticancer drugs such as paclitaxel, docetaxel, vincristin, etoposide, camptothecin, irinotecan are clinically available; however due to the rapid development of resistance to chemotherapeutic drugs and their side effects, novel anticancer drugs of natural sources are under an extensive search for cancer therapy (2,3).

The family Asteraceae which contains over 1600 genera and more than 23000 species is the largest family with rich chemical constituents (4). It has a broad medicinal utilization worldwide in which over 300 species of the family are known to have ethnomedicinal uses for cancer related diseases (5). The number of studies related to the cytotoxicity of Asteraceae plants and their secondary metabolites have

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intensively investigated in recent years. In these studies, sesquiterpene lactones and flavonoids have been principally stated to be responsible compounds for the cytotoxic effects (6–10). These compounds induce apoptosis after disrupting cell cycle of cancer cells in vitro and in vivo and also inhibit angiogenesis and metastasis (11,12).

The genus Conyza Less. belongs to the family Asteraceae and consists of about fifty species all over the world. In Turkey, the genus Conyza is represented by three species in the flora of Turkey, namely C. canadensis (L.) Cronquist, C. bonariensis (L.) Cronquist, and C. albida Willd. ex. Sprengel (13,14). C. canadensis (syn. Erigeron canadensis L.), known as “Canadian fleabane” or “horseweed”, is native throughout of North America and is also widespread in Europe. It is an annual plant erecting 10 to 180 cm high, with sparsely hairy stems (15). C. canadensis was reported to be used for its diuretic, antibacterial, anti-inflammatory, tonic, astringent, antihaemorrhagic properties as well as for the treatment of diarrhea and dysentery in folk medicines (16–18). In addition, a decoction of the plant is used anticancer purposes in North America (19).

The phytochemical studies on C. canadensis have so far pointed out to presence of terpenes, acetylene derivatives, flavonoids, benzoic acid derivatives, alkaloids, essential oils, sphingolipids, fatty acids and sterols (20–26). Among them, C10 acetylenes such as diyn-ene (e.g. E-lachnophyllum methyl ester) and ene-diyn-ene (e.g. matricaria methyl ester isomers), and C10 lactones (e.g. BZ-matricaria-γ-lactone) are typical constituents of the genus (20,26).

C. canadensis have been demonstrated to exert several biological activities such as cytotoxic, antifungal, antibacterial, antiviral, anti-inflammatory, antioxidant, and antiagregant (26–33). In previous studies, the extracts from C. canadensis were reported to have prominent cytotoxic effects on various cancer cell lines (26,34–37). To the best of our knowledge, there is no scientific report available in support of the cytotoxic effect of C. canadensis on A549 and H1299 human lung cancer cells. The aim of the present study was to investigate the possible in vitro cytotoxic effect of the extracts from the aerial parts and roots of C. canadensis in human lung adenocarcinoma cell lines (A549 and H1299).

**EXPERIMENTAL**

**Chemicals**

In the extraction procedure, methanol, n-hexane, chloroform and n-butanol were of analytical grade and were purchased from Merck Co. (Darmstadt, Germany). Analytical thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F254 plates (Art. 5554, Merck). The plates sprayed with anisaldehyde reagent [76% methanol (Merck) and 19% ortho-phosphoric acid (Riedel-De Haën, Buchs, SG Switzerland), 5% p-anisaldehyde (Merck)], 30% H2SO4 (Merck) solution in MeOH (Merck) and 1% vanillin-H2SO4 solution [vanillin (Boehringer Mannheim, Mannheim, Germany) and H2SO4 (Merck)].

**Plant material**

Conyza canadensis (L.) Cronquist was collected from Balcova, Izmir, Turkey, in the flowering-fruit stage, in November 2013. The plant was identified by Prof. Dr. Meçit Vural from the Department of Botany, Faculty of Science, Gazi University. A voucher specimen (F. Ayaz 29) has been deposited at Herbarium of Gazi University (G AZI), Ankara, Turkey.

**Preparation of extracts**

39.52 g powdered aerial parts (CCH) and 40.25 g roots (CCR) of the plant were extracted with 80% methanol by stirring at 40°C for 6 h three times (3x300 mL). Following filtration, the combined methanol extracts were evaporated in vacuo at 40°C to dryness. The concentrated MeOH extracts (100 mL) were further fractionated by successive solvent extractions with n-hexane (3x100 mL), chloroform (3x100 mL) and n-butanol saturated with H2O (3x100 mL) in a separatory funnel. Each extract as well as remaining aqueous phase (R-H2O) after solvent extractions was evaporated to dryness under reduced pressure to yield "n-Hexane extract" (0.04 g for CCH, 0.29 g for CCR), "CHCl3 extract" (0.14 g for CCH, 0.30 g for CCR), "n-BuOH extract" (2.63 g for CCH, 1.86 g for CCR) and "R-H2O extract" (1.03 g for CCH, 2.83 for CCR), respectively.

**Phytochemical analysis**

1 mg/mL of C. canadensis extracts were applied to silica gel plates. The n-hexane and CHCl3 extracts were developed with the mixture of n-hexane:acetone (7:3) as a mobile phase. TLC plates were evaluated under UV light at 254 and 366 nm for the determination of fluorescent compounds. Anisaldehyde reagent and 30% H2SO4 were sprayed to the plates to visualize the separated compounds and then plates were heated for 5 min at 100°C. Terpenes were appeared in pink, purple and green coloration with anisaldehyde reagent. In addition, terpenes also showed red coloration under UV 254 nm sprayed with 30% H2SO4 after heating for 5 min at 100°C. The n-butanol and R-H2O extracts were developed in a mixture of solvent system CHCl3:MeOH:H2O (61:32:7) and then sprayed with 1% vanillin-H2SO4 solution. The plates were heated for 5 min at 100°C before examined under UV light. Flavonoids detected as yellow and orange zones on the plates (38).

**Cell culture**

A549 and H1299 human lung adenocarcinoma cells were kindly provided by Prof. Hakan Akça (Pamukkale University, Faculty of Medicine, Denizli, Turkey). A549 and H1299 cells were cultured in RPMI 1640 medium supplemented with penicillin G (100 U/mL), streptomycin (100 mg/mL), L-glutamine, and 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2.

**Cytotoxicity assay**

For the Sulforhodamine B (SRB) assay, which is routinely performed by National Cancer Institute (NCI) for in vitro drug screening, n-hexane, chloroform, n-butanol and remaining
water extracts were added to 96-well plates to make up a final concentration range of 1.56 µg/mL to 100 µg/mL by serial dilutions (six two-fold dilutions). Then, A549 and H1299 cells were seeded at a density of 5x10^3 cells per well of 96-well plates. Subsequently, cells were incubated with various concentrations of the aerial parts and roots extracts for 48 h. The assay was terminated by the addition of ice-cold 50% (w/v) trichloroacetic acid. SRB 0.4% (w/v) in 1% (v/v) acetic acid staining was then performed. The bound dye was extracted using 10 mM unbuffered Tris and optical density was measured at 564 nm with an ELISA plate reader (FLASH Scan S12, Analytik Jena, Germany). Viability of treated cells was calculated in reference to the untreated control cells by using the following formula:

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\text{Cell viability (%) = } \left[ 100 \times \left( \frac{\text{Sample Abs}}{\text{Control Abs}} \right) \right].
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RESULTS AND DISCUSSION

The cytotoxic effect of the extracts from the aerial parts and roots of *C. canadensis* on lung cancer cell lines (A549 and H1299) were first investigated by the SRB assay after treating cells with increasing doses of extracts (1.56 µg/mL-100 µg/mL) for 48 h. It was found that the extracts inhibited growth of cells in a dose-dependent manner and prominently reduced the cell viability at the 100 µg/mL. The cytotoxic effects after the treatment with the extracts against human lung adenocarcinoma cell lines were shown in Figures 1a, 1b, 2a and 2b.

In order to compare antigrowth effects of various concentrations of the aerial parts and roots extracts of *C. canadensis*, the root extracts exhibited relatively higher antigrowth effects than the aerial parts extracts. The strongest cytotoxic activity was detected for the *n*-hexane extract of the roots with IC₅₀ values 94.73 and 84.85 µg/mL on A549 and H1299 cell lines, respectively. Overall, the antigrowth effects of the extracts were not dependent on the cell line. Considering the fact that A549 expresses wild type p53 and H1299 is p53 null, it can be stated that the resulting antigrowth effects of the extracts are p53 independent (39). This may actually be favorable because majority of cancers have mutated p53, thereby these extracts would still be active against even p53-mutated ones.

To the best of our knowledge, this is the first attempt rationalizing the cytotoxic effect of *C. canadensis* on A549 and H1299 human lung cancer cells. Nevertheless, a few
studies demonstrated earlier cytotoxic effect of the extracts of *C. canadensis* of different origin having varying levels of inhibition on various cancer cell lines (26,34-37). For instance; *n*-hexane, chloroform and aqueous MeOH extracts partitioned from MeOH extract of the aerial parts, flowers and roots of *C. canadensis* and the H₂O extracts prepared from the residual plant materials were investigated for their cytotoxic properties on HeLa, MCF-7 and A431 cell lines using the MTT assay. The *n*-hexane phase of the roots exhibited markedly antigrowth effects on the cell lines (62.4-70.1%) at 10 µg/mL, and the CHCl₃ phase of the roots demonstrated moderate antiproliferative activity (39.3-47.9%) at the same concentration (34). According to the bioactivity-guided fractionation of the *n*-hexane and chloroform phases of the methanol extract from the roots of *C. canadensis*, two new unusual C₁₀ γ-dihydropyranone derivatives (conyzapyranone A and conyzapyranone B), as well as 2 γ-lactone acetylene derivatives (e.g. 4E,8Z-matricaria-γ-lactone), triterpenes, sterols (e.g. spinasterol), a hydroxy fatty acid and a flavonoid were isolated. Among them, conyzapyranone B, 4E,8Z-matricaria-γ-lactone and spinasterol were found to have remarkable antiproliferative activity against HeLa, MCF-7 and A431 cell lines (26). In other study, cytotoxic activities of petroleum ether, ethyl acetate and methanol extracts of the aerial parts of *C. canadensis* were investigated on Hep-2 using methylene blue assay at 24, 48 and 72 h of incubation. At 72 h of incubation, the most active extracts were found to be ethyl acetate and petroleum ether extracts with IC₅₀ values 45 and 50 µg/mL, respectively (36). In another study, erigeronol, a new triterpene derivative, was isolated from *C. canadensis* as a potent cytotoxic compound with IC₅₀ value of 7.77±0.47 µg/mL on melanoma B16 cell line by the MTT method (37). In these studies, triterpenes, C₁₀ acetylene derivatives and dihydropyranones have mainly found as effective cytotoxic constituents in *C. canadensis* (26,37). In our study, terpenes and flavonoids were principally detected in the root extracts according to the preliminary phytochemical analysis. The present investigation represents a preliminary screen for the cytotoxic effect of *C. canadensis* in human lung cancer cell lines. In accordance with the National Cancer Institute Guidelines, extracts with IC₅₀ values <20 µg/mL were accepted as active (40). This study resulted in moderate cytotoxic activity compared to the previous studies against the selected cell lines which might be attributed to usage of different cell lines as well as the diverse phytochemical composition in the extracts. The present results are also in accordance with ethnomedicinal uses of the plant reported by Hartwell (Hartwell, 1968).

In conclusion, we provided the first evidence for cytotoxic effects of *C. canadensis* against A549 and H1299 cancer cell lines although it shows the cytotoxic activity at relatively higher doses.

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