

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

## *Conyza canadensis* (L.) Cronquist'in İnsan Akciğer Kanseri 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-H<sub>2</sub>O) extracts fractionated 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 IC<sub>50</sub> 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

### ÖZ

Asteraceae familyası bitkileri antikanser aktivite potansiyeline sahip olmalarından dolayı büyük ilgi görmektedir. Bundan dolayı, bu çalışmada, *Conyza canadensis* (L.) Cronquist'in (Asteraceae) insan akciğer kanseri hücre hatlarına karşı (A549 ve H1299) sitotoksik etkisi ilk kez test edilmiştir. *C. canadensis*'in kökleri ve toprak üstü kısımlarının metanol ekstresinden fraksiyonlanan *n*-hekzan, kloroform, *n*-butanol ve kalan sulu (R-H<sub>2</sub>O) ekstraktlarının sitotoksik etkisi Sülfürdamini B (SRB) yöntemiyle incelenmiş ve % canlılık oranı hesaplanmıştır. Sonuçlar *C. canadensis* ekstraktlarının kanser hücreleri üzerinde doza bağlı olarak sitotoksik aktiviteye sahip olduğunu göstermiştir. Kök ekstraktları nispeten toprak üstü kısımlarından daha yüksek aktivitede bulunmuştur. En aktif ekstre A549 ve H1299 hücre hatları üzerine IC<sub>50</sub> değerleri sırasıyla 94.73 ve 84.85 µg/mL olan köklerin *n*-hekzan ekstresi olarak tespit edilmiştir. Bu sonuçlar *C. canadensis*'in akciğer kanseri hücrelerinde orta derecede sitotoksikite gösterdiğini belirtmektedir. Bu durum bitkinin terapötik amaçlar için değerlendirilebileceğini göstermektedir.

**Anahtar kelimeler:** Asteraceae, *Conyza canadensis*, Sitotoksik aktivite, Akciğer kanseri, Sülfürdamini B testi

### 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, antihemorrhagic 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, C<sub>10</sub> acetylenes such as diyn-ene (e.g. *E*-lactonophyllum methyl ester) and ene-diyn-ene (e.g. matricaria methyl ester isomers), and C<sub>10</sub> lactones (e.g. 8*Z*-matricaria- $\gamma$ -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 F<sub>254</sub> 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% H<sub>2</sub>SO<sub>4</sub> (Merck) solution in MeOH (Merck) and 1% vanillin-H<sub>2</sub>SO<sub>4</sub> solution [vanillin

(Boehringer Mannheim, Mannheim, Germany) and H<sub>2</sub>SO<sub>4</sub> (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. Mecit 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 (GAZI), 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 (3×300 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 (3×100 mL), chloroform (3×100 mL) and *n*-butanol saturated with H<sub>2</sub>O (3×100 mL) in a separatory funnel. Each extract as well as remaining aqueous phase (R-H<sub>2</sub>O) 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), "CHCl<sub>3</sub> 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-H<sub>2</sub>O 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 CHCl<sub>3</sub> 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% H<sub>2</sub>SO<sub>4</sub> 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% H<sub>2</sub>SO<sub>4</sub> after heating for 5 min at 100°C. The *n*-butanol and R-H<sub>2</sub>O extracts were developed in a mixture of solvent system CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (61:32:7) and then sprayed with 1% vanillin-H<sub>2</sub>SO<sub>4</sub> 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% CO<sub>2</sub>.

### 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  $\mu\text{g/mL}$  to 100  $\mu\text{g/mL}$  by serial dilutions (six two-fold dilutions). Then, A549 and H1299 cells were seeded at a density of  $5 \times 10^3$  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:

$$\text{Cell viability (\%)} = [100 \times (\text{Sample Abs}) / (\text{Control Abs})].$$

## 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  $\mu\text{g/mL}$ -100  $\mu\text{g/mL}$ ) 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  $\mu\text{g/mL}$ . 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  $\text{IC}_{50}$  values 94.73 and 84.85  $\mu\text{g/mL}$  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

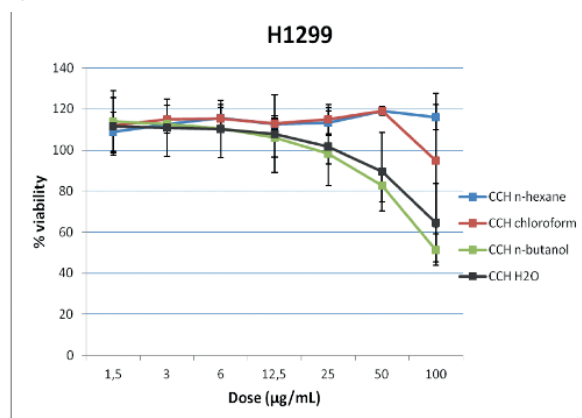


Figure 1a. The cytotoxic effects with different concentrations of the aerial parts extracts of *C. canadensis* on H1299 cell lines

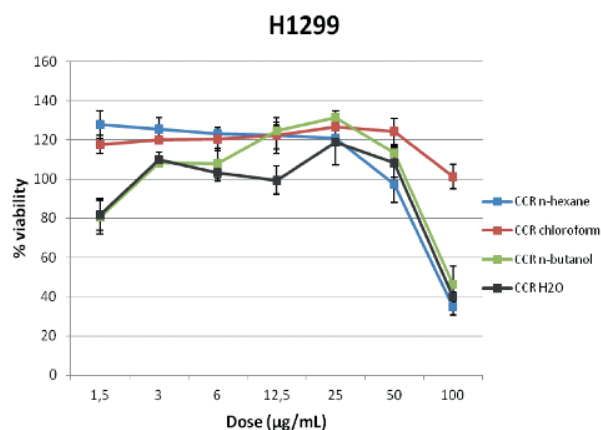


Figure 2a. The cytotoxic effects with different concentrations of the root extracts of *C. canadensis* on H1299 cell lines

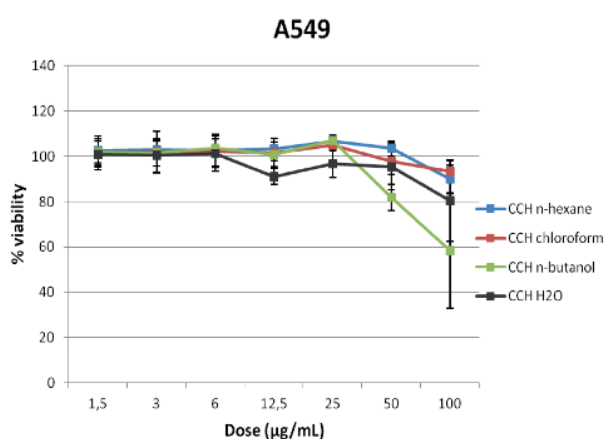


Figure 1b. The cytotoxic effects with different concentrations of the aerial parts extracts of *C. canadensis* on A549 cell lines

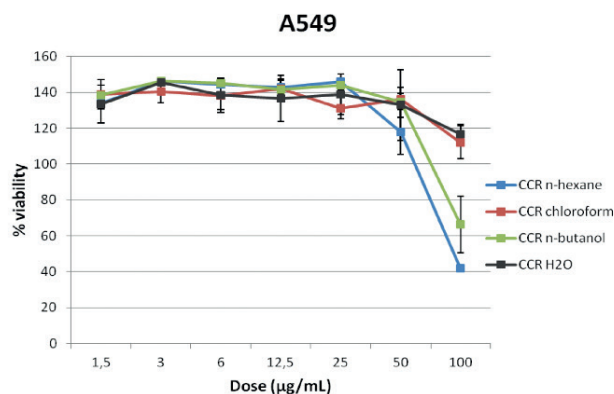


Figure 2b. The cytotoxic effects with different concentrations of the root extracts of *C. canadensis* on A549 cell lines

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 H<sub>2</sub>O 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 CHCl<sub>3</sub> 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 C<sub>10</sub> 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 IC<sub>50</sub> 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 IC<sub>50</sub> value of 7.77±0.47 µg/mL on melanoma B16 cell line by the MTT method (37). In these studies, triterpenes, C<sub>10</sub> 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 IC<sub>50</sub> 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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