Dandelion Root Extract Affects the Proliferation, Survival and Migration of Cervical Cancer Cell Lines

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Research Article

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Abstract

Background

Taraxacum officinale has been shown to exert diverse biological activities, in someones cancer cell lines. Also in traditional medicine have been its used fresh latex to treat warts (non-malignant HPV infection); however, its effect over cervical cancer cell lines is yet to be explored. The aim was to evaluate the effects of a root extract of *Taraxacum officinale* on the proliferation, survival and migration of cervical cancer cell lines.

Methods

Ethanollic extract was obtained from *T. officinale* roots (R-EtOH). Caski (HPV16), HeLa (HPV18), C33A (HPV-) cervical cancer cell lines (CC) and keratinocytes HaCaT (HPV-) were used. Viability and inhibitory concentration values were determined by the MTT assay. Clonogenic assay was used to evaluate the survival. Effect on cell migration was evaluated by wound healing assay. Apoptotic response was observed using the TUNEL and Annexin V/7AAD assay.

Results

R-EtOH extract showed a dose-dependent cytotoxic effect on all cell lines. The number and size of the colonies, mainly in CC, decrease with higher concentrations of extract. R-EtOH effectively inhibits cell migration, and causes a higher percentage of apoptotic cells.

Conclusion

This study reports, for the first time, the antitumor potential of dandelion root extract (R-EtOH) on CC. Our results showed that R-EtOH affect proliferation, survival and cell migration. In addition, R-EtOH leads to apoptosis in all CC cells; thus, it is a promising extract for future studies which will allow to evaluate its usefulness in cervical cancer treatment as in other cancer types.

Background

Cervical cancer, one of the most common gynecological tumors, is the third leading cause of cancer-associated deaths among women worldwide with an estimated 530,000 new cases annually and 270,000 deaths. Approximately 85% of worldwide deaths from cervical cancer occur in underdeveloped or developing countries, and the death rate is 18 times higher in low-income and middle-income countries when compared with wealthier countries.[1] In Argentina, 5000 women per year are still diagnosed with cervical cancer and 2,000 die from this tumor [2].

Human papillomavirus (HPV) infection is predominantly responsible, nearly 100%, for the incidence of cervical cancer [3]. According to statistical analyzes, among the 15 high-risk (HR) HPV genotypes, HPV16 and HPV18 are responsible for 75% of cervical cancer cases [4]. While low-risk (LR) HPV genotypes, such as HPV-6 and HPV-11, are associated with non-malignant manifestations of infection, for example, low-grade squamous intraepithelial lesions, anogenital warts, condyloma acuminata and others [5]

There are different treatment methods with their advantages and disadvantages. Therefore, choosing a treatment option is never easy and the choice will be influenced by multiple factors [6–8]. One of the options is chemotherapy; however, the main problems concerning chemotherapeutic agents are severe adverse effects and multiresistance formations [9].

In that sense, natural therapeutic agents have become important for the development of new treatment strategies that could be deployed in cancer therapy. Phytochemical compounds obtained from extracts of plant roots, bulbs, barks, leaves, stems and other plant parts have shown promising potential as anti-cancer drugs, and as lead compounds in the synthesis of new drugs [10].

In traditional medicine, fresh "dandelion" latex (*Taraxacum officinale* G. Weber ex F.H. Wigg) is sometimes used to treat warts (manifestations of HPV infection), although without proven efficacy [11, 12]. *T. officinale* is a widespread flowering herbaceous perennial plant which belongs to the Asteraceae family with wide global distribution. Prominent constituents in dandelion include sesquiterpene lactones, triterpenes, several phenolic acids, flavonoids and coumarin [13, 14]. It is regarded as a nontoxic herb that can be potentially exploited for its choleric, diuretic, antirheumatic, and anti-inflammatory properties [15]. Recent studies showed a strong anti-cancer activity of a dandelion root extract. Some authors found that this extract is able to induce a rapid activation of the death-receptor mediated extrinsic pathway of apoptosis in human leukemia and pancreatic cancer cells. In addition, this extract would be cancer cell selective, as the same treatment is not detrimental to non-cancer cells [16–19]. Moreover, new studies have reported antitumor activity of *T. officinale* in cell lines of pediatric cancer, colon cancer and gastric cancer [20–22].

It is known that cervical cancer is initially asymptomatic, later transforming into high-grade squamous intraepithelial lesions and invasive cancer. Thus, the findings of the anti-tumor role of *T. officinale* mentioned above, allow as to thinking that bioactive components of this species could have activity in the treatment of lesions of different severity, caused by the HPV.

The aim of this study was to evaluate the effects of a *T. officinale* root extract on the proliferation, survival and migration of cervical cancer cell lines infected with human papillomavirus.

Methods

Collection of *T. officinale*

The vegetal material was collected in Córdoba city, Argentina (between 31°22'47.75 "S, 64°8'46.34" W and 31°22'43.84 "S, 64° 8'24.49" W), in accordance with the national guidelines of Argentina. The species was identified by PhD Gloria Barboza, in the Museum of Botany of Facultad de Ciencias Exactas Físicas y Naturales-Universidad Nacional de Córdoba, where a sample of herbarium was deposited (CORD00027086).

Obtaining root extract of *T. officinale* (R-EtOH)

Individuals of dandelion were transported to the laboratory, washed with water in order to remove all traces of dust, and then dried in an oven stove at 28°C, limiting its exposure to the light. Once the material was dried, roots were separated from aerial parts and subsequently ground. An extraction with ethanol was carried out by means of a Soxhlet apparatus. Extractive liquids were separated from the plant material which was concentrated to dryness by using a rotary evaporator under reduced pressure and at moderate temperatures. A stock solution in dimethylsulfoxide [100 mg/mL] was prepared from which subsequent tests were performed.

Cell lines

The following cell types were used: CaSki (HPV16+), HeLa (HPV18+), C33A (HPV-) cervical cancer cell lines and human immortalized keratinocytes HaCaT (HPV-). This last cell line was used as a control. CaSki and HaCaT cells were maintained in RPMI 1640 medium (Gibco™, Thermo Fisher Scientific), while HeLa and C33A were kept in Dulbecco's modified Eagle's medium (DMEM Gibco™, Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS, NATOCOR®, Argentina) and 40 µg/mL gentamicin. All cell lines were provided by the Cell Culture Laboratory of Instituto de Virologia Dr. Jose Maria Vanella, Argentina and were preserved in culture flasks at 37°C with 5% CO₂ in a humidified incubator.

Cell viability assay

Cell viability and inhibitory concentration values [IC₂₀] and [IC₅₀] were determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay. This assay allows to assess the cytotoxic potential of R-EtOH extract, since only mitochondrial dehydrogenases formed in viable cells are able to reduce tetrazolium salt into an insoluble formazan product [23]. Briefly, 1x10⁵ cells/mL were seeded in 96-well plates at 37°C with 5% CO₂ for overnight incubation and treated with different concentrations of R-EtOH (10-1000 µg/mL) for 48 hours in their respective media, supplemented with 2% FBS. The cells were then incubated in a serum-free medium that contained MTT at a final concentration of 0.5 mg/mL for 1 hour [24]. The crystals formed in intact cells were solubilized in 100 µL of MTT solution (10% of triton X-100, 10% of 0.1N HCl, 80% isopropanol), and the absorbance was measured at 570 nm by using a microplate reader (BioTek ELx800, USA). All samples were assayed in triplicate, and values were normalized to untreated controls. Analysis of results was done with software Origin Pro 8.6.

Clonogenic Survival Assay

Clonogenic assay allows us to estimate cell survival, based on the cell's regenerative capacity after having been exposed to an extract. Cells (200 cells/well) were placed in 12-well plates and incubated for 48 hours in RPMI 1640 or DMEM with 10% FBS at 37°C and 5% CO₂ in a humidified incubator. Then, the aforementioned medium was discarded and the cells were treated with [IC₂₀] and [IC₅₀] R-EtOH in culture medium with 2% FBS for 2 hours. Culture medium with 2% FBS was used for the untreated control. Subsequently, the medium with R-EtOH was also discarded, washed with phosphate buffered saline (PBS), and then medium supplemented with 10% FBS in 3% methylcellulose was added. After 7 days, it was fixed with methanol and stained with 0.1% violet crystal. The number of visible colonies was counted using an optical microscope with a 40x magnification. It was interpreted as a colony whose clone comprises 50 cells or more. The results were expressed as surviving fraction (SF) [25].

$$\text{Plating efficiency (PE)} = \frac{\text{number of colonies counted}}{\text{number of cells plated}} \times 100$$

$$\text{Surviving Fraction (SF)} = \frac{\text{PE of treated cells}}{\text{PE of untreated cells}}$$

Migration assay

Cell migration capacity was calculated by wound healing assay. Cells were seeded at a density of 2 x10⁵ cells/mL, placed into 12-well plates and incubated for 48 hours reaching 100% confluence, then they were treated with [IC₂₀] R-EtOH in culture medium with 2% FBS for 48 hours. Culture medium with 2% FBS was used for the untreated control. The next day, artificial wounds in each one of the cell lines were made with 10µL tips, they were washed three times with PBS and incubated in a serum-free medium. Wounds were observed at 0, 6, 12, 18 and 24 hrs, with the OLYMPUS IX81 microscope to 20X. Average extension of the wound closure was evaluated by measuring the area of the wound by the ImageJ/Fiji software, version 1.46 (<http://imagej.nih.gov/ij/>) [26].

2.7 Identification of cell apoptosis by means of Hoechst staining and TUNEL assay

Cells were seeded at a density of approximate 1 x10⁵ cells/mL, in 96 well plates. The next day, they were treated with [IC₂₀] and [IC₅₀] R-EtOH in culture medium with 2% FBS, in triplicate and for 48 hours. Culture medium with 2% FBS was used for the untreated control. For terminal deoxynucleotidyl transferase dUTP nick-end labeling, TUNEL assay was performed using the In Situ Cell Death Detection Kit, TMR red TUNEL (Roche Diagnostics, Sigma-Aldrich). Cells were fixed in 4% paraformaldehyde and then they were washed with PBS. Subsequently, cells were permeabilized with 0.1% Triton X-100, in 0.1% sodium citrate for 2 min on ice and then they were washed with PBS. The cells were incubated with 50 µL TUNEL reaction mixture at 37°C for 60 min in a dark humidified atmosphere. Images were captured with an OLYMPUS IX81 fluorescence microscope, and subsequently they were evaluated with ImageJ/Fiji software, version 1.46 (<http://imagej.nih.gov/ij/>). Hoechst 33258 staining (Sigma-Aldrich) was used to visualize the nucleus.

Kinetic of apoptotic cell death via Annexin VFITC/7AAD assay.

Cells were seeded at a density of 2×10^5 cells/mL placed into 12-well plates and incubated reaching 100% confluence. Then they were treated with $[IC_{20}]$ R-EtOH in culture medium with 2% FBS for 24 and 48 hours. Culture medium with 2% FBS was used for the untreated control. As a following step, cells were trypsinized (0.15% trypsin) to lift adherent cells from the plates; they were washed twice with FBS and once with PBS and finally suspended in culture medium. Staining was carried out with Kit I for detection of apoptosis FITC annexin V, according to the manufacturer's instructions (BD Pharmingen™). Subsequent to the staining stage, the percentages of viable, apoptotic and necrotic cells were analyzed using the FACSCanto™ II (BD Biosciences) flow cytometer, and data were interpreted with the FlowJo X software, version 10.0.7r2.

Statistical analysis

All experiments were repeated at least three independent times. Statistical analyses were performed using GraphPad Prism 6.1 and Origin Pro 8.6 software. Statistical tests included the Students T-test and Fisher-Test in which $p < 0.05$ or less was considered significant.

Results

Effect of R-EtOH on cell viability

We investigated the effect of R-EtOH on cervical cancer cells viability by-comparing with non-cancerous cells through the reduction method of MTT. The inhibitory concentrations $[IC_{20}]$ and $[IC_{50}]$ are shown in Table 1. It was found that R-EtOH causes a reduction in dose-dependent cell viability, and that fisher's test showed significant differences in the cytotoxicity curve for the CaSki and C33A cells with respect to the concentrations in HaCaT. Effective concentrations of R-EtOH found for cervical cancer cells were lower than in HaCaT (Table 1). Therefore, in order to obtain a greater effect on tumor cells we decided to use concentrations of $[IC_{20}]$ and $[IC_{50}]$, obtained in HaCaT cell line, for the following assays in all cell lines.

Influence of R-EtOH on cell growth using a clonogenic assay.

Table 1: R-EtOH $[IC_{20}]$ and $[IC_{50}]$ values for different cell lines

Cell Line	R-EtOH		
	IC ₂₀ [*] [μg/mL]	IC ₅₀ ^{**} [μg/mL]	F-Test ^{***}
HaCaT	151.6 ± 13.5	228.1 ± 8.0	
Hela	108.1 ± 20.2	201.8 ± 12.3	>0.05
Caski	94.5 ± 0.8	161.1 ± 0.7	<0.05
C33A	22.3 ± 3.8	37.1 ± 3.7	<0.05

Data are mean values from three independent experiments $\bar{x} \pm SD$ performed in triplicate. (*) **IC₂₀**: concentration that reduced the viable cells to 20%. (**) **IC₅₀**: concentration that reduced the viable cells to 50% (***) **F-Test**: The IC values found for CC cell lines were compared with the corresponding findings in HaCaT cell. $p < 0.05$ or less was considered significant

Since the clonogenic assay is useful for testing the proliferative capability of cells by virtue of the cell's ability to undergo sufficient proliferation to form a colony, it is especially suitable for assessing long-term effects of cell treatments (e.g., a few weeks after treatment). In particular, as the cell metabolic state and clonogenic potential are not necessarily parallel events, the clonogenic assay can add valuable and distinct information to that provided by the MTT assay and may be seen as complementary [27]. We observed that the reduction in the ability to form colonies in cervical cancer cells was significantly greater than in HaCaT cells (Fig. 1).

The inhibitory effect of R-EtOH on cells migration capacity

Besides cell proliferation, migration is an important characteristic of tumor cells [20]. Thus, we performed wound healing assays to assess the impact of R-EtOH on cancer cell migration. It was observed that treatment with R-EtOH inhibited the ability to migrate into the wound in all cell lines, this affects mainly cervical cancer cells, unlike the untreated control group (Fig. 2 a. and b). At the end of the assay HeLa cells had a lower migration capacity than CaSki cells, this could be because the latter are cells of CC metastasis, so they would have greater migration capacity than HeLa.

R-EtOH induces apoptosis in cervical cancer cells

In order to determine whether the cell death induced by R-EtOH was apoptotic, we used the specific TUNEL assay (Fig 3a). Significant differences were observed in the percentage of apoptotic cells between CC and HaCaT for each concentration. HaCaT cells showed a slight apoptotic effect after treatment with R-EtOH, but this was not statistically significant (Fig. 3b). It was observed that with higher doses of R-EtOH the percentage of apoptotic cells in cervical cancer cells significantly increased (Fig 3b). In [IC₂₀] there were significant differences between CaSki-HeLa and CaSki-C33A, but there were not differences between HeLa and C33A. However, for [IC₅₀] there were significant differences between all CC lines,

mainly affecting CaSki then C33A and finally HeLa (Fig. 3b). This result suggests that R-EtOH contains bioactive components which were effective to induce apoptosis in cervical cancer cells.

Kinetic of apoptotic cell death via Annexin VFITC/7AAD assay

Stages of apoptosis were quantified using Annexin V as a marker for apoptosis and 7-amino-actinomycinD (7AAD) as a marker of necrosis. In this assay, cells in the early stages of apoptosis stain Annexin V–positive and 7AAD-negative (upper left quadrant, Q1), whereas cells in the later stages of apoptosis stain Annexin V–positive and 7AAD-positive (upper right quadrant, Q2). Viable cells stain negative for both Annexin V and 7AAD (lower left quadrant, Q4) and necrotic cells stain Annexin V–negative and 7AAD-positive (lower right quadrant, Q3) (Figure 4a).

As shown in Fig. 4a, the majority of untreated cells were viable (lower left quadrant, Q4). There were significant differences between the percentages of apoptosis of all the CC lines with respect to HaCaT. After 24 hours of R-EtOH [IC_{20}] treatment, we observed an increase in the percentage of apoptotic cells, mainly in early apoptosis (Q1). This increase was significantly different for all CC cells when compared to untreated cells (Fig 4b). After 48 hours of treatment, there was a drastic reduction in viability in CC cells (Q4) and a significant increase in the percentage of apoptosis for CaSki and C33A cells when compared to the 24 hour treatment. Although after 24 hours there were not significant differences in the percentages of apoptosis between CC cells, after 48 hours significant differences between all CC lines were observed, being the highest percentages in CaSki, then C33A and finally HeLa cells (Fig 4b). Although after 48 hours an increase in the percentage of necrotic cells is observed, the fact that cells progress through early apoptosis in a time-dependent manner before staining positive for 7AAD suggests that positive 7AAD staining is indicative of cells dying as a result of apoptosis and not necrosis.

Discussion

Cervical cancer is responsible for 10–15% of cancer-related deaths in women worldwide, where HPV is the causative agent in over 99% of the cases. Although vaccines for HPVs and improvements in early screening have successfully reduced the mortality rate, cervical cancer is still considered a major public health problem in developing countries [28]. Although there is a diversity of treatments for lesions caused by HPV, recent studies report that about one out of six treated women (16%) manifested at least one persistent High Risk HPV type that was associated with recurrent or residual High Squamous Intraepithelial Lesion disease [29]. Therefore, the search for new treatments is of great importance. In recent years, antitumor properties of dandelion extracts have been reported; however, there is little evidence of its effects on cervical cancer.

In MTT assay, we revealed a decrease in dose-dependent cell viability and greater cytotoxicity in CaSki and C33A cells with respect to HaCaT. However, we did not find significant differences between HeLa and HaCaT cells in this assay. R-EtOH showed a great cytotoxic effect, since [IC_{50}] values found were lower than reported in other studies [20–22]. This may be due to the nature of different cancer cell lines or to

the fact that R-EtOH might contain a higher concentration of bioactive compounds, since the other reports refer to aqueous extracts.

Our results confirmed that exposure to higher concentrations of R-EtOH significantly inhibits the proliferation and colony formation of cervical cancer cells. It is known that any substance that inhibits the clonogenic progression of tumor cells could be considered a potential anticancer therapeutic agent [30]. Analysis of wound healing showed the ability of R-EtOH to inhibit cell migration, showing greater effect in cancer cells. This effect has also been reported by other authors in different cancer cell lines treated with dandelion extracts [20, 22, 31]. HaCaT cells treated with R-EtOH were able to migrate more freely to the wound area than cancer cell lines, showing selectivity of R-EtOH to cancer cells. This inhibition of migration, caused by R-EtOH, could reduce the ability of cancer cells to metastasize to secondary sites.

The loss of balance between cell proliferation and apoptosis is a hallmark that increases the failure of tumoral cells to be eliminated through apoptosis. One essential strategy for cancer therapy is to activate apoptotic pathways in the tumor cells [32]. The Annexin-V/7AAD and TUNEL assays confirmed that the decrease in cell viability is a result of apoptotic processes induced by R-EtOH treatment. Extension of treatment periods (24–48 hrs) as well as the increment of concentration ($[IC_{20}]-[IC_{50}]$), increased significantly the apoptosis rate in cancer cell lines.

Our findings provide other authors' reports with additional information concerning the antitumor properties of *T. officinale*, which turns this plant species into an important target in the search for new compounds that can be useful in cancer treatment [16, 20–22].

Conclusion

This study reports, for the first time, the antitumor potential of dandelion root extract (R-EtOH) against cervical cancer cell lines. Our results showed that dandelion root extract (R-EtOH) contains bioactive components that affect proliferation, survival and cell migration. In addition to this, it leads cervical cancer cells to the programmed cell death. Therefore, this is a promising extract for future studies which will allow to evaluate the usefulness of its compounds in the treatment of cervical cancer as well as in other cancer types.

Declarations

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Writing – review & editing: VENEZUELA R. F., KONIGHEIM B. S., CUFFINI C. G.

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References

1. Small W, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, et al. Cervical cancer: A global health crisis. *Cancer*. 2017;123:2404–12.
2. Arrossi S, Curotto M, Thouyaret L, Paolino M, Cuberli M, Laudi R. Manual para la implementacion del test de VPH en contexto Programático. 2016.
<http://www.msal.gob.ar/images/stories/bes/graficos/0000000906cnt-2016-11-08-manual-para-la-implementacion-test-vph-en-contexto-programatico.pdf>.

3. Harder T, Wichmann O, Klug SJ, van der Sande MAB, Wiese-Posselt M. Efficacy, effectiveness and safety of vaccination against human papillomavirus in males: A systematic review. *BMC Med.* 2018;16:1–14.
4. Melnikow J, Henderson JT, Burda BU, Senger CA, Durbin S, Soulsby MA. Screening for Cervical Cancer With High-Risk Human Papillomavirus Testing: A Systematic Evidence Review for the U.S. Preventive Services Task Force. Rockville, MD: Agency for Healthcare Research and Quality; 2018.
5. Steben M, Tan Thompson M, Rodier C, Mallette N, Racovitan V, DeAngelis F, et al. A Review of the Impact and Effectiveness of the Quadrivalent Human Papillomavirus Vaccine: 10 Years of Clinical Experience in Canada. *J Obstet Gynaecol Canada.* 2018;:1–11. doi:10.1016/j.jogc.2018.05.024.
6. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N, et al. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2017;28 Supplement 4:v72–83.
7. Park IU, Introcaso C, Dunne EF. Human Papillomavirus and Genital Warts: A Review of the Evidence for the 2015 Centers for Disease Control and Prevention Sexually Transmitted Diseases Treatment Guidelines. *Clin Infect Dis.* 2015;61 Suppl 8:S849–55.
8. Workowski K, Berman S. Genital Warts. Sexually Transmitted Diseases Treatment Guidelines 2010. Centers for Disease Control and Prevention. 2018. <http://www.cdc.gov/std/treatment/2010/genital-warts.htm>. Accessed: October 25, 2018.
9. Chinembiri TN, Du Plessis LH, Gerber M, Hamman JH, Du Plessis J. Review of natural compounds for potential skin cancer treatment. 2014.
10. Goyal S, Gupta N, Chatterjee S, Nimesh S. Natural Plant Extracts as Potential Therapeutic Agents for the Treatment of Cancer. *Curr Top Med Chem.* 2016;17:96–106.
11. Guarrera PM. Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). *Fitoterapia.* 2005;76:1–25.
12. Shannon F, Sasse A, Sheridan H, Heinrich M. Are identities oral? Understanding ethnobotanical knowledge after Irish independence (1937–1939). *J Ethnobiol Ethnomed.* 2017;13:1–19.
13. Schütz K, Carle R, Schieber A. Taraxacum-A review on its phytochemical and pharmacological profile. *J Ethnopharmacol.* 2006;107:313–23.
14. Mišek M, Marcinčáková D, Legáth J. Polyphenols Content, Antioxidant Activity, and Cytotoxicity Assessment of Taraxacum officinale Extracts Prepared through the Micelle-Mediated Extraction Method. *Molecules.* 2019;24:1025. doi:10.3390/molecules24061025.
15. González-Castejón M, Visioli F, Rodriguez-Casado A. Diverse biological activities of dandelion. *Nutr Rev.* 2012;70:534–47.
16. Chatterjee SJ, Ovadje P, Mousa M, Hamm C, Pandey S. The Efficacy of Dandelion Root Extract in Inducing Apoptosis in Drug-Resistant Human Melanoma Cells. *Evidence-Based Complement Altern Med.* 2011;2011:1–11. doi:10.1155/2011/129045.
17. Ovadje P, Chatterjee S, Griffin C, Tran C, Hamm C, Pandey S. Selective induction of apoptosis through activation of caspase-8 in human leukemia cells (Jurkat) by dandelion root extract. *J*

- Ethnopharmacol. 2011;133:86–91. doi:10.1016/j.jep.2010.09.005.
18. Ovadje P, Chochkeh M, Akbari-Asl P, Hamm C, Pandey S. Selective Induction of Apoptosis and Autophagy Through Treatment With Dandelion Root Extract in Human Pancreatic Cancer Cells. *Pancreas*. 2012;41:1039–47. doi:10.1097/MPA.0b013e31824b22a2.
 19. Ovadje P, Hamm C, Pandey S. Efficient Induction of Extrinsic Cell Death by Dandelion Root Extract in Human Chronic Myelomonocytic Leukemia (CMML) Cells. *PLoS One*. 2012;7:e30604. doi:10.1371/journal.pone.0030604.
 20. Menke K, Schwermer M, Felenda J, Beckmann C, Stintzing F, Schramm A, et al. *Taraxacum officinale* extract shows antitumor effects on pediatric cancer cells and enhance mistletoe therapy. *Complement Ther Med*. 2018;40 June 2017:158–64. doi:10.1016/j.ctim.2018.03.005.
 21. Ovadje P, Ammar S, Guerrero J-A, Arnason JT, Pandey S. Dandelion root extract affects colorectal cancer proliferation and survival through the activation of multiple death signalling pathways. *Oncotarget*. 2016;7:73080–100.
 22. Zhu H, Zhao H, Zhang L, Xu J, Zhu C, Zhao H, et al. Dandelion root extract suppressed gastric cancer cells proliferation and migration through targeting lncRNA-CCAT1. *Biomed Pharmacother*. 2017;93:1010–7. doi:10.1016/j.biopha.2017.07.007.
 23. Sittampalam G, Coussens N, Arkin M, Auld D, Austin C, Bejcek B, et al. *Assay Guidance Manual*. *Assay Guid Man*. 2016;Md:305–36.
 24. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55–63.
 25. Munshi A, Hobbs M, Meyn RE. Clonogenic cell survival assay. *Methods Mol Med*. 2005;110:21–8.
 26. Choudhari AS, Suryavanshi S a., Kaul-Ghanekar R. The Aqueous Extract of *Ficus religiosa* Induces Cell Cycle Arrest in Human Cervical Cancer Cell Lines SiHa (HPV-16 Positive) and Apoptosis in HeLa (HPV-18 Positive). *PLoS One*. 2013;8:1–10.
 27. Finetti F, Moglia A, Schiavo I, Donnini S, Berta GN, Di Scipio F, et al. Yeast-derived recombinant avenanthramides inhibit proliferation, migration and epithelial mesenchymal transition of colon cancer cells. *Nutrients*. 2018;10:1–16.
 28. Yao S, Xu J, Zhao K, Song P, Yan Q, Fan W, et al. Down-regulation of HPGD by miR-146b-3p promotes cervical cancer cell proliferation, migration and anchorage-independent growth through activation of STAT3 and AKT pathways. *Cell Death Dis*. 2018;9:1055. doi:10.1038/s41419-018-1059-y.
 29. Lindroth Y, Bjelkenkrantz K, Forslund O. Spectrum of HPV types before and after treatment of cervical intraepithelial neoplasia grade 2 and 3. *J Clin Virol*. 2017;97 September:38–43. doi:10.1016/j.jcv.2017.10.014.
 30. Rafehi H, Orlowski C, Georgiadis GT, Ververis K, El-Osta A, Karagiannis TC. Clonogenic Assay: Adherent Cells. *J Vis Exp*. 2011;:15–7. doi:10.3791/2573.
 31. Ovadje P, Ammar S, Guerrero J-A, Arnason JT, Pandey S. Dandelion root extract affects colorectal cancer proliferation and survival through the activation of multiple death signalling pathways. *Oncotarget*. 2016;7:73080–100. doi:10.18632/oncotarget.11485.

32. Khazaei S, Esa NM, Ramachandran V, Hamid RA, Pandurangan AK, Etemad A, et al. In vitro antiproliferative and apoptosis inducing effect of *Allium atrovioaceum* bulb extract on breast, cervical, and liver cancer cells. *Front Pharmacol.* 2017;8 JAN:1–16.

Figures

Figure 1

Ability of R-EtOH to inhibit the formation of colonies on cervical cancer cells. Asterisks indicate the significance of p value, comparing cervical cancer cell lines with HaCaT. Student's t-test: (**) p<0.001

Figure 2

Effect of the R-EtOH extract on cell migration a. Microscopy images were observed, at 0, 6, 12, 18 and 24 hrs after artificial wound, with the OLYMPUS IX81 microscope to 20x. C= untreated control, T=treatment with R-EtOH [IC₂₀ 150 µg/mL]. b. Evaluation of artificial wound closure by tumor cell migration, utilizing wound healing assay. Extension percentage of the wound closure. The area of the wound was measured by means of ImageJ/Fiji software, version 1.46. Black Line= untretated control, Gray line= treatment with R-EtOH [IC₂₀].

Figure 3

Evaluation of apoptosis induction by R-EtOH, through the TUNEL assay a. Representative photomicrographs of TUNEL staining. The microscope used is an OLYMPUS IX81 microscope to 40x. b. Percentage of TUNEL positive cells. Asterisks on the columns correspond to differences between CC cells

and HaCaT cells. Asterisks on gray lines correspond to differences between IC20 and IC50 concentrations in each cell line. Asterisks on black lines correspond to different concentrations of IC20 between CC cells. Asterisks on the dark gray lines correspond to different concentrations of IC50 between CC cells. Student's t-test: (*) $p<0.01$, (**) $p<0.001$.

Figure 4

Quantification of apoptosis stages by flow citometry a. Representative pictograms of flow cytometry analysis and of the effect of R-EtOH [IC20] at 24 hrs and 48 hrs on different cell lines are shown for counts of 30000 cells. b. Quantification of Annexin V–positive staining, including both early (Q1) and late (Q2) stage apoptosis. Asterisks in the columns correspond to differences between treated and untreated cells. Asterisks on the columns correspond to differences between CC cells and HaCaT cells, for each exposure time. Asterisks on gray lines correspond to differences between 24 hrs and 48 hrs of treatment in each cell line. Asterisks on black lines correspond to differences at 48 hrs between CC cells. Student's t-test: (*) $p<0.01$, (**) $p<0.001$.