

ANTIPROLIFERATIVE ACTIVITY OF EXTRACTS OF GNAPHALIUM GRACILE H.B.K. AGAINST CANCER CELL LINES

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Abstract

Ethanol and n-hexane extracts obtained from the leaves and inflorescences of *Gnaphalium gracile*, were tested at different concentrations to evaluate their antineoplastic activities on pancreatic, colon, and prostate cancer cell lines by examining mitochondrial function. The polar extracts of both, leaves and inflorescences which contain gnaphalin, quercetin, and 3-methoxy quercetin, exhibited cytotoxicity against every cell line tested with EC₅₀ values ranging between 20.23±1.185 µg/mL and 70.71±1.1419 µg/mL.

The most remarkable values were observed in pancreatic cancer Panc 28 and androgen-dependent prostate LnCaP cells, with EC₅₀ values of 20.23±1.185 and <25µg/mL, and androgen-independent prostate cancer PC-3, colon HCT-116 and pancreatic MIA PaCa cells with values ranging between 28.84±1.1766 and 34.41±1.057 µg/mL. The non-polar extract derived from leaves demonstrated significant cytotoxicity towards colon cancer HCT-116 cells, with an EC₅₀ of 39.46±1.0617 µg/mL.

However, the non-polar extract from the inflorescences did not have an appreciable effect on cell proliferation of any of the cell lines tested except for androgen-independent prostate cancer PC-3 cells with an EC₅₀ of 62.05±1.237 µg/mL. The data obtained support the traditional use of *G. gracile* and suggest the polar extracts from aerial parts, as an interesting source for the development of novel antineoplastic agents.

Keywords: *Gnaphalium gracile*, anti-neoplastic activity, pancreatic cancer, colon cancer, prostate cancer

Introduction

Gnaphalium gracile H.B.K is a species that belongs to the family Asteraceae. In equatorial regions it grows between 2000 and 3200 meters above sea level and it has broad synonymy [1]. The genus *Gnaphalium* has worldwide distribution, with the greatest diversity of species found in the Andean regions of South America. In Colombia, these species grow in association with plants belonging to the genus *Achyrocline* with which they share morphological similarities that may lead to identification errors. Plants from these genera also produce similar flavonoids that may confer closely related medicinal properties to species that in turn, farmers use interchangeably for the same medicinal purposes and commonly name vira-vira. Species of the *Gnaphalium* genus are advocated in the treatment of several different diseases, such as, skin infections, bronchial disorders, inflammation, and cancer in different organs [2]. *Gnaphalium elegans* is popularly used against prostate cancer as well as *Gamochaeta purpureum*, a plant also known as vira-vira. Thus, species of several genera belonging to the tribe Inuleae, such as *Gnaphalium*, *Achyrocline* and *Conyza*, are sold in the popular markets of medicinal plants as vira-vira, to treat the same illnesses because they have similar medicinal effects. These properties have been attributed to flavonoids produced as active metabolites that may convey these healing properties. A number of studies have sought to identify the secondary metabolites produced by different species of *Gnaphalium* [3-7]. These reports include the descriptions of flavonoids as diterpenes [8] with an unsubstituted ring B, and methoxylation on various carbons of rings A and C [9-11]. In a recent study, it was found that the minor variations of ring substitution of flavonoid isomers lead to preferential binding to different DNA sequences [11] and specific cytotoxic actions on cancer cell lines with varying differentiation status [12]. A study of the chemistry of the species *Gnaphalium gracile* H.B.K. reports the presence of methoxylated flavonoids, gnaphaline, and others flavonoids with various hydroxylations in their rings, 3-methoxy quercetin and quercetin [13]. Because active metabolites may occur in different parts of the plant, we hypothesized that extracts obtained with

solvents of different polarity from leaves or inflorescences may have differential anticancer properties and effects on cancer cell lines of various carcinomas. In this study we present the cytotoxic effects of polar and apolar extracts from leaves and inflorescences of *G. gracile* H.B.K. on pancreatic, colon, and prostate cancer cells with varying tumorigenic and differentiation status, and determine the plant sources that may lend support to its traditional use in the treatment of cancer.

Methods

Procedure to collect and process the plant material.

Plants of *G. gracile* H.B.K. were collected during the months of July through September in the area surrounding the Tominé reservoir, in the municipality of Guatavita, in Cundinamarca, Colombia. The specimens were identified at the Colombian National Herbarium. Leaves and inflorescences were separated, dried in the shade, and soxhlet extracted with AcOEt. The use of the latter solvent, avoids the extraction of salts and sugars that may interfere with the extraction of the fractions of interest. From 230g of inflorescences is avoided 17g of extract was obtained. Following the same procedure, but separately, 70g of extract was obtained from 645g of leaves and stems. 5g of each extract were fractionated S/L sequentially with petrol and ethanol, to obtain the apolar fractions (in petrol) and polar (in EtOH) of leaves and inflorescences. The fractions were designated as EFapolar, EFpolar, and EHpolar EHapolar for inflorescences and leaves respectively, and were subsequently dried under vacuum. A 10g sample of leaf extract was subjected to column chromatography on SiGel, with mobile phase Petrol: EtOAc 8: 2. Fractions of 50 mL were collected and isolated and identified as stigmaterol, dehydrostigmaterol and gnaphaline in fractions 6 and 12 respectively, the compounds were purified by crystallization in hexane. In the same manner, gnaphalin was identified after isolation from inflorescence extracts. It was found in a higher proportion of about 0.3% of dry material. Column chromatography with mobile phase RP18 and EtOH : water 7:3, using the polar extract of leaves or inflorescences, yielded quercetine, and a mixture of 3-methoxyquercetin and quercetin. The compounds

were identified by comparing their HNMR spectra with those of pure samples of gnapthaline, 3-methoxyquercetin and quercetin (figure 1).

Cell Lines and Culture Conditions

Tumor derived cells that originated in multiple tissue sites, including: colon (Caco-2 and HCT-116), pancreas (MIA PaCa), and prostate (LNCaP and PC-3), were obtained from the American Tissue Type Culture Collection (Manassas, VA), and maintained according to the supplier instructions. The Panc-28 cell line was a gift from Dr. Paul Chiao, at the University of Texas M. D. Anderson Cancer Center [14] and was grown in tissue culture in the same manner as pancreatic cell line MIA PaCa-2, in Dulbecco's modified Eagle's medium with high glucose, supplemented with 10% serum and penicillin/streptomycin (GIBCO, Life Technologies, Carlsbad, CA). All cells were seeded in 48 well plates and allowed to reach 75% confluency before treatment.

Cell Viability Assay

Cells were treated with either vehicle or (EH) polar, (EH) apolar, (EF) polar, (EF) apolar *G. gracile* extracts from leaves and inflorescences respectively, at concentrations of 25, 50, or 125 µg/mL. The dissolution vehicle was dimethyl sulfoxide at a maximum final concentration of 0.4% in the treated well (Sigma- Aldrich, St. Louis, MO). After 24 hours of incubation, 3-(4, 5-methyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich) was added at 500µg/mL/well for 3 hours. Formazan products were solubilized with acidified 2-propanol (0.1N HCl) and 0.1% NP-40. Assays were quantified by reading optical density at a wavelength of 590 nm using a Biotek PowerWave XS2 plate reader (Winooski, VT). Statistical analyses were done in an IBM SPSS statistics 20, and the inhibitory concentration EC50 value was defined as the extract concentration which caused a 50% decrease in the cell viability of the control. Data from assays displaying decrease of cell viability ≥50%, were evaluated by nonlinear regression analysis (GraphPad Prism, La Jolla, CA), and represented as the effective concentration required to decrease 50% of cell viability (EC50).

Results

The polar extract derived from the leaves of *G. gracile* has greater cytotoxic activity against various cancer cell lines when compared to the apolar extract.

A marked loss in cell viability is observed via MTT assays after treatment of pancreatic, colon, and prostate cancer cell lines with the ethanolic extract of *G. gracile* leaves at concentrations between 25 and 125 µg/mL. The highest cytotoxic effect was observed on pancreatic cancer Panc28 cells, and prostate carcinoma androgen-dependent LNCaP cells (figure 1A). Treatment with the apolar leaf extract had a lesser effect on cell viability when compared to the polar extract. However, appreciable cytotoxic activity against colon cancer HCT-116 cells is observed, followed by Panc 28, androgen-independent prostate cancer PC-3, and androgen-dependent LNCaP cells (figure 1B).

The polar extract derived from the inflorescences of *G. gracile* inhibits cell viability on the cell lines tested but not the apolar extract.

The inflorescence polar extract is most effective at inhibiting cell viability of pancreatic cancer Panc 28 and colon cancer HCT-116 cells, with appreciable activity on pancreatic MIA PaCa cancer cells at concentrations between 25 and 125µg/mL (figure 1C). On the contrary, the apolar extract derived from inflorescences has no effect on these cell lines, and only has a modest effect on androgen-independent PC-3 (figure 1D).

The polar and apolar extracts derived from leaves and inflorescences of *G. gracile* have a differential cytotoxic effect on the various cancer cell lines tested

The polar extract from leaves effectively inhibits cell viability in androgen-dependent LNCaP and androgen-independent PC-3 prostate cancer cells, pancreatic cancer MIA PaCa and colon adenocarcinoma CaCo -2 cells (figure 1A). However, the polar extract derived from inflorescences has a minimal effect on these cell lines at the concentrations shown (figure 1C). The apolar extract derived from leaves display marked cytotoxic activity against pancreatic Panc28, MIA PaCa, and colon HCT-116 cancer cells (figure 1C). On the contrary, the apolar extract from inflorescences has no effect on these cell lines (figure 1D). Cell viability is greatly diminished in colon cancer CaCo-2 cells by the apolar extract from leaves, whilst the extract

from inflorescences may cause cell proliferation (figures 1C and D).

The polar extracts from leaves and inflorescences of *G. gracile* are highly effective against pancreatic cancer cells Panc28.

The polar extracts are effective against Panc28 and MIA PaCa pancreatic cancer cells (figures 2A and B) with the lowest observed EC₅₀ of 20.23±1.185 µg/mL on Panc28 cells (figure 2C). The inflorescence polar extract is also similarly effective on Panc28 cells with an EC₅₀ of 27.58±1.063 µg/mL, but display a lesser effect on MIA PaCa with an EC₅₀ of 57.54± 1.06 µg/mL. The apolar extracts display low or no effect on cell viability in these cells, except for the leaf extract, which is effective against Panc 28 with an EC₅₀ of 57.88± 1.075 µg/mL (figure 2).

The viability of colon cancer HCT-116 but not CaCo-2 cells is effectively diminished by the polar and apolar extracts from leaves and the polar extract from inflorescences of *G. gracile*.

Certain extracts from *G. Gracile* suppress cell viability more effectively in HCT-116 cells than in CaCo-2 (figure 3A and B). HCT-116 cells treated with the polar extracts from leaves and inflorescences and the apolar extract from leaves display EC₅₀ values of 33.95±1.058, 39.46±1.0617, and 40.78±1.0405 µg/mL. While CaCo-2 values are ~50, 86.24±2.5609, and 65.8±1.0463 µg/mL for the same extracts (figure 3C). The apolar extract from inflorescences has no effect on either cell line (figure 3).

Cell viability in both androgen-independent PC-3 and androgen dependent LnCaP prostate cancer cells is effectively suppressed by the polar extract derived from leaves of *G. gracile*.

The EC₅₀ values of 28.84±1.1766 for PC-3 and <25.00 µg/mL for LnCaP cells suggest a highly effective suppression of cell viability by the polar extract derived from leaves. The apolar extract also exerts a significant effect in PC-3 cells with an EC₅₀ of 48.66±1.1069 µg/mL, and a much lesser effect on LnCaP cells as indicated by an EC₅₀ of 60.14±1.1715 µg/mL. The polar and apolar extracts derived from inflorescences are less effective in these cells.

Discussion

The cytotoxic activity of four crude extracts from leaves and inflorescences of *Gnaphalium gracile* H.B.K. were studied against six human cancer cell

lines derived from pancreas (MIA PaCa, Panc 28), colon (HCT-116, CaCo-2) and prostate (PC-3, LnCaP). Our findings indicate that the differential cytotoxic activity exerted by the extracts from leaves and inflorescences are likely due to the various compounds present in them as related to gene expression profiles in these cell lines. This is particularly evident by the significantly higher activity displayed by the same extracts on colon cancer HCT-116 as compared to CaCo-2 cells. Additionally, the extracts suppressed cell viability in all cells studied at various degrees of activity, except for CaCo-2 cells, which were consistently least affected.

This may be due to the fact that CaCo-2 is a better differentiated cancer cell line with apicobasal polarity similar to normal intestinal cells [15] and that *G. gracile* is considered a non-toxic medicinal plant, recommended to be taken in infusions. Additionally, it has high concentrations of flavonoids, among these, quercetin [13], a recognized pro-apoptotic compound that targets specifically and almost exclusively tumor cells, while sparing normal cells [16, 17].

In our previous studies we have shown plant derived compounds with preferential cytotoxic activity towards cells categorized as highly tumorigenic [10]. Aldehyde dehydrogenase (ALDH) is a specific marker of subpopulations of cancer-initiating cells in a tumor, and an indicator of tumorigenic status in cancer cell lines [18-21]. The effect *G. gracile* extracts on cell viability is observed in cells categorized as highly tumorigenic as well as those that are not, suggesting the presence of compounds with varying targets. For example, the polar leaf extract (EH polar) is most effective against Panc28, LnCaP, PC-3, HCT-116, and MIA PaCa, with half maximal effective concentrations between (20.23±1.185 and 34.41±1.057µg/mL). Panc28, HCT-116, and MIA PaCa express high levels of ALDH and are categorized as highly tumorigenic [22-25], but not LnCaP and PC-3 cells [26]. These half maximal effective concentrations are the lowest obtained among all extracts tested. Thus, the polar leaf extract (EH) has the highest activity against the cell lines tested. On the contrary, the apolar extract from inflorescences (EF) has the least activity with no effect on pancreatic or colon cancer cells, suppressing cell viability only on PC-3 cells at a half

maximal effective concentration of 62.07 ± 1.237 $\mu\text{g/mL}$. Indeed, this extract contains a high concentration of low polarity substances such as aromatic monoterpene compounds and fatty acids, as well as low concentrations of flavonoids that may be responsible for the observed weak activity on PC-3 prostate cancer cells

The cytotoxic activity exerted by the *G. gracile* crude extracts reported here is significantly higher as compared to other reports of plants from the genus *Gnaphalium* commonly advocated to have antineoplastic properties. Extracts from the leaves, stems, and inflorescences *Gnaphalium spicatum* are reported to have IC_{50} values of $>250 \mu\text{g/mL}$ for most cancer cells tested, with the most effective values observed in the ethanolic extract obtained from the roots with IC_{50} values ranging between 46 – 215 $\mu\text{g/mL}$ for cells lines tested [27].

The polar extracts from leaves and inflorescences, as well as the apolar extracts differ greatly in activity towards the tumor cell lines tested. This may be the result of the presence of compounds at various concentrations, as well as to differential distribution of compounds to certain parts of the plant. Further research is needed to isolate and determine the distribution and concentration of these compounds in the plant, as well as determine their specific targets and mechanisms of action responsible for their antineoplastic properties.

Acknowledgments

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References

1. Rzedowski, G. C. de y J. Rzedowski. Flora fanerogámica del Valle de México. 2a ed. Instituto de Ecología y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Pátzcuaro, Michoacán, México. 2001.
2. García-Barriga H. Flora Medicinal de Colombia Nacional I, Editor. Bogotá, Colombia. 1975
3. Escarria S, Torrenegra R, Angarita B. Colombian plants of the genus *Gnaphalium*. *Phytochemistry*. 1977; 16: 1678.
4. Torrenegra RD. Química Y actividad biológica de algunas plantas promisorias colombianas. *Revista Latinoamericana de Química*. 2000; 28.
5. Torrenegra R, Escarria S, Achenbach H. Plantas Colombianas del Genero *Gnaphalium* (II) *Revista Latinoamericana de Química*. 1979; 10: 83-84.
6. Torrenegra R, Escarria S. Flavonoides de *Gnaphalium pellitum*. *Revista Latinoamericana de Química*. 1978; 8: 101.
7. Guerreiro E, Kavka J, Giordano O. 5,8-dihydroxy-3,6,7-trimethoxyflavone from *Gnaphalium gaudichaudianum*. *Phytochemistry*. 1982; 21: 2601-2602.
8. Bohlmann F, Ziesche J. Neue diterpene aus *Gnaphalium*-arten. *Phytochemistry*. 1980; 19: 71-74.
9. Torrenegra RD, Pedrozo J. Estudio Quimiotaxonomico de los generos *Gnaphalium* y *Achyrocline* *Revista Asociacion Colombiana de Ciencias Biologicas*. 1984; 2: 39-44.
10. Torrenegra R, Pedrozo J, Rojas C, Carrizosa S. Plantas Colombianas del genero *Gnaphalium* (IV) *G. rufescens* y *G. antennaroides* *Revista Latinoamericana de Química*. 1987; 18, 116-118.
11. Duran E, Ramsauer VP, Ballester M, Torrenegra RD, Rodriguez OE, et al. Qualitative analysis of sequence specific binding of flavones to DNA using restriction endonuclease activity assays. *Biopolymers*. 2013; 99: 530-537.
12. Thomas CM, Wood RC, 3rd, Wyatt JE, Pendleton MH, Torrenegra RD, et al. Anti-neoplastic activity of two flavone isomers derived from *Gnaphalium elegans* and *Achyrocline bogotensis*. *PLoS One*. 2012; 7: e39806.
13. Torrenegra R, Ricardo A, Pedrozo J, Fuentes O. Flavonoids from *Gnaphalium gracile* H.B.K. *Journal of Crude Drug Research*. 1989; 27: 22.
14. Sclabas GM, Fujioka S, Schmidt C, Evans DB, Chiao PJ. NF-kappaB in pancreatic cancer. *Int J Gastrointest Cancer*. 2003; 33: 15-26.
15. Gilbert T, Rodriguez-Boulan E. Induction of vacuolar apical compartments in the Caco-2 intestinal epithelial cell line. *J Cell Sci*. 1991; 100 (Pt 3): 451-458.
16. Park MH, Min do S. Quercetin-induced downregulation of phospholipase D1 inhibits proliferation and invasion in U87 glioma cells. *Biochem Biophys Res Commun*. 2011; 412: 710-715.

17. Du G, Lin H, Wang M, Zhang S, Wu X, et al. Quercetin greatly improved therapeutic index of doxorubicin against 4T1 breast cancer by its opposing effects on HIF-1 α in tumor and normal cells. *Cancer Chemother Pharmacol*. 2010; 65: 277-287.
18. Lee SH, Ryu JK, Lee KY, Woo SM, Park JK, et al. Enhanced anti-tumor effect of combination therapy with gemcitabine and apigenin in pancreatic cancer. *Cancer Lett*. 2008; 259: 39-49.
19. Shin SY, Hyun J, Yu JR, Lim Y, Lee YH. 5-Methoxyflavanone induces cell cycle arrest at the G2/M phase, apoptosis and autophagy in HCT116 human colon cancer cells. *Toxicol Appl Pharmacol*. 2011; 254: 288-298.
20. Erhart LM, Lankat-Buttgereit B, Schmidt H, Wenzel U, Daniel H, et al. Flavone initiates a hierarchical activation of the caspase-cascade in colon cancer cells. *Apoptosis*. 2005; 10: 611-617.
21. Torres F, Quintana J, Estevez F. 5,7,3'-trihydroxy-3,4'-dimethoxyflavone-induced cell death in human leukemia cells is dependent on caspases and activates the MAPK pathway. *Mol Carcinol* 2010; 49: 464-475.
22. Neumeister V, Agarwal S, Bordeaux J, Camp RL, Rimm DL. In situ identification of putative cancer stem cells by multiplexing ALDH1, CD44, and cytokeratin identifies breast cancer patients with poor prognosis. *Am J Pathol*. 2010; 176: 2131-2138.
23. Lin L, Liu Y, Li H, Li PK, Fuchs J, et al. Targeting colon cancer stem cells using a new curcumin analogue, GO-Y030. *Br J Cancer*. 2011; 105: 212-220.
24. Visus C, Wang Y, Lozano-Leon A, Ferris RL, Silver S, et al. Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8(+) T cells. *Clin Cancer Res*. 2011; 17: 6174-6184.
25. Schumacher M, Hautzinger A, Rossmann A, Holzhauser S, Popovic D, et al. Potential role of P-gp for flavone-induced diminished apoptosis and increased adenoma size in the small intestine of APC(min/+) mice. *Cancer Invest*. 2011; 29: 396-404.
26. Karna P, Gundala SR, Gupta MV, Shamsi SA, Pace RD, et al. Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells in vitro and in vivo. *Carcinogenesis*. 2011; 32: 1872-1880.
27. Callacondo-Riva D, Quispe-Mauricio A, Lindo-Gamarra S, Vaisberg AJ. Actividad citotóxica del extracto etanólico de *Gnaphalium spicatum* "keto keto" en cultivos de líneas celulares tumorales humanas *Rev Peru Med Exp Salud Publica*. 2008; 25: 380-385.

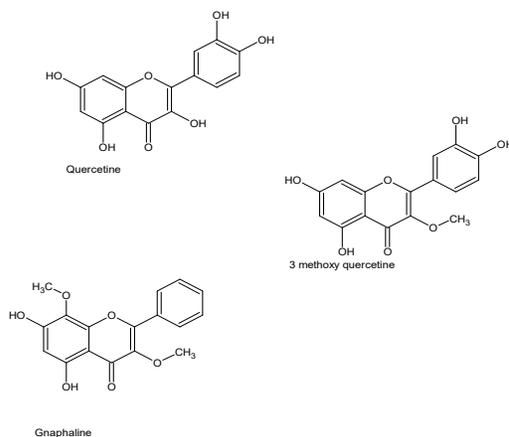


Figure 1. Molecular structures of Gnaphalin, 3-methoxy quercetin and quercetin.

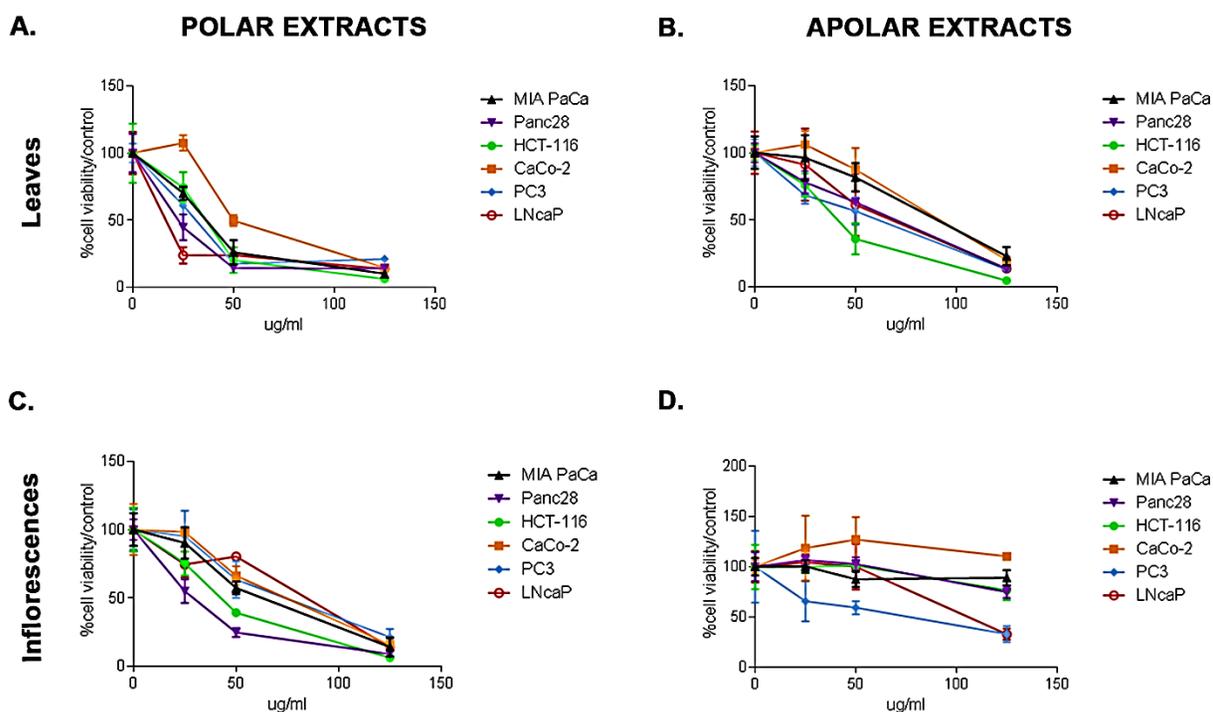


Figure 2. Comparison of the effects of polar and apolar extracts derived from leaves and inflorescences on pancreatic, colon, and prostate cancer cells. The effects of the polar and apolar extracts from leaves (1A and 1B) and inflorescences (1C and 1D) on pancreatic (Mia Paca and Panc 28), colon (HCT 116 and Caco 2), and prostate (PC-3 and LNCaP) cancer cells were determined by MTT assay and are represented as a percent of the control absorbance at a wavelength of 590 nm. All data were collected at 24 h after treatment. Data shown are from representative experiments ($n = 3$). Values are expressed as mean \pm SE, * $p < 0.05$, significant difference between control and assayed concentrations for each extract.

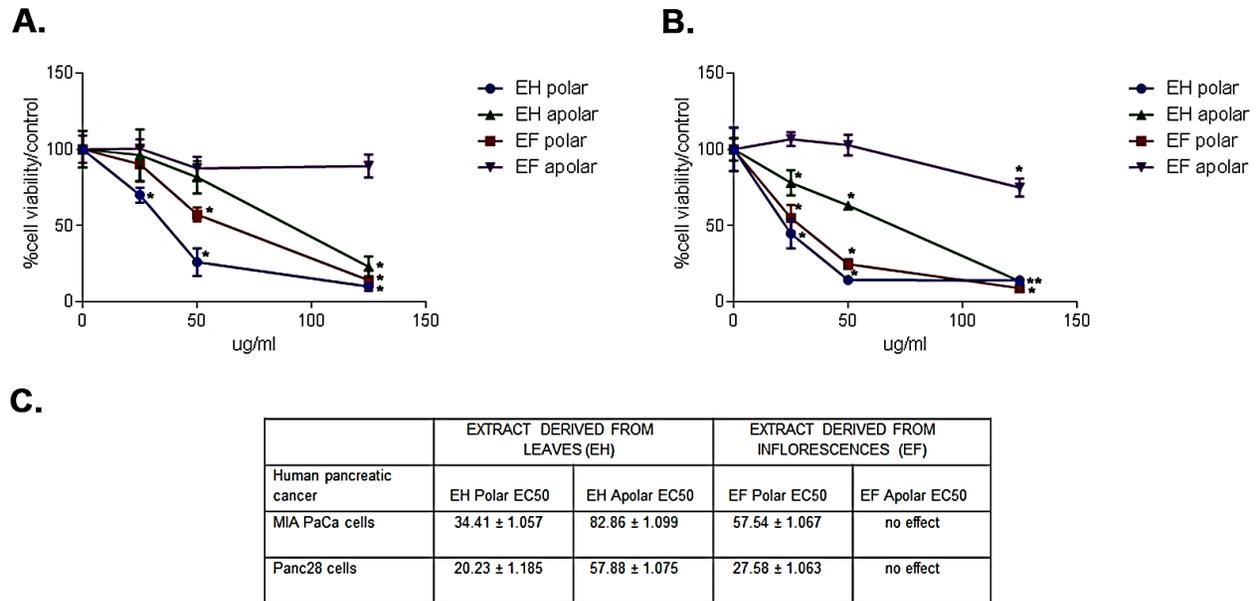


Figure 3. The effects of polar and apolar extracts derived from leaves (EH) and inflorescences (EF) on pancreatic cancer cells. Poorly differentiated MIA PaCa (figure 2A), and better differentiated Panc 28 (figure 2B) pancreatic cancer cells were treated with 25, 50 or 125 $\mu\text{g/ml}$ of polar and apolar EH and EF extracts. The effects of the extracts on cell viability were determined 24 hours after treatment via MTT assay and are represented as a percent of the control absorbance at a wavelength 590nm. Data shown are from representative experiments ($n = 3$). Values are expressed as mean \pm SE, * $p < 0.05$, significant difference between control and assayed concentrations for each extract. C. Half maximal effective concentration (EC_{50}) \pm SE for treatment with polar and apolar extracts from leaves and inflorescences on pancreatic carcinoma cells. The values were estimated by non-linear regression analysis.

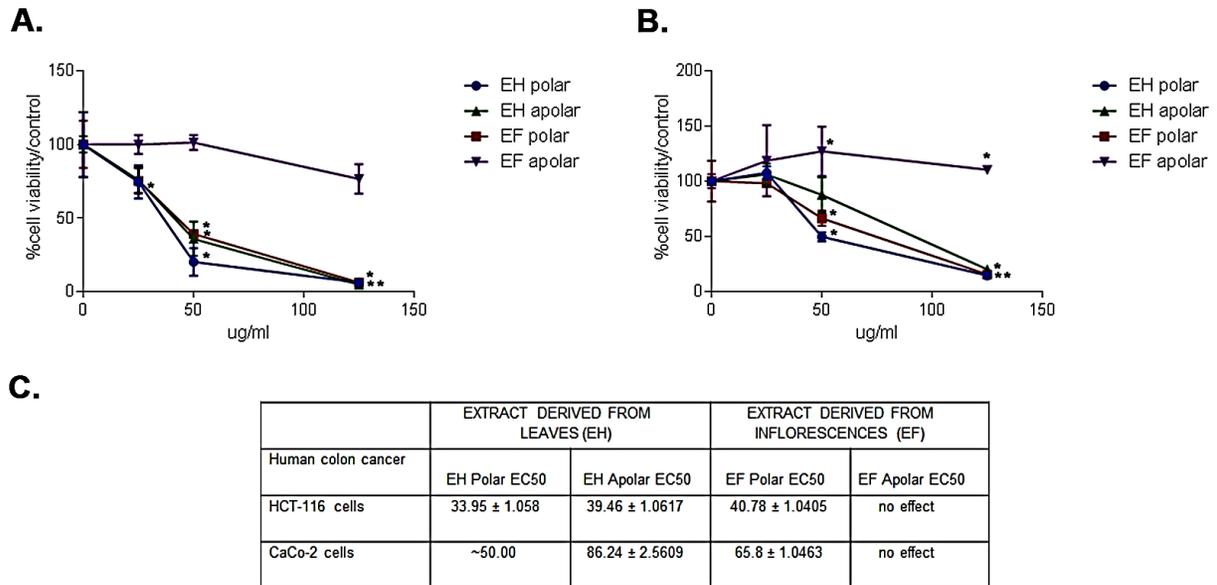


Figure 4. The effects of polar and apolar extracts derived from leaves (EH) and inflorescences (EF) on colon cancer cells. Poorly differentiated HCT-116 (figure 3A), and better differentiated CaCo-2 (figure 3B) colon cancer cells were treated with polar and apolar EH and EF extracts at concentrations of 25, 50 or 125 $\mu\text{g/ml}$. The effects of the extracts on cell viability were determined by MTT assay 24 hours after treatment, and are represented as a percent of the control absorbance at a wavelength 590nm. Data shown are from representative experiments ($n = 3$). Values are expressed as mean \pm SE, * $p < 0.05$, significant difference between control and assayed concentrations for each extract. C. Half maximal effective concentration (EC₅₀) \pm SE for treatment with polar and apolar extracts from leaves and inflorescences on colon cancer cells. The values were estimated by non-linear regression analysis.

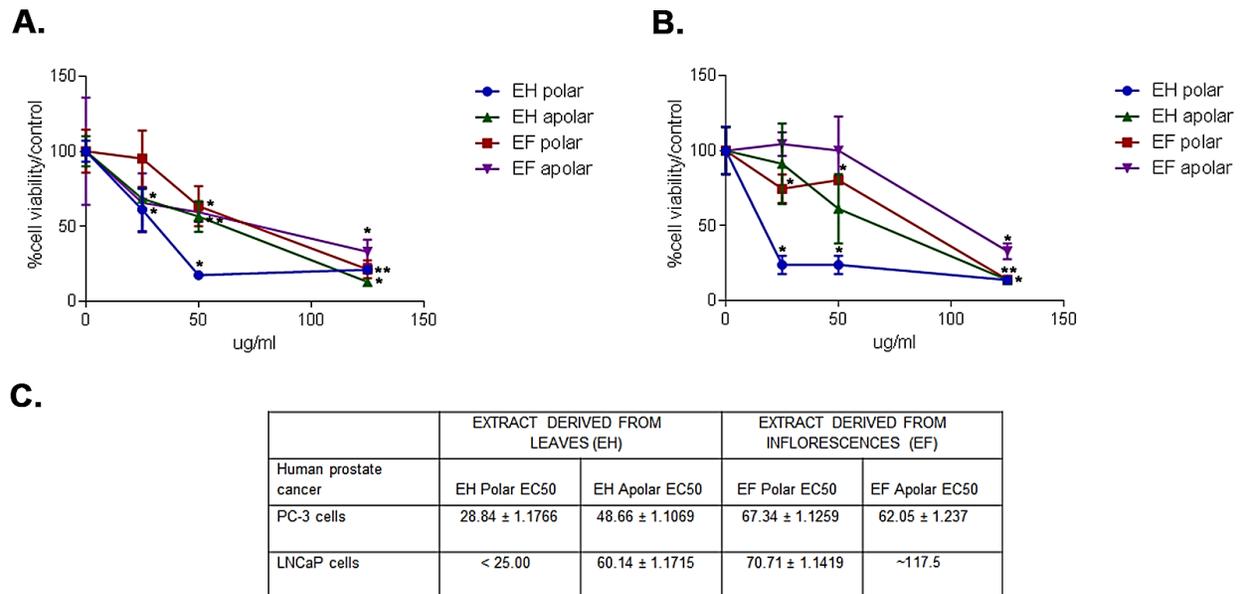


Figure 5. The effects of polar and apolar extracts derived from leaves (EH) and inflorescences (EF) on prostate cancer cells. Androgen-independent PC3 (figure 4A), and androgen-dependent LNCaP (figure 4B) prostate cancer cells were treated with polar and apolar EH and EF extracts at concentrations of 25, 50 or 125 µg/ml. The effects of the extracts on cell viability were determined by MTT assay 24 hours after treatment and are represented as a percent of the control absorbance at a wavelength 590nm. Data shown are from representative experiments (n = 3). Values are expressed as mean ± SE, * p<0.05, significant difference between control and assayed extracts at specified concentrations. C. Half maximal effective concentration (EC₅₀) ±SE for treatment with polar and apolar extracts from leaves and inflorescences on colon cancer cells. The values were estimated by non-linear regression analysis.