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RESEARCH

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Bioactive potential of tropical highland apple (*Malus domestica* cv. Anna) crude extract: opportunities for food waste revalorization

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Abstract

Background: The third most produced fruit crop in the world, apples (*Malus domestica* Borkh) are one of the most accessible and widely consumed fruits, with known benefits for human health. Although they are a temperate crop, apple cultivation has been successfully implemented in lower latitudes. Tropical highland Costa Rican apples (cv. Anna) have shown high total polyphenol content, and cytotoxic effects against human cancer cell lines. However, most reports originate from purified fractions, obtained using methods which are not easily translatable for commercial applications. In this study, we prepared a polyphenol-rich bioactive extract from Costa Rican Anna apples, using food-grade solvents and simple techniques, aiming towards easy translation of the extraction protocols to small local producers.

Results: The whole apple crude extract (AE) was investigated for its total polyphenol content, general phytochemical profile, cytotoxicity against human breast and lung cancer cells, and regenerative potential in murine skin fibroblast monolayers. The AE showed total polyphenol amounts comparable to that obtained using more refined extractions in previous studies, and exhibited cytotoxic activity against human breast (MCF7) and lung (NCI-H460) cancer cells, and inhibition of cell proliferation in the scratch-wound-healing assay.

Conclusions: Food-grade simple protocols were successful for obtaining a polyphenol-rich bioactive extract from Costa Rican Anna apples. The easy-to-implement extraction protocols and biochemical tests could provide a source of bioactive phytochemicals to be used in circular production systems.

Keywords: Apple, *Malus domestica* cv. Anna, Phytochemical profile, Cytotoxicity

Background

Apple fruits (*Malus domestica* Borkh, Rosaceae) are worldwide accessible and low-cost fruits with high nutritional value, diverse bioactive phytochemical content, and several health-promoting properties confirmed in clinical trials, including antioxidant, anti-inflammatory, cardioprotective and neuroprotective effects, gastrointestinal benefits, and modulatory activities in the glucose and lipid metabolism [1, 2]. The health benefits of dietary apple intake have been attributed, at least partially, to their antioxidant polyphenol content [3]. Antioxidant plant polyphenols have been shown to reduce the risk of developing chronic diseases associated with the accumulation of age-related oxidative damage [4–8]. The phytochemical composition and bioactive activities of apples have been described elsewhere [2, 3, 9–12].

After bananas and watermelons, apples were listed as the third most produced fruit crop in the world in 2020, reaching over 86 million tons, with China as the main producer (46.9%), followed by the United States (5.4%),

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Turkey (5.0%), Poland (4.1%), and India (3.2%) [13]. Although they are traditionally a temperate crop, apples have been increasingly adapted to cultivation at low latitudes, with higher temperatures and longer photoperiods [14]. Tropical highland conditions may also provide high solar irradiation, which is known to promote flavonoid accumulation and diversity in plants [15]. Accordingly, high levels of UV irradiation have been shown to increase anthocyanin and flavonol content in fruit peel in apples [16].

One of the most prominent apple varieties cultivated in tropical high-altitude settings is *M. domestica* cultivar Anna (Golden Delicious x Red Hadassiya), a low chilling requiring variety created in Israel in 1963 [14, 17]. Anna apples cultivated in Costa Rica highlands have shown higher and more diverse total polyphenol content in peel extract than other cultivars from temperate regions, as well as cytotoxic effects against gastric (AGS) and colon (SW-620) human carcinoma cells [18, 19].

In this study, we prepared a whole fruit crude extract from Anna apples cultivated in the same Costa Rican highland region, using food-grade solvents and simple techniques, aiming towards easy translation of the extraction protocols to small local producers. The whole apple extract was evaluated for total polyphenol content, general phytochemical profile, cytotoxic activity against human breast (MCF7) and human lung (NCI-H460) cancer cells, and regenerative potential in NIH-3T3 murine skin fibroblast monolayers.

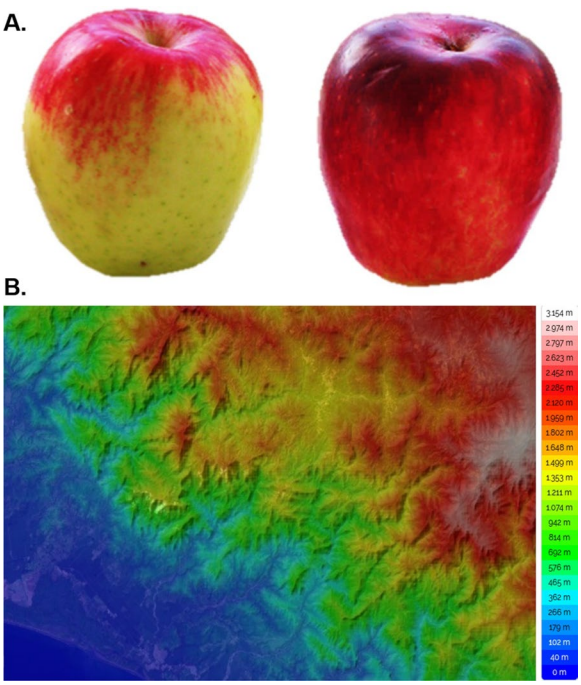

Fig. 1 **A**. Early ripe (left) and ripe (right) apple fruits (*Malus domestica* cv. Anna) from San Marcos de Tarrazú, San José, Costa Rica (collected under permit R-CM-ITCR-002–2021-OT, CONAGEBIO, MINAE, Costa Rica). Locally grown fruits may be recognized by the green-yellow spots on their peel, and their tangy acidic flavor. **B**. Topographic map of the Costa Rican region where the cultivars are grown, which locates at about 1500 m.a.s.l. Geographically, it is located between coordinates 83°58′16" to 84°9′28" west longitude, and 9°26′46" to 9°43′7" north latitude [20]

Methods

Apple extract (AE) preparation

Apple fruits (*Malus domestica* cv. Anna) were purchased in a ripe state (Fig. 1) from local producers located in San Marcos, Tarrazú, San José, Costa Rica (permit R-CM-ITCR-002–2021-OT). Whole fruits (without seeds) were rinsed in water, sliced, freeze-dried (Bench Top FDB-8602, OPERON), grinded (1 mm, MF-10 basic IKA, WERKE), and preserved at −20 °C until extraction. The powdered material was extracted (1:10, plant material to solvent) by three consecutive water–ethanol macerations at room temperature, followed by ethanol elimination in a vacuum evaporator (40 °C, R-300, BUCHI). The identity of all fruits collected was authenticated by Botanist M.Sc. Elizabeth Arnáez-Serrano, Biology Department, Costa Rica Institute of Technology and were previously confirmed with the support of the Costa Rican National Herbarium [19].

Phytochemical analysis

Preliminary phytochemical screening was evaluated qualitatively by colorimetric, foaming, and precipitation reactions [21, 22], including detection of flavonoids (Shinoda test), steroids and terpenoids (Liebermann-Buchard test), alkaloids (Dragendorff test), and saponins (foam test).

High-performance Thin Layer Chromatography (HPTLC) was performed using the freeze-dried AE powder dissolved in methanol (1 mg mL^{-1}). The method was developed using a CAMAG HPTLC system (CAMAG, Muttenz, Switzerland). Sample application (10 µL) was done on a 20 × 10 cm HPTLC glass plate coated with Silica gel F_{254} (Merck, Darmstadt, Germany). Samples were applied to the HPTLC plate with the aid of the CAMAG Automatic Sample Applicator 4 (ATS4) as 8 mm bands. The first application position (x axis) was 20 mm and the sample bands were applied at a distance of 8 mm from the base of the HPTLC plate (application position y). The distance between tracks was 11 mm. Chromatography development was done in a CAMAG Automatic Developing Chamber 2 (ADC 2) with the following settings: 30 s pre-drying, 20 min saturation with filter paper, humidity control with $MgCl_2$ (10 min, 33% relative humidity),

70 mm migration distance (solvent front position), 5 min drying time, 10 ml of mobile phase for HPTLC development and 25 mL of mobile phase for HPTLC chamber saturation. The mobile phase was comprised of ethyl acetate: water: formic acid (100: 30: 20). The chromatograms were exposed to UV light (254 nm and 366 nm) and white light. The derivatization of the chromatograms with the natural product reagent (2-aminoethyl diphenyl borinate), anisaldehyde reagent, phosphomolybdic acid, fast blue salt B and the Dragendorff reagent were performed with the CAMAG Derivatizer. If the derivatization process required heating, The CAMAG TLC Plate Heater III was used. All reagents were purchased from SIGMA; prepared and applied according to the CAMAG Derivatizer instructions. Pre- and post-derivatization, images were taken, processed, and documented with the CAMAG TLC Visualizer. All HPTLC steps were programmed and monitored with the CAMAG VisionCATS software (version 2.5). Image comparison views were created with this software as well.

Total phenolic content (TPC) in the apple extract (AE) was determined using a modified Folin-Ciocalteu spectrophotometric method as described by Rojas-Garbanzo et al. [23]. Briefly, AE were incubated with Folin-Ciocalteu reagent and sodium carbonate solution (75 g/L) at 50 °C for 15 min. The absorbance was measured at $\lambda = 620$ nm (FLUOstar OPTIMA, BMG LABTECH). TPC was calculated by comparing against an external calibration curve of gallic acid (10–80 mg GAE L^{-1}, $r^2 = 0.9909$). Results were expressed as µg of gallic acid equivalents (GAE) per gram of AE. TPC are shown as the mean ± SD ($n = 3$).

Cell culture

The cell lines used in this study were NIH-3T3 (ATCC CRL-1658™, murine skin fibroblasts, passages 51 to 54), NCI-H460 (ATCC HTB-177™, human lung carcinoma, passages 46 to 50), and MCF7 (ATCC HTB-22™, human breast adenocarcinoma, passages 18 to 21). NIH-3T3 were cultured in DMEM (4.5 g L^{-1}, GIBCO) and NCI-H460 cells were cultured in RPMI (GIBCO). Both DMEM and RPMI media were supplemented with 10% fetal bovine serum (FBS, SIGMA), 2% L-glutamine (4 mM, GIBCO), 1% sodium pyruvate (0.11 mg mL^{-1}; SIGMA) and 1% antibiotics (1×10^4 IU mL^{-1} penicillin and 1×10^4 µg mL^{-1} streptomycin; GIBCO). The same supplemented DMEM was used for culturing MCF7 cells, with additional 10 µg mL^{-1} insulin (SIGMA). All cells were maintained at 95% humidity and 5% CO_2 at 37 °C.

Cytotoxicity by MTT assay

Cells lines NIH-3T3, NCI-H460, and MCF7 were seeded onto 96-well plates (1×10^5 cells cm^{-2}) and treated for 24 h with increasing concentrations of the apple extract (AE) diluted in the respective culture medium. Cell viability was measured by the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay as described by Calvo-Castro et al. [24]. The formazan crystals were dissolved in ethanol 95%, and the absorbance was measured at 570 nm (FLUOstar OPTIMA, BMG LABTECH). Cell viability was normalized as relative percentages in comparison to untreated controls. The half-maximal inhibitory concentration (IC50) for each extract in each cell line was determined from dose–response linear dispersion curves. Data is shown as the mean ± SD ($n = 3$). Normality was tested with the Kolmogorov–Smirnov test and D'Agostino-Pearson test. Differences between AE treatments in the three cell lines were analyzed by two-way ANOVA followed by a Bonferroni post-test. All statistical analyses were performed using the software package Graph-Pad Prism (version 8.01; GraphPad Software, USA).

Scratch wound healing assay

NIH-3T3 fibroblasts were seeded on 24-well plates (1×10^5 cells cm^{-2}). Confluent monolayers were scraped using a standard 1 mL micropipette tip, leaving an empty and homogeneous cross-shaped area [25], followed by treatment for 72 h with non-cytotoxic AE concentrations (38 and 58 µg GAE mL^{-1}). The lesion was photographed by light microscopy every 24 h, and percentage wound area over time was calculated using Image J (https://www.imagej.nih.gov/ij/). Data is shown as the mean ± SD ($n = 3$).

Results

Phytochemical analysis of the apple extract (AE)

The absence of bands in the HPTLC when exposed to UV light at 366 nm (Fig. 2, lane 1) and the few bands in the phosphomolybdic acid derivatization at visible light (Fig. 2, lane 5) suggest low compound diversity in the sample. Nonetheless, polyphenol and flavonoid content in the AE was confirmed by positive Folin-Ciocalteu and Shinoda tests, respectively (Table 1). Moreover, the HPTLC derivatized with the natural product reagent (Fig. 2, lane 2), presented two intense dark blue bands (at Rfs of 0.08 and 0.15) while exposed to UV light at 366 nm, common for phenolic compound. These bands were derivatized with phosphomolybdic acid and returned a blue color at visible light (Fig. 2, lane 5). This

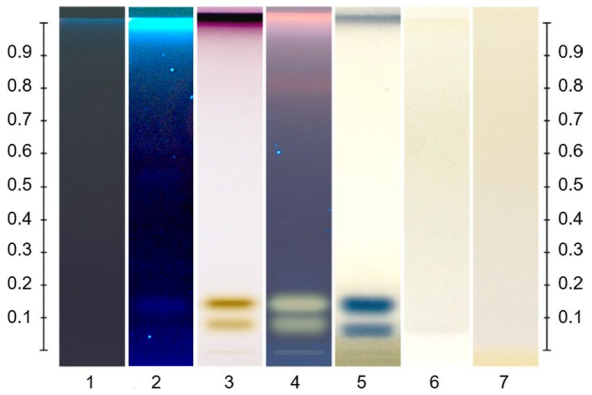

Fig. 2 High-performance Thin Layer Chromatography (HPTLC) of whole apple (*Malus domestica* cv. Anna) freeze-dried AE powder (1 mg mL^{-1}) (Image Comparison View, CAMAG). The HPTLCs were developed with UV light at 366 nm (1); or derivatized and developed with the natural products reagent and at 366 nm (2); with anisaldehyde at visible light (3); with anisaldehyde at 366 nm (4); with phosphomolybdic acid at visible light (5), with Fast Blue B Salt staining at visible light (6); and with Dragendorff reagent at visible light (7)

indicates that such substances are probably glycones. Together, the results for these bands suggest the presence of anthocyanins.

The extract was negative for containing alkaloids, saponins, steroids and terpenoids (Table 1). The absence of red-blue bands after treatment with anisaldehyde at visible light (Fig. 2, lane 3) and at 366 nm (Fig. 2, lane 4) confirms the lack of triterpenes and steroids in the sample. The lack of bands after staining with the Dragendorff reagent at visible light (Fig. 2, lane 7) confirms the absence of alkaloids in the sample. The lack of bands with the Fast Blue B Salt derivatization at visible light (Fig. 2, lane 6) could indicate the absence of aflatoxins in the extract.

Table 1 General physicochemical composition of the whole apple (*Malus domestica* cv. Anna) extract (AE)

Parameter	Apple extract (AE)
Humidity (%)*	76 ± 2
Fats and oils (mg kg^{-1})*	868 ± 45
Protein (%)*	0.06 ± 0.01
Ashes (%)*	0.47 ± 0.08
Sodium (mg kg^{-1})*	23.8 ± 0.5
Soluble solids (° Brix)*	16.1
Total polyphenols (μg GAE g^{-1})	767.40 ± 65.83
Flavonoids (Shinoda test)	Positive (red coloration)
Saponins (foam test)	Negative (no foam)
Steroids and terpenoids (Liebermann-Buchard test)	Negative (no color)
Alkaloids (Dragendorff test)	Negative (no precipitate)

*Analyzed by food official methods of analysis of the association of official analytical chemists (AOAC) at Centro de Investigación y de Servicios Químicos y Microbiológicos (CEQUIATEC), Costa Rica.

Cytotoxicity of the apple extract (AE)

The effect of the AE on the viability of human cancer cells MCF7 (breast cancer) and NCI-H460 (lung cancer), as well as NIH-3T3 murine skin fibroblasts, was tested (MTT assay) after treatment for 24 h. The AE was most effective against MCF7 cells, requiring a half-maximal inhibitory concentration (IC50) 6.5 times and 3.9 times lower than in the H460 and 3T3 cell lines, respectively (Fig. 3).

Scratch wound healing assay

The effect of AE on wound healing was tested by monitoring the regeneration of a simulated lesion on a monolayer of murine skin fibroblasts (NIH-3T3) (Fig. 4). While the untreated control regained full confluence (98.71 ± 2.24%) after 48 h, treatment with AE (in concentrations below the respective IC50) resulted in delayed wound regeneration, showing a prolonged effect

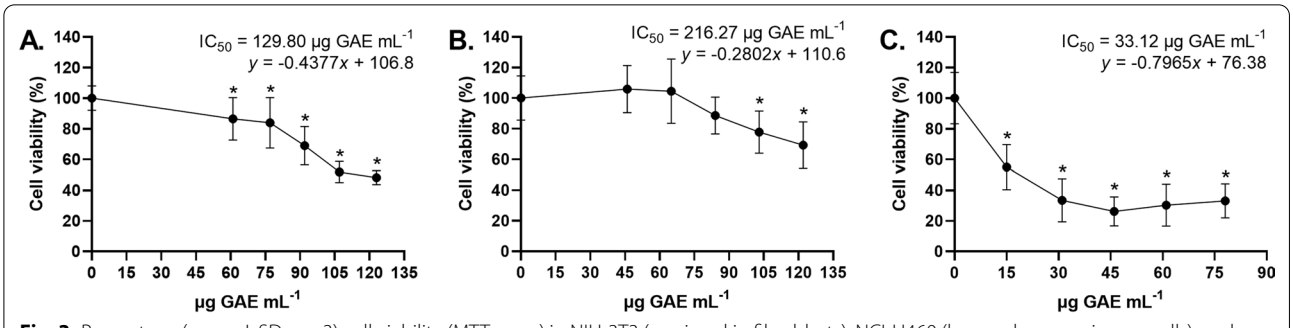

Fig. 3 Percentage (mean ± SD, $n = 3$) cell viability (MTT assay) in NIH-3T3 (murine skin fibroblasts), NCI-H460 (human lung carcinoma cells), and MCF7 (human breast adenocarcinoma) cells, treated with whole apple (*Malus domestica* cv. Anna) extract (1 mg ml^{-1}) for 24 h. **A**. NIH-3T3 (0, 61, 77, 92, 107, 123 μg GAE ml^{-1}). **B.** NCI-H460 (0, 46, 65, 84, 102, 123 μg GAE ml^{-1}). (C) MCF7 (0, 15, 31, 46, 61, 77 μg GAE ml^{-1}). Statistical differences relative to the untreated controls were determined by one-way ANOVA followed by a Dunnett´s post-test (*$p < 0.05$) (Graph-Pad Prism v. 8.01)

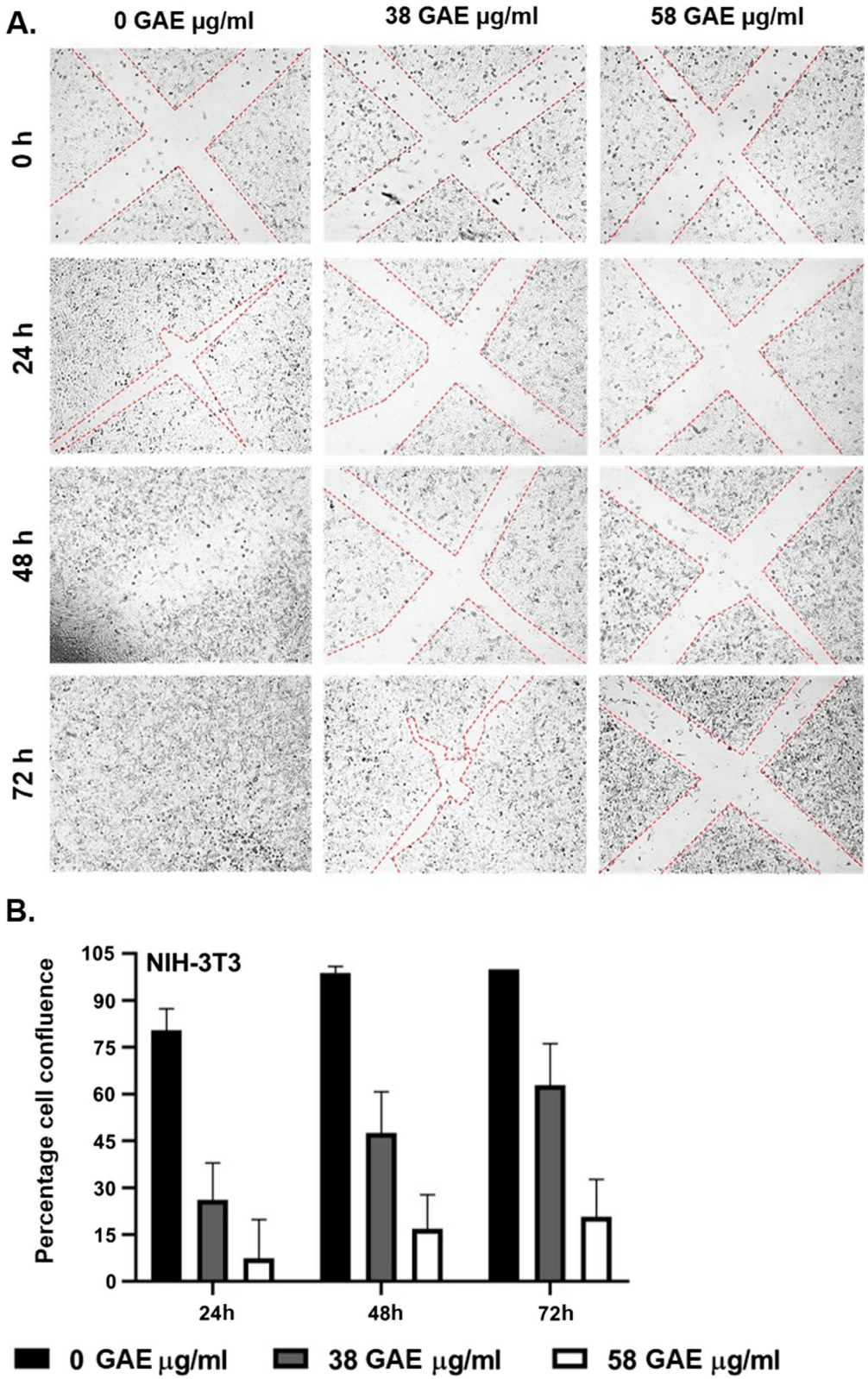

Fig. 4 Scratch Wound Healing assay on scraped NIH-3T3 (murine skin fibroblasts) monolayers treated with non-cytotoxic whole apple (*Malus domestica* var. Anna) extract concentrations (38 and 58 µg GAE ml^{-1}) for 72 h. **A**. Representative photograph (10x) of the lesions over time. **B**. Percentage (mean ± SD, $n = 3$) cell confluence in the lesion over time (Graph-Pad Prism v. 8.01)

even after 72 h at the highest concentration, with only 20.72 ± 11.97% lesion restoration.

Discussion

In this study, food-grade tropical highland whole apple (*Malus domestica* var. Anna) crude extract (AE) obtained by ethanol extraction showed slightly higher total polyphenol content (767.40 ± 65.83 mg GAE g^{-1}) than previous studies using apples from the same source (up to 619.6 ± 19.5 mg GAE g^{-1}, methanol-based extraction) [18, 19]. The discrepancy is probably due to different extraction and analytical techniques, and natural fruit variation caused by seasonal cultivation, ripeness, and storage conditions. Nonetheless, the presence of phenolic compounds in the AE was confirmed by HPTLC, Folin-Ciocalteu and Shinoda tests (Table 1, Fig. 2). Moreover, the phytochemical analysis also showed no evidence of alkaloids, saponins, steroids, terpenoids and aflatoxins in the AE (Table 1, Fig. 2), supporting the potential use of whole apple crude extract for human intake in future applications.

The anti-cancer potential of apple extracts has been thoroughly documented [9, 10]. In this study, the AE exhibited cytotoxic effects in low concentrations against human breast (MCF7; IC_{50} = 33.12 µg GAE mL^{-1}) and human lung (NCI-H460; IC_{50} = 216.26 µg GAE mL^{-1}) cancer cells (Fig. 3), adding to the cytotoxicity (MTT assay) reported by Navarro-Hoyos et al. [19] for an apple skin extract from the same source, against human gastric (AGS; IC_{50} = 167.22 ± 10 µg extract mL^{-1}) and human colon (SW-620; IC_{50} = 295.93 ± 29 µg extract mL^{-1}) cancer cells.

Comparably, Tow et al. [26] showed that considerable amounts of polyphenols (up to 539.84 ± 8.90 mg polyphenols GAE g^{-1}, extracted with 50% ethanol) with cytotoxic activities (against cervical HeLa, liver Hep-G2, and colon HT-29 human cancer cells) can be recovered from frozen apple industrial byproducts. Considering that over 20 million tons of apple residues from agroindustry are produced per year globally [27], food-grade simple protocols as described in this investigation could be implemented for obtaining relevant amounts of total polyphenols from apple sources, including underutilized waste material, which could be converted into high-value recycled products with nutritional, medicinal, and cosmetic applications [27–30]. Although apple production in tropical highland regions is not as extensive as in temperate latitudes [13], circular economy strategies should be encouraged for the upcoming challenges on food security and human health in the near future.

On the other hand, non-cytotoxic doses of the AE did not improve the regeneration of a simulated lesion on a monolayer of murine skin fibroblasts (NIH-3T3); on the contrary, the AE seemed to delay or inhibit the regeneration process. The scratch-adjacent cells treated with AE remained attached to the flask, but with reduced migration into the simulated lesion in comparison to the control, and in an apparent dose-dependent manner. Given that we only monitored the surface area covered with cells over 72 h, future experiments should quantify cell amounts, doubling times and viability, over an extended period of time, and after removing the AE treatment and reverting to control conditions. Even though this observation speaks against possible regenerative applications, and while the effect might only be cytostatic, the prolonged inhibition of cell migration or proliferation at relatively low concentrations supports the chemopreventive potential of the AE. Future studies should also explore the impact of gastrointestinal digestion of the extracts on the biological effects reported here, and consider other forms of administration.

Conclusions

In this study, we verified that a food-grade whole fruit crude extract from tropical highland Costa Rican Anna apples showed similar total polyphenol content than previously reported in more refined extractions [18, 19], and exhibited cytotoxic activity against human breast (MCF7) and lung (NCI-H460) cancer cells. Overall, the simple food-grade extraction protocol and easy-to-implement low-cost phytochemical tests could be put into practice by small producers for obtaining relevant amounts of bioactive polyphenols from apple materials, aiming towards their conversion into added-value products in circular production systems.

Abbreviations

AE: Whole apple crude extract; HPTLC: High-performance thin layer chromatography; TPC: Total phenolic content; GAE: Gallic acid equivalents; MTT: 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide citotoxicity assay; SD: Standard deviation; ANOVA: Analysis of variance.

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Author contributions

LACC and JCGG prepared the extracts. MLV, CCC and GZF conducted the experiments. EAS and KSZ collected the fruits and managed the collection permits. L.A.C.C. wrote the first draft of the manuscript, and all authors read, edited, and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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References

1. Vallée Marcotte B, Verheyde M, Pomerleau S, Doyen A, Couillard C (2022) Health benefits of apple juice consumption: a review of interventional trials on humans. Nutrients 14:1–21. https://doi.org/10.3390/nu14040821
2. Oyenihi AB, Belay ZA, Mditshwa A, Caleb OJ (2022) " An apple a day keeps the doctor away ": the potentials of apple bioactive constituents for chronic disease prevention. J Food Sci. https://doi.org/10.1111/1750-3841.16155
3. Feng S, Yi J, Li X, Wu X, Zhao Y, Ma Y, Bi J (2021) Systematic review of phenolic compounds in apple fruits: compositions, distribution, absorption, metabolism, and processing stability. J Agric Food Chem 69:7–27. https://doi.org/10.1021/acs.jafc.0c05481
4. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L (2005) Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 45:287–306. https://doi.org/10.1080/1040869059096
5. Magrone T, Perez de Heredia F, Jirillo E, Morabito G, Marcos A, Serafini M (2013) Functional foods and nutraceuticals as therapeutic tools for the treatment of diet-related diseases. Can J Physiol Pharmacol 91:387–396. https://doi.org/10.1139/cjpp-2012-0307
6. Alissa EM, Ferns GA (2012) Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. J Nutr Metab 2012:569486. https://doi.org/10.1155/2012/569486
7. Nichenametla SN, Taruscio TG, Barney DL, Exon JH (2006) A review of the effects and mechanisms of polyphenolics in cancer. Crit Rev Food Sci Nutr 46:161–183. https://doi.org/10.1080/10408390591000541
8. Zhou Y, Zheng J, Li Y, Xu D-PP, Li S, Chen Y-MM, Bin L-B (2016) Natural polyphenols for prevention and treatment of cancer. Nutrients 8:E515. https://doi.org/10.3390/nu8080515
9. Nezbedova L, McGhie T, Christensen M, Heyes J, Nasef NA, Mehta S (2021) Onco-preventive and chemo-protective effects of apple bioactive compounds. Nutrients 13:4025. https://doi.org/10.3390/nu13114025
10. Patocka J, Bhardwaj K, Klimova B, Nepovimova E, Wu Q, Landi M, Kuca K, Valis M, Wu W (2020) *Malus domestica*: A review on nutritional features, chemical composition, traditional and medicinal value. Plants 9:1408. https://doi.org/10.3390/plants9111408
11. Puértolas-Balint F, Schroeder BO (2020) Does an apple a day also keep the microbes away? The interplay between diet, microbiota, and host defense peptides at the intestinal mucosal barrier. Front Immunol 11:1164. https://doi.org/10.3389/fimmu.2020.01164
12. Shehzadi K, Rubab Q, Asad L, Ishfaq M, Shafique B, Ali MM, Ranjha N, Mahmood S, Mueen-Ud-Din G, Javaid T, Sabtain B, Farooq R (2020) A critical review on presence of polyphenols in commercial varieties of apple peel, their extraction and health benefits. J Biog Sci Res 6:1–8. https://doi.org/10.46718/jbgsr.2020.06.000141
13. FAO (2021) FAOStat. Food and Agriculture Organization of the United Nations. http://www.fao.org. Accessed 25 May 2022
14. Jackson JE (2000) Apple production at low latitudes. In: Erez A (ed) Temperate fruit crops in warm climates. Springer Science+ Business Media Dordrecht. https://doi.org/10.1007/978-94-017-3215-4
15. Warner R, Wu B-S, MacPherson S, Lefsrud M (2021) A review of strawberry photobiology and fruit flavonoids in controlled environments. Front Plant Sci 12:611893. https://doi.org/10.3389/fpls.2021.611893
16. Henry-Kirk RA, Plunkett B, Hall M, McGhie T, Allan AC, Wargent JJ, Espley RV (2018) Solar UV light regulates flavonoid metabolism in apple (*Malus x domestica*). Plant Cell Environ 41:675–688. https://doi.org/10.1111/pce.13125
17. Cepeda M. A, Vélez-Sánchez JE, Balaguera-López HE (2021) Analysis of growth and physicochemical changes in apple cv. Anna in a high-altitude tropical climate. Rev Colomb Ciencias Hortícolas 15:e12508. doi:https://doi.org/10.17584/rcch.2021v15i2.12508
18. Navarro M, Moreira I, Arnaez E, Quesada S, Azofeifa G, Vargas F, Alvarado D, Chen P (2018) Polyphenolic characterization and antioxidant activity of *Malus domestica* and *Prunus domestica* cultivars from Costa Rica. Foods 7:15. https://doi.org/10.3390/foods7020015
19. Navarro-Hoyos M, Arnáez-Serrano E, Quesada-Mora S, Azofeifa-Cordero G, Wilhelm-Romero K, Quirós-Fallas MI, Alvarado-Corella D, Vargas-Huertas F, Sánchez-Kopper A (2021) HRMS Characterization, antioxidant and cytotoxic activities of polyphenols in *Malus domestica* cultivars from Costa Rica. Molecules 26:7367. https://doi.org/10.3390/molecules26216493
20. Multi-Error-Removed Improved-Terrain DEM (MERIT DEM) (http://hydro.iis.u-tokyo.ac.jp/~yamadai/MERIT_DEM/). In: Yamazaki D, Ikeshima D, Tawatari R, Yamaguchi T, O'Loughlin F, Neal JC, Sampson CC, Kanae S, Bates PD (2017) A high-accuracy map of global terrain elevations. Geophys Res Lett, https://doi.org/10.1002/2017GL072874
21. Harborne JB (1984) Phytochemical methods. A guide to modern techniques of plant analysis. Springer Dordrecht. https://doi.org/10.1007/978-94-009-5570-7
22. Krishnaswamy NR (2003) Chemistry of natural products: a laboratory handbook. Universities Press, Hyderabad
23. Rojas-Garbanzo C, Pérez AM, Vaillant F, Pineda-Castro ML (2016) Physicochemical and antioxidant composition of fresh peach palm (*Bactris gasipaes Kunth*) fruits in Costa Rica. Braz J Food Technol 19:e2015097. https://doi.org/10.1590/1981-6723.9715
24. Calvo-Castro L, Syed DN, Chamcheu JC, Vilela FMP, Pérez AM, Vaillant F, Rojas M, Mukhtar H (2013) Protective effect of tropical highland blackberry juice (*Rubus adenotrichos* Schltdl.) against UVB-mediated damage in human epidermal keratinocytes and in a reconstituted skin equivalent model. Photochem Photobiol 89:1199–1207. https://doi.org/10.1111/php.12104
25. Grada A, Otero-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V (2017) Research techniques made simple: analysis of collective cell migration using the wound healing assay. J Invest Dermatol 137:e11–e16. https://doi.org/10.1016/j.jid.2016.11.020
26. Tow WW, Premier R, Jing H, Ajlouni S (2011) Antioxidant and antiproliferation effects of extractable and nonextractable polyphenols isolated from apple waste using different extraction methods. J Food Sci 76:163–172. https://doi.org/10.1111/j.1750-3841.2011.02314.x
27. Guardia L, Suárez L, Querejeta N, Rodríguez Madrera R, Suárez B, Centeno TA (2019) Apple waste: a sustainable source of carbon materials and valuable compounds. ACS Sustain Chem Eng 7:17335–17343. https://doi.org/10.1021/acssuschemeng.9b04266
28. Duan Y, Mehariya S, Kumar A, Singh E, Yang J, Kumar S, Li H, Awasthi MK (2021) Apple orchard waste recycling and valorization of valuable product-a review. Bioengineered 12:476–495. https://doi.org/10.1080/21655979.2021.1872905
29. Pascoalino LA, Reis FS, Prieto MA, Barreira JCM, Ferreira ICFR, Barros L (2021) Valorization of bio-residues from the processing of main portuguese fruit crops: From discarded waste to health promoting compounds. Molecules 26:2624. https://doi.org/10.3390/molecules26092624
30. Zhang F, Wang T, Wang X, Lü X (2021) Apple pomace as a potential valuable resource for full-components utilization: a review. J Clean Prod 329:129676. https://doi.org/10.1016/j.jclepro.2021.129676

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