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The therapeutic potential of *Physalis longifolia* against various carcinomas

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ABSTRACT

Our research highlights the nutraceutical potential of the fruits of *Physalis longifolia* Nutt. (Solanaceae) in antitumor therapy. Recently we reported the isolation of withaferin A, along with 22 other withanolides from the aerial parts of *P. longifolia*. Herein, we present our biological, ethnobotanical, and phytochemical investigations on the edible fruit (known as "wild tomatillo" or "long leaf groundcherry") of the species. Specifically, dried fruit extract was examined for its chemical composition and biological activity. Explorative chromatography analysis of the fruit extract indicated the presence of withaferin A, which was further confirmed by subsequent HPLC analysis. The therapeutic potential of orally administered fruit extract was also investigated, where gavage treatment induced a 60% volume reduction in triple negative breast carcinomas (MDA-MB-468LN) in an experimental mouse model. This encouraging data indicate the potential of *P. longifolia* fruits as a dietary supplement, while also supporting the previously reported pharmaceutical potency of semi-synthetic withalongolide derivatives as promising chemotherapeutic candidates for antitumor therapies.

Contents

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1. Introduction

Withanolides are highly oxygenated steroids derived from a C_{28} ergostane skeleton [1]. The first compound reported in this class was isolated in 1964, and subsequently named withaferin A (**1**) (Fig. 1) [1]. Since then, successive phytochemical investigations have resulted in the isolation of almost an additional 900 withanolides [1]. In recent years, withanolides have gained significant scientific interest due to their antitumor capacities and structural diversity [1]. These compounds are predominantly reported in the Solanaceae plant family, where the primary sources of natural withanolides are found in the extensively studied *Datura*, *Jaborosa*, *Physalis*, and *Withania* genera [1]. Previously, we reported the anti-proliferative potential of a series of diversified withanolides isolated from several members of these genera, which include *Datura wrightii* Regel [2] *Jaborosa caulescens* var. *bipinnatifida* Gillies and Hook. [3], *Physalis hispida* (Waterf.) Cronquist [4], *Physalis longifolia* Nutt. [5–7], and *Withania somnifera* Dunal [8,9]. Such extensive research afforded a withanolide library that was utilized as part of an extensive structural activity relationship study. The resulting data revealed that the presence of a Δ^2-1-oxo functionality in the A-ring of the withanolide structural scaffold was a requirement for the anti-proliferative properties of these compounds [10]. Subsequent stability studies revealed that bioactive withanolides containing such moieties were susceptible to Michael addition degradations in methanolic solutions [11]. Previously, we observed that gavage treatment of *P. longifolia* fruit induced a 1–2 week delay in colorectal tumor growth in a tumorigenesis murine model [12]. In continuing this research, we utilized HPLC analysis to determine the presence of anti-proliferative withanolides in *P. longifolia* fruit (Fig. 2). In addition, herein we present the bioactivity (*in vivo* and *in vitro*) of *P. longifolia* fruits in a triple negative breast carcinoma (TNBC) preclinical animal model (Fig. 3–6).

2. The ethnobotany and phytochemistry of *P. longifolia*

P. longifolia Nutt., "wild tomatillo" or "longleaf groundcherry", is a perennial herbaceous species that produces numerous pea-sized yellow-green ripe fruits that taste similar to an under-ripened strawberry [13]. This species occurs from northern Mexico throughout the continental U.S. and into southern Canada [13].

Physalis is believed to have originated in Mexico, and today there are at least 75 known species in the genus [14]. Among them, there are 29 *Physalis* species in the United States, including the non-native *P. longifolia*, which was first introduced to California, where it is currently listed as a noxious weed [15]. However, the berries have a long history of ethnobotanical usage by several North American indigenous tribes (Zuni, Rio Grande, Acoma, Laguna, San Felipe, and Hopi Pueblos) and were either boiled or consumed fresh [16,17]. In addition, traditional cultivation of the species was reported in Zuni gardens [16,18].

Fruits from related *Physalis* species, such as the commercially available *Physalis* ixocarpa and *Physalis* peruviana, have gained gastronomy popularity, where they are routinely utilized in confectionery and green "tomatillo" sauces [13]. Furthermore, previous phytochemical analysis identified numerous antioxidant constituents in these *Physalis* fruits, where anthocyanins were found to be present in *P. ixocarpa* [19]; while carotenoids, as well as withanolides were observed in *P. peruviana*; respectively [20,21]. These studies strongly suggested that the fruits of related *Physalis* species, such as *P. longifolia*, are worthy of similar investigations.

Species identifications are difficult within *Physalis* and many of the identifications related to historical uses were not very accurate. We know that *P. longifolia* was largely used for both food and medicine, but accurate species data were not generally recorded and clearly similar species were confused.

Such historical ethnobotanical data stimulated our initial phytochemical investigation on the above ground biomass of *P. longifolia*, which resulted in the isolation of an array of novel [withalongolides A–P; **2–6**, **10–13**, and **16–22**] as well as known [2,3-dihydrowithaferin A (**8**), 3β-methoxy-2,3-dihydrowithaferin A (**9**), sitoindoside IX (**7**), viscosalactone B (**14**), withaferin A (**1**), 2,3-dihydro-3β-*O*-sulfate withaferin A (**15**), and 3α,6α-epoxy-4β,5β,27-trihydroxy-1-oxowitha-24-enolide (**23**), respectively] withanolides (Fig. 1) [5,6]. The initial MTS assay-based anti-proliferative evaluation of these compounds identified that a Δ^2-1-oxo functionality in ring A; in conjunction with either a 5β,6β-epoxy or 5α-chloro-6β-hydroxy moiety in ring B; as the minimum structural requirements for withanolides to produce potent cytotoxic activity [10]. Subsequent semi-synthetic experiments were conducted on the most anti-proliferative constituents of the species, such as compound **2**, which generated a highly potent withanolide derivative (**24**) (Fig. 1) [7]. Additional cell proliferation assay testing revealed that withanolides **1**, **2**, and **24** produced nanomolar IC_{50} values (**1**: 290, 4000, 2000, 800, 200 nM; **2**: >10,000, 5100, 5300, 3300 nM, >10,000 nM; and **24**: 98 nM; 810 nM; 140 nM; 2200 nM; 410 nM) against melanoma (B16F10 and SKMEL-28), human oral cavity carcinoma (JMAR, and MDA-1986) and normal fetal fibroblast (MRC-5) cells, respectively [5].

In depth stability analysis revealed that potent cytotoxic withanolides containing a Δ^2-1-oxo functionality in ring A (which includes **1**, **2**, and **24**) were susceptible to degradation through Michael addition reactions to form withanolide artifacts [11]. With this in mind, further MTS assay-based testing was conducted on the *P. longifolia* derived withanolides that contained a Δ^2-1-oxo functionality in ring A, where nanomolar concentrations of these withanolides were observed to stimulate cell cycle arrest (from *G*1/*G*0 to *G*2/M phase) through the induction of the apoptotic pathway. Specifically, these data revealed that **1** (369, 31, and 750 nM), **2** (570, 119, and 12,700 nM), and **24** (117, 29, and 1580 nM), produced potent IC_{50} values against adrenocortical carcinoma (Yl and SW13) cells compared to normal fetal fibroblasts (MRC-5), respectively [22]. In addition, it was observed that withanolides **1**, **2** and **24** modulated the expression of several adrenocortical carcinoma proteins in a dose dependent manner, such as Jagged 1, MAPK, and Akt/mTOR pathway proteins [22]. This cumulative biological data advocated the use of **24** as the positive control for future research.

The combined bioassay, ethno-botanical, and phytochemical data strongly suggested that the *P. longifolia* fruit warranted further investigation. As such, the fruit was examined in colorectal tumor growth in an experimental mouse model, where preliminary results indicated that gavage treatment of the fruit induced a 1–2 week delay in tumor growth [12].

In this present investigation, HPLC analysis was utilized to determine the presence of anti-proliferative withanolides in extracts of the fruit. The efficacy of the fruit at targeting human carcinoma cells *in vitro* (oral cavity: JMAR; and triple negative breast: MDA-MB-231) as well as *in vivo* (triple negative breast: MDA-MB-468LN) was examined.

3. Material and methods

3.1. Chemicals and plant material

Wild *P. longifolia* seed samples were collected in 2010 from Stafford county in Kansas. These seeds were sown in the University of Kansas medicinal plant research garden, to produce 2-month-old plant samples, which were harvested to produce the fruits that were utilized in this study. All plant materials were collected,

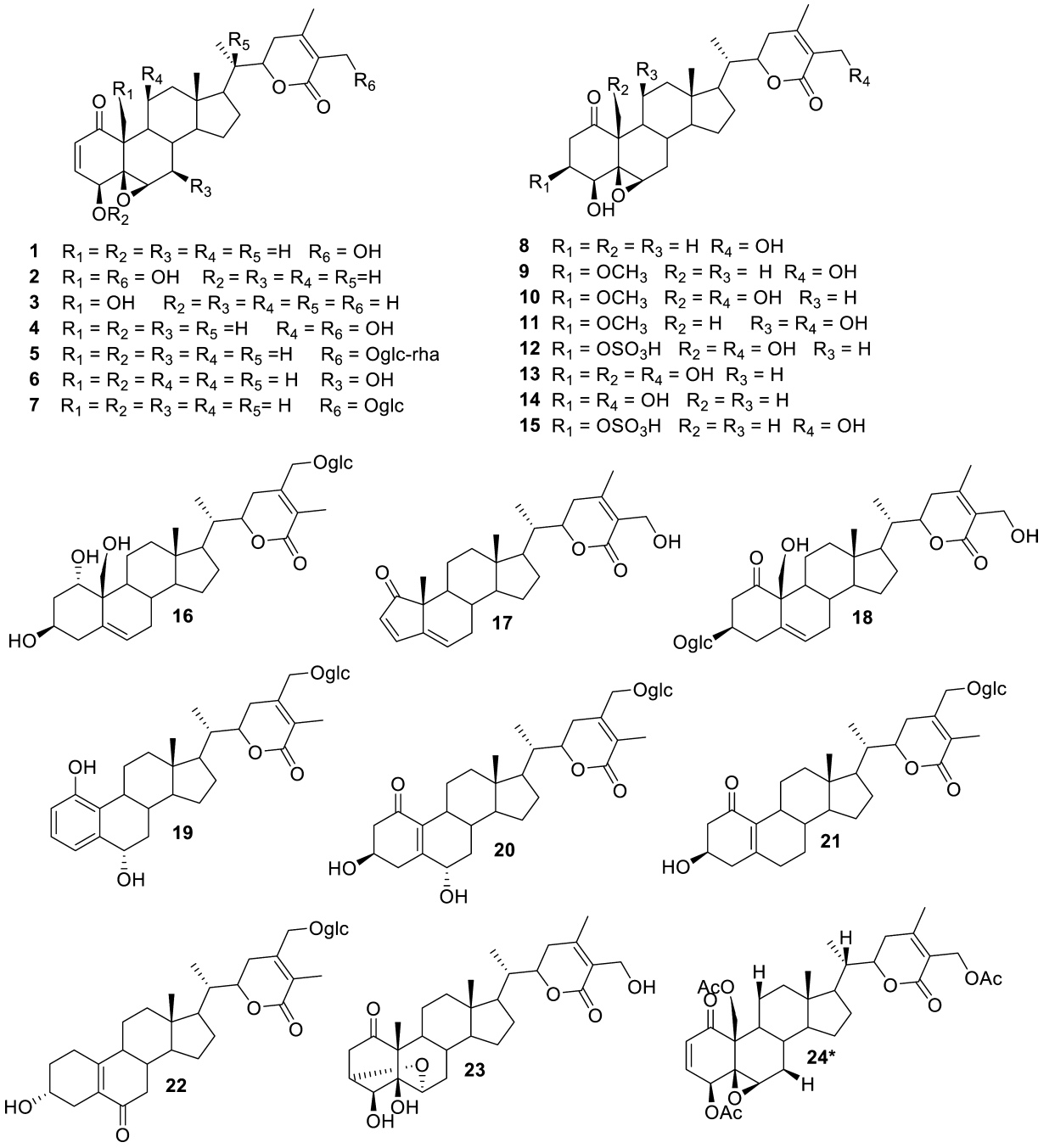

Fig. 1. Withanolides isolated from *Physalis longifolia* (**1**–**23**), and a semi-synthetic* withanolide derivative (**24**).

cultivated and authenticated by the Kindscher laboratory at the Kansas Biological Survey, University of Kansas, Lawrence, KS, United States. Voucher specimens (KK1830083011) were deposited in the R.L. McGregor Herbarium of the University of Kansas. Analytical (dichloromethane, hexane, and methanol) and HPLC grade (acetonitrile and methanol) solvents were purchased from Fisher Scientific Co. (Fair Lawn, NJ); whereas deionized water filtered through a Millipore Milli-Q A10 system (Millipore Corp., Bedford, MA) produced HPLC grade water.

3.2. Sample preparation

Freeze-dried *P. longifolia* fruit (0.7 kg) was extracted for 72 h at r. t. in (1:1) dichloromethane – methanol solution (fruit extract, **FE**, 48.5 g). This extract was utilized for bioassays and *in vivo* studies, in conjunction with the semi-synthetic positive control, **24**, which was prepared from **2** as previously described [5].

3.3. HPLC analysis

The chromatographic separation was performed on an IRIS IProSIL120-5 C18 AQ column (4.6 × 250 mm, 5.0 mm), connected to an Agilent 1200 series system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, an auto-sampler and a PDA detector, and ChemStation software. Comprehensive absorption analysis of the sample, by the PDA detector across a 190–400 nm wavelength range, revealed 220 nm as the optimal analytical detection wavelength. Chromatographic separation was

achieved in a water (A)-acetonitrile (B) mobile phase by linear gradient (30–80% B; 0–18 min) followed by isocratic (80% B; 18–25 min) elution at a constant flow rate of 1.0 mL/min. Peak assignments were made by comparing retention times and characteristic absorption spectra from the PDA with those of the authentic standard, **1**. For compositional analysis, a 30 mg sample of fruit extract (**FE**) was completely solubilized with 10 mL deionized water, partitioned twice with equal volumes of hexane to remove the significant lipid content, and the resulting aqueous layer was dried to yield an enriched fruit extract (**EFE**) containing more polar constituents (15 mg). In addition, the enriched fraction sample was spiked with three different concentrations of purified **1**, which confirmed the presence of **1** in the fruit extract (Fig. 2).

3.4. Bioassays

3.4.1. Cell lines and reagents

Validated human head and neck (JMAR) and human triple negative breast (MDA-MB-468LN and MDA-MB-231) cancer cell lines were grown in 2D culture at 37 °C in a humidified atmosphere of 5% CO_2 in air. MDA-MB-468LN cells were cultured in minimal essential medium (MEM)-alpha, (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Sigma–Aldrich, St. Louis, MO), 1% penicillin/streptomycin (Life Technologies, Grand Island, NY), and 2% L-glutamine 200 mM; (Life Technologies, Grand Island, NY). JMAR and MDA-MB-231 cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 2% L-glutamine, 1% MEM-vitamin (100×; Hyclone, Logan, UT), and 1% MEM nonessential amino acids (Sigma–Aldrich, St. Louis, MO). L-buthionine-sulfoximine (BSO) and n-acetyl cysteine (NAC) were obtained from (Sigma–Aldrich, St. Louis, MO).

3.4.2. Cell proliferation assay

The MDA-MB-231 and JMAR cell lines were seeded in 96-well plates, at a density of approximately 3000 cells/well. The cells were allowed to adhere for 6 h and then treated with varying concentrations of either **FE** or **24**. CellTiter-Glo luminescent assay (Promega, Madison, WI) was utilized to measure viability of cells based on quantification of the ATP levels. CellTiter-Glo assay reagent (50 μL/well) was added 72 h-post treatment, luminescent signals were quantified after equilibration (10 min incubation at r.t.) using a BioTek Synergy Neo plate reader (BioTek, Winooski, VT), and IC_{50} values were calculated using GraphPad Prism 5 software.

3.4.3. Immunoblot analysis

JMAR and MDA-MB-231 cells grown to 60–80% confluence were treated for 24 h with varying concentrations of either **24** or **FE**. Cells were collected post-treatment, washed with 1 × PBS solution and then lysed as previously described [22]. In brief, post-lysis cells were centrifuged at 14,000 rpm for 20 min, the supernatant was collected, and protein levels were quantified using BCA Protein Assay (Thermo Scientific, Rockford, IL). Approximately 20 μg of protein per sample was separated on 8–14% SDS-PAGE gels and then transferred on to a nitrocellulose membrane (Midwest Scientific, St. Louis, MO, USA). The membranes were blocked in 5% milk for 1 h, washed, and probed overnight at 4 °C with appropriate dilutions of primary antibodies [23]. The blots were then washed thrice with PBS-T (PBS with 0.2% Tween 20) and probed with appropriate horseradish peroxidase (HRP) conjugated secondary antibodies (Santa Cruz Biotechnology, Dallas, TX) at r.t. for 1 h. The membranes were washed again with PBS-T, and the bands were visualized using either Super Signal West Pico or Femto chemiluminescence reagent (Thermo Scientific, Rockford, IL) on an X-ray film (Midwest Scientific, St. Louis, MO).

3.4.4. In vivo *breast cancer tumor model*

All animal studies were conducted according to the protocol approved by the Committee on Use and Care of Animals at the University of Michigan. MDA-MB-468LN aggressive TNBC cells were prepared at a concentration of 3×10^6 cells per 100 μL in a 1 × PBS solution and were injected under isoflurane anesthesia into the mammary fat pad of 6 week old female athymic Nu/Nu mice (Harlan Laboratories, Indianapolis, IN). Tumor size was measured thrice weekly, and the tumor volumes were calculated

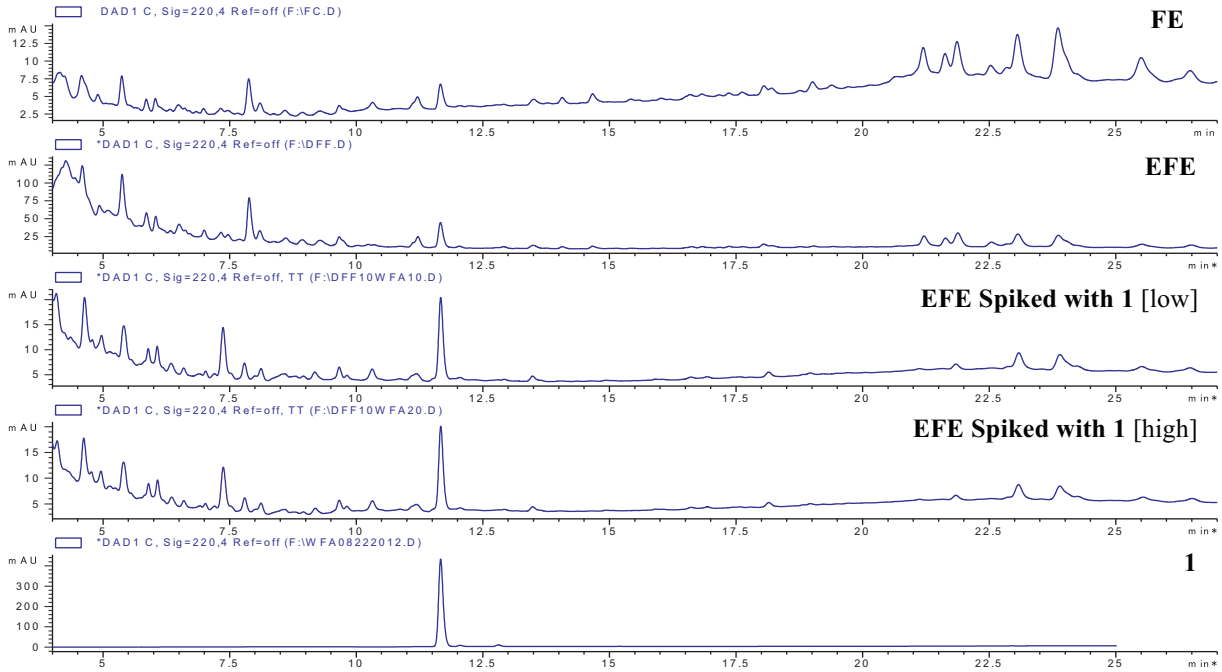

Fig. 2. HPLC profiles of *Physalis longifolia* fruit extract (**FE**), enriched-fruit extract (**EFE**), withaferin A (**1**), and the **EFE** spiked with increasing concentrations of **1**.

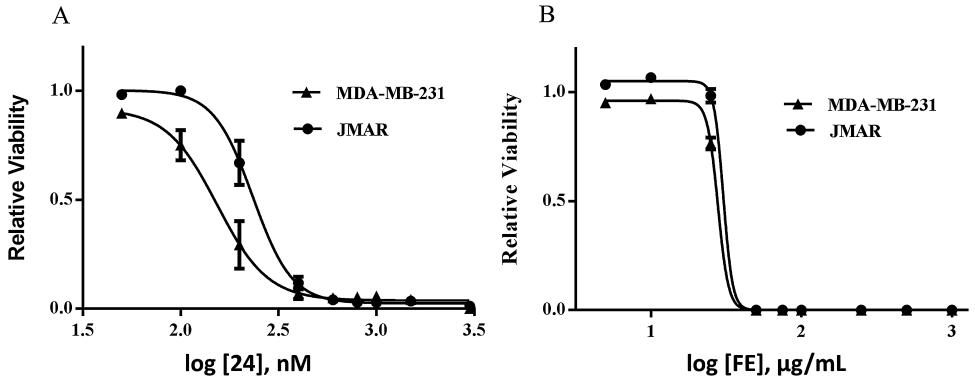

Fig. 3. Anti-proliferative effects of increasing concentrations of **24** (Fig. 3A) or *Physalis longifolia* fruit (**FE**,Fig. 3B) extract on JMAR and MDA-MB-231 cells *in vitro* after 72 h.

as previously described [24]. When the tumor volume reached approximately 4×4 mm or 5 mm in any direction, mice were randomized into either the control or the **FE** treatment groups (7–8 mice/arm). The mice were dosed by oral gavage at 10 mg/kg/day for 21 consecutive days, and followed for an additional 6 weeks post treatment. The **FE** stock solution was made up at 100 mg/kg in DMSO, aliquoted, and stored at −20 °C to prevent artifact formation. A dosing solution was prepared fresh before each administration at 2.5 mg/mL (**FE** stock diluted in sterile water). This provided an oral dose of 100 µL for a 25 g mouse and was adjusted accordingly based on animal mass. Placebo animals were dosed with a vehicle control (DMSO in sterile water only). Animals were euthanized humanely if: (1) tumor volume exceeded 2000 mm^3; (2) significant tumor ulceration was observed; or (3) animal body conditioning score demonstrated significant deterioration as per unit for laboratory animal medicine (ULAM) at the University of Michigan protocol. The two tailed *p*-values were calculated using Microsoft Excel software.

4. Results and discussion

The initial HPLC profile fingerprint of the *P. longifolia* fruit extract (**FE**) displayed a weak peak with the same retention time of **1**. In an effort to better visualize and enrich this peak, the defatted fruit fraction (**EFE**) was prepared, as previous investigations have shown that a significant amount of lipid is present in other *Physalis* fruits, such as *P. peruviana* [21]. The main peaks present in the HPLC profile of this enriched fraction (**EFE**) were also present in the crude fruit extract (**FE**) (Fig. 2). Subsequently, the enriched fraction was spiked with varying concentrations of **1**, thus confirming the presence of **1** in the fruit (Fig. 2).

Potent cell proliferation reduction *in vitro* (JMAR and MDA-MB-231) was observed post treatment (72 h) with **24** (Fig. 3A) and **FE** (Fig. 3B). Specifically, **FE** produced IC_{50} values of 30.26 ± 20.46 µg/mL and 27.88 ± 11.07 µg/mL; whereas **24** produced IC_{50} values of 233.9 ± 18.4 nM and 153.6 ± 24.3 nM; in JMAR and MDA-MB-231 cells, respectively.

In addition, it was observed that the treatment of cells with **FE** or **24** stimulated the apoptotic pathway, through induction of oxidative stress and depletion of Akt/mTOR pathway proteins. This supports previous studies that have shown that **1** and related withanolides exert their anti-proliferative properties through the modulation of several key oncogenic proteins, which include the Akt/mTOR signaling pathway proteins [23–27].

In this present study, decreased levels of EGFR, Akt, and mTOR were noted post-treatment (24 h) in both JMAR (by 31.6%, 72.2%, and 15.9% with 0.5 µM of **24**; to 96.4%, 99.2%, and 86.4% with 2.5 µM of **24**) and MDA-MB-231 (by 81.7%, 97.5% and 40% with 0.5 µM of **24**; to 98.8%, 99.9% and 87% with 2.5 µM of **24**) cells (Fig. 4A). Similarly, **FE** solutions (50 µg/mL) also diminished EGFR, Akt and mTOR protein expression post-treatment (24 h) in both JMAR (by 19.83%, 47.01%, and 32.61%) and MDA-MB-231 (45.08%, 54.45%, and 0%) cells (Fig. 4B). Significant protein expression fold increases in HSP32 (37.85 and 94.3) and HSP70 (2.25 and 5.39)

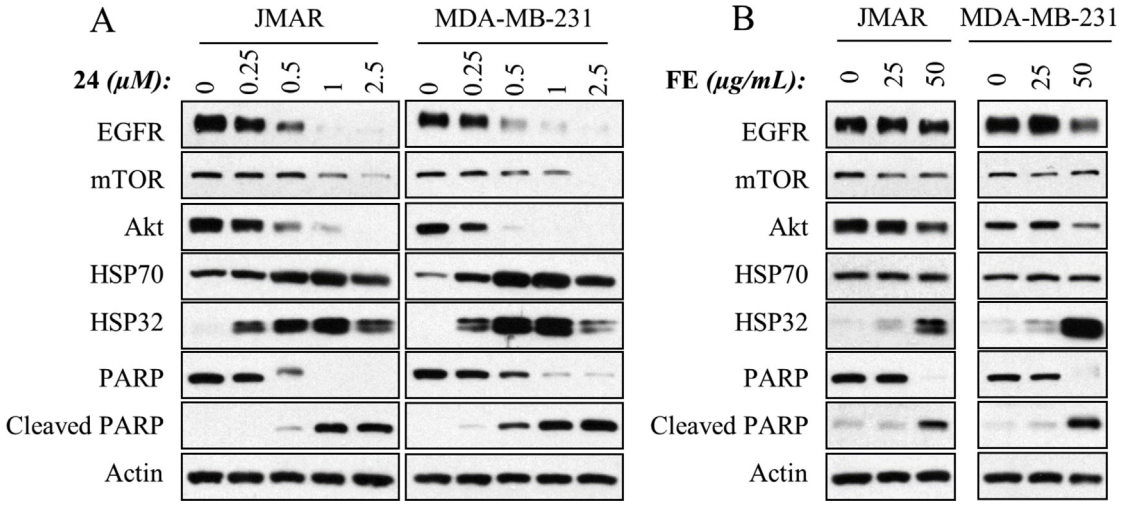

Fig. 4. Western blot analysis of an array of proteins including those of the Akt/mTOR pathway, heat shock response and apoptosis after treatment with either **24** (Fig. 4A) or *P. longifolia* fruit (**FE**, Fig.4B). JMAR and MDA-MB-231 cells were treated with varying concentrations of either **24** or **FE** for 24 h, and the cellular proteins were isolated and analyzed by Western blotting. Actin was used as a loading control.

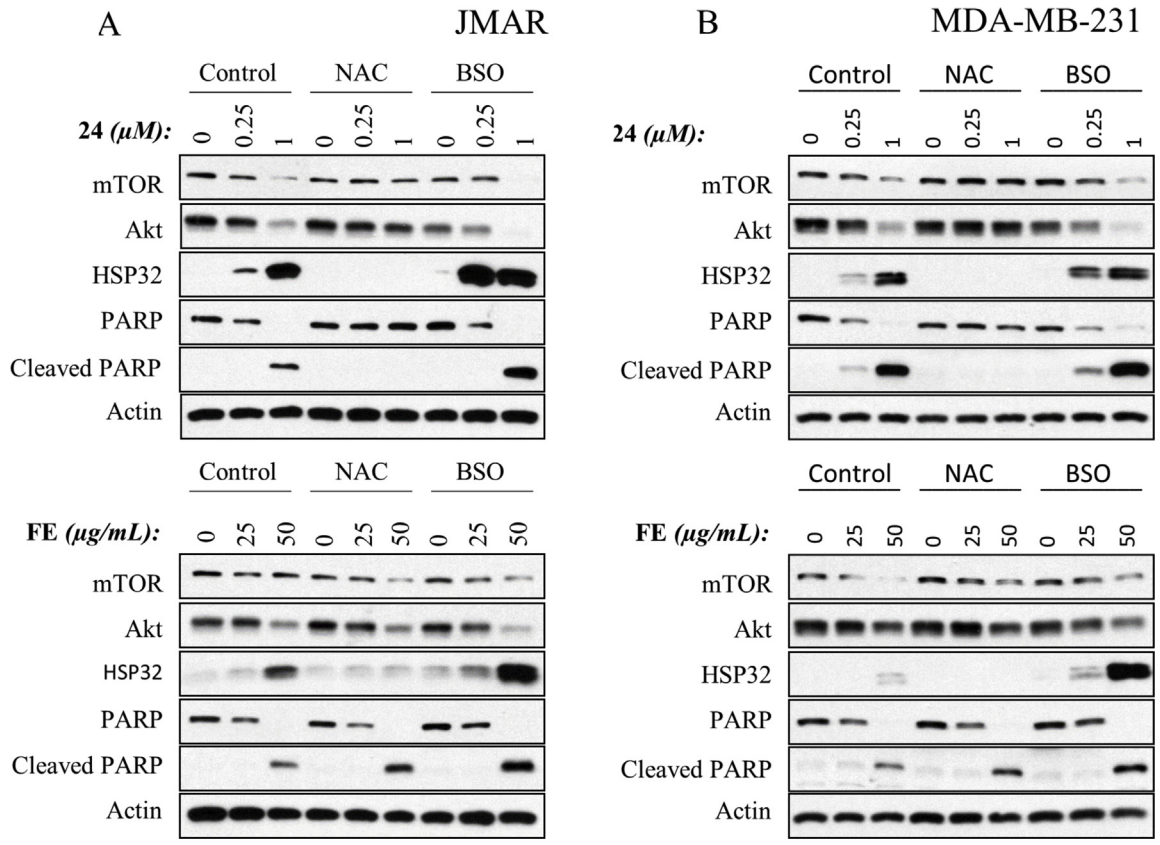

Fig. 5. Pre-treatment of JMAR (Fig. 5A) and MDA-MB-231 (Fig. 5B) cells with L-buthionine-sulfoximine (BSO) enhances **24** and *Physalis longifolia* fruit (**FE**) extract anti-proliferative and pro-apoptotic activity which was in-part blocked by NAC pre-treatment. The cells were pre-treated with either BSO for 24 h or NAC for 1 h and then treated with either **24** or **FE** and analyzed for an array of proteins by immunoblot analysis. Actin was used as a loading control.

were observed after **24** 1.0 μM treatments in JMAR and MDA-MB-231, respectively. Similarly, protein expression fold increases of HSP32 (34.96 and 35.79) were observed after **FE** 50 μg/mL treatments in JMAR and MDA-MB-231, respectively. In each case, the induction of the apoptotic pathway was evaluated by PARP cleavage starting at concentrations of 0.5 μM of **24**, and 50 μg/mL of **FE**.

Furthermore, cells pre-treated (24 h) with 500 μM of the gamma-glutathione (GSH) synthetase inhibitor, L-buthionine-sulfoximine (BSO), increased the efficacy of **24** and **FE**. Conversely, cells pre-treated for 1 h with 5 mM of the thiol antioxidant, NAC, abrogated the activity of **24** and **FE**. Western blot analysis confirmed that BSO enhanced the mechanism of action of **24** and **FE** in JMAR (Fig. 5A) and MDA-MB-231 (Fig. 5B) cells, where decreased Akt expression as well as increased expressions of HSP32 and PARP cleavage were observed at low drug concentrations (0.25 μM **24**, and 25–50 μg/mL **FE**). Though NAC pre-treatment completely nullified the anti-proliferative and pro-apoptotic effects of **24**; it failed to prevent **FE**-mediated PARP cleavage, as well as the depletion of Akt and mTOR proteins. This suggests that **FE** contains constituents that maybe activating a secondary mechanism that is independent of thiol oxidation. Such encouraging *in vitro* data suggested that *in vivo* studies might produce similar results. Subsequent *in vivo* studies with a TNBC [MDA-MB-468LN] orthotopic murine model revealed that **FE** treatment demonstrated a greater reduction (>39% and 60% by day 21 and 62; with $p < 0.04$ and $p < 0.05$ versus controls, respectively) in tumor volume compared to vehicle control animals (Fig. 6) without any observed global animal toxicity as noted by weight change or decrease in body condition score. It is also important to note that the concentration of fruit extract required to achieve physiologic effect *in vitro* is similar to the concentrations that were tested in our *in vivo* studies. Additionally this concentration is lower than that of the reported *W. somnifera* extract concentrations utilized in clinical trials [28].

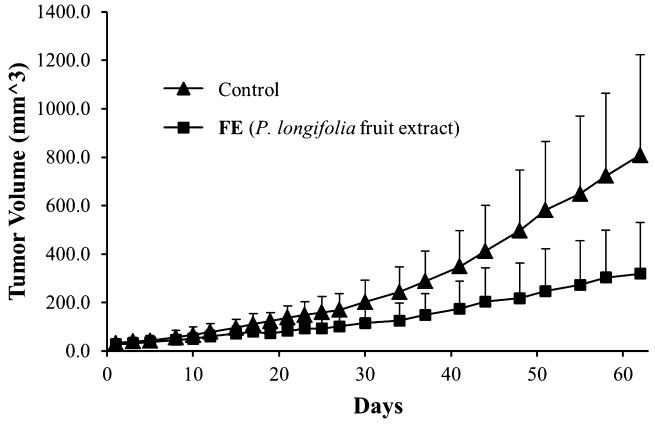

Fig. 6. *In vivo* tumor growth curves: *Physalis longifolia* fruit (**FE**) extract mediated reduction in tumor volume in triple negative breast cancer orthotopic murine model (MDA-MB-468LN) compared to vehicle control groups.

5. Conclusions

The presence of withaferin A (**1**) in *P. longifolia* fruit was confirmed by HPLC analysis utilizing the matrix spike method, where retention times and UV absorptions were compared (Fig. 2). Previously, we have shown that **1** and **24** inhibit RET phosphorylation and the mTOR signaling pathway proteins in MTC cells. Our

most recent data, presented here for the first time, further explores that mechanism. Specifically, the fruit extract, **FE**, is highly potent at reducing cell viability, inducing apoptosis through modulation of EGFR/Akt/mTOR proteins, and through induction of oxidative stress *in vitro* in head and neck carcinomas, as well as breast cancer cell lines. In addition, the data demonstrate that *in vitro* activity can be efficiently translated *in vivo*, as **FE** gavage treatment induced a 60% volume reduction in a TNBC (MDA-MB-468LN) orthotopic murine model.

Combined, these encouraging data – in conjunction with the observed *in vivo* reduction of tumor volume without significant toxicity – highlight the potential of *P. longifolia* fruits as a dietary supplement, and suggests that future research on the fruits of this species should focus on the identification of its major constituents. Furthermore, the data also supports the previously reported pharmaceutical potential of semi-synthetic withalongolide derivatives, such as **24**, as promising chemotherapeutic candidates for antitumor therapy.

Acknowledgements

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References

[1] Zhang HP, Cao CM, Gallagher RJ, Timmermann BN. Antiproliferative withanolides from several solanaceous species. Nat. Prod. Res. 2014;28(22):1941–51, doi:http://dx.doi.org/10.1080/14786419.2014.919286. 24871278.

[2] Zhang H, Bazzill J, Gallagher RJ, Subramanian C, Grogan PT, Day VW, Kindscher K, Cohen MS, Timmermann BN. Antiproliferative withanolides from *Datura wrightii*. J. Nat. Prod. 2013;76(3):445–9, doi:http://dx.doi.org/10.1021/np300766p. 23252848.

[3] Zhang H, Cao CM, Gallagher RJ, Day VW, Montenegro G, Timmermann BN. Withanolides from *Jaborosa caulescens* var. *bipinnatifida*. Phytochemistry 2014;98:232–5, doi:http://dx.doi.org/10.1016/j.phytochem.2013.11.005. 24314746.

[4] Cao CM, Zhang H, Gallagher RJ, Day VW, Kindscher K, Grogan P, Cohen MS, Timmermann BN. Withanolides from *Physalis hispida*. J. Nat. Prod. 2014;77(3):631–9, doi:http://dx.doi.org/10.1021/np400953n. 24456028.

[5] Zhang HP, Samadi AK, Gallagher RJ, Araya JJ, Tong XQ, Day VW, Cohen MS, Kindscher K, Gollapudi R, Timmermann BN. Cytotoxic withanolide constituents of *Physalis longifolia*. J. Nat. Prod. 2011;74(12):2532–44, doi:http://dx.doi.org/10.1021/np200635r. 22098611.

[6] Zhang HP, Motiwala H, Samadi A, Day V, Aubé J, Cohen M, Kindscher K, Gollapudi R, Timmermann B. Minor withanolides of *Physalis longifolia*: structure and cytotoxicity. Chem. Pharm. Bull. 2012;60(10):1234–9, doi:http://dx.doi.org/10.1248/cpb.c12-00305. 23036966.

[7] Motiwala HF, Bazzill J, Samadi A, Zhang H, Timmermann BN, Cohen MS, Aubé J. Synthesis and cytotoxicity of semisynthetic withalongolide A analogues. ACS Med. Chem. Lett. 20134(11), doi:http://dx.doi.org/10.1021/ml400267q. 24273633.

[8] Tong X, Zhang H, Timmermann BN. Chlorinated withanolides from *Withania somnifera*. Phytochem. Lett. 2011;4(4):411–4, doi:http://dx.doi.org/10.1016/j.phytol.2011.04.016. 22125584.

[9] Zhang HP, Hagan K, Patel O, Tong XQ, Day VW, Timmermann BN. 6α,7α-Epoxy-5α-hydroxy-1-oxo-22R-witha-2,24-dienolide(withanolide B), 5β,6β-epoxy-4β,20-dihydroxy-1-oxo-22R-witha-2,24-dienolide(withanolide D), and 4β,27-dihydroxy-1-oxo-22R-witha-2,5,24-trienolide(5,6-deoxywithaferin A) in roots of *Withania somnifera*: isolation and their crystal structures. J. Chem. Crystallogr. 2014;44(3):169–76, doi:http://dx.doi.org/10.1007/s10870-014-0499-1.

[10] Zhang H, Samadi AK, Cohen MS, Timmermann BN. Anti-proliferative withanolides from the Solanaceae: a structure-activity study. Pure Appl. Chem. 2012;84(6):1353–67, doi:http://dx.doi.org/10.1351/PAC-CON-11-10-08. 24098060.

[11] Cao CM, Zhang H, Gallagher RJ, Timmermann BN. Withanolide artifacts formed in methanol. J. Nat. Prod. 2013;76(11):2040–6, doi:http://dx.doi.org/10.1021/np400296s. 24152046.

[12] Gallagher RJ, Zhang H, Cao CM, Cohen MS, Corbett S, Kindscher K, Timmermann BN. Fruits of *Physalis longifolia* inhibit tumor growth in colorectal cancer. Planta Med. 2013;79(10):869, doi:http://dx.doi.org/10.1055/s-0033-1348734.

[13] Kindscher K, Long Q, Corbett S, Bosnak K, Loring H, Cohen M, Timmermann BN. The ethnobotany and ethnopharmacology of wild tomatillos, *Physalis longifolia* Nutt., and related *Physalis* species: a review. Econ. Bot. 2012;66(3):298–310, doi:http://dx.doi.org/10.1007/s12231-012-9210-7.

[14] Whitson M, Manos PS. Untangling *Physalis* (Solanaceae) from the physaloids: a two-gene phylogeny of the Physalinae. Syst. Bot. 2005;30(1):216–30.

[15] http://www.invasive.orgInvasive Plant Atlas of the United States [Internet] The University of Georgia (GA) : Warnell School of Forestry and Natural Resources and College of Agricultural and Environmental Sciences - Dept. of Entomology. [cited 2011 July 31]. Available from:

[16] Castetter EE. Uncultivated native plants used as food – ethnobotanical studies in the American Southwest. Univ. New Mexico Biol. Series 1935;4(1):57–9.

[17] Hough W. Environmental interrelations in Arizona. Am. Anthropol. 1898;11(5):133–55, doi:http://dx.doi.org/10.1525/aa.1898.11.5.02a00000.

[18] Stevenson MC. Ethnobotany of the Zuñi Indians. SI-BAE Annu. Rep. 1915;30:39–64.

[19] Gonzalez-Mendoza D, Grimaldo-Juarez O, Soto-Ortiz R, Escoboza-Garcia F, Hernandez JFS. Evaluation of total phenolics, anthocyanins and antioxidant capacity in purple tomatillo (*Physalis ixocarpa*) genotypes. Afr. J. Biotechnol. 2010;9(32):5173–6.

[20] Ramadan MF. Bioactive phytochemicals, nutritional value, and functional properties of cape gooseberry (*Physalis peruviana*): a overview. Food Res. Int. 2011;44(7):1830–6, doi:http://dx.doi.org/10.1016/j.foodres.2010.12.042.

[21] Puente LA, Pinto-Muñoz CA, Castro ES, Cortés M. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: a review. Food Res. Int. 2011;44(7):1733–40, doi:http://dx.doi.org/10.1016/j.foodres.2010.09.034.

[22] Subramanian C, Zhang H, Gallagher R, Hammer G, Timmermann B, Cohen M. Withanolides are potent novel targeted therapeutic agents against adrenocortical carcinomas. World J. Surg. 2014;38(6):1343–52, doi:http://dx.doi.org/10.1007/s00268-014-2532-0. 24763440.

[23] Grogan PT, Sleder KD, Samadi AK, Zhang HP, Timmermann BN, Cohen MS. Cytotoxicity of withaferin A in glioblastomas involves induction of an oxidative stress-mediated heat shock response while altering Akt/mTOR and MAPK signaling pathways. Invest. New Drugs 2013;31(3):545–57, doi:http://dx.doi.org/10.1007/s10637-012-9888-5. 23129310.

[24] Cohen SM, Mukerji R, Samadi AK, Zhang X, Zhao H, Blagg BS, Cohen MS. Novel C-terminal Hsp90 inhibitor for head and neck squamous cell cancer (HNSCC) with *in vivo* efficacy and improved toxicity profiles compared with standard agents. Ann. Surg. Oncol. 2012;19(Suppl. 3):S483–90, doi:http://dx.doi.org/10.1245/s10434-011-1971-1. 21837531.

[25] Hahm ER, Moura MB, Kelley EE, Van Houten B, Shiva S, Singh SV. Withaferin A-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species. PLOS One 2011;6(8):e23354, doi:http://dx.doi.org/10.1371/journal.pone.0023354. 21853114.

[26] Koduru S, Kumar R, Srinivasan S, Evers MB, Damodaran C. Notch-1 inhibition by withaferin-A: a therapeutic target against colon carcinogenesis. Mol. Cancer Ther. 2010;9(1):202–10, doi:http://dx.doi.org/10.1158/1535-7163.MCT-09-0771. 20053782.

[27] Oh JH, Kwon TK. Withaferin A inhibits tumor necrosis factor-alpha-induced expression of cell adhesion molecules by inactivation of Akt and NF-kappaB in human pulmonary epithelial cells. Int. Immunopharmacol. 2009;9(5):614–9, doi:http://dx.doi.org/10.1016/j.intimp.2009.02.002. 19236958.

[28] Auddy B, Hazra J, Mitra A, Abedon B, Ghosal S. A standardized *Withania somnifera* extract significantly reduced stress-related parameters in chronically stressed humans: a double-bind, randomized, placebo-controlled study. J. Am. Nutraceutical. Assoc. 2008;11(1):50–6.