# Poha Berry (*Physalis peruviana*) with Potential Anti-inflammatory and Cancer Prevention Activities

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## Abstract

*The Daniel K. Inouye College of Pharmacy, during a historic event in Spring 2016, graduated the first two students in the Pacific region to earn a PhD in pharmaceutical sciences at the University of Hawai'i at Hilo. The college offers PhD programs in these five disciplines: Cancer Biology, Medicinal Chemistry, Pharmaceutics, Pharmacognosy, and Pharmacology. One of the Pharmacognosy dissertations focused on plant-derived natural products with potential anti-inflammatory and cancer chemopreventive activities.* Physalis peruviana *(Pp) L. originated in tropical South America. It has become naturalized and is found readily on the Island of Hawai'i. The edible fruits are commonly known as cape gooseberry or poha in Hawai'i. In part of our study, three new withanolides, physaperuvin G (***1***), physaperuvins I-J (***2-3***), along with four known withanolides, namely, 4β-hydroxywithanolide E (***4***), withaperuvin C (***5***), and physalactone (***6***), coagulin (***7***) were isolated from the aerial parts of* P. peruviana*. In addition, two known compounds, phyperunolide F (***8***), and withanolide S (***9***), were isolated and identified from the poha berry fruits. The structures and absolute stereochemistry of new compounds from poha were elucidated by several spectroscopy methods: Nuclear Magnetic Resonance (NMR) spectroscopy, X-ray diffraction, and mass spectrometry analyses. All isolated poha compounds (aerial parts and fruits) were evaluated for their anti-inflammatory activity with lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells, and tumor necrosis factor alpha (TNF-α)-activated nuclear factor-kappa B (NF-κB) with transfected human embryonic kidney cells 293. Most of the isolated natural compounds showed activity with these assays. Additional studies were performed with models of colon cancer. Specifically, 4β-hydroxywithanolide E (4HWE) inhibited the growth of colon cancer monolayer and spheroid cultures. The compound induced cell cycle arrest at low concentrations and apoptosis at higher concentrations. These data suggest the ingestion of poha berries may have some effect on the prevalence of colon cancer. Additionally, poha isolates compounds were evaluated for their growth inhibitory effects with U251MG glioblastoma and MDA-MB-231 breast cancer cells that harbor aberrantly-active signal transducer and activation of transcription 3 (STAT3), compared to normal NIH-3T3 mouse fibroblasts. This work has led to the filing of three provisional patents with the University of Hawai'i Office of Technology Transfer and Economic Development.*

## Introduction

Although heart disease remains the leading cause of death nationwide, the death rate resulting from heart disease has significantly diminished over the years, and cancer has overtaken heart disease as the leading cause of death in 22 states in the United States.[1] The American Cancer Society estimates that 1,685,210 new cancer cases will be diagnosed this year (2016), and 595,690 Americans will die of cancer.[2] Among the types of cancers, lung and colorectal cancers account for the greatest number of deaths. Additionally, prostate and breast cancers are reported commonly in men and women, respectively.[2] In 2012, statistical data collected by the National Cancer Institute and the Centers for Disease Control and Prevention showed melanoma, oral, uterus, and pancreatic cancers affect Hawai'i residents to a greater extent than other states.[3,4] About 380 Hawai'i residents were diagnosed with melanoma in 2013.[3] The rate of new melanoma cases diagnosed among Whites in Hawai'i is almost triple the national average.[3] Oral cancer is also more common on Hawai'i Island than other areas nationwide, and this leads to higher-than-average mortality rate in Hawai'i County. However, breast and prostate cancers are still the most commonly diagnosed cancers in Hawai'i County. In particular, the Native Hawaiian population has higher-than-average cancer rates; in this regard, males had a 21% higher cancer death rate, and women had a 37% higher death rate, as compared to other groups.[3]

Conventional treatment for cancer includes radiation, surgery and chemotherapy. Often, cancer chemotherapeutic drugs are not selective and can cause toxicity and/or severe side effects, precluding effective treatment.[5] Since cancer continues to be a major public health problem and conventional cancer chemotherapy treatments have not controlled the incidence of most cancer types, new approaches for cancer treatment are critically needed. Recent detailed studies of the mechanism of action and pathogenesis of cancer has led to the discovery of new key molecular and signaling pathways amenable for targeted therapy.[5] The targeted therapy approach has shifted from using cytotoxic compounds that cause tumor regression to molecular target-based drug discovery.[6] The challenge is to identify key molecular targets that underlie the malignant behavior of a cancer cell, and to target these directly. The mutated phenotype in a cancer cell is driven by the pattern of genes expressed in the cell; therefore, much attention has focused on the abnormal activation of transcription factors that regulate genes controlling proliferation, survival, self-renewal, and invasion. Two such oncogenic transcription factors are Signal Transducer and Activator of Transcription 3 (STAT3) and Nuclear Factor-kappa B (NF-κB).[7,8]

**Oncogenic Transcription Factors**

NF-κB transcription factors and signaling pathways are central coordinators in innate and adaptive immune responses.[9] NF-κB regulates numerous physiological processes including cellular proliferation, development, differentiation, immunity, apoptosis, inflammation, and metabolism.[7] NF-κB proteins are present in the cytoplasm in association with inhibitory proteins (inhibitors of NF-κB).[10] NF-κB is activated rapidly in response to a wide range of stimuli, including pathogens, pro-inflammatory cytokines, such as tumor-necrosis factor (TNF-α), and interleukin-1.[7,11] Park and Hong indicated that aberrantly active NF-κB pathways may be associated with many different types of human cancers.[12]

It is generally known that normal STAT3 activation is transient in keeping with cellular requirements for proliferation, development, apoptosis, and inflammation. In contrast, aberrant STAT3 activity dysregulates growth and survival, promotes angiogenesis,[13] migration and invasion of tumor cells, and induces tumor-immune tolerance.[14] In turn, constitutive STAT3 activation is a molecular abnormality that is causally linked to cancer aggressiveness. As mentioned above, both NF-κB and STAT3 signaling pathways have integrated roles in inflammatory responses that promote cancer development and growth. Recently, the roles of NF-κB and STAT3 in a variety of cancers including breast, colon, gastric and liver cancers have been heavily studied.[12] The activation and interaction between STAT3 and NF-κB plays a vital role in control of the communication between cancer cells and inflammatory cells.[13,15] These two major transcription factors regulate the ability of pre-neoplastic and malignant cells to resist apoptosis-based tumor-surveillance and regulating tumor angiogenesis and invasiveness.[7] Thus, it is of interest to focus on specific therapies to target NF-κB and STAT3 inhibition in malignant cancers. To explore and exploit these findings for cancer therapy, we set out to search for plant-derived natural products that could simultaneously block the effects of NF-κB and STAT3. These dual STAT3/NF-κB inhibitors may lead to unique strategies for cancer therapy. The aim of this work was to identify bioactive natural products derived from *P. peruviana* as STAT3 and NF-κB inhibitors, and inhibitors of nitric oxide (NO) production via inducible nitric oxide synthase (iNOS).[16] Compounds with these activities can also serve as chemical probes to uncover STAT3-NF-κB-dependent molecular events that are important for the cancer phenotype.

**Regulation of the expression of Inducible Nitric Oxide Synthase (iNOS)**

Nitric oxide (NO) is a free radical that is synthesized from L-arginine in a reaction catalyzed by a family of nitric oxide synthase (NOSs) enzymes. It has beneficial antiviral, antitumor, antimicrobial, and immunomodulatory effects.[17] However, induction of aberrantly-active iNOS can lead to detrimental effects. For example, abnormal production of NO is involved in the inflammatory process and carcinogenesis.[17] Three different nitric oxide synthase enzymes are involved in NO production: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Among them, the iNOS gene has been found consistently associated with chronic inflammation, tumor production,[18,19] and metastasis.[20] Signal transducer and activator of transcription 1 (STAT-1α), is a transcription factor specific for the interferon (IFN) pathway and plays a vital role in mediating IFN-dependent biological responses,[17] such as tumor surveillance[21] and cell growth control.[22] Activation of the transcription factors NF-κB and STAT-1α leads to activation of the iNOS promoter and appear to be an essential step for iNOS induction in a majority of cells.[17] NO modulates different cancer-related events including angiogenesis, apoptosis, cell cycle, invasion, and metastasis.[23] Therefore, inhibition of NO production has significant therapeutic potential and numerous possibilities for cancer chemoprevention.[20] As an example, Granados-Principal and coworkers (2015)[24] revealed that L-$N^G$-monomethyl arginine citrate (L-NMMA), an NO inhibitor, exhibited decreasing tumor growth and enhanced survival rates in a triple-negative breast cancer (TNBC) mouse model.[24]

**Solanaceae (*Physalis peruviana* L.), aka Poha Berry**

Natural products obtained from plants have been the source of many useful anticancer drugs. The Department of Pharmaceutical Sciences at the Daniel K. Inouye College of Pharmacy (DKICP) has conducted research as ongoing collaboration to discover plant-derived cancer chemoprevention and anticancer agents (collaborators: Founding Dean Professor John M. Pezzuto, DKICP and Dr. James Turkson, Professor and Program Director Cancer Biology and Natural Products Program from the University of Hawai'i Cancer Center). The tropical plant *P. peruviana* L. originated in tropical South America and is a member of the Solanaceae family. We are investigating *P. peruviana* as a possible source of anti-inflammatory and anticancer agents. In Hawai'i, *P. peruviana* has become naturalized and is found and collected on the Island of Hawai'i, in Pepeekeo, in open mountain slopes at elevations between 1,500 and 4,000 feet. The plant consists of fruit, leaves, and stems. Commonly known as cape gooseberry, poha, ground cherry, and husk tomato, the fruit contains many seeds, and is juicy, sweet, and tangy, with a high content of vitamin C, carotenoids, and bioflavonoids with antioxidant properties. The berries are eaten fresh or used in making jam. Locally, the fruit is eaten fresh, preserved as jam, or prepared in pies, or ice-cream. A number of ethnic and cultural groups employ the leaves, stems, and fruits of *P. peruviana* in medicinal folkoric medicine(s) for the treatment of asthma, abdominal ailments in children, constipation, diuretic, glaucoma, headache, jaundice, reducing swelling and inflammation, postpartum pain, skin diseases, and as a vermifuge.[25-29] *P. peruviana* fruit juice has also shown reno- and hepato-protective effects against acute renal and liver injury models in rats,[30,31] with no apparent adverse effects.[32] A poultice from the leaves and stems is commonly applied over wounds and skin infections.[33]

Although other *Physalis* species have been studied, more than 30 withanolide derivatives have been isolated from *P. peruviana*.[34-37] There are only a few detailed reports on the bio-

active anti-inflammatory compounds from *P. peruviana*, and no report(s) of withanolides with NO and STAT3 inhibitory activity. Our initial study showed that extracts of the fruits, and aerial parts of *P. peruviana* decreased the activities of aberrantly-active NF-κB and STAT3, two key redox-regulated transcription factors that control cellular and disease processes. Our objective was to identify and discover compounds that specifically block either one or both of these transcriptions factors, and inhibit NO production.

## Methods

**Plant materials extraction, isolation, and structure determination:** Collection and preparation of plant materials, extraction and isolation were performed as described previously.[16,38] The structures of new compounds and other analogues were determined by spectroscopic methods, including 1D and 2D NMR, and mass spectrometry.[16,38] The absolute configuration of compound (**1**) was confirmed using single-crystal X-ray diffraction analysis, as well as supported from NOESY experiments.[16,38] The organic extracts of *P. peruviana* aerial parts and fruits, and compounds, were evaluated using cancer chemoprevention bioassays: (a) inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells; (b) tumor necrosis factor alpha (TNF-α)-induced NF-κB activity using stable transfected human embryonic kidney cells 293.[39,40]

**Inhibition of TNF-α-induced NF-κB activity:** This assay was performed using 293/NF-κB-Luc HEK cells (Freemont, CA, USA) as described previously.[39] All chemicals were purchased from BD Biosciences, USA. NF-κB activity was measured with a luciferase kit (Madison, WI, USA) using a LUMIstar Galaxy Luminometer (BMG Labtechnologies, Durham, NC, USA) according to the manufacturer's instructions. Data were calculated as % inhibition relatively to DMSO control. Samples which showed more than 50% inhibition at the test concentration 20 μg/mL were tested for dose-dependence to determine $IC_{50}$ values (half-maximal inhibitory concentration). A NF-κB inhibitor was used as a positive control: *N*-tosyl-L-phenylalanylchloromethyl ketone (TPCK), $IC_{50}$= 5.09±0.6 μM. Sulphorhodamine B (SRB) cytotoxicity was performed in parallel to avoid false positive results.

**NO inhibition assay:** In this assay, potential to inhibit inducible nitric oxide synthase (iNOS) was evaluated with lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells as described previously.[40] Samples showing more than 50% inhibition at a concentration 20 μg/ml were tested at three-fold serial dilutions to find the $IC_{50}$ values. L-$N^G$-Monomethyl arginine citrate (L-NMMA) was used as a positive control ($IC_{50}$ = 23.5 μM, BD Biosciences, USA). The SRB assay was performed simultaneously to test the cytotoxic effect of samples.

## Results and Discussion

In our study, the organic crude extracts of *P. peruviana* (aerial parts and fruits) exhibited significant inhibitory activities on both NF-κB activity and nitrite oxide generation at a concentration of 20 μg/mL, and were less cytotoxic in these anti-inflammatory assays.[16,38] Therefore, bioassay-guided fractionation of extracts of *P. peruviana* (aerial parts) were performed, and yielded three new withanolides, physaperuvins G (**1**), I (**2**), J (**3**), and four known compounds, 4β-hydroxywithanolide E (**4**), withaperuvin C (**5**), physalactone (**6**), and coagulin (**7**) (Figure 1).[16,38] In addition, two known compounds, phyperunolide F (**8**) and withanolide S (**9**), were isolated from poha fruits (Figure 2).

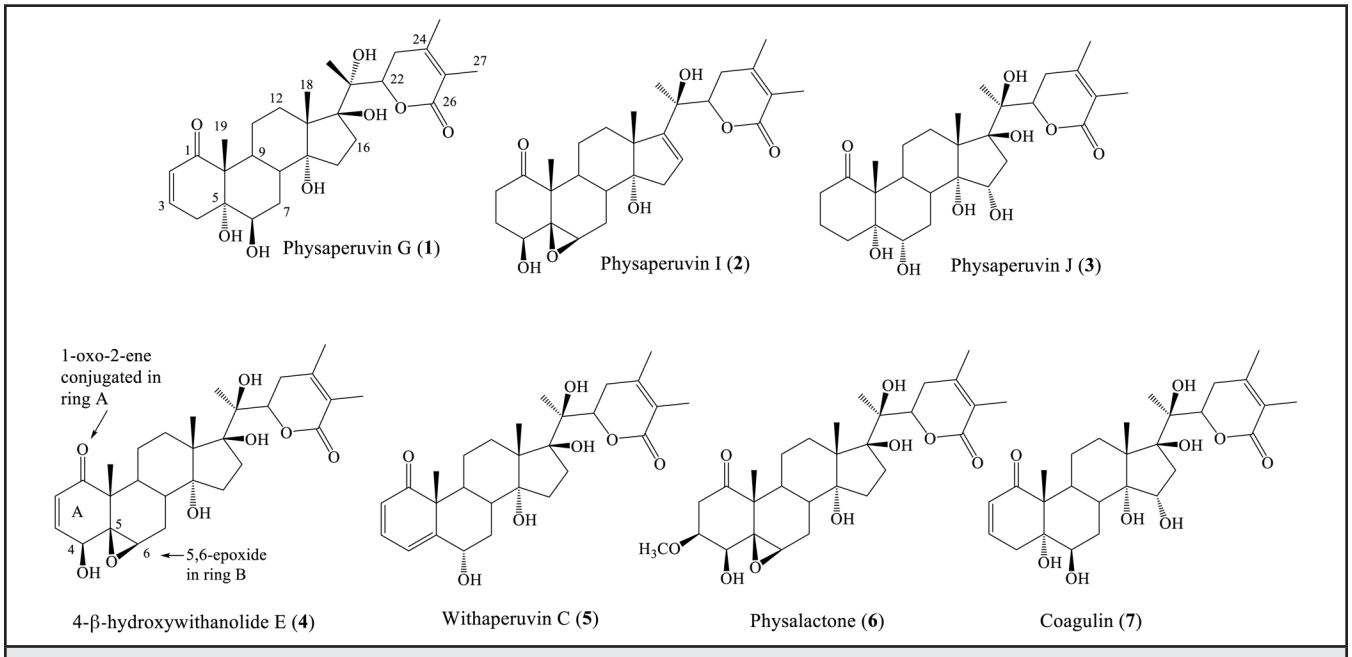


Figure 1. Structures of withanolides isolated from the aerial part of *P. peruviana*.

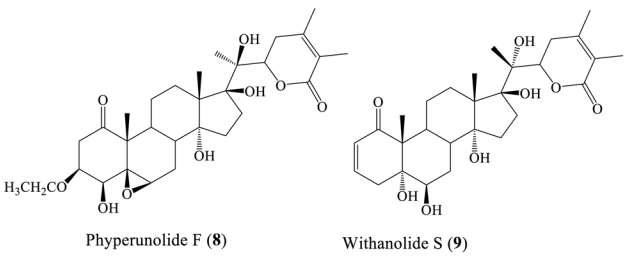


Figure 2. Structures of withanolides isolated from the fruits of *P. peruviana*.

**Biological Evaluation**

**Anti-inflammatory Activity**

The results of these assays indicate that several anti-inflammatory withanolides, namely, 4β-hydroxywithanolide E (**4**), withaperuvin C (**5**), physalactone (**6**), and phyperunolide F (**8**), purified from the aerial parts and fruits of *P. peruviana*, have potent TNF-α-induced NF-κB inhibitory activity, displaying $IC_{50}$ values ranging of 0.04-5.6 μM (Table 1).[16,38] The remaining withanolides were either moderately active ($IC_{50}$ 8.9–31.2 μM) or inactive. The half-maximal inhibitory concentration ($IC_{50}$) is reflective of the efficacy of a compound in inhibiting a specific biological function (TNF-α-induced NF-κB activity). The potency of 4β-hydroxywithanolide E (**4**) was greater than that of *N*-tosyl-L-phenylalanylchloromethyl ketone (TPCK), a positive control used for NF-κB activity ($IC_{50}$ 5.09 μM) (Table 1).

Oh and coworkers (2008)[41] demonstrated that withaferin A (**10**) inhibits inflammation through inhibition of iNOS gene expression and NO production via inactivation of protein kinase B, also known as Akt, and subsequently down-regulating of NF-κB activity. Therefore, withanolides may have general potential for cancer chemoprevention due to their anti-inflammatory properties. Furthermore, Kleinert and coworkers (2003)[17] found that activation of the transcription factors NF-κB and STAT-1α could lead to activation of the iNOS promoter, and this appears to be an essential step for iNOS induction in most cells. Based on previous studies, inhibition of NF-κB activity correlated with inhibition of NO-production. Consequently, the anti-inflammatory effect of withanolides was tested against inhibition of NO production with LPS-treated murine macrophage RAW 264.7 cells. 4β-Hydroxywithanolide E (**4**), withaperuvin C (**5**), and physalactone (**6**) showed the highest NO-inhibitory activity against LPS-induced nitric oxide release, with $IC_{50}$ values in the range of 0.32-2.4 μM (Table 1). In this test, cytotoxicity was observed with 4β-hydroxywithanolide E (**4**) at the 50% inhibitory concentration (Table 1). Some molecular mechanisms underlying the cytotoxic effects of 4β-hydroxywithanolide E (**4**) include the generation of damaged DNA, production of reactive oxygen species, and induction of apoptosis.[42] In contrast, withaperuvin C (**5**), physalactone (**6**), and phyperunolide F (**8**), inhibited NO production with $IC_{50}$ values of 2.3-6.2 μM without apparent cytotoxicity to host cells (Table 1, % survival at concentration of 50 μM for **5**, **6**, and **8** were 79.3 –100.0%). Since the potency of these compounds were greater than that of L-$N^G$-monomethyl arginine citrate, a positive control for iNOS ($IC_{50}$ 23.5 μM) (Table 1), withaperuvin C (**5**), physalactone (**6**), and phyperunolide F (**8**) appear to be attractive leads for further studies.

Poha fruit

Table 1. Results of anti-inflammatory activities of purified compounds from *P. peruviana*.[16,38]

| Compounds | NF-κB assay | | | Nitrite assay | | |
|---|---|---|---|---|---|---|
| | % Inhib.[a] | $IC_{50}$ (μM) | % Surv.[b] | % Inhib.[c] | $IC_{50}$ (μM) | % Surv.[d] |
| Physaperuvin G (**1**) | 65.8 ± 4.9 | 31.2 ± 3.3 | 89.6 ± 10.1 | 26.4 ± 2.7 | ND | 84.1 ± 3.0 |
| Physaperuvin I (**2**) | 43.7 ± 4.0 | ND[e] | 81.9 ± 0.5 | 64.7 ± 4.4 | 14.6 ± 1.2 | 100.0 ± 1.3 |
| Physaperuvin J (**3**) | 60.4 ± 2.9 | 10.4 ± 3.6 | 83.9 ± 2.2 | 47.7 ± 2.0 | ND | 100.0 ± 4.0 |
| 4β-Hydroxywithanolide E (**4**) | 99.5 ± 0.1 | 0.04 ± 0.03 | 88.6 ± 10.1 | 99.4 ± 0.1 | 0.32 ± 0.02 | 50.1 ± 13.3 |
| Withaperuvin C (**5**) | 85.0 ± 4.4 | 5.6 ± 2.11 | 100.0 ± 8.0 | 99.2 ± 0.7 | 2.4 ± 0.2 | 79.3 ± 1.2 |
| Physalactone (**6**) | 64.4 ± 2.9 | 2.1 ± 0.23 | 73.3 ± 7.9 | 97.9 ± 2.0 | 2.3 ± 0.2 | 90.7 ± 5.0 |
| Coagulin (**7**) | 70.2±8.1 | 8.9 | 82.2 ± 3.9 | 67.1 ± 2.2 | 16.7 ± 0.3 | 100.0 ± 6.3 |
| Phyperunolide F (**8**) | 86.8±1.8 | 0.06 | 100 ± 8.0 | 88.4 ± 0.8 | 6.2± 1.6 | 100.0 ± 0.7 |
| Withanolide S (**9**) | 41.7± 3.8 | ND | | 9.4 ± 2.6 | ND | 100.0 ± 2.3 |
| TPCK[f] | | 5.09 | | | | |
| L-NMMA[g] | | | | | 23.5 | |

[a]% Inhibition of NF-κB at 50 μM. [b]% Survival at concentration of 50 μM. [c]% Inhibition of NO production at 50 μM. [d]% Survival at concentration of 50 μM. [e]ND, Not determined. [f]Positive control for NF-κB. [g]Positive control for NO.

**Anti-cancer Activity**

Of cancers affecting both men and women, colorectal cancer is the second leading cause of cancer deaths in the United States. Although a number of studies have been reported concerning the potential anti-cancer activity of withanolides, investigations with models of colon cancer are scarce. Using a small panel of colon cancer cell lines, we observed promising growth inhibitory effects following treatment with 4β-hydroxywithanolide E (**4**).[42] In addition to demonstrating activity with cells cultured as monolayers, the compound was found to mediate a significant response with three-dimensional spheroid cultures. Based on these data, we became interested in the mechanism by which (**4**) blocked colon cancer cell growth, and more detailed investigations were performed with HT-29 cells in culture (Figure 3).[42]

The mode of action facilitated by (**4**) was found to be dose-dependent. At higher concentrations, the cells underwent apoptosis. At lower doses, those considered a greater interest since the concentration required to inhibit growth of cultured cells is ~0.1 $\mu$M, a complex array of responses were observed. First, the level of $p21^{Waf1/Cip1}$, a cyclin-dependent kinase inhibitor, was enhanced, and simultaneously, the levels of several cell cycle-related proteins were reduced. In addition, the levels of Hsp90 client proteins were downregulated, nuclear sirtuin 1 (SIRT1) was increased, and histone H3 acetylated at lysine 9 was decreased. The expression of 21 genes was altered based on analysis of an array of cell cycle-related genes. Of particular note, the level of *PTGS2* (prostaglandin-endoperoxide synthase 2), which is known to be associated with poor prognosis, was decreased, and this correlated with reduced protein levels of cyclooxygenase-2 (COX-2). In sum, these data indicate that 4β-hydroxywithanolide E (**4**) functions by a unique multimodal mechanism of action and advanced testing would be worthwhile.

Withaferin A (**10**) (Figure 4) is a withanolide derivative which is very closely related to 4β-hydroxywithanolide E.[43] Withaferin A (**10**) is isolated from *Withania somnifera* which is a popular Ayuverdic herb that has the ability to reduce tumor cell proliferation while increasing overall animal survival time.[43] In addition, it has been shown to enhance the effectiveness of radiation therapy while potentially mitigating undesirable side effects.[43] Withaferin A (**10**) has been shown to have preclinical effects on human breast cancer *in vitro* and *in vivo*.[44] A report showed that withaferin A inhibited interleukin-6 (IL-6)-inducible activation of STAT3 in breast cancer cells.[45] It triggers apoptosis, which largely inhibits cell migration/invasion of breast cancer cells, even after IL-6-induced activation of STAT3.

Cysteine proteases are important proteins for therapeutic targeting of tumors and inflammatory disease.[51,52] It is known that Michael-acceptor reactions form covalent bonds with the active site of cysteine proteases to elicit a biological effect.[49,50] This Michael-reaction is the nucleophilic addition of a carbanion or another nucleophile such as a sulfhydryl group to an α,β-unsaturated carbonyl compound. Structure-activity relationship also revealed that both 4β-hydroxywithanolide E (**4**) (Figure 1) and withaferin A (**10**) (Figure 4) contain Michael-acceptor moieties, such as present in 4β-hydroxy-5β,6β-epoxy-


Figure 3. Journal cover featuring 4β-hydroxywithanolide E (4): Park EJ, Sang-ngern M, Chang LC, Pezzuto JM. Induction of cell cycle arrest and apoptosis with downregulation of Hsp90 client proteins and histone modification by 4β-hydroxywithanolide E isolated from *Physalis peruviana*. Mol. Nutr. Food Res. 2016; 60: 1482-1500. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

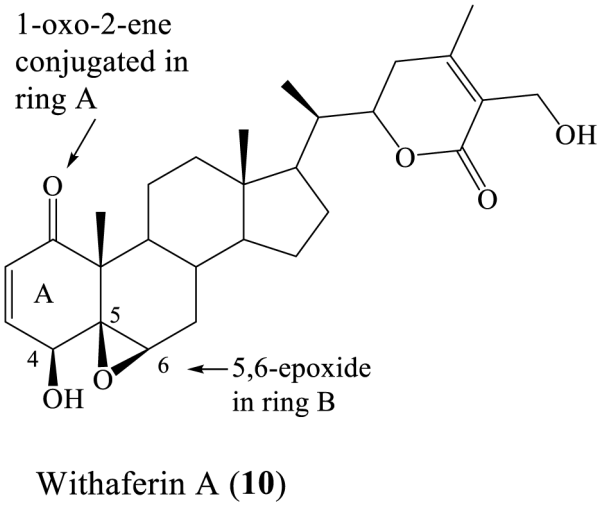


Figure 4. Structure of withaferin A isolated from *Withania somnifera*.

2-en-1-one that are attributable to cytotoxic activity.[46-48] The α,β-unsaturated ketone moiety in withaferin A (**10**) that could exhibit Michael-acceptor reaction, may bind directly to various cellular nucleophiles and thereby lead to a loss in selectivity.

Physalactone (**6**) contains an α,β-unsaturated enoate moiety (a *δ*-lactone ring substituent at the C-17 position) and therefore may be capable of a Michael-acceptor reaction. However, in our study, the thiol-reactivity of physalactone (**6**) in NMR trapping experiments with cysteamine showed that the side chain enoate signal remained unchanged after the cysteamine addition. Furthermore, our data (Table 1) demonstrated lack of cytotoxic effects of certain withanolides. Therefore, withanolides such as physalactone (**6**) and phyperunolide F (**8**) derivatives from poha berries, deserve further studies to determine the mechanism of action.

## Conclusions

As modern medicine continues to expand, so do the uses of botanical medicines. Tropical plants continue to yield new and interesting lead agents with potential for future drug development or as herbal remedies. The phytochemical and biological evaluation of *P. peruviana* collected in Pepeekeo, Hawai'i, has provided several new lead active compounds that could be developed further as new cancer chemoprevention and cancer chemotherapeutic agents. Through this research effort, we have identified withanolide derivatives as the main components in the aerial parts and poha berries of *P. peruviana*. Among the isolates, compounds 4β-hydroxywithanolide E (**4**), withaperuvin C (**5**), physalactone (**6**) from aerial parts, and phyperunolide F (**8**) from poha berries, block NF-κB transcription factor and inhibit NO production. Specifically, physalactone (**6**) and phyperunolide F (**8**) appeared to be the most promising leads. These compounds are more potent than a positive control, L-$N^G$-monomethyl arginine citrate ($IC_{50}$ 23.5 $\mu$M), with less or no cytotoxicity at 50 $\mu$M. This lead could be useful for development of novel anti-inflammatory and cancer chemoprevention agents. 4β-Hydroxywithanolide E (**4**) exhibited potent antiproliferative activity with the HT-29 human colorectal cancer cell line.[42] The compound showed G0/G1 cell cycle arrest at lower concentration and induced apoptosis at higher concentrations. Furthermore, 4β-hydroxywithanolide E (**4**) modulated oncogenic proteins by downregulating Hsp90 client proteins and exerted epigenetic modification by decreasing acetylation of histone H3. In sum, our studies may help to elucidate potential mechanisms facilitated by the traditional use of *P. peruviana* as anti-inflammatory and anticancer herbs.

## Future Directions

Future studies with these bioactive withanolides are needed to define the effects of the various structural features on anti-inflammatory and anticancer activities. For example, the re-isolation of sufficient quantities of promising compounds [4β-hydroxywithanolide E (**4**), physalactone (**6**), phyperunolide F (**8**)] for more advanced mechanistic studies as well as antitumor studies with animal models would be of interest. Work of this type may also lead to a better understanding of the molecular mechanisms of the cooperative roles of NO and NF-κB in cancer etiology and offer greater opportunities for the design of new chemopreventive and chemotherapeutic approaches. In addition, the potential beneficial effects of using *P. peruviana* as whole herbs should be explored.

Translational research is needed to determine if *P. peruviana* can mediate any beneficial responses in humans, and to determine an optimal dosage range for achieving these effects. As an herb, *P. peruviana* might be used in conjunction with radiation therapy or chemotherapy to ameliorate toxicity, which speaks to its potential role in integrative oncologic care. Model systems for the evaluation of health claims made for herbal remedies derived from poha berries including cancer chemoprevention, anti-inflammatory, antioxidant, and reduction of oxidative stress may be explored. The identification of bioactive and less cytotoxic constituents in poha berry will promote the use of the plant in Hawai'i as an herbal remedy in anticancer and cancer chemoprevention regimens. Finally, improving and promoting local products in the State of Hawai'i could be particularly important in enhancing the economic well-being of Hawai'i, and possibly leading to sustainable drug development or as an herbal remedy.

## Conflict of Interest

None of the authors identify any conflict of interest.


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