Proliferative Activities of Benguet Legume Cultivars on a Breast Epithelial Cell Line

Cielo Mae D. Marquez
Conrad Allan C. Chong
Leomir A. Diaz
Harold M. Carag
Michael C. Velarde*
Institute of Biology
College of Science
University of the Philippines Diliman

John Carlo F. Dela Cruz
Dhennis T. Versoza
Noel S. Quiming
Marilou G. Nicolas
Natural Products and Peptidomics Laboratory
Department of Physical Sciences and Mathematics
College of Arts and Sciences
University of the Philippines Manila

ABSTRACT

Legumes are extensively cultivated around the globe for human consumption and may contain potential estrogenic activities that interfere with normal physiology and pathophysiology. However, different cultivars grown in different regions of the world have different activities that may provide different nutritional value. Hence, in this study, legume cultivars from Benguet, a major source of legumes and other highland vegetables in Northern Philippines, were evaluated for their proliferative effects in the breast epithelial cancer cell line MCF7. Ethyl acetate extracts from *Phaseolus vulgaris* L. and *Vigna unguiculata* (L.) Walp., but not from *Tamarindus indica* L. and *Pisum sativum* L., induced slight proliferative effects on MCF7 cells at a low dose but reduced cell number at a higher dose. The proliferative effect of the extracts is likely estrogen receptor (ER)-dependent, as the same legume extracts only displayed inhibitory effects in the ER-negative MDA-MB-231 cells. Similar proliferative effects of *P. vulgaris* and *V. unguiculata* ethyl acetate extracts were reflected in their similar HPLC profiles, which is distinct from the HPLC profile of the *T. indica* ethyl acetate extract. Overall, our findings show that certain legumes from Benguet have slight proliferative activities in MCF7 cells, implying their potential estrogenic activities.

Keywords: beans, extracts, peas, Philippine plants, seeds

* *Corresponding Author*

INTRODUCTION

Legumes such as common beans (*Phaseolus vulgaris* L.) and peas (*Vigna unguiculata* L., *Pisum sativum* L.) are grown and consumed across the world. In temperate and semitropical climates, *P. vulgaris* are cultivated and harvested for their edible seeds. In fact, there are a wide variety of cultivated beans in the world, including navy beans, kidney beans, and black turtle beans (Ganesan and Xu 2017). Several bean and pea cultivars are also grown in the Philippines and are typically found in the Filipino diet. These include cowpeas (*V. unguiculata*), which are locally called paayap, garden peas (*P. sativum*) called sitsaro, and other common beans (*P. vulgaris*) such as string beans (sitaw) and Baguio beans (bitsuelas).

Legumes contain several phytoestrogens that exhibit estrogen-dependent cell proliferation in estrogen receptor (ER)-containing cells (Mostrom and Evans 2018). These phytoestrogenic compounds include flavonoids and isoflavonoids, which contain phenolic structures that mimic the phenolic structures of the female hormone estradiol. Because of their structural similarities to estrogen, these compounds can bind to estrogen receptors to elicit a response such as cell proliferation or growth inhibition (Vitale et al. 2013). However, different cultivars of common beans (*P. vulgaris* L.), such as those grown in France, Mexico, Brazil, Peru, Italy and Mediterranean countries, have different amounts and compositions of phenolic compounds (Heimler et al. 2005; Díaz-Batalla et al. 2006; Luthria and Pastor-Corrales 2006; Kalogeropoulos et al. 2010; Ranilla et al. 2010; Ombra et al. 2016; Weidner et al. 2018). In turn, this results in different levels of proliferative and anti-proliferative activities on selected cancer cell lines (Ombra et al. 2016). For example, the crude extract from Indian *P. vulgaris* L. cultivar black turtle bean has an IC_{50} value of 50 µg/mL in MCF7 cells (Kumar et al. 2017), while the methanolic extract from Spanish dark *P. vulgaris* L., A.C. Tolosana has an IC_{50} value of 31.5 µg/mL on the same breast cancer cell line (López et al. 2013).

Legume consumption is thought to decrease cancer risk and inhibit breast cancer growth. For instance, women who consume common beans and lentils at least twice per week have reduced risk of developing breast cancer by 24% than women who ate beans and lentils less than once a month (Adebamowo et al. 2005). Similarly, women with high intake of dietary beans, such as garbanzos, pinto, kidney, black, red, lima and black-eyed peas, have a reduced risk of breast cancer by 20% (Sangaramoorthy et al. 2018). Mortality rates due to breast cancer are also lowest in countries with the greatest bean consumption (Correa 1981). However, results

are inconsistent as some studies find no association between dietary legume intake and reduced risk in several cancer types (Fung et al. 2005; Shannon et al. 2005). Nonetheless, animal models show that feeding rats with common beans twice or more per week reduces their risks of breast cancer from 95% to 67% (Thompson et al. 2009). Consumption of dry beans also decreases cancer multiplicity and tumor burden in rats in a dose-dependent manner (Thompson et al. 2008).

Legumes are commonly grown and consumed in the Philippines. Particularly, Benguet is a major producer of beans, peas, and other highland vegetables in Northern Philippines. Because the effects of legumes on breast cancer cell proliferation may vary depending on the cultivar used, this study analyzed the proliferative activities of extracts from legumes obtained from Benguet. This study also attempted to relate the observed proliferative activity of the legume extracts to their chemical profile by High Performance Liquid Chromatography (HPLC).

METHODS

Plant Collection, Preparation, and Extraction

Legumes were obtained from Benguet State University and La Trinidad Trading Outpost, La Trinidad, Benguet, Philippines on a dry season. Seed samples were washed, oven dried at 40 °C for four hours, and homogenized using a standard mechanical grinder. The powdered samples were passed through a 2 mm sieve mesh, and approximately 50 grams from each sample were placed into separate clean jars. Samples were defatted through maceration using 150 mL *n*-hexane (AR., RCI Labscan, AR1083-G4L) for 48 hours and subsequently filtered using vacuum filtration. Then, the defatted powder was completely dried in the fume hood for one hour or until the *n*-hexane was completely evaporated. The powder was transferred to another clean jar and extracted twice using ethyl acetate followed by methanol (HPLC Grade, Duksan, UN1230) for 48 hours on each extraction. The extracts were again filtered using vacuum filtration and the solvents were evaporated from the extract through rotary evaporation at 40 °C temperature and 70 kPa using the rotary evaporator system (IKA, RV 8 S99) paired with piston vacuum pump (Welch, 2534C-02 A). The dried extracts were transferred into glass dram vials and were stored at -40 °C until further use. A small portion of each extract was dissolved in an appropriate solvent (i.e., DMSO) to serve as stock solutions for the succeeding experiments.

DNA Extraction and Sequencing

Identification of their bean cultivars (*P. vulgaris* and *V. unguiculata*) were confirmed using DNA barcoding. Each legume seed (100 mg) was subjected to DNA isolation using ZYMO DNA Extraction Kit (Catalog D6005) following manufacturer's protocol with some modifications (Zymo Research). Powdered legume seeds were added to lysis tubes, followed by the addition of bashing beads and lysis buffer. Lysis tubes were rigorously mixed for 30 minutes in a speed cell disruptor (Disruptor Genie) to homogenize the seeds. After addition of 0.4 mg/mL proteinase K, samples were then incubated at 60 °C for one hour followed by centrifugation at 10,000 x g for three minutes. Supernatants were filtered into collection tubes and DNA was subsequently eluted from filter columns. The extracted DNA from each legume was transferred into a 1.5 mL tube and stored at -20 °C. DNA quantification was carried out using NanoDrop 2000 (Thermo Scientific). PCR amplification was performed in a BioRad T100 thermal cycler using the nuclear internal transcribed spacer (ITS2) and rbcL region as described previously (Li *et al.* 2011; Gu *et al.* 2013). The primer pairs for ITS2 and rbcL are as follows: ITS2-F: 5'-ATG CGA TAC TTG GTG TGA AT-3'; ITS2-R: 5'-GAC GCT TCT CCA GAC TAC AAT-3'; rbcL-F: 5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3'; rbcL-R: 5'-GAA ACG GTC TCT CCA ACG CAT-3'. PCR cycles for ITS2 consisted of an initial heating step for five minutes at 94 °C, 40 cycles at 94 °C for 30 seconds, annealing step at 56 °C for 30 seconds, extension step at 72 °C for 45 seconds, and a final extension for 10 minutes at 72 °C. The PCR cycles for rbcL consist of initial heating step for five minutes at 95 °C, 35 cycles at 95 °C for one minute, with annealing temperature of 50 °C for one minute, an extension step at 72 °C for one minute, and a final extension for six minutes at 72 °C. PCR products were run on a 1% agarose gel at 120 V for 20 mins, and were then purified using PCR purification kit before submitting for sequencing at Macrogen Laboratory, Korea.

Sequence Alignment and Phylogenetic Analysis

DNA sequences were trimmed and cleaned prior to sequence alignment using MEGA10 software (https://www.megasoftware.net). Sequences were manually refined by visual inspection before performing structural analysis. Phylogenetic affiliation of the genes was determined by alignment to the NCBI non-redundant database (www.ncbi.nlm.nih.gov) using the BLASTn algorithm. Phylogenetic analyses were done in MEGA software, applying Maximum likelihood with best fit DNA model for ITS2 while Neighbor Joining analysis model was applied for the rbcL.

Morphometric and Principal Component Analysis

Using a caliper, four morphometric variables (i.e., length, width, thickness, and length/width ratio) of legume seeds were measured. Principal Component Analysis (PCA) was conducted using Past3 to identify and differentiate the clustering of the bean cultivars collected from La Trinidad, Benguet.

Cell Culture and Treatments

The breast cell lines MCF7 and MDA-MB-231 used in this study were previously obtained from American Type Culture Collection (ATCC, Manassas, VA). MCF7 cells were maintained in Minimum Essential Medium (Life Technologies 11095-080), supplemented with 0.01% (v/v) gentamicin sulfate amphotericin B (Lonza CC-4081J), 10% Fetal Bovine Serum (FBS) (Life Technologies 10500-064), and 1% insulin-transferrin-selenium A (ThermoFisher 51300-044). The breast cell line MDA-MB-231 was used for comparison and were grown using Dulbecco's Minimum Essential Medium (Life Technologies 12800-017), 10% FBS and 0.01% (v/v) gentamicin sulfate amphotericin B. Both cell lines were maintained in 25 cm^2 or 75 cm^2 culture flasks and kept in an incubator at 37 °C in 5% CO_2, 95% humidified air. Both cell lines were passaged every 3-4 days.

For cell proliferation experiments involving the estrogen-responsive (ER) MCF7 cells, a steroid-depleted medium was prepared with the following components: DMEM without phenol-red (Life Technologies 31053-028), 10% hormone-free charcoal-dextran stripped FBS, and 0.01% (v/v) gentamicin sulfate amphotericin B. MCF7 cells were seeded onto a 96-well microtiter plate using the steroid-depleted medium, and allowed to grow for 24 hours. High (30 µg/mL) and low (3 µg/mL) dose of the extracts in the presence of 10 nM estradiol (E2) were dissolved in steroid-depleted medium and then incubated with MCF7 cells for 72 hours to determine their effect on cell proliferation. Cells were also treated with equivalent concentrations of DMSO to serve as vehicle control. Because plant extracts with IC50 values of ≤ 30 µg/mL are considered active against cancer cell lines based on the National Cancer Institute, USA (NCI, USA) guidelines (Suffness and Pezzuto 1990), this concentration (30 µg/mL) was set as the upper limit and a 10-fold decrease (3 µg/mL) served as the lower limit for the screening of the proliferative capacity of the legume cultivars. A similar assay was performed in non-ER-responsive triple negative MDA-MB-231 cells using normal maintaining medium without estradiol. Cell proliferation was measured by MTT assay as described previously with modifications (Mosmann 1983). This assay is based on the ability of viable cells to reduce the yellow MTT dye into purple formazan crystals. Briefly, spent medium was

removed from each well and replaced with 20 µl of the MTT dye. After four hours, 100 µl of DMSO was added to dissolve the crystals and plate was read at 570 nm using Varioskan Flash multimode reader. All assays were done in three independent trials with technical duplicates per trial.

Solid-Phase Extraction (SPE)

A standard solid-phase extraction protocol with OASIS® PRiME HLB Extraction Cartridges (186008718; 6 cc volume, 500 mg bed size) paired with Rocker® 300 vacuum pump was used for the SPE clean-up. Briefly, this consisted of a conditioning step using 6 mL of methanol and 6 mL of water at 6 mL/min; a loading step of 2 mL of the sample dissolved in methanol at a flow rate of 2 mL/min; a washing step with 6 mL water at 6 mL/min; and two elution steps with 6 mL each of methanol at 6 mL/min. Wash partition was collected and freeze dried into powder using Labconco Freezone 2.5 Freeze Dryer (7670540) paired with Vacuubrand Chemistry Hybrid Pump RC6 at -52 °C temperature and 0.240 m Barr pressure, while the elution partition was collected and dried using rotary evaporation.

HPLC Profiling of Legume Extracts

The High-Performance Liquid Chromatography (HPLC-UV) Shimadzu system consisting of a pump (Shimadzu Nexera X2, LC-30AD), a pump controller (CBM-20A), a degassing unit (DGU-20A5R), an autosampler (Nexera X2, SIL-30AC) and a photodiode array detector (Nexera X2, SPD-M30A) was used for the chromatographic analysis of the crude extracts. The mobile phase of the HPLC analysis was composed of water with 0.1% trifluoroacetic acid (solvent A) and acetonitrile with 0.1% trifluoroacetic acid (solvent B) (Merck, 1.08178.0050) at a flow rate of 0.9 mL/min following the gradient system: 5% solvent B at 0 minute, gradually increasing solvent B concentration to 95% to 45 minutes and maintaining 95% solvent B concentration up to 55 minutes. C18 Reverse Phased Column Purospher® STAR RP-18 endcapped (5 µm) LiChroCART® 150-4.6 (Merck) was used as the column for the HPLC Analysis. The absorbance was extracted at 190 nm and peaks of interest are scanned at 320 nm ± 4 nm. Injection volume was 10 µL and sample run was performed in duplicate. Samples from the SPE clean-up procedure was prepared by dissolving the sample with HPLC grade methanol at 5 mg/mL and was filtered using syringeless filter device GE Mini-Uniprep™ Nylon Filter with Polypropylene Housing 0.45 µm pore size (Whatman™, UN203NPUNYL). Shimadzu LabSolutions ver. 5.81 software was used for the data processing and acquisition of the HPLC profile.

Data Processing and Statistical Analyses

All data parameters were expressed as means ± SEM. Two-way ANOVA test with Tukey posthoc analysis was used to evaluate differences among multiple groups. Differences with *p*-values <0.05 were considered significant. All statistical analyses were calculated in GraphPad Prism 7.

RESULTS

Identification of Benguet Legume Species

We performed morphological identification and DNA barcoding to confirm the identity of legumes obtained from La Trinidad, Benguet. *T. indica* and *P. sativum* were easily identified based on morphology (Figure 1), but the identities of the white and maroon beans as *P. vulgaris* and the black and brown-eyed beans as *V. unguiculata* were further confirmed by their ITS sequences (Figure 2A). To further distinguish the morphological characteristics of the *P. vulgaris* and *V. unguiculata*, we also performed PCA on these legume species. Using seed morphometrics as variables, PCA revealed that the two different species, *P. vulgaris* and *V. unguiculata*, clustered into two separate groups, while the cultivars of each species were more difficult to distinguish based on seed size (Figure 2B). Nonetheless, the different cultivars clustered into smaller groups, suggesting that there may be subtle differences (in addition to color) between the different cultivars.

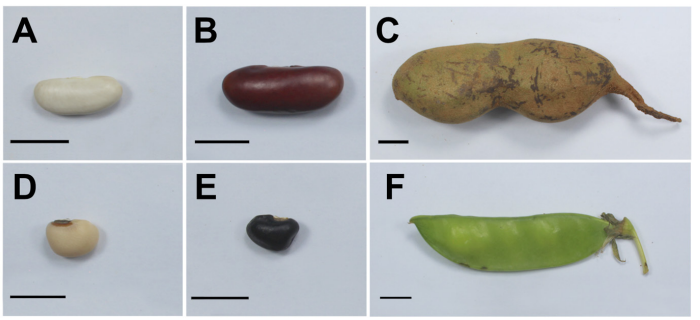


Figure 1. Gross seed morphology of Benguet legumes. Six legumes from La Trinidad, Benguet were identified as (A) *Phaseolus vulgaris* white and (B) maroon, (C) *Tamarindus indica,* (D) *Vigna unguiculata* brown-eyed and (E) black, and (F) *Pisum sativum*. Horizontal scale bar for each image represents 1 cm.

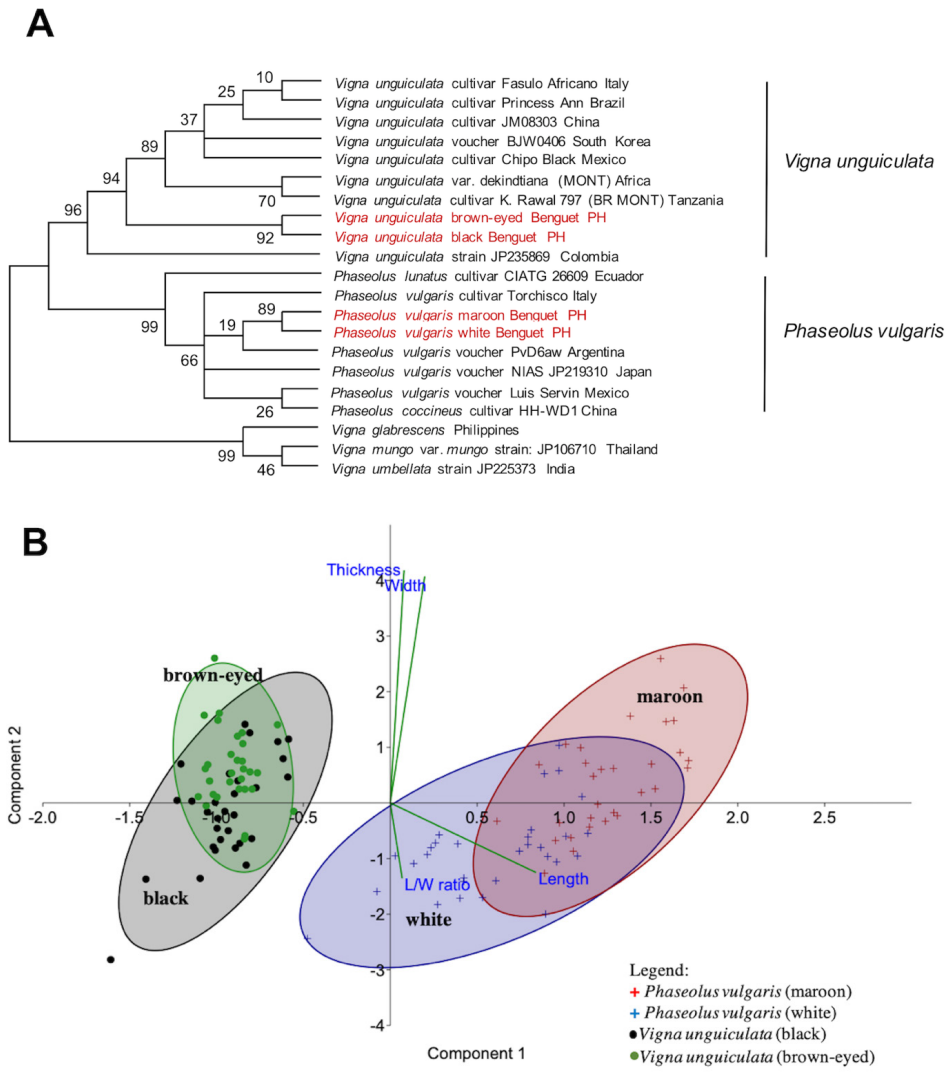
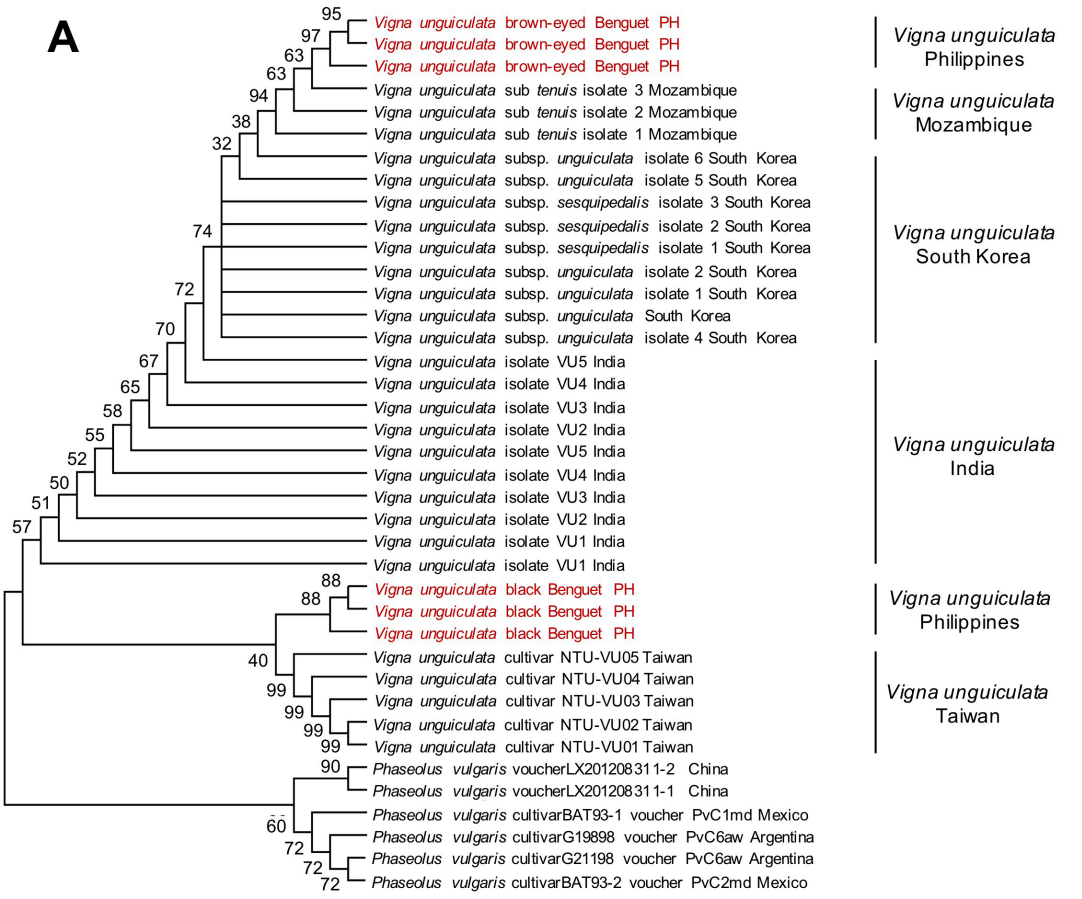


Figure 2. Clustering of Benguet legumes by species using ITS2 sequences and principal component analysis (PCA). (A) Phylogenetic analysis using maximum likelihood of ITS2 sequences from *V. unguiculata* and *P. vulgaris* cultivars obtained from La Trinidad, Benguet. The percentage of replicate trees in which the associated taxa clustered together in 1,000 bootstrap is shown next to the branches. Values less than 20% were omitted. (B) PCA plot of seed parameters of Benguet legume cultivars (n = 30 per cultivar) revealing four groups with two distinct clusters. *V. unguiculata* brown-eyed (green) and black (black) clustered separately from *P. vulgaris* white (blue) and maroon (red).

Further DNA analysis using rcbL sequences revealed that the *V. unguiculata* black Benguet legumes clustered with Taiwan and Indian cultivars, while the *V. unguiculata* brown-eyed Benguet legumes clustered with cultivars from Mozambique, South Korea, China, and Italy (Figure 3A). Interestingly, the *P. vulgaris* white and maroon Benguet beans tend to cluster together, with a closer phylogenetic relationship with the Argentina and Mexico cultivars (Figure 3B). This confirms that *P. vulgaris* white and maroon legume cultivars are more genetically related to each other than to *V. unguiculata* Benguet black and brown-eyed legume cultivars.



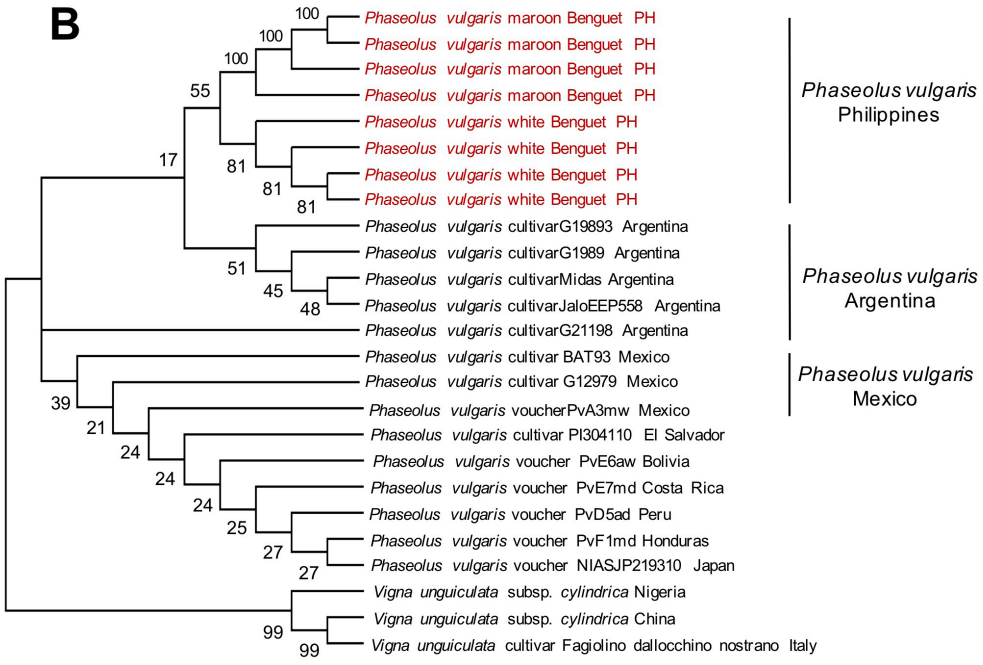


Figure 3. Clustering of Benguet legumes by species using rbcL sequences. Phylogenetic analysis using neighbor joining of (A) *rcbL* sequences from *V. unguiculata* (n=3) and (B) *P. vulgaris* (n=3) cultivars obtained from La Trinidad, Benguet. The percentage of replicate trees in which the associated taxa clustered together in 10,000 bootstrap is shown next to the branches. Values less than 20% were omitted.

Differential Proliferative Effects of Legume Extracts on Breast Cell Lines

Previous studies showed that common beans inhibit growth in ER-containing cells. Hence, legumes grown in Benguet were tested to determine their effects on the proliferation of ER-expressing breast cell line MCF7. At a high dose, methanol extracts of *P. vulgaris* white and maroon and *V. unguiculata* brown-eyed significantly decreased cell number in MCF7 cells, while the *V. unguiculata* black, *T. indica*, and *P. sativum* did not significantly reduce cell proliferation in MCF7 cells (Figure 4A). In contrast, only a high dose of hexane extracts of the *P. vulgaris* white and *V. unguiculata* black significantly inhibited cell proliferation in MCF7 cells (Figure 4B). Except for both black and brown-eyed *V. unguiculata,* high doses of ethyl acetate extracts also significantly decreased cell number in MCF7 cells (Figure 5A). Low doses of all methanol and hexane extracts from six Benguet cultivars do not have significant proliferative effects in MCF7 cells.

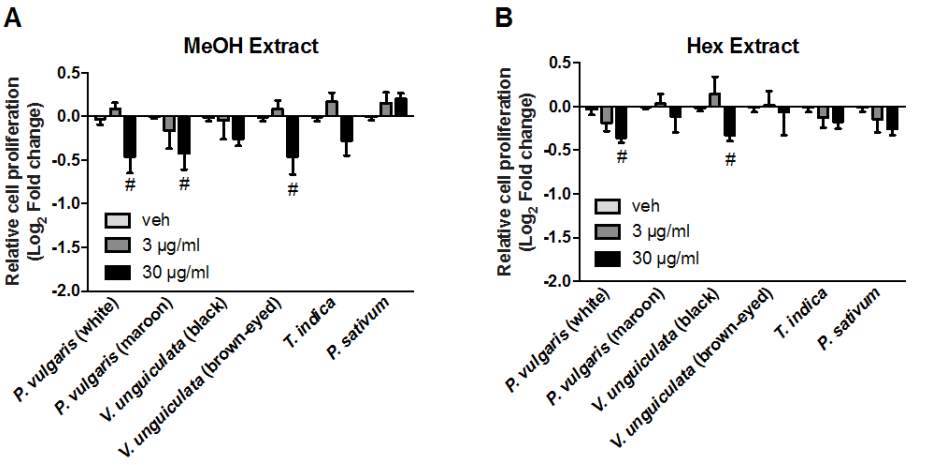


Figure 4. Decreased cell proliferation in MCF7 cells treated with methanol and hexane extracts from select Benguet legumes. Relative cell proliferation reported as $\log_2$ fold change (means ± SEM) in MCF7 cells treated with low (3 µg/mL) and high (30 µg/mL) dose of (A) methanol and (B) hexane extracts from different legumes for 72 hours. Relative cell proliferation was measured by MTT and normalized to vehicle (veh) control. Asterisk (*) and # indicate significant difference relative to vehicle control at $p<0.05$ by two-way ANOVA followed by Tukey post hoc analysis.

While several legume extracts reduced cell proliferation in MCF7 cells at high doses, low doses of the ethyl acetate extracts from both *P. vulgaris* and *V. unguiculata,* but not *T. indica* and *P. sativum*, increased cell proliferation in MCF7 (Figure 5A). This suggests that exposure to low doses of *P. vulgaris* and *V. unguiculata* beans may have slight proliferative effects on breast MCF7 cells. To test if this is specific to ER-containing cells, we then treated ER-negative cell line MDA-MB-231 with the same extracts without estradiol. Interestingly, at low doses, the slight proliferative effects of these extracts on MCF7 were not observed in MDA-MB-231 (Figure 5B). Furthermore, these extracts reduced cell number of MDA-MB-231 at higher doses, similar to that in MCF7 cells. Except for *P. vulgaris* maroon and *T. indica,* all ethyl acetate extracts significantly decreased the proliferation of these cells at high concentrations. These findings suggest that *P. vulgaris* and *V. unguiculata* from Benguet may contain compounds that induce mild proliferation in ER-containing MCF7 cells at low dose and, at high dose, inhibit proliferation of both breast cancer cell lines.

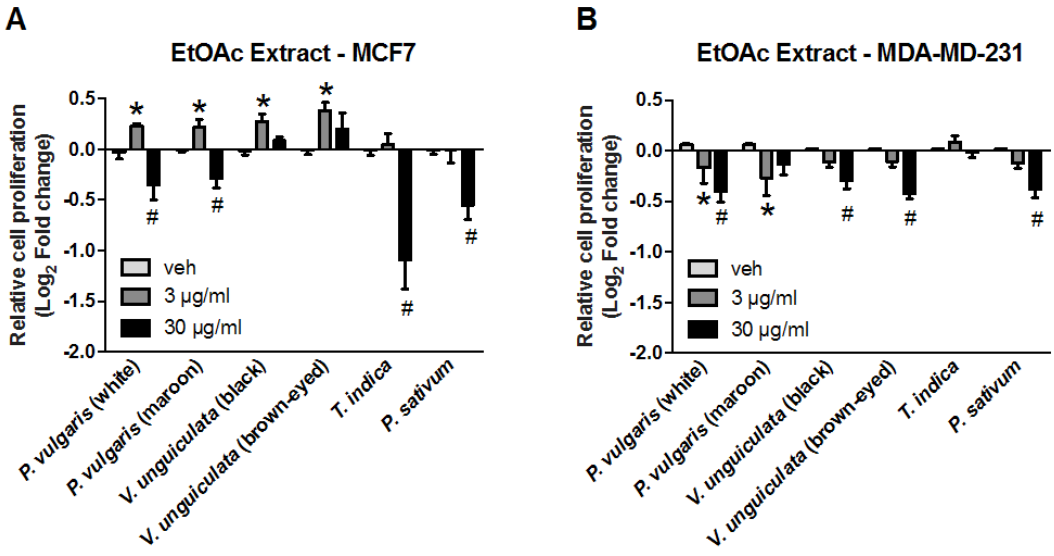


Figure 5. Altered cell proliferation in MCF7 and MDA-MB-231 cells treated with ethyl acetate extracts from select Benguet legumes depending on dose. Relative cell proliferation reported as $\log_2$ fold change (means ± SEM) in (A) MCF7 or (B) MDA-MB-231 cells treated with low (3 µg/mL) and high (30 µg/mL) dose of different legume extracts for 72 hours. Relative cell proliferation was measured by MTT and normalized to vehicle (veh) control. Asterisk (*) and # indicate significant difference relative to vehicle control at $p<0.05$ by two-way ANOVA followed by Tukey post hoc analysis.

Similar HPLC Profiles of Legume Extracts

Because ethyl acetate extracts exhibited very interesting results with respect to cell proliferation, HPLC profiling was then focused on these extracts. Among *P. vulgaris* and *V. unguiculata* cultivars, four peaks were observed to be consistently present with retention times at around 15.6 minutes, 16.5 minutes, 17.2 minutes and 21.4 minutes (Figure 6). Several distinct peaks appeared between 20 minutes and 35 minutes elution time across all four extracts that can be used to differentiate the cultivars from one another. In contrast, the peaks obtained from *T. indica* extract was distinct from *P. vulgaris* and *V. unguiculata*. Because *P. vulgaris* and *V. unguiculata* but not *T. indica* ethyl acetate extracts showed mild proliferation in MCF7 cells, the common peaks found in *P. vulgaris* and *V. unguiculata* but not *T. indica* may be further studied to identify the possible compounds responsible for inducing cell proliferation in ER-containing MCF7 but not MDA-MB-231 cells.

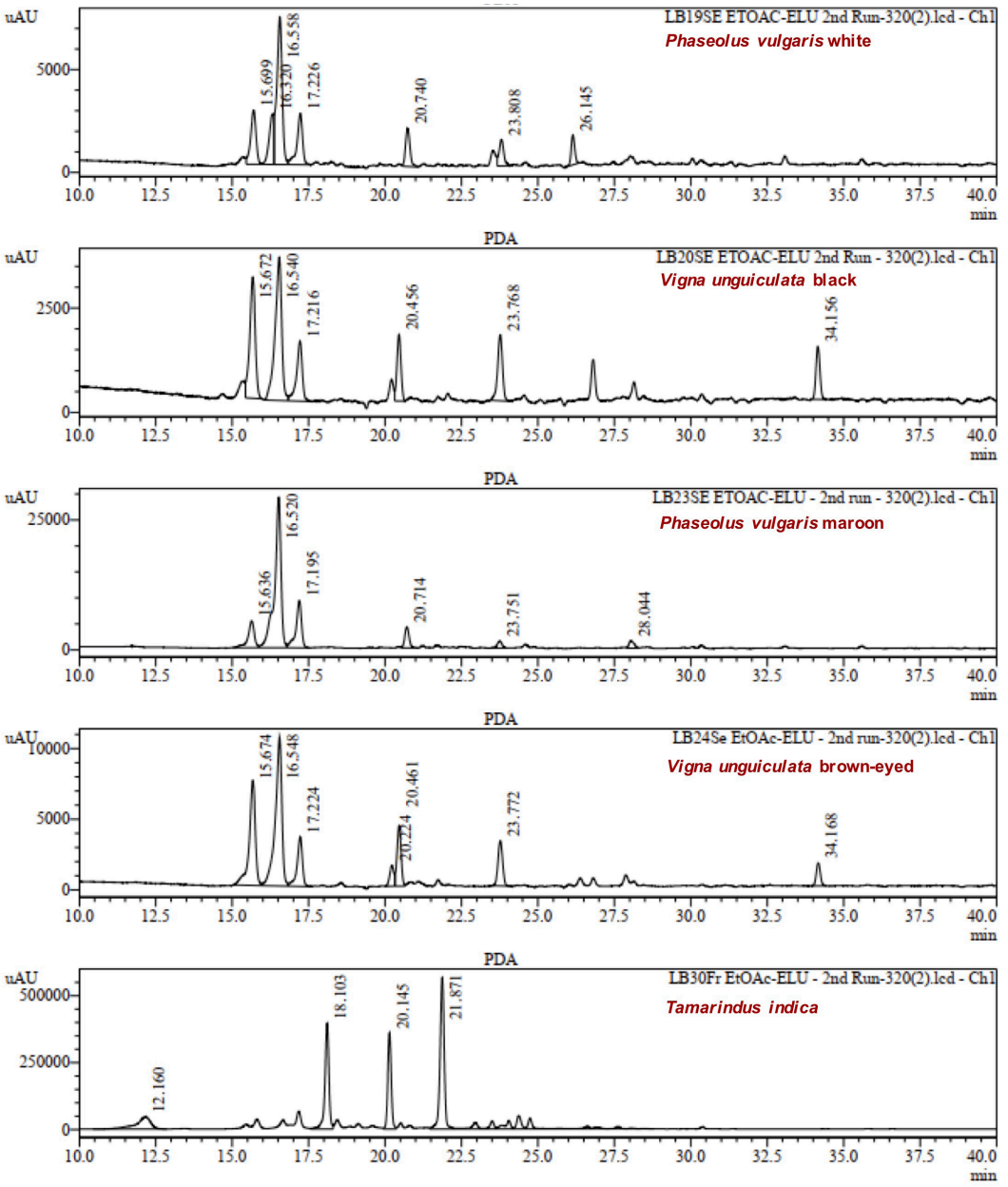


Figure 6. Comparable HPLC chromatograms in *P. vulgaris* and *V. unguiculata* vs. *T. indica* ethyl acetate extracts. Retention times from HPLC runs of ethyl acetate legume cultivar extracts after SPE are shown. All chromatograms were analyzed at 320 nm absorbance. Chromatographic conditions: Purospher® STAR RP-18 endcapped LiChroCART® (C18, 5 µm, 150 mm - 4.6 mm i.d.). Mobile phase, gradient elution with water/acetonitrile (5% to 95% over 45 minutes); flow rate 0.9 mL/min. Autosampler temperature 15 °C; Sample concentration 5 mg/mL; injection volume at 10 µL, in duplicate.

DISCUSSION

This study demonstrated the differential effects of legume extracts in the estrogen-responsive MCF7 and non-estrogen-responsive MDA-MB-231 cell lines. We showed that a low dose of ethyl acetate from *P. vulgaris* and *V. unguiculata* significantly increased cell proliferation in MCF-7 but not MDA-MB-231 cells, while a high dose of these extracts exhibited anti-proliferative activities in both cell lines. *P. vulgaris* and *V. unguiculata* also revealed a similar HPLC profile, which may contribute to their common proliferative effects. While this study did not focus on isolating active compounds present in the extracts, results from this study offer preliminary findings on the effects of the crude extracts as a whole. Further chemical analysis of the actual compounds found in these legumes and their impact on cell proliferation are needed to determine their mechanism of action.

Legumes contain a mixture of several phytoestrogens including genistein and diadzein, which promote cell proliferation in ER-expressing cells (Zava and Duwe 1997; Dampier et al. 2001; Ju et al. 2006; Vitale et al. 2013; Mostrom and Evans 2018). Hence, it is possible that phytoestrogens in *P. vulgaris* and *V. unguiculata* ethyl acetate extracts analyzed in this study may have contributed to the increased cell proliferation in the ER-expressing MCF7 cells. However, it is still possible that other non-estrogenic compounds may have influenced cell proliferation in MCF7 cells. Studies on the proliferative effects of legume cultivar extracts on other ER- and non-ER-expressing cell lines may be considered in the future.

The two cell lines MCF7 and MDA-MB-231 represent two distinct breast cell line models with distinct phenotypes. While both cell lines were derived from pleural effusions, MCF7 cells express luminal epithelial markers and are epithelial in nature, relative to the invasive and more mesenchymal MDA-MB-231 cells (Mladkova et al. 2010). These differences may influence their proliferative responses to treatment. Indeed, an herbal mixture from *Scutellaria barbata* affects cell proliferation of breast cancer cells by regulating specific phases of the cell cycle depending on the cell phenotype (Marconett et al. 2010). Hence, the increased cell proliferation in MCF7 but not in MDA-MB-231 after treatment with *P. vulgaris* and *V. unguiculata* ethyl acetate extracts in this study may also be attributed to the difference in cell phenotypes. It will be interesting to investigate whether these extracts are indeed only proliferative to epithelial types of breast cancer and not to invasive mesenchymal types, as this can potentially benefit patients with advanced cancer.

The ethyl acetate extracts from *P. vulgaris* and *V. unguiculata* in this study showed differential response when treated with low versus high dose. This differential

response suggests that legumes contain several phytochemicals that may collectively work together to influence cell proliferation. Indeed, this differential response depending on dose was also observed in crude extracts of licorice roots, which increased cell proliferation in MCF7 cells at a low dose through an ER-dependent mechanism and inhibited proliferation at a high dose through an ER-independent manner (Hu et al. 2009). It is possible that legumes in this study may also contain some mild pro-proliferative components that are masked by anti-proliferative compounds when used at high amounts. Hence, identifying the different components that promote or inhibit cell proliferation will provide insights into how these components collectively affect the overall breast cancer cell proliferation. Future studies can then be directed to separating the anti-proliferative from the proliferative components of the extract to help develop functional foods that reduce breast cancer cell growth with minimal side effects.

Overall, we showed that common beans (*P. vulgaris*) and cowpeas (*V. unguiculata*), but not *T. indica* and *P. sativum*, from Benguet contain bioactive compounds with potential pro- and anti-proliferative activities in breast cancer cell lines, suggesting that consumption of these legumes may potentially have positive and negative impact on the pathogenesis of breast cancer depending on the amount of intake and the type of cancer present. Hence, future in vivo studies may investigate the contribution of the level of legume consumption on the promotion of ER+ and ER- breast cancers.

ACKNOWLEDGMENTS

This work was funded by the University of the Philippines Diliman, Office of the Vice President for Academic Affairs, Emerging Interdisciplinary Research Program (OVPAA-EIDR, EIDR-C08-006, MCV & NSQ).

REFERENCES

Adebamowo CA, Cho E, Sampson L, Katan MB, Spiegelman D, Willett WC, Holmes MD. 2005. Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. Int J Cancer. 114(4):628-633.

Angeles-Agdeppa I, Sun Y, Denney L, Tanda KV, Octavio RAD, Carriquiry A, Capanzana MV. 2019. Food sources, energy and nutrient intakes of adults: 2013 Philippines national nutrition survey. Nutr J. 18(1):1-12.

Correa P. 1981. Epidemiological correlations between diet and cancer frequency. Cancer Res. 41:3685-3689.

Dampier K, Hudson EA, Howells LM, Manson MM, Walker RA, Gescher A. 2001. Differences between human breast cell lines in susceptibility towards growth inhibition by genistein. Br J Cancer. 85(4):618-624.

Díaz-Batalla L, Widholm JM, Fahey GC, Castaño-Tostado E, Paredes-López O. 2006. Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.). J Agr Food Chem. 54 (6):2045-2052.

Fung TT, Hu FB, Holmes MD, Rosner BA, Hunter DJ, Colditz GA, Willett WC. 2005. Dietary patterns and the risk of postmenopausal breast cancer. Int J Cancer. 116(1):116-121.

Ganesan K, Xu B. 2017. A critical review on polyphenols and health benefits of black soybeans. Nutrients. 9(5):455.

Gu W, Song J, Cao Y, Sun Q, Yao H, Wu Q, Chao J, Zhou J, Xue W, Duan J. 2013. Application of the ITS2 region for barcoding medicinal plants of Selaginellaceae in Pteridophyta. PLoS ONE. 8(6):2-9.

Heimler D, Vignolini P, Dini MG, Romani A. 2005. Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. J Agr Food Chem. 53(8):3053-3056.

Hu C, Liu H, Du J, Mo B, Qi H, Wang X, Ye S, Li Z. 2009. Estrogenic activities of extracts of Chinese licorice (*Glycyrrhiza uralensis*) root in MCF-7 breast cancer cells. J Steroid Biochem Mol Biol. 113(3-5): 209-216.

Ju YH, Fultz J, Allred KF, Doerge DR, Helferich WG. 2006. Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice. Carcinogenesis. 27(4):856-863.

Kalogeropoulos N, Chiou A, Ioannou M, Karathanos VT, Hassapidou M, Andrikopoulos NK. 2010. Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. Food Chem. 121(3):682-690.

Key TJ, Allen NE, Spencer EA, Travis RC. 2002. Evidence for diet as a risk factor for cancer. Lancet. 360 861-868.

Kotepui M. 2016. Diet and risk of breast cancer. Wspolczesna Onkol. 20(1):13-19.

Kumar S, Sharma VK, Yadav S, Dey S. 2017. Antiproliferative and apoptotic effects of black turtle bean extracts on human breast cancer cell line through extrinsic and intrinsic pathway. Chem Cent J. 11(1):56.

Li FW, Kuo LY, Rothfels CJ, Ebihara A, Chiou WL, Windham MD, Pryer KM. 2011. rbcL and matK earn two thumbs up as the core DNA barcode for ferns. PLoS ONE [Internet]. [cited 2019 July 12] 6(10):e26597. Available from: https://doi.org/10.1371/journal.pone.0026597

López A, El-Naggar T, Dueñas M, Ortega T, Estrella I, Hernández T, Gómez-Serranillos MP, Palomino OM, Carretero ME. 2013. Effect of cooking and germination on phenolic composition and biological properties of dark beans (*Phaseolus vulgaris L.*). Food Chem. 138(1):547-555.

Luthria DL, Pastor-Corrales MA. 2006. Phenolic acids content of fifteen dry edible bean (Phaseolus vulgaris L.) varieties. J Food ComposAnal. 19(2-3):205-211.

Marconett CN, Morgenstern TJ, San Roman AK, Sundar SN, Singhal AK, Firestone GL. 2010. BZL101, a phytochemical extract from the *Scutellaria barbata* plant, disrupts proliferation of human breast and prostate cancer cells through distinct mechanisms dependent on the cancer cell phenotype. Cancer Biol Ther. 10(4): 397-405.

Mladkova J, Sanda M, Matouskova E, Selicharova I. 2010. Phenotyping breast cancer cell lines EM-G3, HCC1937, MCF7 and MDA-MB-231 using 2-D electrophoresis and affinity chromatography for glutathione-binding proteins. BMC Cancer [Internet]. [cited 2019 November 26] 10:449. Available from: https://doi.org/10.1186/1471-2407-10-449

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Methods. 65(1-2): 55-63.

Mostrom M, Evans TJ. Phytoestrogens. In: Gupta RC, editor. Veterinary Toxicology. 3rd ed. London, UK: Academic Press; c2018. p. 817-833.

Ombra MN, D'Acierno A, Nazzaro F, Riccardi R, Spigno P, Zaccardelli M, Pane C, Maione M, Fratianni F. 2016. Phenolic composition and antioxidant and antiproliferative activities of the extracts of twelve common bean (*Phaseolus vulgaris* L.) endemic ecotypes of Southern Italy before and after Cooking. Oxid Med Cell Longev. [Internet] [cited 2019 June 28] 12:1-12. Available from: https://doi.org/: 10.1155/2016/1398298

Pedro MRA, Benavides RC, Barba CVC. 2006. Dietary changes and their health implications in the Philippines. FAO Food Nutr Pap. 84:205-257.

Ranilla LG, Kwon YI, Genovese MI, Lajolo FM, Shetty K. 2010. Effect of thermal treatment on phenolic compounds and functionality linked to type 2 diabetes and hypertension management of Peruvian and Brazilian bean cultivars (*Phaseolus vulgaris* L.) using in vitro methods. J Food Biochem. 34(2):329-355.

Sangaramoorthy M, Koo J, John EM. 2018. Intake of bean fiber, beans, and grains and reduced risk of hormone receptor-negative breast cancer: the San Francisco Bay Area breast cancer study. Cancer Med. 7(5):2131-2144.

Shannon J, Ray R, Wu C, Nelson Z, Dao LG, Li W, Hu W, Lampe J, Horner N, Satia J, et al. 2005. Food and botanical groupings and risk of breast cancer: a case-control study in Shanghai, China. Cancer Epidem Biomar. 14(1):81-90.

Singh PN, Fraser GE. 1998. Dietary risk factors for colon cancer in a low-risk population. Am J Epidemiol. 148(8):761-774.

Suffness M, Pezzuto JM. Assays related to cancer drug discovery. In: K Hostettmann. Methods in plant biochemistry: assays for bioactivity. Volume 6. London, c1990. p. 71-133.

Thompson MD, Thompson HJ, Brick MA, McGinley JN, Jiang W, Zhu Z, Wolfe P. 2008. Mechanisms associated with dose-dependent inhibition of rat mammary carcinogenesis by dry bean (*Phaseolus vulgaris*, L.). The J Nutr. 138(11):2091-2097.

Thompson MD, Brick MA, McGinley JN, Thompson HJ. 2009. Chemical composition and mammary cancer inhibitory activity of dry bean. Crop Sci. 49(1):179-186.

Vitale DC, Piazza C, Melilli B, Drago F, Salomone S. 2013. Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet. 38(1):15-25.

Weidner S, Król A, Karamać M, Amarowicz R. 2018. Phenolic compounds and the antioxidant properties in seeds of green- and yellow-podded bean (*Phaseolus vulgaris* L.) varieties. CyTA - J Food. 16(1):373-380.

Zava DT, Duwe G. 1997. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. Nutr Cancer. 27(1):31-40.

———

Cielo Mae D. Marquez is a Science Research Specialist at the Regenerative Biology Research Laboratory of the Institute of Biology, University of the Philippines Diliman. She completed her Master's Degree in Biology (Cell and Molecular Biology) in the same institute. Her research interests are on natural products and cancer biology.

John Carlo F. Dela Cruz is a Science Research Specialist of the Natural Products and Peptidomics Laboratory, University of the Philippines Manila. He finished his Bachelor's Degree in Biochemistry at the University of the Philippines Manila. His research interests are on natural products and chromatography techniques. He is also working with antimicrobial assays.

Conrad Allan C. Chong is a former Science Research Specialist at the Institute of Biology, University of the Philippines Diliman. He received his Master's Degree in Oncological Sciences at the University of Utah School of Medicine.

Leomir A. Diaz is a Senior Science Research Specialist who also serves as a Post-Award Grant and Procurement Officer of the Regenerative Biology Research Laboratory at the Institute of Biology, University of the Philippines Diliman. He previously obtained his Master's Degree in Marine Science (Marine Biotechnology) from the Marine Science Institute, UP Diliman.

Harold M. Carag is a University Research Assistant II at the Institute of Biology, University of the Philippines Diliman. He received his Master's Degree major in Botany and minor in Sociology at the University of the Philippines Los Baños. His research interests include ethnobotany and taxonomy of endemic food and medicinal flora of the Philippines.

Dhennis T. Versoza is a Research Associate at the Natural Products and Peptidomics Laboratory, University of the Philippines Manila. He completed his Master's Degree in Pharmacy major in Pharmaceutical Chemistry from the College of Pharmacy, University of the Philippines Manila. His research interest is the purification and isolation of natural products.

Noel S. Quiming <nsquiming@up.edu.ph> is an Associate Professor at the Department of Physical Sciences and Mathematics, College of Arts and Sciences, University of the Philippines Manila. His research interests include natural products chemistry and chromatographic separation.

Dr. Marilou G. Nicolas is the head of the Natural Products and Peptidomics Laboratory at the University of the Philippines Manila. Her research interests are in the field of metabolomics and peptidomics.

Dr. Michael C. Velarde <mcvelarde@up.edu.ph> is an Associate Professor and head of the Regenerative Biology Research Laboratory at the Institute of Biology, University of the Philippines Diliman. His research interest includes cell cycle regulation and steroid hormone signaling.