Effect of UV radiation (365 nm) to the production of antimitotic compounds in *Arachis hypogaea* (peanut) roots

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Abstract

Arachis hypogaea, commonly known as peanut, is a legume reported to contain phytoalexins that are potentially antimitotic. Phytoalexins are substances produced by plants as a response to numerous biotic and abiotic stresses, such as fungal and viral infections, ultraviolet (UV) radiation and ultrasonic exposure. The present study assessed the efficacy of UV radiation in inducing the production of phytoalexins through the *Allium cepa* root tip technique. Crude root ethanol extracts of *A. hypogaea* plants exposed and unexposed to UV radiation (3 mg/mL and 5 mg/mL), and tap water were compared. Results showed that the 5 mg/mL concentration of the UV-exposed plants was the only treatment which showed significant antimitotic potential compared to other treatments. However, optimum concentration ranges between 5 to 10 mg/mL only. In conclusion, exposure of the *A. hypogaea* plants to UV radiation aids the increase of production of antimitotic compounds present in *A. hypogaea* roots.

Keywords: *Arachis hypogaea, Allium cepa root tip technique, ultraviolet (UV) radiation, phytoalexins, mitotic index (MI)*

Introduction. *Arachis hypogaea* (peanut) is an herbaceous plant in the legume or bean family, currently grown mainly for its seeds and oil, and is one of the 19 major crops of the Philippines. The province of Iloilo, particularly, shares 7.9 percent of the national total, and therefore, has one of the largest area harvested of peanut in 2014 [1]. Peanuts, which are high in protein and vitamin B and E, are often incorporated into the diet. Studies show that peanuts contain antiviral, anti-inflammatory, anticancer, antihypertension, antiproliferative [2], cardiovascular disease protection, and neuroprotection [3].

The number of cases of chronic diseases, like hypertension, heart disease, arthritis, has not ceased in increasing exponentially [4]; thus, the interest in the presence and availability of compounds in plant materials that may contain bioactive properties has increased. Peanut roots, which contain several bioactive compounds that are beneficial to the body [5], are of greatest interest. This part of the peanut plant contains coutaric acid which is reported to exhibit antioxidant, anti-inflammatory, and antimicrobial activity [6, 7]. The p-coumaric acid, caffeinic acid, and 4-methoxycinnamic acid are found in the peanut roots [8]. Root mucilage from peanut plants were investigated, and prenylated stilbenes, a type of phytoalexin, were identified. Other isolated compounds were trans-resveratrol, medicarpin, t-arachidin-1, tarachidin-2, t- arachidin-3, t-3'-isopentadienyl-3,5,4'-trihydroxystilbene, and 4-(3-methyl-but-1-enyl)-3,5 - dimethoxy4'- hydroxy - trans-stilbene [8].

Phytoalexins are substances produced by plants as response to biotic stresses, like fungal infection, or insects, and abiotic stresses, such as injury, ultrasound, and ultraviolet (UV) light [9]. The major role of UV-irradiation in triggering the production of stilbenes has been widely studied since 1977, as reported [10]. Accumulation of resveratrol was observed after being subjected to slicing, followed by incubation for 48 hr in the dark [11], after being exposed to UV light [12, 13], wounding, and exposure to compounds. Ultraviolet light exhibited the most efficacy in assisting the production of resveratrol among all of the stresses applied, increasing the resveratrol content 200-fold [14]. While, incubation time of 24 to 48 h was found to be the optimal postharvest stress condition to produce the highest amount of the said major stilbene found in peanut roots [15], the said increase was not affected by the distance of the UV and the time of exposure [16].

Resveratrol, specifically, showcased antiproliferative activity in the cells by disrupting microtubule dynamics, which helps the division of cells [17]. Extensive research has been done in the production of resveratrol and other phytoalexins in grape peels but not on other respondents [18, 19]. While numerous phytoalexins have been identified to inhibit mitosis, studies have only tested them directly as isolated compounds [20, 21]. None of the previous research has examined the root crude extract of the UV-irradiated peanut *Arachis hypogaea* plant in order to determine its effect on the mitotic activity of *Allium cepa* root tips.

The antimitotic activity exhibited by resveratrol is similar to that of other antimitotic drugs. This disrupts the mitotic spindle assembly, and the microtubules, which play a major role in various biological functions such as mitosis, cell motility, and intracellular transport, inhibiting mitotic activity or cell division [12, 22]. Antimitotic drugs target the inhibition of cell proliferation by primarily blocking mitosis, which requires an intense control of microtubule dynamics [12]. Microtubule toxins such as taxol, other taxanes, and the vinca alkaloids are few of the traditional antimitotic agents which have been

proven successful in the clinic; however, resistance and toxicity have limited their efficacy [23, 24]. Resistance to taxanes and vinca alkaloids is caused by structural alterations in tubulin, decreased polymerization ratio, alterations in the expression of microtubule-associated proteins, and the efflux activity of the P-glycoprotein and the multidrug-resistance- protein MRPI which decreases their intracellular accumulation. Toxicity is also a major concern because microtubule-targeting agents, aside from killing tumor-dividing cells, are affecting the normal dividing cells resulting in myelosuppression due to impaired cycling of bone marrow cells. Peanuts, for one, are enriched with health benefits such as antioxidant properties [5], among others which can be found in its roots, making it a potential source of phytochemical compounds to be used for development of new drugs.

This study mainly aimed to evaluate the efficacy of UV light, a known abiotic stress which induces the production of phytoalexins, by comparing the degree of antimitotic potential of the crude root ethanolic extract of *Arachis hypogaea* which has been exposed to UV radiation to the antimitotic activity of the crude root ethanolic extract of *A. hypogaea* which has not been exposed to UV radiation through the *Allium cepa* (onion) root tip technique. Specifically, this study aimed to:

(i) Count the cells undergoing the stages of mitosis (prophase, metaphase, telophase, anaphase), and interphase seen on the onion root tips exposed to the treatments through *Allium cepa* root tip technique;

(ii) Calculate the mitotic index of the onion root tip exposed to the treatments using the *Allium cepa* root tip technique; and,

(iii) Determine the significant difference between the mitotic indices of the onion root tip exposed to different treatments through independent sample t-test.

This study only evaluated the effect of UV radiation exposure to the production of antimitotic compounds in *Arachis hypogaea* roots through the onion root tip technique. Treatments were each prepared in 3 mg/mL and 5 mg/mL concentrations only. The specific potential antimitotic compounds and their corresponding concentration in the peanut root crude ethanolic extract, and the antimitotic potential of other peanut parts was not further determined in this research. The effects of various other biotic and abiotic stress, like fungal infection, chopping, or slicing, to the mitotic index of the onion roots, was not assessed. In addition, due to the unavailability of a 254 nm UV light in any local hardware shop, a 365 nm was used.

Methods. Peanut seeds were bought from Iloilo Central Market and were grown in 4 in x 4 in x 7 in plastic pots and organic rice hull soil obtained from an agricultural shop in Brgy. Fundidor, Molo, Iloilo City. The UV light bulb (365 nm) used for exposure was purchased from ACE Hardware. The 95% ethanol solvent for the ethanol extraction, and the aceto-orcein stain, glass slides, and glass covers for antimitotic assay were acquired from Patagonian Enterprises, 20 E. Lopez Jayme St., Jaro, Iloilo City, 5000 Iloilo. The magnetic stir bar and Whatman no. 1 filter for the ethanol extraction, ten 250-mL beakers, 10 test tubes, forceps, 0.7% H_2O_2, HCl (1N), and a compound microscope with 400x magnification (Motic SFC-100 Series) were requested and borrowed from the PSHS-WVC Laboratories. At least 15 onions weighing 3 to 4 grams and toothpicks were bought from the local market for the growing of onion root tips.

Cultivation of Peanut Plants from Seeds. Four peanut seeds were planted per pot, summing up to a total of 640 peanut seeds grown in 160 plastic pots. The pots were initially filled three-fourths way with rice hull compost soil, with the four peanuts seeds placed in a square orientation. An inch of soil was then added. These pots were situated in an area wide enough for all pots to receive equal amount of sunlight. These were grown in a controlled environment given equal amount of sunlight and water for 30 days before transfer of half of the batch for UV exposure.

Exposure of Peanut Plants to UV Radiation. Eighty plastic pots containing a total of 320 peanut *Arachis hypogaea* plants were transferred to a 2 x 2 x 1 m growing tent made from reflective fabric for the exposure process. The rest of the plants were allowed to grow under normal and controlled conditions. Peanut plants exposed to UV radiation were positioned with an equal distance of 15 cm between each plant and 1 m from the UV light. The UV lights were positioned directly above the plants to ensure equal exposure with an equal distance of 15 cm between each light. The peanut plants were exposed to 365 nm UV light at constant distance for 15 minutes and were left incubated for 36 hours [15].

Crude Ethanol Extraction of Dried and Powdered Peanut Roots. Hairy roots of peanut plants which have been exposed to UV radiation and those which have not were collected and subjected to air drying. Dried samples were ground into a fine powder using a grinder [25]. The dried and homogenized roots were extracted with 95% ethanol:water at a nominal ratio of 9:1 (%v/w) by stirring with a magnetic stir bar for 1 hat room temperature. The slurry was allowed to settle for 24 h, and the supernatant was passed through a Whatman no. 1filter paper. Subsequently, the filtrate was extracted under reduced pressure at boiling temperature (>78°C) using a rotary evaporator. Extracts were stored at 4°C [21].

Culturing of Allium cepa Root Tip. Fifteen commercial equal-sized *Allium cepa* bulbs weighing 3 to 4 g were used. The bulbs were carefully unscaled, placed on top of beakers filled with tap water and allowed to germinate in room temperature. After 48 hours, the onion bulbs were treated with 0.7% H2O2 for an hour. After the H2O2 treatment, onion bulbs with root tips which have grown up to 2 to 3 cm were washed for an hour and were transferred to beakers containing the different treatments (3 mg/mL and 5 mg/mL crude ethanolic extracts of both peanut plants exposed and unexposed to UV radiation, and tap

water) for 72 h [26]. Assignation of bulbs to treatments was randomized.

Antimitotic Assay. After the completion of treatment, the roots (approximately 1 to 2 cm) were excised and collected, and immediately fixed in 3:1

(ethanol:acetic acid) for 24 hours [27]. Root tips were hydrolysed in 1N HCl for 15 min at 60°C and stained with 2% orcein stain. After the removal of root caps from well-stained root tips, a millimeter of the mitotic zones was immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover glass and examined microscopically [28]. Stained root tips were observed under 100x magnification for different stages of cell division [29]. Five hundred cells were analysed per root tip [30], summing up to 4500 cells per treatment. The number of cells in each stage of cell division i.e. prophase, metaphase, anaphase, or telophase, and including the cells in interphase were counted and recorded. The average mitotic index of 3 root tips for each treatment was calculated.

$$mitotic\ index = \frac{Total\ number\ of\ dividing\ cells\ (P+M+A+T)}{Total\ number\ of\ cells\ examined} \times 100\%$$

Statistical Analysis. With the aid of the Statistical Package for the Social Science (SPSS) software, independent sample t-test was used to compute for the differences between the mean of the mitotic indices of the onion root tips exposed to the crude root ethanol extract of UV-exposed peanut plants and the mean of the mitotic indices of the onion root tips exposed to the crude root ethanol extract of peanut plants not exposed to UV. Each of these means was then compared to the mean of the mitotic indices of the onion root tips grown in tap water using the t-test for differences in means.

Results and Discussion. The UV-irradiated *Arachis hypogaea* plant in 5 mg/mL concentration (UV5) exhibited the lowest overall average mitotic index (MI) of 0.095, followed by tap water treatment (0.134), UV-irradiated *A. hypogaea* plant in 3 mg/mL concentration (Treatment UV3) with 0.198, then Treatment No UV 5 or unexposed *A. hypogaea* root crude ethanol extract in 5 mg/mL concentration (0.222), and lastly, unexposed *A. hypogaea* root crude ethanol extract in 3 mg/mL concentration (Treatment No UV3) with an overall average MI of 0.239 (see Figure 1).

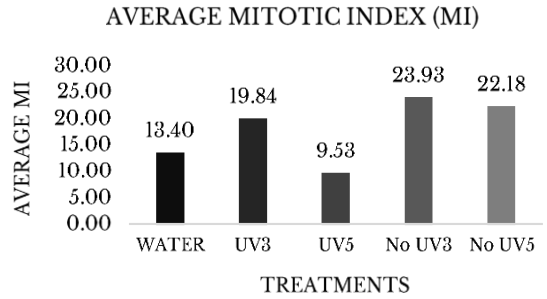

Figure 1. Graph of the overall average MI of untreated setup, and the four crude root ethanol extracts of *Arachis hypogaea* treatments

Treatment UV 5 had the lowest MI, inhibiting cellular division the most, while NO UV 3 had the highest MI with the highest rate of cellular proliferation. Three of the setups had a higher MI, namely UV3, NO UV3, and NO UV5, than the untreated setup where onion root tips were only exposed to tap water. The compounds found in the crude ethanol extracts of these treatments suggest the presence of cell proliferating properties which encourage cellular division to the onion root tip. On the other hand, the UV5 setup had a lower MI compared to the untreated. This suggests the presence of sufficient antimitotic compounds enough to counteract other cell proliferating compounds found in the extract and to significantly inhibit mitosis in the onion root tip.

In the order of treatments which had the highest mitotic index, Treatment NO UV 3 had the highest. This is due to no external stress of UV radiation being added to the setup. Without the significant presence of antimitotic compounds, the treatment's concentration of 3 mg/mL does not show a significant importance to its mitotic activity when treatments of unexposed peanut plants are used. Thus, as exhibited by the data gathered, as the concentration of the extract increases to 5 mg/mL with Treatment NO UV 5, no significant decrease in its mitotic index was observed.

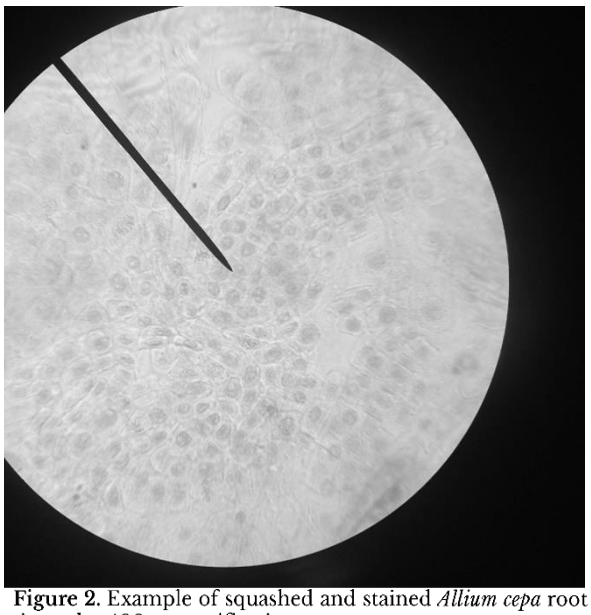

Figure 2. Example of squashed and stained *Allium cepa* root tip under 400x magnification

As UV radiation is added to the setup, no significant decrease in the mitotic index of the onion root tip from Treatments NO UV 3 to UV 3 was observed; however, a significant decrease in mitotic index was observed between Treatments NO UV 5 and UV 5. This exhibits the increase of antimitotic compounds extracted from the peanut root as it was exposed to UV radiation. The mitotic index is inversely proportional to the amount of antimitotic compounds in the extract.

Treatments UV 3 and UV 5 showed a significant decrease in their mitotic index as the concentration of

the treatment increases. This relates to the importance of UV radiation in inducing the production of antimitotic compounds. Treatments unexposed to UV radiation had no significant difference as their concentration increases however a significant difference was observed in Treatments exposed to UV radiation. As aforementioned, UV radiation exhibits increased production of antimitotic compounds; thus, resulting to a greater significance increase in the amount of antimitotic compound present as the concentration increases. As the concentration of the crude ethanolic extract of peanut roots increases in the solution to which the onion root tips are exposed to, the concentration of antimitotic compounds present also increases attributing to the aforementioned significant decrease in mitotic index.

Previous studies have shown the significance of UV radiation as an abiotic stress to induce the production of antimitotic compounds. Studies reported an increase of trans-resveratrol in table grapes in response to UV-irradiation [31, 32]. These compounds are classified as phytoalexins which are produced by plant tissues in response to external stress. Compounds such as resveratrol found to exhibit antimitotic properties induced by UV-irradiation are also found in peanuts [33]. Most of these compounds are identified to be non-polar. Thus, ethanol, a non-polar solvent, was used to extract peanut roots. These antimitotic phytoalexin compounds were perhaps obtained in the extraction although further phytochemical testing is necessary to confirm this.

Thus, UV radiation significantly induces the production of antimitotic compounds at an optimum concentration ranging from 5 mg/mL to 10 mg/mL exhibited by the significant decrease of the mitotic index between Treatment No UV 5 and UV 5. The antimitotic potential of the treatment and its production of antimitotic compounds are inversely proportional to the onion root tip's mitotic index. Concentrations of extracts also significantly affects the antimitotic activity of treatments exposed to UV radiation shown by the significant decrease in mitotic index between Treatments UV 3 and UV 5. Among all the treatments, only Treatment UV 5 exhibited significant antimitotic activity to the onion root tip compared to the control untreated setup of tap water.

Error Analysis. Possible errors which arose during the conduct of the data gathering may be attributed to the identification of the stages of mitosis when computing for the mitotic index. In the ethanol extraction of peanut roots, incomplete evaporation of solvents may have also affected the antimitotic performance of the crude ethanolic extracts to the onion root tips. Based on the study's results, confirming that UV radiation induces the production of antimitotic compounds, further research may be done to identify these specific compounds present in the crude ethanol extract of *Arachis hypogaea* roots, and how it may be utilized.

Conclusion. Ultraviolet radiation, an abiotic stress, exhibited significant efficacy in decreasing the mitotic activity of the exposed onion root tips. However, optimum concentration is greater than or equal to 5 mg/mL but less than 10 mg/mL. The mitotic activity in the onion root tips significantly decreases as the concentration of the crude root ethanol extract of *A. hypogaea* plant exposed to UV radiation increases. As hypothesized, UV-irradiated *A. hypogaea* crude root ethanol extract in 5 mg/mL concentration (Treatment UV5) had the lowest average mitotic index for its increased production of antimitotic compounds resulting from its exposure to UV light and to its higher concentration.

Recommendations. To further expand the knowledge on the current findings, it is recommended that phytochemical analysis should be done in the crude ethanol extract of *Arachis hypogaea* roots should be identified. Other solvents like water, methanol, or dimethyl sulfoxide (DMSO) may also be used. Moreover, the efficacy of other biotic and abiotic stress in increasing the antimitotic compounds present in peanut roots may be compared to the current data where the abiotic stress, UV radiation, was used. The peanut plants are also suggested to be grown either less or more than 30 days to discover the optimum duration of the growing of peanut plants in which there is maximum phytoalexin production. For further study, other variables, such as chromosomal aberration, should be determined in order to provide more accurate evaluation of the antimitotic activity of the extract. Lastly, it is recommended to use a 254 nm ultraviolet light for the exposure of peanut plants.

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