A Method to the Madness

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Last, but certainly not least, I would like to thank God the creator for allowing me to participate in this opportunity and showing me his grace, love, and forgiveness through the places I've seen in Taiwan and the people I've met.

Introduction

Personal Remarks

Growing up in Southern Ohio, my world from an early age was surrounded by a "can-do" work attitude. My parents always tried to push all four of us kids to be our best and achieve greatness. My siblings and I are worlds apart in personalities and working style, but we try to get the work done, and done right. Since our fifth grade year of grade school, all of us became involved in science fair. Science fair peaked our interest and opened our eyes to the world around us.

Ever since my elder sister had the opportunity to become a delegate to the Global Youth Institute in 2006, then a Borlaug-Ruan international intern at the Hybrid Rice Institute in Changsha, China in 2007, I wanted to follow in her footsteps.

In the summer of 2009, I had the chance to go to Aguaytia, Peru on a medical mission trip for GoInternational. There, I learned what it was really like living in a country other than the U.S., experiencing Peru's poorest neighborhoods. While there, my team set up a free healthcare clinic. There were two doctors (each with an interpreter), a dentist (with a team of two assistants and an interpreter) and a pharmacy (two people plus an interpreter). I worked in the pharmacy and also tested urine for urinary tract infections. I also used what little Spanish

training I had to give the patients instructions on how to take their medicine. I learned so much about myself and the world around me in that one week, so when I returned to the U.S. I was on fire. I wrote my first paper for the World Food Prize on land degradation in Peru. I used research and first-hand experiences to convey the need for infrastructure, especially in the eastern part of Peru.

World Food Prize Foundation Involvement

I presented my research paper at the 2009 Ohio Youth Institute, competing with around 20 other students from around Ohio, to become one the 6 delegates to the Global Youth Institute in Des Moines, Iowa. I surpassed my expectations to become one the lucky 6 and got the chance to present my paper to a very esteemed group of individuals. The Global Youth Institute was like nothing I've ever experienced. From the interactive discussion panels, to the workshop where all the delegates helped package meals, to the Hunger Banquet hosted by the interns from 2009, everything was driven by one purpose: to end world hunger and malnutrition. During those three days, I had the chance to interact with the interns from summer 2009. Because I was a sophomore, I knew I could not apply for an internship for summer 2010, but I knew come summer 2011, I'd try.

In 2010, I wrote another research paper to present at the Ohio Youth Institute on education in Brazil, because during the fall semester of 2010, my elder sister studied abroad in Brazil. There I competed again to become a delegate for a second year to the Global Youth Institute. They told me I hadn't needed to write a second paper, or go to the GYI for a second time, to compete for a Borlaug-Ruan internship, so they sent 6 new people to experience what I had the year before. I was glad for the chance to research and present a whole other aspect of food security, and I was glad, even eager for others to experience the GYI as I had the year before.

In December, I applied to become a Borlaug-Ruan International Intern at AVRDC-The World Vegetable Center in Taiwan or India, or ICIPE (African Insect Science for Food and Health) in Kenya.

In January, I found out that I was one of 25 candidates for an interview over skype. The panel of judges asked some difficult questions, but I tried my best to give honest answers. I had prepared for the interview with practice questions and a pep talk from my sister.

In late February, a letter arrived at my home in Ohio from the World Food Prize Foundation. I tore open the envelope to find that I was to be a Borlaug-Ruan international intern at AVRDC-The World Vegetable Center in Taiwan. To put it in the simplest terms, I was ecstatic. My dream of having a part, however small, in the solution to world hunger and malnutrition was going to be a reality.

AVRDC-The World Vegetable Center: Headquarters: Shanhua, Tainan, Taiwan

Figure 1: Claire Huong (Ting Ting), Neoh Weiting, and I jumping in front of the AVRDC-The World Vegetable Center Administration building.

Figure 2: Sunset behind the Administrative building at AVRDC-The World Vegetable Center in Taiwan

Founded in 1971, the Asian Vegetable Research and Development Center is based in Shanhua, Tainan, Taiwan, making it the only international agricultural research institute based in a Chinese-speaking country. What began as the Asian Vegetable Research and Development Center, changed to AVRDC-The World Vegetable Center to encompass the work and centers all over the world. There are several regional centers in countries such as Korea, Uzbekistan, Dubai, Mali, Madagascar, Cameroon, Niger, Thailand, and India serving Asia, Africa, and Oceania, as well as work in Central and South America.

The mission of AVRDC-The World Vegetable Center is "to alleviate poverty and malnutrition in the developing world though the increased production and consumption of safe vegetables." According to avrdc.org, vegetables can: 1) alleviate poverty by creating new jobs and new sources of income for farmers and landless laborers, 2) improve health by providing micronutrients lacking in the diets of poor people, 3) enhance learning and working capacities of adults and children through improved diets and health, and 4) improve the sustainability of food production practices by diversifying cropping systems.

AVRDC-The World Vegetable Center receives around US$18 million from many governments including Australia, France, Germany, Japan, Korea, Philippines, Switzerland, Taiwan, Thailand, United Kingdom, and United States, as well as from institutions, foundations, and the private sector. These include the Asian Development Bank (who started AVRDC-The World Vegetable Center), Rockefeller Foudation, Bill & Melinda Gates Foundation, Asia & Pacific Seed Association, Farm Africa, and the Organic Center for Education and Promotion.

AVRDC-The World Vegetable Center is the only international agricultural research center based in a Chinese-speaking country, it also is the only international research center that always has had "development" in its name and mandate. This is important in the structure of the center, as it ties the world's largest public vegetable gene bank with research and global development and dissemination.

AVRDC-The World Vegetable Center is divided into four themes: Germplasm, Breeding, Production, and Consumption.

Research is divided into unit divisions at AVRDC-The World Vegetable Center, including Nutrition, Biotechnology/Molecular Breeding, Bacteriology, Bulb/Allium, Indigenous Vegetables,

Entomology, Mycology, Pepper, Tomato, and Virology. Other units at the center include Global Technology Dissemination, Communications and Information, and Socioeconomics.

This summer, I had the pleasure to work in the Nutrition unit of AVRDC-The World Vegetable Center, but interacted on a daily basis with scientists from the other units of the research building.

Figure 4: AVRDC-The World Vegetable Center Summer Interns and Staff.

Figure 3: Group Picture of the Nutrition unit (plus those who helped with Bitter Gourd).
Not Pictured: Cynthia Du

Analysis of Phytochemicals in African Nightshades

Abstract

Phytochemicals are mostly secondary metabolites in plants, considered non-essential nutrients regarding human health, but have disease-preventive and protective qualities useful in the human body. There is a need to investigate the phytonutrients in African nightshade, particularly glycoalkaloids to better understand their nutritional contribution and potentially negative effect. Glycoalkaloids are a group of phytochemicals accounting for poisoning or even death around the world. The objective was to establish a method to identify phytochemicals in African nightshades, focusing on glycoalkaloids, and quantify. 40 African nightshade samples were collected from Kenya, Cameroon, and Tanzania and grown in a controlled test plot in Taiwan. All samples were nutritionally evaluated then freeze-dried and stored at -40ºC. Three samples were profiled to establish their phytochemical profile. The samples were extracted using a methanol/ethanol extraction method. These samples were extracted further for the quantification of their respective glycoalkaloids, using a methanol-based extraction then solid-phase extraction, also using methanol as the primary solvent. For the profiling, the samples were evaluated through UPLC-MS analysis using A: 0.1% Formic Acid in Water and B: 0.1% Formic Acid in Acetonitrile as solvents in the mobile phase. For the glycoalkaloid analysis, the samples were also evaluated through the UPLC-MS using A: 10 mM of ammonia acetate in water titrated with formic acid, and B: 20% methanol in water, with an isogradient of 70%A/30%B for 20 minutes. Mass spectroscopy was used to further identify and quantify for profiling and glycoalkaloid analysis. The alkaloids in potato young shoots and eggplant cultivars were identified, and preliminary data was collected for the nightshade cultivars. The methodology was established, but needs to be improved for better resolution.

Introduction

Phytochemicals

Phytochemicals are mostly secondary metabolites in plants. They are non-essential nutrients to human beings, but have protective and disease-preventive properties [12]. Many phytochemicals are part of the plants' natural defense against damage by pests, other plants, the sun, and natural disasters. These plants pass along their protective benefit to the human body.

There are six major actions of phytochemicals in the human body [12]. Antioxidants are phytochemicals (such as carotenoids, flavonoids and polyphenols) that protect cells from oxidative damage by free radicals. The loss of an electron or oxidation produces free radicals, which scavenge other atoms for an electron creating more free radicals, causing oxidative stress and damage to the cells [13]. Antioxidants, by donating an electron without becoming a free radical itself, are capable of stabilizing free radicals before they can react and cause harm, in much the same way that a buffer stabilizes an acid to maintain normal pH [13].

Hormonal actions include stimulation and imitation. Some phytochemicals have the ability to stimulate hormonal actions in the human body, such as isoflavones found in soy, which can imitate human estrogen and is used to reduce menopausal symptoms and osteoporosis [12].

Stimulation of enzymes is a characteristic of indoles found in cabbage, which stimulates enzymes that make human estrogen less effective [12]. Interference of enzymatic activity is

exhibited by terpenes and protease inhibiters. Preventing the multiplication of cancer cells, saponins from beans interfere with cell DNA replication. Also, capsaicin found in hot pepper can protect cells from the destruction by carcinogens [12]. Allicin from garlic has antibacterial effects, another action of phytochemicals [12]. “Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry”[12].

Glycoalkaloids

Glycoalkaloids are naturally occurring phytochemicals that are nitrogen-containing plant steroids each with a carbohydrate side chain attached to the 3-OH position [4]. Glycoalkaloids in the *Solanaceae* family include chaconine, solanine, solasonine, and solamargine. *Solanum* is one of the most important and largest genera of the family *Solanaceae* comprising 84 genera and 3000 species [7]. *Solanum* species that are consumed in Africa and found in vegetable gardens include *S. macrocarpon*, *S. scabrum*, *S. villosum, S. nigrum,* and *S. americanum* [1, 5].

Beside its important role in food*, Solanum* also plays a crucial role in African traditional medicine. They have been traditionally used as an analgesic, antispasmodic, antiseptic, antidysentric, antinarcotic, emollient, diuretic, tonic, soporific, laxative, anticancer, antiulcer, and for disorders of the neuro-vegetative system [5, 7]. Some of these plants, especially *Solanum nigrum*, are considered poisonous in certain areas of the world [4]. Solanum leaves and immature fruits may contain high concentration of glycoalkaloids that can cause poisoning or even death when ingested in sufficient quantities. *Solanaceae* glycoalkaloids (based on a steroid structure bonded to different sugars) may play an important role in the defense mechanism of plants against pests, but they may also be harmful to human health [8].

Some glycoalkaloids can act as pesticides inhibiting enzymes such as acetycholinesterase and butyrylcholinesterase which catalyze the hydrolysis of the neurotransmitter acetylcholine at the synapse in the central nervous system of insects [6]. α-Chaconine and α-solanine are reversible inhibitors of human plasma butyrylcholinesterase [6]. It has been reported that these glycoalkaloids show both acute and chronic toxicity, and have a bitter taste [2]. There have been many studies concerning the glycoalkaloid content of potatoes, tomatoes, eggplants, and nightshades native to the Eastern world. The content {of glycoalkaloids in potatoes} may increase in response to environmental stress conditions such as light, frost and hail damage, and wounding during harvesting or post-harvest handling [2]. Low levels of glycoalkaloids are considered to be necessary for the proper flavoring of potato [9]. In contrast, elevated levels of glycoalkaloids result in a distinct bitter taste followed by a longer-lasting burning sensation in the throat [9]. It has been shown that in potatoes, leaves attain a maximum glycoalkaloid concentration first followed by an even higher concentration in unripe fruits and flowers [6]. African nightshades belong to the solanaceous family which includes potato, tomato, pepper and eggplant. African nightshades have been consumed frequently as a vegetable in most of African countries. However, other parts of the world are not familiar with these crops indigenous to Africa and very few studies have been conducted for African nightshades especially in nutrition and health aspects. There is a need to investigate the phytonutrients in African nightshade, particularly glycoalkaloids to better understand their nutritional contribution and potentially negative effect.

Objective

The overall objective of the project was to establish a protocol to profile major phytochemicals and quantify glycoalkaloids in African nightshades and compare the differences of these phytochemicals among and within African nightshade species obtained from central and eastern African countries.

The work detailed in this report was conducted during a two-month internship work and was part of a project with the specific aim to test various extraction methods and running conditions using UPLC-MS and to establish a protocol for analyses of freeze-dried African nightshade samples for phytochemical profiling and glycoalkaloid content.

Materials and Methods

Part I: Phytochemical profiling:

Plant Materials

A total of 40 African nightshade samples, representing two taste types (sweet and bitter) and seven species were included in the project. The seeds were collected from Cameroon, Kenya, and Tanzania and transferred to AVRDC-The World Vegetable Center in Taiwan with Material Transfer Agreement. Seeds were sowed and planted in an open field at AVRDC-the World Vegetable Center, a southern part of Taiwan in 2006. Leaves were harvested and freeze dried. Nutritional contents were measured previously at AVRDC. Samples were stored at -40ºC.

Extraction

Three samples among the 40 were selected (Table 1). Approximately 50 mg of each sample was weighed in a test tube containing 2ml of solvent. Three solvents including abs ethanol, methanol and 75% methanol in water, were tested. The test tubes were shaken in room temperature (about 25°C) for 2-3 hours. Samples were then centrifuged at 15000 rcf for 5 minutes. The supernatant was filtered and about 2-5 µL each sample was injected into the UPLC-MS.

UPLC Analysis

The LC-MS system includes an Acquity UPLC-PDA (ultra-performance liquid chromatograph- photodiode array detector, Waters Corporation: Milford, MA), equipped with Aquity Tandem Quadrupole Detector (TQD), Empower 3 Chromatography Data Software for UPLC analysis and Mass Lynx for MS analysis. The separation was carried out with an Aquity UPLC BEH C18 column (1.7µm), 3.0 x 100 mm i.d. (Waters, part no. 186004661) at 40ºC, a gradient of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) and flow rate at 0.2 mL/min. Several gradient conditions were tested for the separation of phytochemicals in the samples. The gradient of mobile phase A and B listed in the table below was used for presentation of the profile in this report.

The gradient of mobile phases A and B for UPLC analysis:

Time	%A	%B
0	99	1
3	85	15
25	70	30
35	45	55
53	20	80
65	0	100
70	0	100

Mass Spectroscopy

High-resolution Mass spectroscopy was performed using electrospray MS in positive ion mode. Samples were introduced by the UPLC by loop injection. Nitrogen gas was used as the nebulizer gas. MS source parameters were set with capillary, aperture 1 and cone voltages of 3000, 3 and 30 V, respectively. The desolvation gas and source temperatures were set at 450 oC and 150 oC, respectively, whereas the nebulizer and cone gases flow were respectively set at 800 L/h and 40 L/h. Full scan mass acquisition was performed by scanning an m/z range of 100–1500 Th in a 0.2 s per scan (with an inter-scan time delay of 0.01 s).

Part II: Glycoalkaloid Analysis

Plant Materials

A total of 40 African nightshade samples were used for the extraction. Due to the two month time frame, only 3 samples (Table 1) were included for the quantification of glycoalkaloids. Potato young shoots and eggplant leaves were also included in the study for a comparison of alkaloids. The young shoots of potato was obtained by purchasing potato from markets and putting the tubes at room temperature for several days to weeks to develop green shoots. The eggplant leaves were harvested from the AVRDC field one day before analysis. Both samples were fresh rather than freeze-dried as in nightshade.

Glycoalkaloid Extraction

Approximately 500 mg of nightshade powder was weighed in a test tube with 10 mL of 100% methanol added. The suspensions were sonicated until evenly distributed and then filtered. The clear solution was concentrated with nitrogen air flow to dryness and then dissolved in 5% formic acid in water.

Solid Phase Extraction (SPE Cleanup)

An OASIS HLB extraction cartridge was conditioned with 3mL of 100% methanol, and then equilibrated with 2 mL 5% formic acid in water. The extract was added, and then washed with 2 mL water and 2 mL of 30% methanol in water. The compounds were eluted with 5mL of 100% methanol then concentrated to dryness with nitrogen air flow. The extract was then dissolved in 5mL of 50% methanol in water for LCMS analysis.

Ultra Performance Liquid Chromatography (UPLC) analysis

The LC-MS equipment was the same as described previously. Glycoalkaloid separation was carried out with Aquity UPLC BEH C18 column (1.7µm), 2.1x100mm i.d. (part no. 186002352, Waters) at 40 ºC. 5µl of SPE extracted sample was injected. The mobile phases consisted of A: 10 mM of ammonia acetate in water titrated with formic acid, and B: 20% methanol in water. The flow rate was 0.3mL/min. An isogradient of 70%A/30%B for 20 minutes and 5 minutes wash in between each run was used.

Mass Spectroscopy

High-resolution Mass spectroscopy was performed using electrospray MS in positive ion mode. Samples were introduced by the UPLC by loop injection. Nitrogen gas was used as the nebulizer gas.

Results

This project mainly focused on the development of the method. The following figures show preliminary results, which will be improved, optimizing resolution.

Three nightshade samples were profiled. Figure 4 shows the best optimization at the present time of the three profiles. Figure 5 categorizes the phytochemicals according to Table 2, which shows the range of the UV wavelengths of the major phytochemicals in plants. Each of the nightshade samples shows a unique profile, however the retention times for peaks are similar with differing concentrations. There seems to be a noticeable difference in the flavanoid region of all three samples.

Table 2 is a summary of the nutritional data collected for these three nightshade samples. The nutritional data was calculated in 2007 by AVRDC-The World Vegetable Center Nutrition Unit in Taiwan. Interestingly, the taste of the leaves seem not to have as much of a correlation between the nutritional content as the species.

Figure 6 shows the total ion chromatograph of the two purchased glycoalkaloid standards, along with their respective UV wavelengths from the LC. These standards were chosen because they elute at the beginning (α-solamargine) and at the end (α-solamargine) of the glycoalkaloid region in published results. Though these two standards have similar retention times, wavelengths, and molecular weights they have differences visible with the MS/MS detection.

Figures 7-10 show the positive controls used in the experiment. Figure 7 shows the selected ion responding of the potato glycoalkaloids. Figure 8-10 show the three eggplant samples and their selected ion responding of the glycoalkaloids' respective molecular weights. The potato and eggplant samples showed significant peaks in their responding.

Figures 11-13 show the three nightshade samples' selected ion responding. There are no significant peaks shown by this responding. Better resolution and a MS/MS reading may show small amount of the glycoalkaloids in the African nightshades.

Table 1. African nightshade samples used in the study and their nutrient contents (per 100 g fresh weight) evaluated in 2007

Plot No.	15	17	7
Scientific name	*Solanum villosum*	*Solanum scabrum*	*Solanum scabrum*
Taste	Bitter	Sweet	Bitter
Origin	Kenya	Cameroon	Tanzania
Dry matter, g	9	7.5	7.3
Protein, g	3.68	3.23	3.18
Crude fiber, g	0.99	0.77	0.78
Free sugars, g	0.43	0.3	0.25
Carotenoids, mg			
Violaxanthin	1.71	1.38	1.26
Neoxanthin	0.94	0.87	0.71
Lutein	2.34	2.18	1.8
b-Carotene	1.72	1.37	1.4
α-Carotene	0.06	0.07	0.06

Vitamin C, mg	69	77	79
Calcium, mg	207	197	231
Iron, mg	2.11	2.06	1.83
Antioxidant activity, µmol trolox equivalent	366	490	511
total phenols, mg	91	127	154

Table 2. Major Phytochemicals in African nightshade leaves and their respective wavelengths, for use mainly as a reference when analyzing LC results

Phytochemical Group	Function in Plant	UV Wavelength
Alkaloids	plant defense/antibacterial	205-228
Anthocyanins	antioxidant	520-530
Cartenoids	antioxidant	425-480
Flavanoids	antioxidant	256-288
Phenolic Acids	antioxidant	256-376
Terpenoids	plant defense	243-295

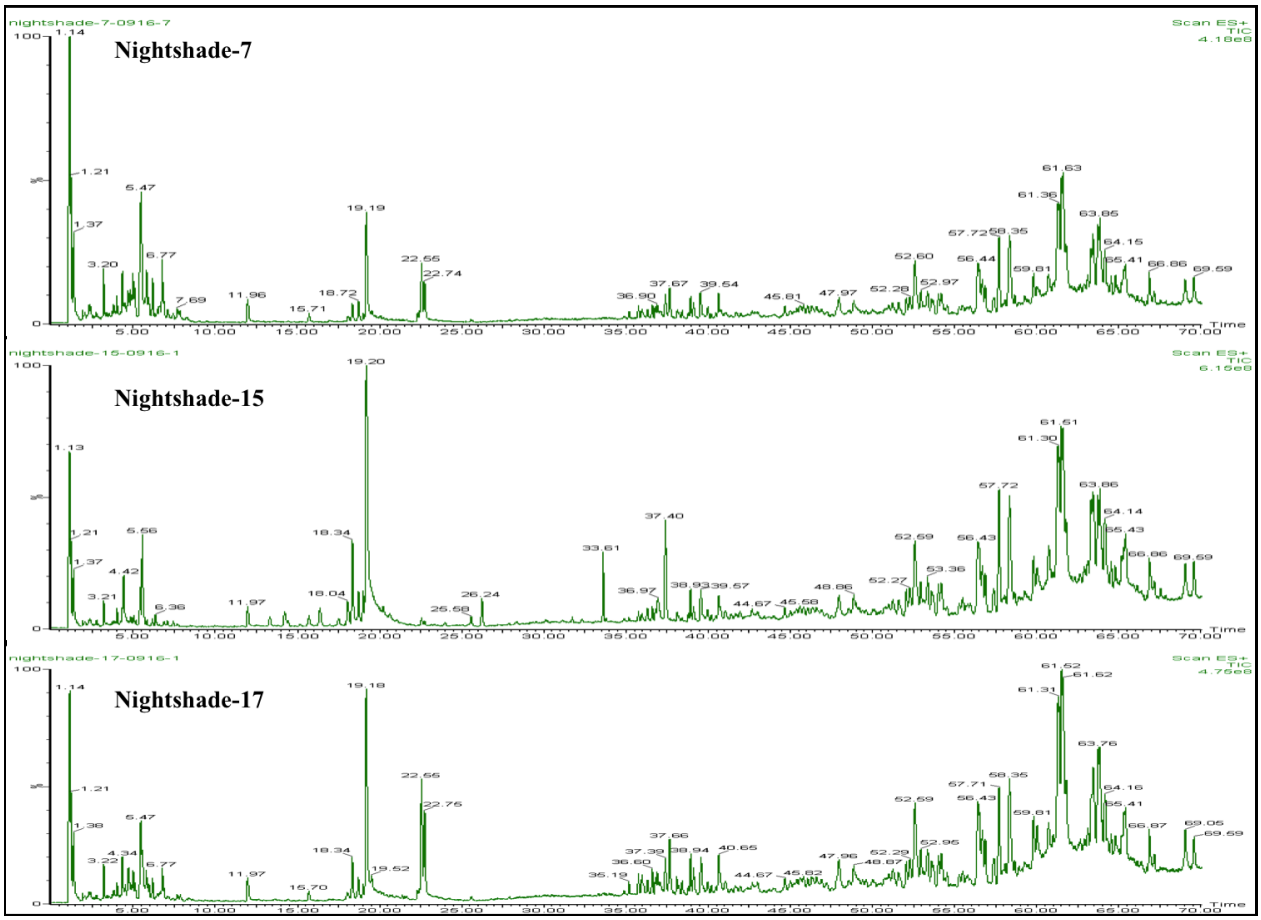

Figure 4. Total ion chromatography of phytochemicals extracted from three African nightshade samples. From the figure, the peak-retention times are quite similar, but the concentration (shown by the peak height).

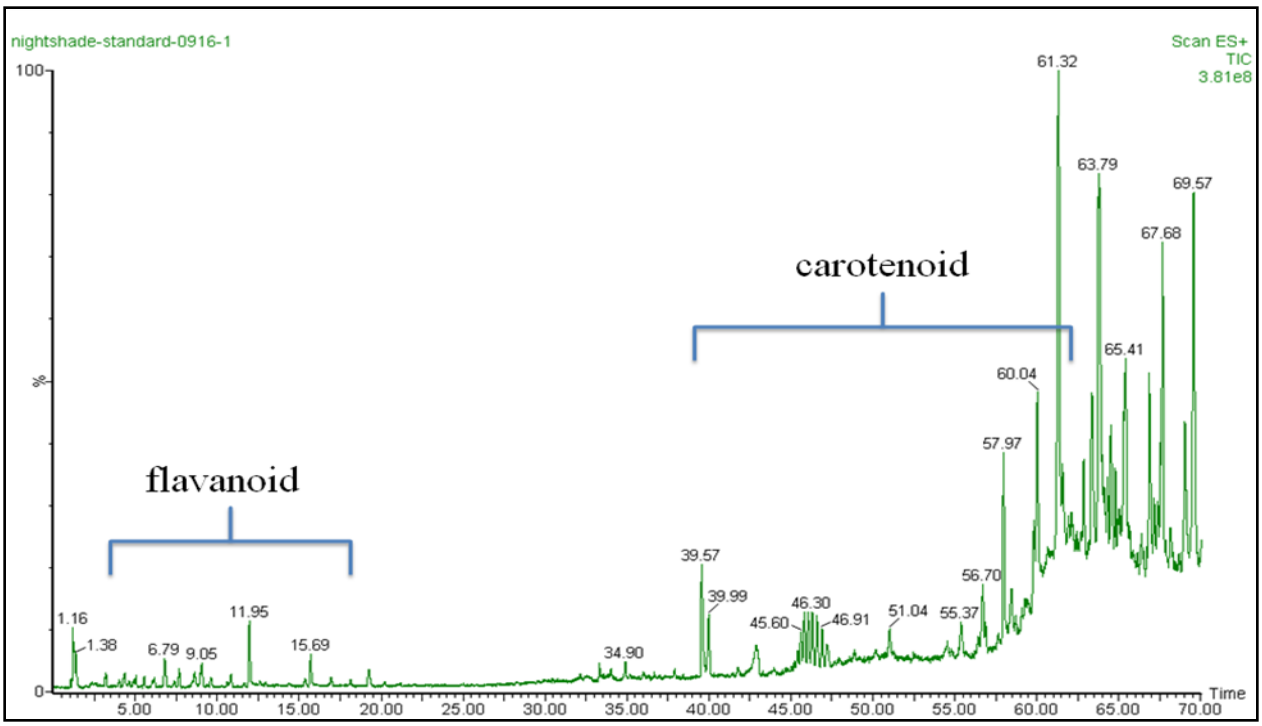

Figure 5: Total ion chromatography of African nightshade (17) and the groups of phytochemicals identified based on their UV/Vis absorption.

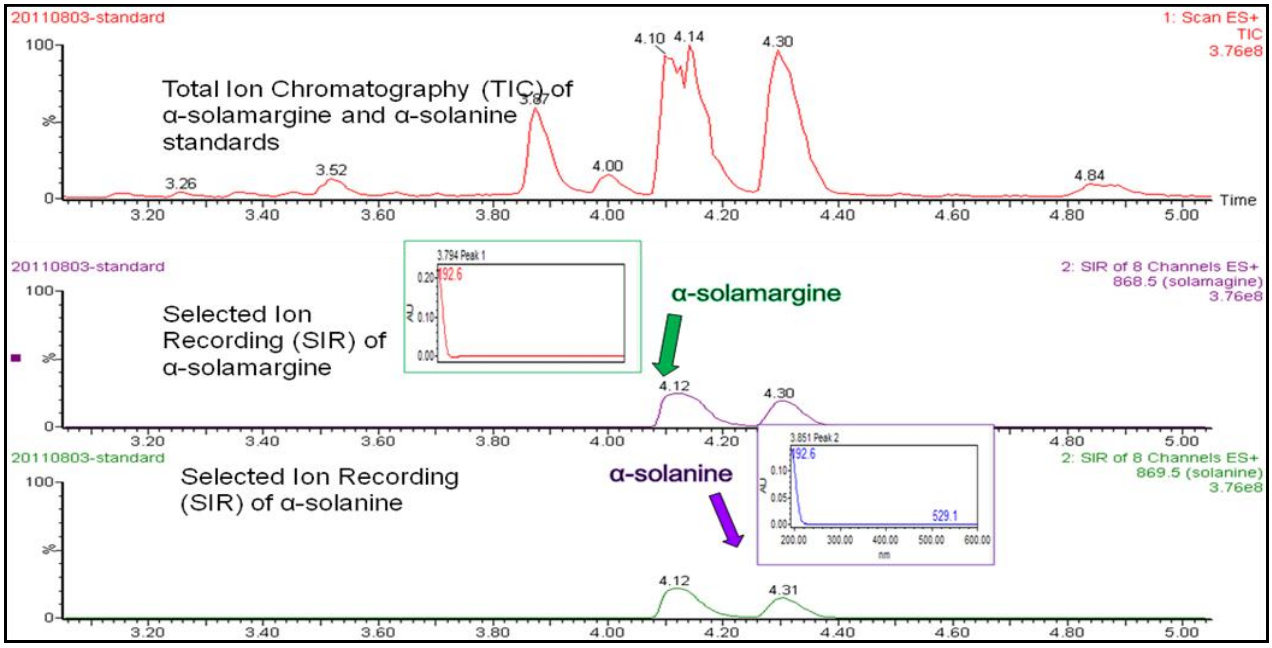

Figure 6: Total ion chromatography of commercial glycoalkaloid standards: α-solanine and α-solamargine with their respective LC peak images including wavelength.

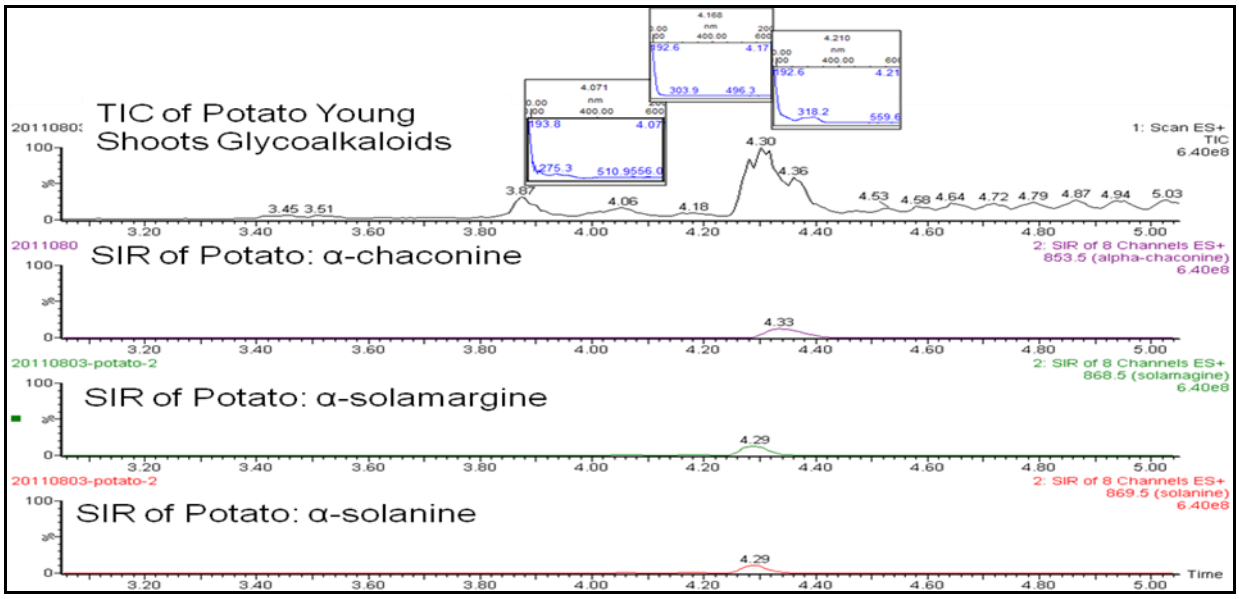

Figure 7: Total and selected ion chromatography of glycoalkaloids extracted from potato young shoots, including LC peak identifications. The SIR or Selected Ion Responding selects for the certain molecular weight of the compound. The compounds to select for were α-chaconine, α-solamargine, and α-solanine.

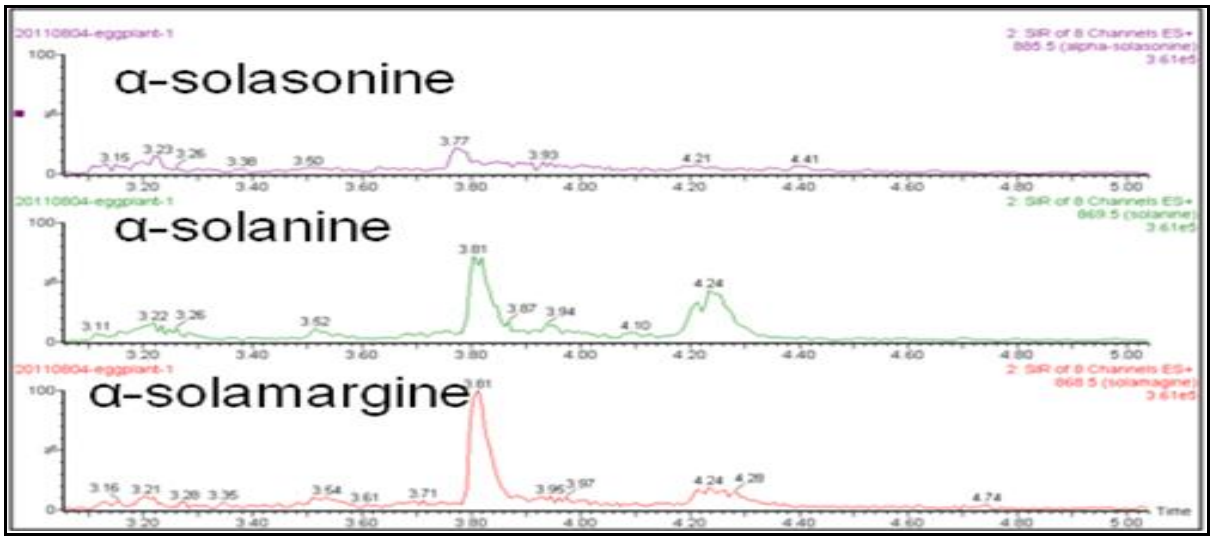

Figure 8. Selected ion chromatoagraphy of glycoalkaloids extracted from eggplant-1 (*Solanum melogena*) leaves, showing peaks at similar retention times for all three glycoalkaloids selected as marker molecular weights (α-solasonine, α-solanine, and α-solamargine)

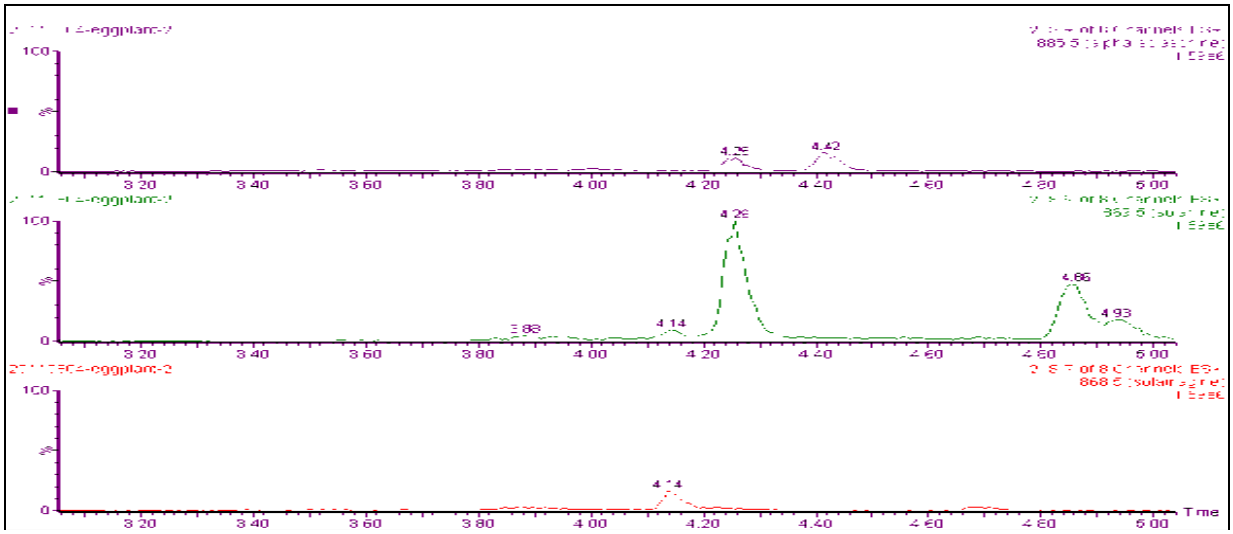

Figure 9 Selected ion chromatography of glycoalkaloids extracted from eggplant-2 (*Solanum aethiopicum*) leaves. Molecular weights used were that of α-solasonine, α-solanine, and α-solamargine.

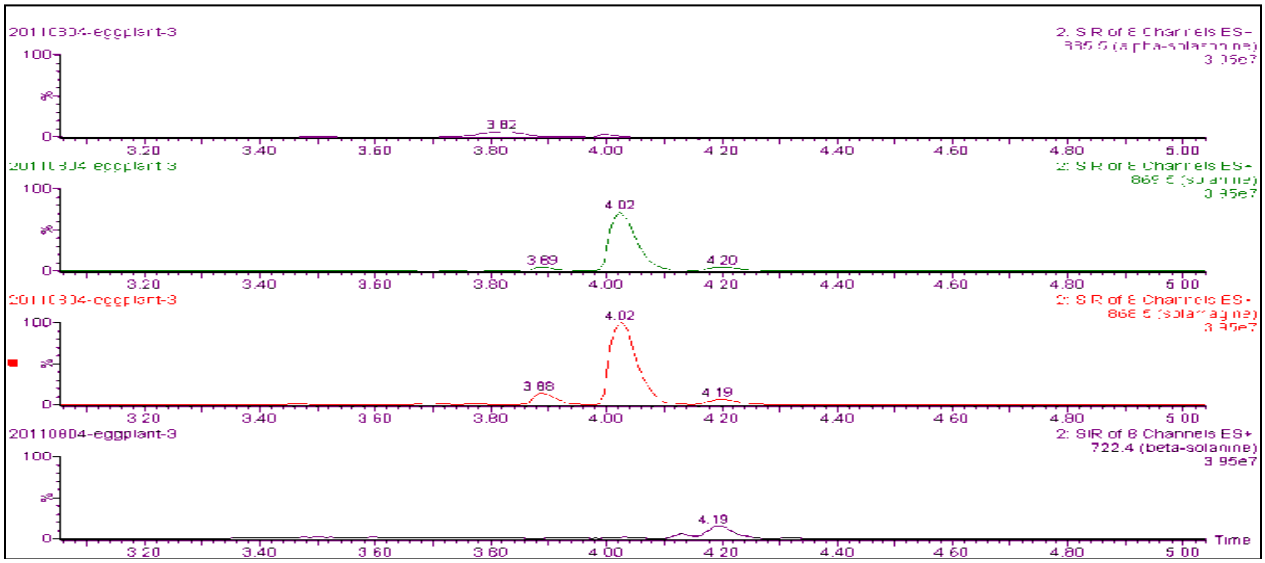

Figure 10: Selected ion chromatography of glycoalkaloids extracted from eggplant-3 (*Solanum macrocarpon*) leaves. Weights used were that of α-solasonine, α-solanine, α-solamargine, β-solamargine.

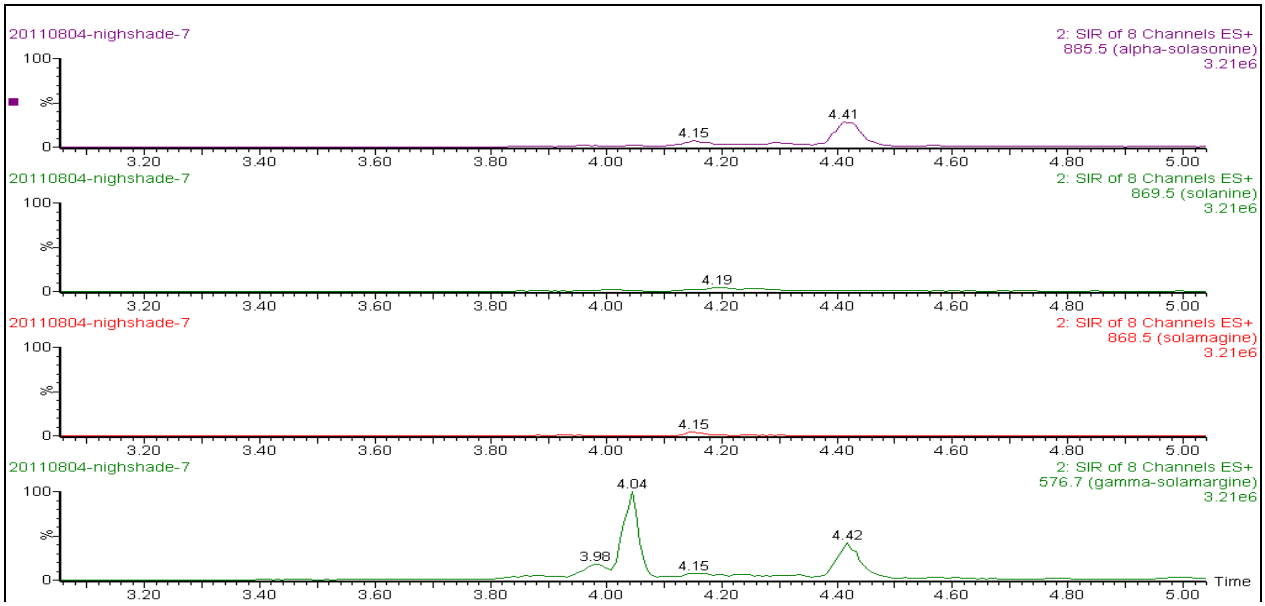

Figure 11: Selected ion chromatoagraphy of glycoalkaloids extracted from African nightshade No 7 (*Solanum scabrum*). Weights used were that of α-solasonine, α-solanine, α-solamargine, γ-solamargine.

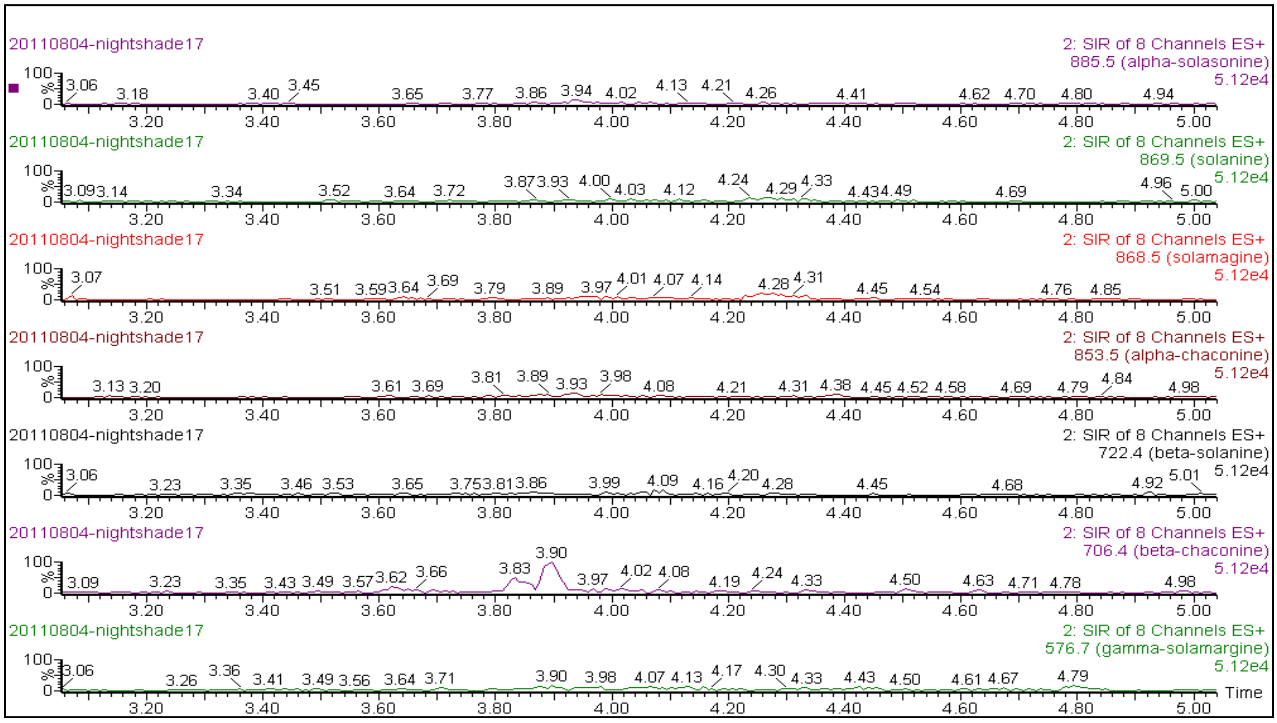

Figure 12: Selected ion chromatoagraphy of glycoalkaloids extracted from African nightshade No 17 (*Solanum scabrum*). Weights used were that of α-solasonine, α-solanine, α-solamargine, α-chaconine, β-solanine, β-chaconine, and γ-solamargine.

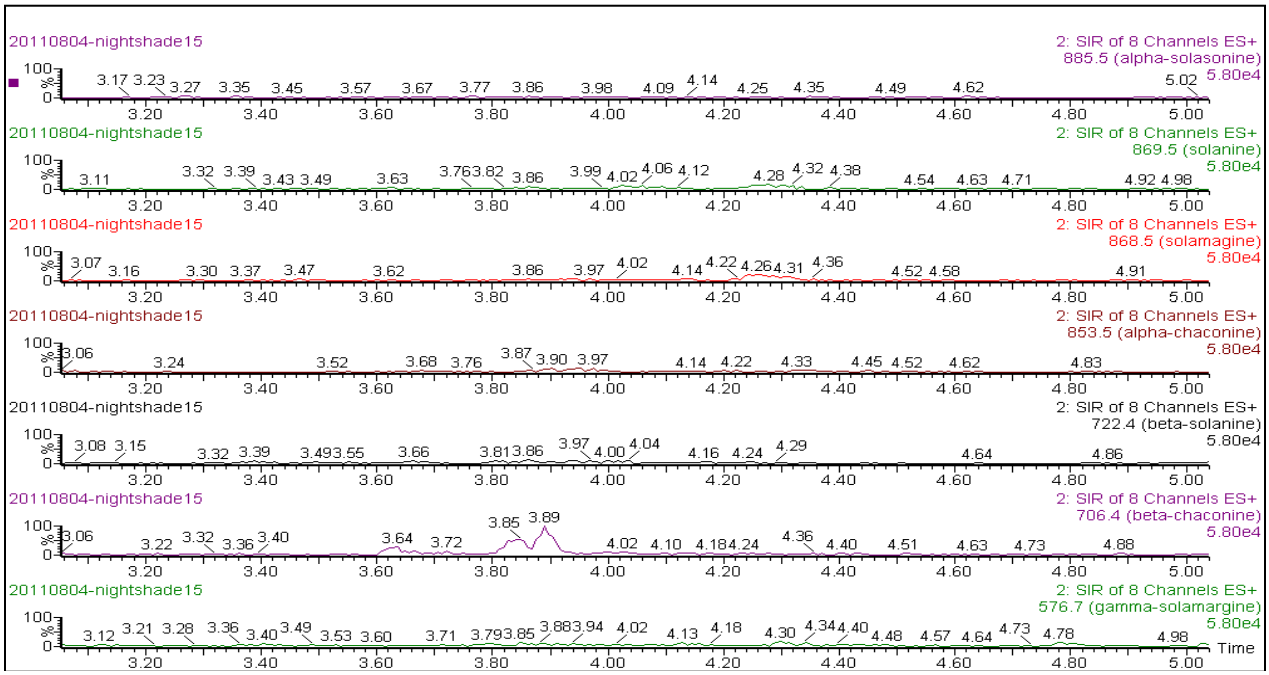

Figure 13: Selected ion chromatoagraphy of glycoalkaloids extracted from African nightshade No 15 (*Solanum villosum*). Weights used were that of α-solasonine, α-solanine, α-solamargine, α-chaconine, β-solanine, β-chaconine, and γ-solamargine.

Conclusion

The alkaloids in potato young shoots and eggplant cultivars were identified, and preliminary data was collected for the nightshade cultivars. The methodology was established, but needs to be improved for better resolution.

Future Improvements

In the future, the procedural plan is to optimize separation and resolution of the methods for phytochemical profiling and glycoalkaloid analysis. Also planned is identifying major phytochemicals in all 40 nightshade samples through profiling. Furthermore, the project plan is to run MS/MS to detect low concentrations of glycoalkaloids in African nightshade to quantify.

Impact on Food Security

African nightshades (*Solanum villosum* and *S. scabrum*) are indigenous to pan Africa region and has been consumed as a vegetable for centuries [14]. People can grow the plants in their farms, collect the leaves from the wild or purchase from local fresh markets for family consumption. The leaves are nutritious, rich in vitamins and minerals. The leaves contain small amount of alkaloids which may have medicinal and health promotion effect. African nightshade has an important role in household food and nutrition security. However, this vegetable was

neglected in the world and confused especially in the western world with the deadly nightshade (*Solanum nigrum*) which is different specie.

This research is part of the AVRDC Vegetable Metabolome project, which gives the world community insight on which vegetables to recommend to people groups around the world for growth in their communities. Part of being secure in the food supply, is being certain that crop is safe and contains the essentials for life. The Vegetable Metabolome projects seek to identify and quantify nutrients in indigenous vegetables as well as conventional vegetables.

Many people in the world are malnutrition. More research of traditional/ local vegetables for their nutritional and anti-nutritional factors is needed. Thus Greater use and consumption of nutrient-rich and safe vegetables would be promoted while precaution may need to be taken if certain IVs contain significant amounts of anti-nutrient factors, such as oxalates, alkaloids, poisonous and bitter-tasting substances.

Looking Back

Figure 14: Group Picture of most of the summer interns at the Malaysian's final presentation

My journey with the World Food Prize essentially began with my elder sister's adventure. As I saw how she was shaped and melded into the person she needed to become, I wanted that same valued experience. What began by wanting her experience morphed very quickly into a desire to change the world, however I could and still can. This fueled my first solo experience out of the country, on a church mission's trip to Aguaytia, Peru. There I helped a medical team by serving the people of Aguaytia and communicating through broken Spanish and hand signals what each patient was to do with the medicine they received at the clinic. I experienced life in rural and urban Peru, with the hardships and progress of their meager sustenance farming and factory life. There I found a passion in life to serve, with my hands, education, and intelligence in feeding the world. This is what really peaked my interest in the World Food Prize. Throughout the three years that I have been a small part of this organization, I haven't felt like a number or a statistic in the fight against hunger, but an integral part of the solution.

Figure 15: Neoh Weiting and I jumping (one of their favorite picture poses) in front of the city of Kaohsiung

Figure 16: Fulfilling one of my many dreams: becoming a hair model for Joyce

In my sophomore year of high school, I wrote a paper on what I viewed in Peru as the largest obstacle to development: infrastructure. From the Ohio WFP Youth Institute in September, I was selected to be one of six to represent Ohio to the Global Youth Institute in Des Moines, Iowa. I was blown away by the various aspects of hunger and the solutions tailored to each country, region, and group. "If you give a man a fish, he will eat for a

day. If you teach a man to fish, he'll eat for a lifetime. But, who controls the river?" This thought-provoking quote is the one thing I heard at GYI 2009 that will stick with me for the rest of my life. It gives voice to the complexity of world hunger; charity and education cannot solve the problem alone. The reworking of entire social groups in one country can have dire circumstances for those countries surrounding it. It must be a global cooperation that defeats hunger or only strife will ensue.

Figure 18: Lab work: Pipetting Methanol into the extraction cartridges

I brought my experiences back the next year to the Ohio WFP Youth Institute, writing a paper on education practices in Brazil. I was conflicted with the results of the institute; on one hand, I was not chosen as a delegate from Ohio for a second time, but on the other, it gave a chance to another candidate to experience what I had experienced the previous year. I realized that I was being somewhat selfish, because I thought being a delegate was everything. It is a fantastic opportunity, one I would never turn down, but I needed to be subservient for another's sake. In the greater scheme of things, it's not about me; it's about building an army of hunger-fighters or those who are moved to defeat world hunger and poverty through innovative solutions. Once I realized this, it opened my eyes to the world around me.

Figure 17: Posing with a beautiful lotus flower

I applied for the Borlaug-Ruan International Internship, never thinking I would be selected. The World Food Prize had already honored our family four years before, selecting my elder sister as a BR intern to China. When I was notified of the selection, I was ecstatic, to say the least. I started planning what I'd study, who I'd see, where I'd go. In the excitement, I almost forgot to see the 'big picture' and reason for this opportunity.

About a week and a half before the intern orientation, I was involved in a serious car crash. I had been going approximately 5 mph over the speed limit on a back road leading to my home, when I swerved to miss two oncoming vehicles. I lost control and rolled my car. This accident put the internship and the rest of my life in perspective. I've been placed on this Earth for a purpose. Now, I knew why God had honored me with the involvement of the World Food Prize and Borlaug-Ruan International Internship program.

Figure 19: Lin Shou, a lady from Communications, and I having a pulping party, i.e. separating Bitter Gourd into its components

Figure 20: Taiwanese men replacing the hood that was blown up

Throughout my summer in Taiwan, I met inspiring people, experienced exceeding human kindness and matured as a person during this time. Whether I was separating bitter gourds into their components, teaching the Malaysian interns to ice skate, traveling to Penghu, Sun Moon Lake, and Taipei with a German PhD, eating traditional Taiwanese food on my supervisor's day off, participating in a Taiwanese religious ceremony in Kaohsiung, becoming a tour guide for several Taiwanese interns at Anping, being on television at the lotus ponds with the Malaysian interns, swimming after dark with all the interns at AVRDC, or watching "romantic" (zombie) movies with coworkers, I experienced multiple aspects of the culture and people of Taiwan and I will always hold this experience in high esteem.

I will never forget my experience in the laboratory, as I mingled with doctorates and interns learning and teaching in turn. I learned laboratory techniques as we battled contamination in the experiment, translated Indian-English into English for my supervisor, and taught my fellow interns about the UPLC-MS as they taught me about their protein extractions, biochemical tests, and other related aspects of their research.

Figure 23: A group picture of most of the Taiwanese interns at the Funkoo bar singing KTV

The whole country, Taiwan, seemed like it was where I belonged. I grew to love their paradoxes: being so afraid of getting skin cancer that they would not walk out in the sun without a UV-protected umbrella, but having their whole families (sometimes five or six people) on one scooter and driving about 80 km/hour. I learned to enjoy their differences while they embraced mine. We shared many memories singing Taiwanese, American, and Korean pop songs in KTVs, as well as sharing our political views in and outside the workplace. While I was in Taiwan, I attended two very different churches. I experienced Catholicism at the church in Shanhua, where I felt out of place, the only time I was in Taiwan. Mostly, it was awkward, only because I thought if it were an English service, there'd be Europeans or Americans attending. I was sorely mistaken, but after the initial shock, I attempted to fit in as I attended the only Catholic service I've ever been to! The other church in Tainan, was nothing but Chinese folks, was an awesome experience. My friend Jen from the Nutrition unit took me to her church and translated for me. Afterwards, we interacted with all the people from the church over a take-out lunch.

Figure 24: Jen's pastor at the Chinese-speaking church and I

Figure 25: On the Maokong Gondola in Taipei

While in Taiwan, I interacted with renowned researchers, farmers, and just normal people on a level I had never experienced. I learned more about myself than I could ever dream. Where the car accident left no doubt as to the reason I was placed on this Earth for some grand purpose, the experience in Taiwan put that purpose into words. I found my purpose for now: to let go of things that don't matter or inconsequential. As Mark Twain once said, "Twenty years from now, you will be more disappointed by the things you didn't do than the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails. Explore. Dream. Discover."

Citations

[1] *AVRDC*, 2011. “Africa: African Nightshade.” http://avrdc.org/index.php?id=108

[2] Chuda, Yoshihiro.; Tsuda, Shogo.; Ohara-Takada, Akiko.; Kobayashi, Akira.; Suzuki, Kazuaki.; Ono, Hiroshi.; Yoshida, Mitsuru.; Nagata, Tadahiro.; Kobayashi, Shoichi.; Mori, Motoyuki. “Quantification of Light-Induced Glycoalkaloids, α-Solanine and α-Chaconine, in Four Potato Cultivars (*Solanum tuberosum* L.) Distributed in Japan by LC/MS.” *Food Sci. Technol. Res.,* 2004, *10 (3)*, 341-345.

[3] Eldridge, Arthur C.; Hockridge, Mary E. “High-Performance Liquid Chromatographic Separation of Eastern Black Nightshade (*Solanum ptycanthum*) Glycoalkaloids.” *J. Agric. Food Chem.* 1983, *31*, 1218-1220.

[4] Friedman, Mendel. “Analysis of biologically active compounds in potatoes (*Solanum tuberosum*), tomatoes *(Lycoperisicon esculentum*), and jimson weed (*Datura stramonium*) seeds.” *Journal of Chromatography A.,* 2004, *1054*, 143-155.

[5] Infonet-Biovision. “African Nightshade.” http://www.infonet-biovision.org/print/ct/142/crops

[6] Friedman, Mendel. “Potato Glycoalkaloids and Metabolites: Roles in the Plant and in the Diet.” *J. Agric. Food Chem.,* 2006, *54*, 8655-8681.

[7] Mohy-ud-din, Ayesha.; Khan, Zaheer-ud-din.; Ahmad, Mashtaq.; Kashmiri, Muhammad Akram. “Chemotaxonomic Value of Alkaloids in *Solanum nigrum* Complex.” *Pak. J. Bot.,* 2010, *42 (1)*, 653-660.

[8] Sanchez-Mata, Maria.; Yokoyama, Wallace E.; Hong, Yun-Jeong.; Prohens, Jaime. “α-Solasonine and α-Solamargine Contents of Gboma (*Solanum macrocarpon* L.) and Scarlet (*Solanum aethiopicum* L.) Eggplants.” *J. Agric. Food Chem.* 2010, *58*, 5502-5508.

[9] Slanina, P. “Solanine (Glycoalkaloids) in Potatoes: Toxicological Evaluation.” *Fd. Chem. Toxic.,* 1990, *28 (11)*, 759-761.

[10]Dao, Lan.; Friedman, Mendel. *J. Agric. Food Chem.* 1996, *44* (8), 2287-2291

[11] Sotelo, Angela.; Serrano, Beatriz., *J. Agric. Food Chem.,* 2000, *48* (6), 2472-2475

[12] *Phytochemicals*. “What are Phytochemicals?”: Top Cultures, Belgium. Available: www.phytochemicals.info

[13] Food InSight, “Functional Food Fact Sheet: Antioxidants.” 2009. Available: http://www.foodinsight.org/Resources/Detail.aspx?topic=Functional_Foods_Fact_Sheet_Antioxidants

[14] PROTA. 2004. Plant Resources of Tropical Africa 2: vegetables. In: Grubben, G. J. H., Denton, O. A., Messiaen, C. M., Schippers, R. R., Lemmens, R. H. M. J., Oyen, L. P. A. (eds), PROTA Foundation, Wageningen, Netherlands.