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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

MANGIFERIN AS A BIOMARKER FOR MANGO ANTHRACNOSE RESISTANCE

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

Herma Pierre

2015

To: Dean Michael Heithaus
College of Arts and Sciences

This thesis, written by Herma Pierre, and entitled Mangiferin as a Biomarker for Mango Anthracnose Resistance, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

Yong Cai

David N. Kuhn

Kateel Shetty

Krishnaswamy Jayachandran, Major Professor

Date of Defense: July 2, 2015

The thesis of Herma Pierre is approved.

Dean Michael Heithaus
College of Arts and Sciences

Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2015

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DEDICATION

I dedicate this to my mother, Marie H. Dugé. Mèsi pou sipò ou. Ou te gen yon gwo sipò plis pase de dènye ane yo epi mwen vrèman apresye ou. Mwen renmen ou!

--Foufoune

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To Lisa Mosser, thank you for your invaluable expertise and for trusting me and allowing me to operate the machinery in your lab. I learned a great deal working with you and your input was truly valuable in my thesis. If I didn't know what area in chemistry to pursue before working on my thesis in your lab, I certainly know it now.

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ABSTRACT OF THE THESIS

MANGIFERIN AS A BIOMARKER FOR

MANGO ANTHRACNOSE RESISTANCE

by

Herma Pierre

Florida International University, 2015

Miami, Florida

Professor Krishnaswamy Jayachandran, Major Professor

Mangos (*Mangifera indica* L.) are tropical/subtropical fruits belonging to the plant family Anacardiaceae. Anthracnose is the most deleterious disease of mango both in the field and during postharvest handling. It is most commonly caused by the *Colletotrichum gloeosporioides* complex. Mangiferin, a xanthanoid compound found in at least twelve plant families worldwide (Luo *et al*., 2012), is present in large amounts of the leaves and edible mangos. Even though this compound plays a pivotal role in the plant's defense against biotic and abiotic stressors, no correlations been made between the compound and mango anthracnose resistance.

Mangos were collected, grouped according to their countries of origin, and evaluated for their mangiferin concentrations at four different stages of development. Extracts of interest were then tested against different strains of *C. gloeosporioides*. The results demonstrated that mangiferin concentrations are significantly different at different stages in fruit development. The antifungal assays were inconclusive.

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I. INTRODUCTION

Mangos (*Mangifera indica* L.) are tropical/subtropical fruits that belong to the plant family Anacardiaceae. Known as the "king of fruits", thousands of cultivars exist worldwide and mangos are one of the world's most important fruit crops (Morton, 1987). Extensive plant breeding has led to the generation of over a thousand cultivars whose fruits show a pronounced diversity in color, shape, flavor, and texture (Berardini *et al.*, 2005) and its global production exceeded 26 million tons in 2004 (Barreto *et al.*, 2004). The dry season stimulates the formation of mango blossoms. The wet, highly humid (>95%) season that follows stimulates the proliferation and development of a deleterious disease known as anthracnose (Nelson, 2008; Chang *et al*, 2012). Post-harvest losses attributable to this disease are substantial (Nelson 2008).

Anthracnose is the most deleterious disease of mango both in the field and during postharvest handling. It is most commonly caused by a complex of fungi called the *Colletotrichum gloeosporioides* complex (*C. gloeosporioides* Penz. and Sacc. In Penz; *C. asianum*; and the nom. nov. *C. queenslandicum*), and in some cases, caused by *C. acutatum* J. H. Simmonds. Fruits without anthracnose lesions are not guaranteed to be anthracnose-free since the infections are quiescent (Chang *et al*, 2012; Karunanayake *et al.*, 2011; Weir *et al*., 2012).

Symptoms include dark lesions on the leaves, panicles, and petioles of the mango tree as well as sunken, prominent dark spots on the fruits. The dark spots on fruits typically coalesce and (ultimately) penetrate deep within the fruit which results in extensive rotting. As of 2008, the most resistant varieties in Florida were Zill and Haden; the most susceptible varieties were Irwin, Sensation, Kent, Keitt, and Tommy Atkins

(Nelson 2008). The resistance of some varieties to *C. gloeosporioides* has been attributed to the high concentrations of antifungal 5-substituted resorcinols in the peel and latex of mango (Karunanayake *et al.*, 2011; Knödler *et al*., 2007).

Alkylresorcinols are compounds found in high concentrations in the peel and latex of mangos (Karunanayake *et al*., 2011; Knödler *et al*., 2007). These compounds have been reported to exhibit antioxidant and antigenotoxic properties. Recent studies have shown that alk(en)ylresorcinol composition consists of a complex pool of C15-, C17-, and C19-substituted resorcinols with various degrees of unsaturation (Knödler *et al*., 2007).

Mangiferin is a xanthanoid compound found in at least twelve plant families worldwide (Luo *et al*., 2012). Present in large amounts in the leaves and edible mangos, this compound has been evaluated for numerous pharmacological applications including antibacterial, antitumor, anti-HIV, antioxidative, and antiviral (Masibo, He, 2008; Singh *et al*., 2012). Also, mangiferin protects the plant from various forms of biotic and abiotic stress (Luo *et al*, 2012). Even though mangiferin plays a pivotal role in the plant's defense against biotic and abiotic stressors, no correlations been made between the compound and mango anthracnose resistance.

The objectives of the present project were to (1) Determine the mangiferin content in the fruit exocarp at four different stages of fruit development of mangos originating in four different regions; (2) Determine if there was a statistical difference in mangiferin content between the different regions of origin; and (3) Determine if there was a correlation between mangiferin content and mango anthracnose resistance *via* antifungal assays against *C. gloeosporioides* Penz. and Sacc. In Penz.

Once the mangiferin content was quantified, the data were analyzed *via* a repeated measures analysis of variance (Repeated Measures ANOVA) to determine if there was a statistical difference in mangiferin content among regions as well as among mangiferin content at different stages of development with the two independent factors being *Region* and *Stage of Development*.

Extracts of interest (containing incredibly high and low amounts of mangiferin) were analyzed for any antifungal capabilities against four different strains of *C. gloeosporioides* Penz. and Sacc. In Penz. A two-way ANOVA was utilized to determine if there was a statistical difference between antimicrobial activity between the four different strains of *C. gloeosporioides* Penz. and Sacc. In Penz. as well as between the extracts applied to the fungi.

Mangiferin operates as a defensive compound against biotic and abiotic stressors. Consequently, it is probable that the compound is produced in higher amounts in regions where UV rays are at high levels and in areas that are conducive for microbial growth *i.e.,* areas in and around the Equator. Therefore, it was hypothesized that the mangiferin content would the highest in Region 4 (Pacific/South America/West Indies) and the lowest in Region 2 (Florida/Hawaii/Israel) during all four stages of development and that there would be a negative correlation between fungus growth and mangiferin concentrations. The control cultivars 'Neelum' and 'Zebda' were reportedly highly susceptible and highly resistant to mango anthracnose, respectively. As such, it was hypothesized that the mangiferin content would be considerably low and considerably high, respectively.

II. MANGOS

2.1 Introduction

Mangos (*Mangifera indica* L.) are tropical/subtropical fruits that belong to the plant family Anacardiaceae. These fruits are famous for their delicious taste, intense peel color, strong aroma, and high amounts of bioactive compounds (*e.g.,* phenolic compounds, minerals, and β-carotene. According to literature, mangos originated approximately 4000 years ago from the Indo-Burmese region (Luo *et al*, 2012). The Persians carried mangos to East Africa around the 10th Century A.D. then the Portuguese introduced it to West Africa and Brazil around the 16th Century. After being established in Brazil, the species was carried to Barbados in 1742 then to the Dominican Republic (Morton, 1987). Extensive plant breeding has led to the genesis of hundreds of cultivars worldwide. In fact, over the last 500 years, mangos have become well-established in tropical locations in the United States (*e.g.,* Hawaii and Florida) and Florida is considered a secondary center for genetic diversity (Schnell *et al*., 2006). The different accessions of mango show diversity in color, shape, flavor, seed size, and composition (Berardini *et al.*, 2005).

Mangos are grown commercially in more than 87 countries and its global production exceeded 26 million tons in 2004 (Barreto *et al*, 2004). According to the FAO, in 2010, India—the world's largest producer of mangos—produced approximately 15 million tons of mangos and had a 40.48% share in the world's total production, followed by China which produced approximately 4.35 million tons of mangos and had an 11.72% share in the world's total production, and Thailand which produced approximately 2.55 million tons of mangos.

2.2 Mango Chemical Constituents

Mangos are known to be excellent sources of different essential vitamins (*e.g.,* β-carotene, ascorbic acid, niacin, and riboflavin). The chemical composition of mangos depends on the variety, location of cultivation, and stage of maturity of the mango plant (Luo *et al*., 2012). Typically, the mango pulp consists of gallic acid, mangiferin, quercetin glycosides, and tannins (Schieber *et al*., 2000)

2.3 Mango Fruit Development

Mango fruits exhibit simple sigmoid-type growth patterns and have three stages of growth. Stage I consists of the first two weeks from the fruit set and is characterized by a slow growth rate. Stage II consists of weeks 3 through 8 and is characterized by an increased growth rate. Stage III consists of weeks 13 through 16. In this stage, growth occurs at a slower rate until maturity. The duration of each stage varies by cultivar and is influenced by climatic, edaphic, and cultural factors (Ponce de León *et al*., 2000).

III. MANGIFERIN

3.1 Structural Information

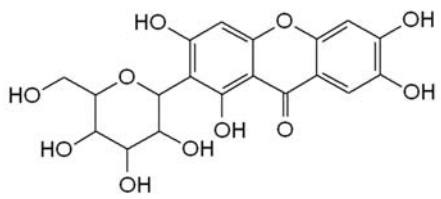


Figure 1. Mangiferin

Mangiferin (1,3,6,7-Tetrahydroxyxanthone-C_2-β-D-glucoside) (**Figure 1**) is a xanthanoid found in the plant species *Mangifera indica* and *Anemarrhena asphodeloides*. With a molecular weight of 422 g/mol, mangiferin is widely distributed in approximately twelve higher plant families (Luo *et al*., 2012). This potent fungistatic compound is present in the leaves, stem bark, fruits, roots, and heartwood of mango trees (Chakrabarti, 2011; Rao *et al*., 2008; Singh *et al*., 2012). It is an important secondary metabolite that protects the plant against various biotic and abiotic stressors (Luo *et al*., 2012).

Polyphenols are known to capture free radicals *via* donating hydrogen atoms or electrons which neutralizes free radicals. The bioactivity of polyphenols may be related to its ability to chelate metals and inhibit lipooxygenases (Stoilova, *et al*., 2008). Lipooxygenases (*i.e*., oxidoreductases) are of the enzyme class 1.13.11. These iron-containing enzymes catalyze the dioxygenation of polyunsaturated fatty acids in lipids containing a cis, cis-1,4-pentadiene structure. Lipooxygenases act on the CH-OH group of donors and act on single donors with the incorporation of molecular oxygen (Enzyme, 2011). It has been reported that the synthesis and accumulation of phenolic compounds impart disease resistance in plants (Chakrabarti, 2011).

Arising from two different aromatization pathways—the Shikimate and Ketate (Acetate) pathways (Wauthoz *et al*., 2007)—mangiferin possesses eight hydroxyl groups: four are within the glucopyranosyl system and the other four are directly engaged on the xanthone skeleton (Gómez-Zaleta *et al*., 2006). With its fused ring formation, free rotation about the C-C bonds does not occur. When packing mangiferin, stabilization is credited to the hydrogen bonds of the hydroxyl (-OH) hydrogens present in the molecule and by crystallization of water molecules (hydroxyl acts as a hydrogen donor and acceptor) (da Cruz Jr. *et al*., 2008). It can be assumed that this characteristic attributes to the high melting point (270°C) of mangiferin.

3.1.1 The Xanthone Backbone.

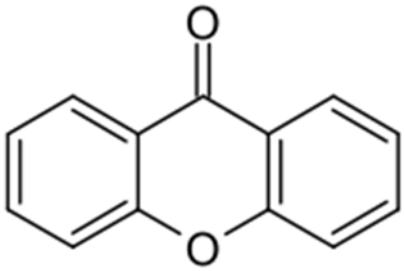


Figure 2. Xanthone Backbone.

As mentioned above, mangiferin is partly composed of a xanthone backbone (**Figure 2**). Xanthone is a heterocyclic compound with a boiling point of 350°C and a melting point of 174°C. These heat stable molecules are one of the most potent antioxidants known. In fact, these "super antioxidants" are thought to be more potent than vitamins C and E (Masibo, He, 2008). An unsubstituted xanthone ring is not known to occur in nature (Thomasek, Crawford, 1986).

Figure 4. An example of a quinone: 1,4-Benzoquinone.

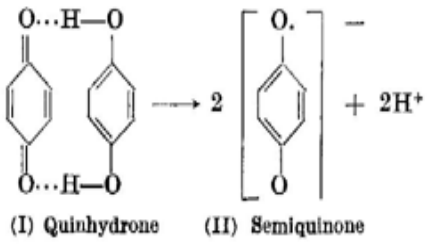


Figure 3. Semiquinone radical.

3.1.2 The Catechol Moiety.

Mangiferin bears a catechol moiety (pyrocatechol or 1,2-dihydroxybenzene) (**Figure 5**) which is a pharmacophore with a well-established anti-oxidant property. Pharmacophores are a set of structural features in a molecule recognized at a receptor site responsible for a molecule's biological activity (Volpato *et al*., 2009). Catechol moieties scavenge highly reactive species (*e.g*., peroxynitrile). Mangiferin activity (which involves the enzyme polyphenol oxidase along with the catechol moiety) gives rise to oxidized products like semiquinone radicals and quinones (**Figures 3** and **4**). These products present potential toxicity owing to their oxidant capacities. Semiquinones are potentially toxic compounds, especially in virtue of their ability to arylate protein thiol groups. Quinones are weak electrophiles that can deplete cellular thiols *via* the Michael Addition reaction to form dithioethers and *via* peroxide-mediated oxidation to form disulfides. Catechol can readily condense to form heterocyclic compounds. For instance, catechol produces quinones with the addition of cyanide (CN). Catechol moieties are present in numerous flavonoids and in catecholamines. Small amounts of

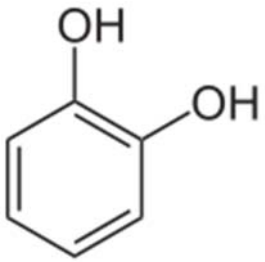


Figure 5. Catechol moiety.

catechol also occur in fruits and vegetables along with the enzyme polyphenol oxidase (reacts to form benzoquinone) (Andreu *et al*., 2005).

3.2 Mangiferin Identification

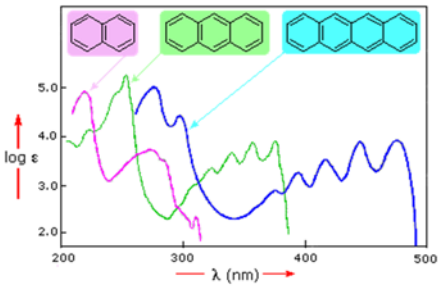


Figure 6. Example of the effect of conjugation on absorbance.

Mangiferin can be identified using an array of techniques such as high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), UV-*vis* spectroscopy, the Folin Ciocalteau method, and mass spectrometry (MS). The present project utilizes HPLC to separate the components of the extract, UV-*vis* spectroscopy to confirm the presence of the compound of interest, and MS to identify the mangiferin and confirm its presence in the extract.

Ultraviolet-*visible* (UV-*vis*) spectroscopy is an analytical technique involving absorbance spectroscopy in the ultraviolet-visible spectral range. Absorbance of radiation is measured as a function of frequency or wavelength because of its interaction with a sample. Absorbance occurs with conjugated compounds. The extent and location of conjugation determines the type of absorption that occurs. It also affects the amount of energy (in the form of waves) that is absorbed by the compound (for an example, see **Figure 6**).

Mangiferin has lambda maxes at 240nm, 230-260nm, 317nm, and 366nm (**Figure 7**). Absorbance at 240nm is the result of the medium energy π-π^* transition of the aromatic ring. Absorbance at 230-260nm is the result of a π-π^* transition in the s-trans

enone system by the cyclic β-diketone presence. Absorbance at 317nm is related to the n-π^* transitions of the aromatic ring. Absorbance at 366nm is caused by the intramolecular charge-transfer absorption (Gómez-Zaleta *et al*. 2006).

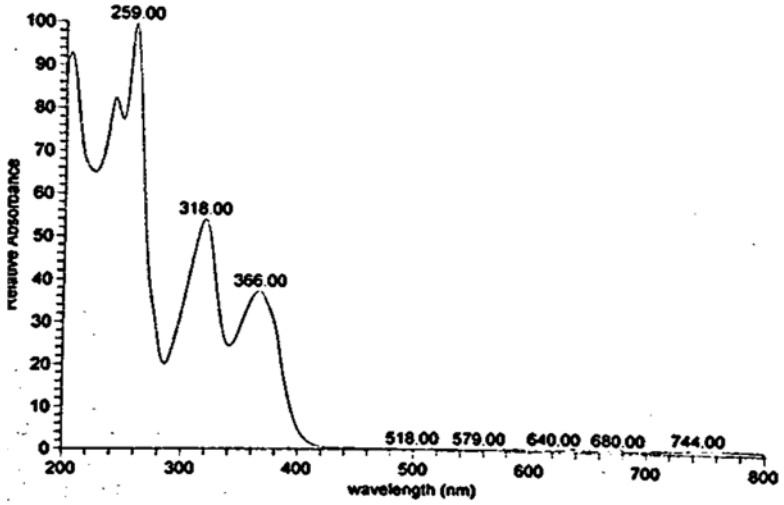


Figure 7. UV absorbance of mangiferin.

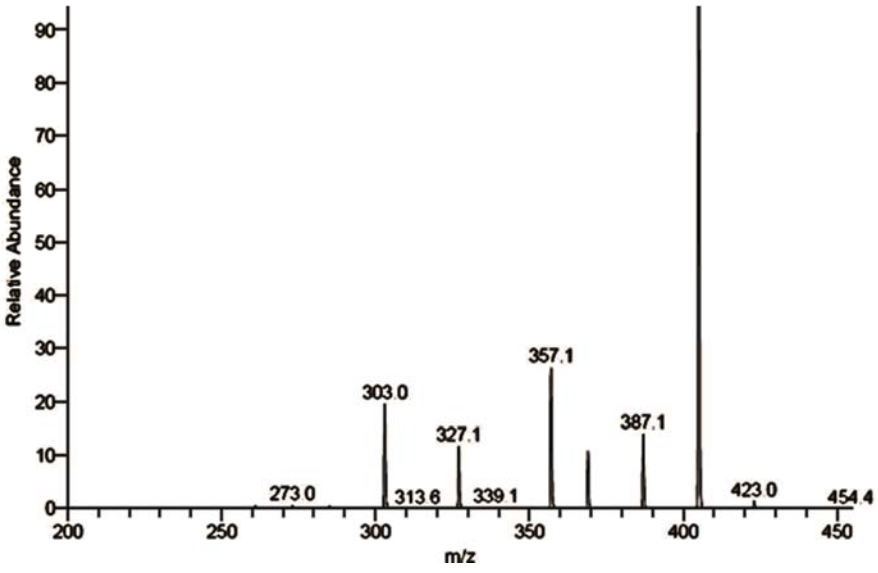


Figure 8. Mangiferin MS/MS fragmentation pattern.

Mass spectrometry is an analytical technique that helps to identify the amount and type of analytes present in a sample by measuring the mass-to-charge (*m/z*) ratio and abundance of gas-phase ions. Atoms or molecules in a sample can be identified by correlating known masses to identified masses or a characteristic fragmentation pattern.

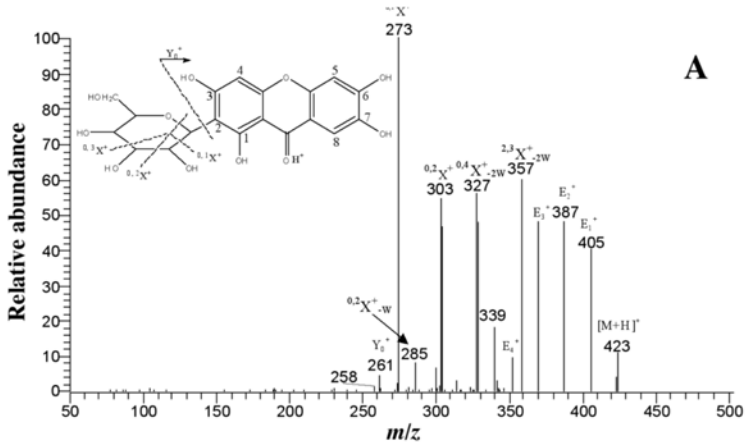


Figure 9. Proposed Fragmentation Pattern of Mangiferin by Sun *et al.* (2009).

Mangiferin has a parent peak at 423 *m/z*, a base peak at 405 *m/z*, and characteristic peaks at 387 *m/z*, 357 *m/z*, 327 *m/z*, and 303 *m/z* (**Figure 8**). The fragments 405 *m/z* and 387 *m/z* may be because of the hydrolysis of the hydroxyl groups that comprise the catechol moiety. The other fragments can be explained by the proposed fragmentation scheme constructed by Sun *et al.* (2009) (**Figure 9**).

3.3 Biological Role in the Plant

Mangiferin is synthesized in the leaves of mango trees and stored in the bark. When a species is inflicted with any form of stress (*e.g.,* cut injury or infection by a pathogen), mangiferin is produced along with its aglycone, norathyriol, and accumulates in the injured organs. Mangiferin is a major constituent of young leaves as well as the

bark of mango trees. It is also utilized in forming new shoots and leaves and is transferred to growing regions during flushing. The compound also serves as a micronutrient carrier molecule.

3.4 Report on Mangiferin's Role Against Fusarium spp.

Mangiferin is a potential biochemical indicator for screening mango genotypes to malformation disease (*Fusarium mangiferae* Britz., M.J. Wingf. & Marasas). Whenever *Fusarium* spp. is present, mangiferin halts the ingress of the fungi into host organelles by oxidizing into polymeric quinones (along with gallic acid conjugates). These quinones cause the collapse of adjoining cells. The collapsing of adjoining cells removes the nutrition source of the fungus and therefore prevents the multiplication of the fungus. Mangiferin also stops the breakdown of host starch. This process removes the nutrition source the pathogen requires to grow and sporulate. Mangiferin stimulates fungal growth at lower concentrations and inhibits growth at higher concentrations. In fact, malformation-resistant mango cultivars have been reported to have higher mangiferin contents. Inversely, if mangiferin accumulates excessively at the site of infection, this results in the manifestation of disease symptoms *via* a wide range of biological and physiological aberrations in the host cells. Accumulated mangiferin causes (1) an increased IAA (indole-3-acetic acid) content which results in an increase in vegetative growth, (2) increased chlorophyll content which causes malformed shoots or panicles to look greener, (3) increased photosynthesis which leads to more carbohydrate synthesis, (4) reduced respiration and amylase activity which causes carbohydrate accumulation, thereby disturbing the C/N ratio, (5) reduced catabolism which increases longevity, and

(6) reduced transpiration which causes a high moisture content. Mangiferin also induces significant morphological and physiological changes in the *Fusarium* pathogen, thereby creating a new physiological race (Chakrabarti, 2011).

IV. MANGO ANTHRACNOSE

Figure 10. Healthy mango (left) and mangos infected with anthracnose (right) (Nelson, 2008).

Anthracnose is the most deleterious disease of mango trees both in the field and during postharvest handling. It is most commonly caused by the teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk (anamorph: *Colletotrichum gloeosporioides* Penz. and Sacc. In Penz) and in some cases caused by *C. acutatum* J. H. Simmonds. Spores germinate and form penetration pegs soon after landing on the peel of unripe fruits. Dormant hyphae remain in intercellular spaces of the peel and cause no damage until the onset of germination during the ripening process. Germination is induced by the release of ethylene by the host. Ethylene is a ripening hormone which induces formation of appressoria (flattened, hyphal "pressing" organ), spore germination, and branching of germ tubes. As such, further development of the fungus is halted until ripening occurs.

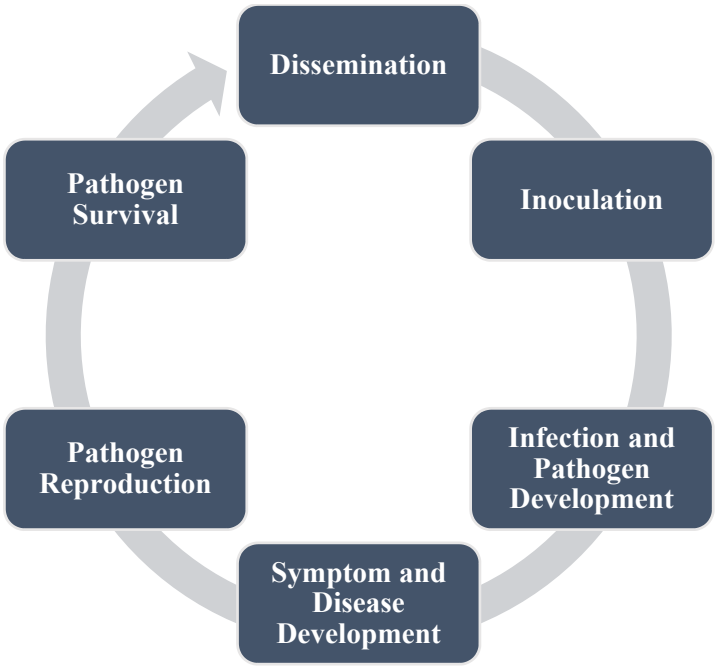


Figure 11. *Colletotrichum gloeosporioides* infection cycle (Nelson, 2008).

Fruits without anthracnose lesions are not guaranteed to be anthracnose-free (Chang *et al*, 2012). Symptoms include black, sunken, rapidly proliferated lesions on organs affected by the fungus.

Colletotrichum gloeosporiodes Penz and Sacc. is the anamorphic stage (asexual stage) of the pathogen. Its life cycle (**Figure 6**) begins with dissemination. Spores (or *conidia*) of the pathogen are dispersed passively *via* splashing rain or irrigation water. Inoculation then follows. It involves the landing of spores on infection sites (*e.g.,* leaves, panicles, and fruit). In young fruit and immature tissue, when the spores are inoculated, they germinate, penetrate through the tissue cuticle and epidermis, and ramify the tissues. On more mature fruit, infections penetrate the tissue cuticle but the disease remains quiescent until the fruits begin to ripen. Disease development becomes apparent when black, sunken, lesions develop on infected organs and rapidly spread. Sticky masses of

conidia form in acervuli (tiny cushion-like structure consisting of a mass of hyphae-bearing asexually produced spores) on symptomatic tissue and the cycle begins again (Nelson, 2008).

The dry (cold) season stimulates the formation of mango blossoms. The wet, highly humid (>95%) season that follows stimulates the proliferation and development of a deleterious disease known as anthracnose (Nelson, 2008; Chang *et al*, 2012). Post-harvest losses as a result of this disease are substantial (Nelson 2008).

The *C. gloeosporioides* strains that affect mangos are genetically and pathologically distinct populations of this species. The mango population has not been found on other tropical fruit crops and is highly virulent only on mangos (*i.e.,* the strains have restricted host ranges) (Nelson 2008). It has been reported that the cultivars that are very susceptible to mango anthracnose include 'Willard', 'Neelum', 'Pirie', and 'Kensington'. The varieties reported to be resistant include 'Saigon', 'Carrie', 'Paris' and 'Zebda' (Litz, 2009; Nelson, 2008).

V. MATERIALS AND METHODS

5.1 Materials

5.1.1 Sample Preparation

During sample preparation the following apparatus and reagents were utilized: an analytical balance, 50mL disposable centrifuge tubes (Fisher Scientific cat#: 05-539-12), liquid nitrogen, dewar vessels, metal mortars, ceramic pestles, funnels, spatulas, and surgical steel blades

5.1.2 Sample Processing

During sample processing the following apparatus and reagents were utilized: 50:50 acetonitrile:water, 15mL disposable centrifuge tubes, 5mL Class A volumetric pipettes, a vortex, a sonicator, a shaker, and a centrifuge.

5.1.3 Sample Fortification

During sample fortification the following apparatus and reagents were utilized: 2mL and 4mL Class A volumetric pipettes, 50mL volumetric flasks, brown bottles, 50:50 acetonitrile:water, and a 5000ppm mangiferin stock solution in 50:50 acetonitrile:water.

5.1.4 Instrumental Analysis

During instrumental analysis the following apparatus and reagents were utilized: Thermo Scientific UltiMate™ 3000 UPLC equipped with a photodiode array detector, Thermo Scientific LTQ Heated Electrospray Ionizer (HESI) coupled with a Line Ion Trap Mass Spectrometer; analytical column: Zorbax 300SB-C18 250 x 4.6mm (5µm), 0.1%

formic acid in HPLC-grade water, 0.1% formic acid in acetonitrile, 15mL disposable centrifuge tubes, autosampler vials, 50:50 acetonitrile:water; and 0.5, 1, 2, 3, 8, and 10mL Class A volumetric pipettes.

5.1.5 Antifungal Assay

The following apparatus and reagents were used: potato dextrose agar, deionized water, 1 L bottles, 1000µL pipettes, concentrated lactic acid (85%), petri dishes, and an autoclave.

5.2 Sampling

Schnell *et* al. (2006) constructed a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram for 10 geographical groups that contained at least four genotypes of *Mangifera indica* as well as two other *Mangifera* species (**Figure 5)** using Rogers' distance that were derived from microsatellite marker allele frequencies. The *Mangifera* species were separated into three clusters and the *M. indica* species was further separated into four clusters. Populations

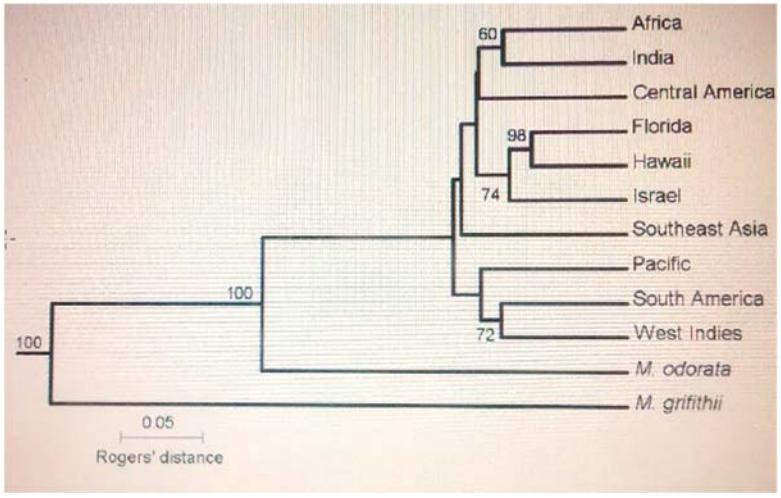


Figure 12. Dendrogram of ten geographical groups of mangos (Schnell *et al.*, 2006).

from Africa, India, and Central America clustered into one group, Florida, Hawaii, and Israel in another group, Southeast Asian populations in their own cluster, and the Pacific, South America, and the West Indies populations in a group. The *M. indica* clusters in the dendrogram were utilized as a template to group samples into four "regions". Using a random number generator, 16 cultivars were randomly selected (**Table 1**)—4 from each region—from the cultivars located at the United States Department of Agriculture-Agricultural Research Service/Subtropical Horticulture Research Station that produced enough fruit for collection. In addition to these cultivars, accessions that were reported to be highly susceptible and highly resistant to anthracnose were sampled: *Neelum* and *Zebda*, respectively. These varieties were put into a group labeled "Region 0".

Samples were collected at four different stages of the mango fruit development. Approximately 8 weeks post-fruit set, two fruit were randomly collected from each tree, appropriately labeled, and stored in a -80°C to await analysis. These samples were at "Stage I" of development. The process was repeated every two weeks over a six week period and those samples were at stages II through IV of development, respectively. A total of 128 fruit were collected.

Table 1. Cultivars analyzed and their origins.

Region	Locations	Cultivar
1	Pacific South America West Indies	Peach
		Pere Louis
		Turpentine #7
		Toledo
2	Southeast Asia	Nam Tan Teen
		Nam Doc Mai
		Myatryaut
		Katar Rum Rung
3	Florida Hawaii Israel	Tommy Atkins
		Fukuda
		Bombay
		Maya
4	Africa India Central America	Sabre
		Diab
		Bullock's Heart
		Jehangir

5.3 Method Validation: Limit of Detection and Limit of Quantitation Determination

Method validation is the process used to confirm than an analytical procedure utilized for a specific test is suitable for its intended use.

5.3.1 Limit of Detection and Limit of Quantitation Determination

The Limit of Detection (LOD) is the lowest quantity of an analyte that can be distinguished from the absence of that analyte (*i.e.,* a blank). The Limit of Quantitation (LOQ) is the limit at which the difference between two values can be distinguished. To determine the LOD and LOQ, a technique recommended by the International Conference on Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2B Methodology guideline on analytical method validation was used. The LOD and LOQ was determined both "out-of-matrix" and "in-matrix". The results acquired for the "out-of-matrix" and "in-matrix" standards was used to determine if there was any enhancement or suppression due to the matrix.

5.3.2 "Out-of-Matrix" Determination

The following standards of mangiferin were prepared in 50:50 Acetonitrile: Water: 50ppm, 150ppm, 250ppm, 350ppm, and 500ppm. The five standards were injected in triplicate on the UPLC-ESI-MS (Injection volume: 1µL). Using Microsoft Excel®, a regression of the peak areas of the standards was acquired. From the statistics, the LOD and LOQ for mangiferin was determined using the following equations:

Equation 1. Limit of Detection Calculation.

$$LOD = 3.33(\frac{\sigma}{S})$$

Equation 2. Limit of Quantitation Calculation.

$$LOQ = 10(\frac{\sigma}{S})$$

where **σ** is the standard deviation of the y-intercept and **S** is the slope of the line.

5.3.1 "In-Matrix" Determination

Five extracts were prepared as directed by the analytical procedure. In microliter amounts, each extract was spiked, each at different levels for final concentrations of 50ppm, 150ppm, 250ppm, 350ppm, and 500ppm, respectively. The five spiked extracts were injected in triplicate on the UPLC-ESI-MS (Injection volume: 1µL). Using Microsoft Excel®, a regression on the data was acquired. From the statistics, the LOD and LOQ for mangiferin was determined using **Equation 1** and **Equation 2**.

5.4 Method Validation: Determination of Linearity and Range

Linearity is the ability of a method to elicit test results that are directly—or by a well-defined mathematical transformation—proportional to an analyte's concentration within a given range. The range is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the analytical procedure as written.

The range of the analytical procedure was established to be from the Limit of Quantitation (LOQ) to 10 times LOQ. The linearity was then determined across the entire range of an analytical method by preparing five concentrations of mangiferin to be analyzed (LOQ, 2LOQ, 5LOQ, 7LOQ, and 10LOQ). The concentrations were analyzed

in triplicate by the UPLC-ESI-MS (Injection volume: 1µL). A regression analysis was prepared using Microsoft Excel®.

5.5 Method Validation: Accuracy Determination

Accuracy is the measure of exactness of an analytical method, the closeness of agreement between the conventional, or the true value or accepted reference value and the observed value. In other words, it is a measure of systematic errors. Accuracy is measured by the percentage of analyte recovered from spiked samples.

A group of blank samples were prepared for accuracy determination. The group consisted of a solvent blank, three replicates of the matrix blank, and three replicates of the matrix blank spiked at five levels (LOQ, 2LOQ, 5LOQ, 7LOQ, 10LOQ). A total of 18 samples were prepared for analysis.

Fortified standards were prepared and used to spike the matrix blanks prior to the extraction. Samples were extracted and analyzed using the analytical procedure being validated. The average percent recovery was calculated for each spike level as well as the standard deviation and coefficient of variance.

5.6 Technical Procedure

5.6.1 Scope

The extraction method that was used was applicable to the quantitative determination of mangiferin in mango peels. The method had a validated LOD of 13.32

ppm and a LOQ of 39.99 ppm and was validated over a concentration range of 39.99 ppm-399.9 ppm. The method generated approximately 4 mL of organic waste per sample.

5.6.2 Principle

A 1 g sample was extracted with 50:50 acetonitrile:water. The extract was sonicated for 15 min, placed on a shaker overnight, vortexed, and centrifuged for 5 min (4500 rpm). An aliquot of the sample was diluted 1:4, transferred to an autosampler vial, and analyzed by an Ultra High-Performance Liquid Chromatograph coupled with a Heated Electrospray Ionizer-Mass Spectrometer with UV detection at 242nm, 367nm, and 3D Field as well as MS-MS detection of the base peak 405 *m/z*.

5.6.3 Process Description

5.6.3.1 Sample Preparation

I. Mixing and Preparation of Samples

Using a surgical steel blade, viable peel (peel that was unbroken with no fungi visibly growing on it) was removed from the mango fruit. Using a metal mortar and ceramic pestle, the peel was ground into a homogeneous powder in liquid nitrogen. The ground tissue was then transferred into a 50 mL disposable centrifuge tube and stored in a cryogenic freezer to await extraction.

II. Weighing of Sub-Samples

Before weighing sub-samples, the suitability of the balance was verified and the appropriate documentation was recorded in the balance logbook. Once this step was completed, a 50 mL disposable centrifuge tube was placed into a beaker and the analytical balance was tared. One gram of the sample was transferred into the 50 mL centrifuge tube and the weight was recorded on the sample worksheet.

5.6.3.2 Sample Processing

To the sample, 5 mL of 50:50 Acetonitrile was added. The sample was then sonicated for 15 minutes and placed on a shaker overnight (setting: low). The sample was then vortexed and centrifuged for 5 min (setting: 4500 rpm). The supernatant was transferred to a 15 mL disposable centrifuge tube and centrifuged for 5 min (setting: 4500 rpm). Approximately 0.5 mL of the supernatant was then transferred to a HPLC vial and 1.5 mL of 50:50 acetonitrile:water was added to the vial.

5.6.3.3 Sample Fortification

I. Preparation of Fortification Solution

Using an eppendorf pipette, 1.4 mL of a 5000 ppm mangiferin stock solution was transferred to a 50 mL volumetric flask and diluted to mark with 50:50 acetonitrile:water. The concentration was approximately 140 ppm. The

solution was transferred to an appropriate sized brown bottle and labeled. The activity was recorded on the sample worksheet.

II. Preparation of Standard Check

The standard that was used was the LOQ. For LOQ standard preparation, please see section *5.6.3.4.*

III. Determination of Percent Recovery of Mangiferin

Every group that was extracted and analyzed was accompanied by a blank composite sample and a spiked (fortified) sample to determine the percent recovery for that day's extraction. The fortified sample was prepared by spiking a composite sample with 5 mL of the fortified solution prepared in section *4.6.3.3*(**I**). Once the HPLC analysis was completed, the following calculations were used to determine the percent recovery:

Equation 3. Percent Recovery Calculations.

$$Percent\ recovered = \frac{Amount\ found\ (ppm)}{Amount\ spiked (ppm)} x100\%$$

5.6.3.4 Instrumental Analysis

I. Preparation of Bench Standards

Using a class A volumetric pipette, 2 mL of the 5000 ppm stock solution of mangiferin was transferred to a 15 mL disposable centrifuge tube and then 8 mL of 50:50 acetonitrile:water was added to the tube. The concentration was approximately 1000 ppm with a shelf life of 6 months. This solution was the

bench intermediate. This solution was used to create the following bench standards: LOQ, 5LOQ and 10LOQ. The concentrations of the bench standards were approximately 40 ppm, 200 ppm, and 400 ppm, respectively.

To prepare the LOQ standard, 0.5 mL of the bench standard was diluted to 12.5 mL with 50:50 acetonitrile:water. To create the 5LOQ standard, 1.0 mL of the bench intermediate was diluted to 5.0 mL with the same solvent mixture. To create the 10LOQ standard, 2.0 mL of the bench intermediate was diluted to 5.0 mL with the same solvent mixture.

II. System Suitability

Prior to analysis, the system was checked for its ability to analyze samples within the parameters deemed "acceptable" by the user (*i.e.,* suitability). The standard check (see section 5.*6.3.3*(**I**)) was injected three tiems then the RSD of the three injections was calculated. The RSD had to be less than or equal to 5% in order for the system to be suitable. By injecting the standard in triplicate, the reproducibility of the system was determined. Once the RSD for the standard check was less than or equal to 5%, the bench standards (LOQ, 5LOQ, 10LOQ) were injected into the UPLC-MS and a standard curve was created. The R^2 value of the curve had to be ≥ 0.98 in order for the system to be suitable. If these criteria were not met, maintenance was performed and the standard check and standard curve were analyzed again. Sample analysis was not performed until system suitability was achieved.

III. Analysis

The 5LOQ standard was placed after every 5 samples and at the end of analysis. Samples found to be outside of the calibration curve (*i.e.,* the calculated concentrations of the samples were more than 30% of the 10LOQ or less than 30% of the LOQ) were diluted appropriately and the dilution factor was applied to the final amount.

To calculate the amount of mangiferin, the following calculation was used:

Equation 4. Mangiferin Content Calculation.

$$Amount\ of\ mangiferin = \frac{(Amount\ found\ (ppm)\ x\ Dilution\ factor)}{Recovery\ Factor}$$

IV. Analytical Method

Separations were performed on a Thermo Scientific UltiMate™ 3000 UPLC coupled with a Thermo Scientific LTQ Heated Electrospray Ionizer (HESI) Linear Ion Trap Mass Spectrometer was utilized for analysis. The column used was a Zorbax 300SB-C18 column (250 x 4.6mm, 5µm) set at 35°C. Mangiferin was detected in the eluent with a UV photodiode array detector (Thermo Scientific) set at 242nm, 367nm, and 3-D Field. The extract (1µL) was diluted 1:4 and injected into the HPLC-MS. The mobile phase consisted of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) with a flow rate of 0.500 mL/min. The following gradient was utilized over a run time of 19 min: initially 95% A for 1 min; to 100% B in 4 min; held for 4 min; to 95%

A in 7 min; and held for 3 min. The polarity of the mass spectrometer was positive. Two scan events were employed. During the full scan mode, the normalized collision energy was 35.0V, capillary temperature was 320°C, the source fragmentation was off, the data type was profile, and the masses scanned ranged from 115 *m/z* to 455 *m/z*. During the second scan event, the parent mass was identified as 423 *m/z*, the normalized collision energy was 20.0V, the source fragmentation was set at 35.0V, and masses scanned ranged from 115 *m/z* to 430 *m/z*. Peak integration was performed using *Genesis*.

5.6.3.5 Antifungal Assay

Four strains of *Colletotrichum gloeosporioides* Penz. and Sacc. In Penz were obtained from the University of Florida Tropical Research and Education Center (UF-TREC). Extracts from stage 4 of development from the cultivars Zebda, Neelum, Tommy Atkins, and Fukuda were tested in triplicate against these strains *via* disk diffusion assays on acidic PDA plates. Zones of inhibition were documented and a Two-Way ANOVA was implemented to determine if there was a correlation between any antifungal properties exhibited and mangiferin content in the peel extracts of the selected cultivars. To create the acidic PDA plates, 39g of PDA was added to a 1 L bottle followed by 1L of deionized water. The mixture was autoclaved and cooled to 50-60°C. Once the mixture cooled to the appropriate temperature, 1 mL of concentrated lactic acid (85%) was stirred into the mixture. The media were then poured into petri dishes and allowed to solidify before storing in a cool, dark location.

VI. RESULTS

6.1 Method Validation

6.1.1 Determination of the Limit of Detection and Quantitation

To determine the limit of detection (LOD) and limit of quantitation (LOQ), a five-point calibration plot was constructed using standards at the following concentrations: 50ppm, 150ppm, 250ppm, 350ppm, and 500ppm (**Figure 13**). Linearity was achieved with a R^2 value of 0.9518. The LOD was determined to be 61.45 ppm and the LOQ was determined to be 184.5ppm. The standard at the highest level deviated from linearity. As such, another calibration plot was by constructed by omitting the values obtained for the 500ppm standard (**Figure 14**). Linearity was achieved with a R^2 value of 0.9969. The LOD was determined to be 13.32ppm and the LOQ was determined to be 39.99ppm. As such, the bench standards that were prepared to create a calibration plot prior to each HPLC-MS analysis and the standards used to determine the accuracy of the method were at the following levels: 40ppm (LOQ), 200ppm (5LOQ), and 400ppm (10LOQ).

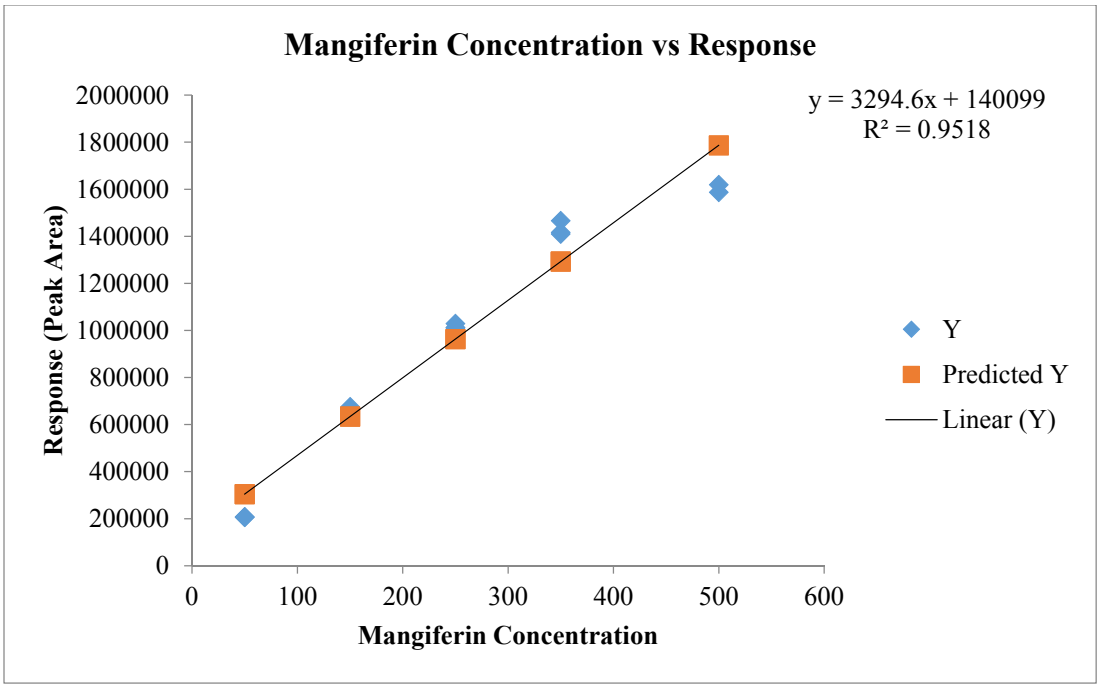


Figure 13. Initial 5-point calibration plot used to determine the limits of detection and quantitation.

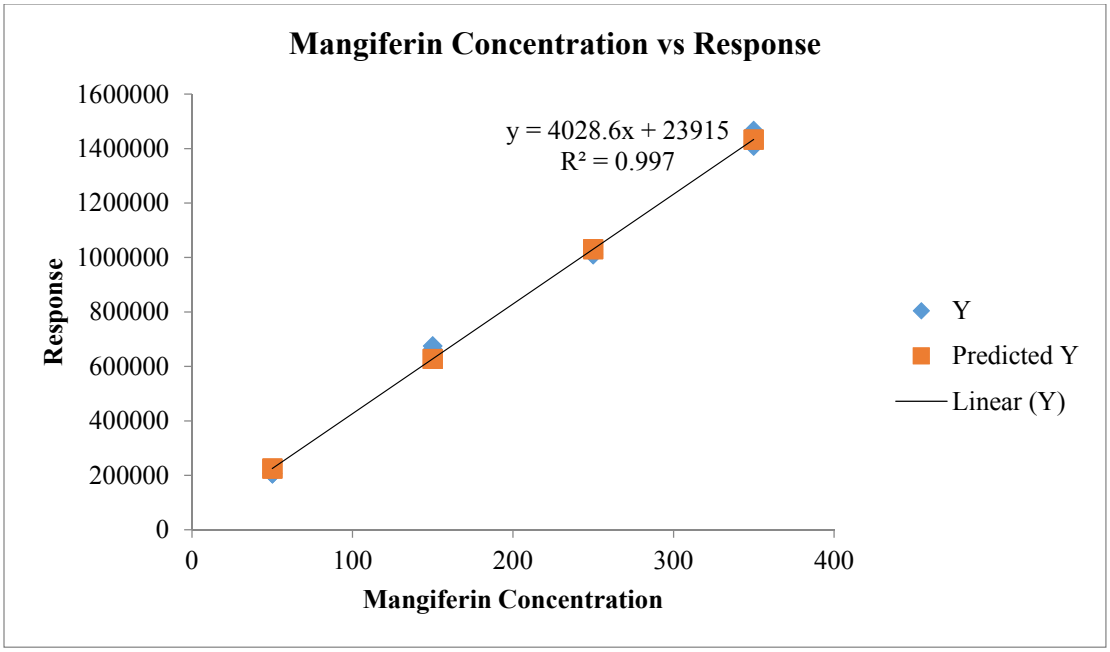


Figure 14. Final calibration plot used to determine the limits of detection and quantitation.

6.1.2 Accuracy Determination

Mango peel composites were spiked at the following levels: LOQ (40 ppm), 2LOQ (80 ppm), 5LOQ (200 ppm), 7LOQ (280 ppm), and 10LOQ (400 ppm) (**Table 2**). The mango peel composites spiked with standards at the LOQ level had the lowest mean percent recovery at 55.49%. Mango peel composites spiked with standards at the 5LOQ level had the highest mean percent recovery at 164%. The average percent recovery for all levels analyzed was 87.37% with a coefficient of variance of 0.56. With the exception of the LOQ, all coefficients of variance values were below 0.15.

Table 2. Percent recoveries of mangiferin at levels over the 40ppm-400ppm range.

	Mangiferin (ppm)	% Recovery	Average	SD	CV
Comp	22.609	--	**21.84991366**	**3.026**	**0.139**

Comp	24.424	--			
Comp	18.516	--			
LOQ	22.996	57.515%	**55.49%**	**0.235**	**0.42**
LOQ	24.815	31.033%			
LOQ	31.149	77.909%			
2LOQ	91.139	113.976%	**113.36%**	**0.012**	**0.01**
2LOQ	91.293	114.168%			
2LOQ	89.518	111.949%			
5LOQ	336.769	168.461%	**164.00%**	**0.042**	**0.03**
5LOQ	326.809	163.479%			
5LOQ	319.944	160.045%			
7LOQ	387.273	129.130%	**116.95%**	**0.163**	**0.14**
7LOQ	369.812	123.308%			
7LOQ	295.153	98.414%			
10LOQ	354.603	88.678%	**83.59%**	**0.045**	**0.05**
10LOQ	327.081	81.795%			
10LOQ	321.049	80.287%			

6.2 Mangiferin Quantification

XCalibur software constructed reports that included a Total Scan PDA chromatogram, a MS/MS chromatogram highlighting the compound of interest, the fragmentation pattern of the compound of interest, and the calibration plot used to quantify mangiferin for each sample analyzed. An example of the report is located in the Appendix.

	Region	Stage 1		Stage 2		Stage 3		Stage 4	
		Average (ppm)	Standard Error	Average (ppm)	Standard Error	Average (ppm)	Standard Error	Average (ppm)	Standard Error
Peach	1	7401.5	3470	2786.1	967.7	2186.7	847.6	817.74	212.1
Pere Louis	1	381.10	306.0	151.02	115.9	194.94	129.1	439.36	162.1
Turpentine #7	1	16187	5316.8	4438.8	587.4	4846.7	1060	2602.4	25.56
Toledo	1	2960.2	60.957	1664.6	276.3	2076.5	667.0	1563.6	232.7
Nam Tan Teen	2	4349.2	645.88	3896.6	5287	616.81	333.9	824.52	231.3
Nam Doc Mai	2	883.63	789.69	356.63	413.2	135.54	133.7	46.526	4.452
Myatryaut	2	735.86	741.84	1093.2	363.2	767.00	588.8	151.74	28.65
Katar Rum Rung	2	1281.8	543.92	2420.0	1560	2023.0	112.0	1318.2	334.8
Tommy Atkins	3	21216	497.47	5663.8	19.57	4611.0	690.0	3701.8	385.5
Fukuda	3	48.813	8.9295	8.4974	0.4314	22.792	1.776	6.3784	0.060
Bombay	3	55.740	16.439	31.758	12.18	35.094	6.245	14.848	7.824
Maya	3	11568	8751.1	3605.4	728.4	2424.3	929.8	1121.9	93.33
Sabre	4	1050.0	318.01	99.613	39.79	1139.6	664.3	201.78	40.06
Diab	4	1318.0	359.05	597.49	263.4	3832.2	186.1	382.12	62.71
Bullock's Heart	4	1968.1	917.84	4404.6	463.7	165.42	49.82	617.67	504.0
Jehangir	4	280.84	70.432	773.96	56.18	600.37	750.6	363.94	197.9
Zebda	0	26735	2561.2	21610	3058	11639	1073	14481	1456
Neelum	0	27491	115.84	13718	1936	14489	1837	16124	286.1

Figure 15. Mean mangiferin content per stage of development.

Mangiferin content varied across cultivars as well as in each stage of development (**Figure 15**). The mangiferin content decreased exponentially as the fruit developed over time. The content was the highest at the first stage of development and the lowest at the fourth stage of development. The test variety with the highest amount of mangiferin with the least amount of variability at each stage of development was 'Tommy Atkins'. This cultivar had average mangiferin concentrations of 21216ppm, 5663.8ppm, 4611.0ppm, and 3701.8ppm during stages 1, 2, 3, and 4 of development, respectively. The test variety with the lowest amount of mangiferin with the least amount of variability at each stage of development was 'Fukuda'. The cultivar had mean mangiferin concentrations of 48.813ppm, 8.4974ppm, 22.792ppm, and 6.3784ppm during stages 1, 2, 3, and 4 of development, respectively. Both cultivars were from Region 3 and originated from Florida and Hawaii, respectively.

The control variety 'Neelum' (which reportedly exhibits high susceptibility to anthracnose) had the highest amount of mangiferin at during the first, third, and fourth stages of development at 27491ppm, 14489ppm, and 16124ppm, respectively with the control variety 'Zebda' (which reportedly exhibits high resistance to anthracnose) following close behind at 26735ppm, 11639ppm, and 14481ppm, respectively. 'Zebda' had the highest amount of mangiferin during the second stage of development at 21610ppm with 'Neelum' following close behind at 13718ppm. 'Neelum' originates from India and 'Zebda' originates from Egypt.

The descriptive statistics for each region at each stage of development can be found in **Table 3** and **Figures 16-19**. Regions that exhibited the highest and lowest concentration of mangiferin varied per stage of fruit development. Region 1 had the highest amount of mangiferin during stages 3 and 4 of development. Region 3 had the highest amount of mangiferin during stages 1 and 2 of development. Region 2 had the lowest amount of mangiferin during stage 3 of development. Region 4 had the lowest amount of mangiferin during stages 1, 2, and 4 of development.

Table 3. Descriptive Statistics of Each Region per Stage of Development.

	Region		Statistic	Std. Error
Stage 1	0	Mean	27112.90207	771.622802
		Std. Deviation	1543.245603	
	1	Mean	6732.53581	2424.687581

		Std. Deviation	6858.052123	
	2	Mean	1812.61280	588.029074
		Std. Deviation	1663.197384	
	3	Mean	8222.12665	3545.340362
		Std. Deviation	10027.736845	
	4	Mean	1154.24887	267.367543
		Std. Deviation	756.229611	
Stage 2	0	Mean	17664.38425	2506.348645
		Std. Deviation	5012.697290	
	1	Mean	2232.00068	605.383562
		Std. Deviation	1712.283288	
	2	Mean	1941.61602	898.898827
		Std. Deviation	2542.469824	
	3	Mean	2327.35815	919.579861
		Std. Deviation	2600.964621	
	4	Mean	1468.91363	651.375213
		Std. Deviation	1842.367321	
Stage 3	0	Mean	13063.97847	1026.486406
		Std. Deviation	2052.972813	
	1	Mean	2168.18295	661.350513
		Std. Deviation	1870.581729	
	2	Mean	885.57657	279.462133
		Std. Deviation	790.438276	
	3	Mean	1773.31209	737.574549
		Std. Deviation	2086.175860	
	4	Mean	1434.41607	556.240825
		Std. Deviation	1573.286638	
Stage 4	0	Mean	15302.22567	638.962904
		Std. Deviation	1277.925808	
	1	Mean	1368.77383	313.613646
		Std. Deviation	887.033344	
	2	Mean	585.23720	203.167681
		Std. Deviation	574.644980	
	3	Mean	1211.24797	572.360990
		Std. Deviation	1618.881350	
	4	Mean	391.37618	92.069223

	Std. Deviation	260.411088	

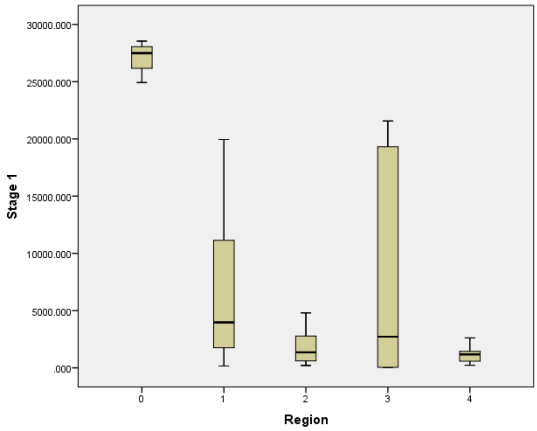


Figure 16. Stage 1: Mangiferin content per region.

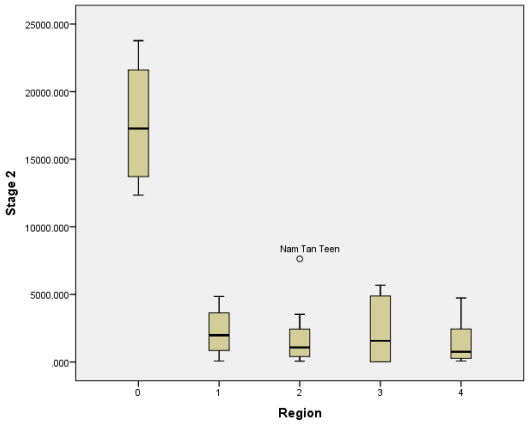


Figure 17. Stage 2: Mangiferin content per region.

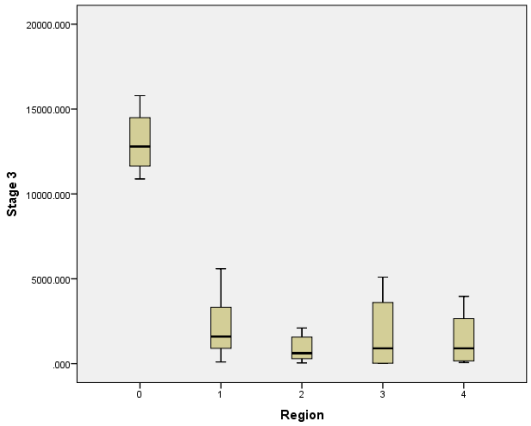


Figure 18. Stage 3: Mangiferin content per region.

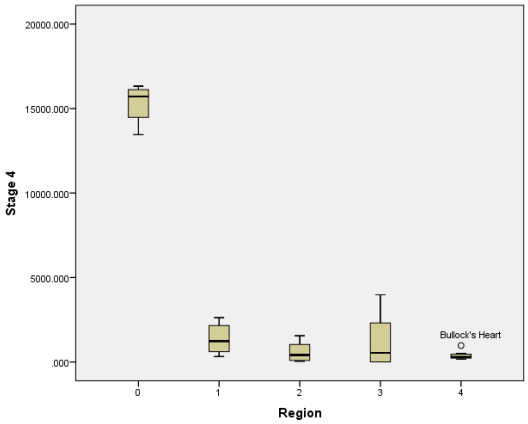


Figure 19. Stage 4: Mangiferin content per region.

6.3: Statistical Analysis: Repeated Measures ANOVA

The quantified mangiferin was analyzed by a repeated measures ANOVA. The data was analyzed by grouping the control cultivars and test cultivars to perform the analysis. The data was grouped according to the stage of development, not region. The descriptive statistics for mangiferin content in each stage of development showed that the data distribution was not normal (**Table 4, Figures 15-22)**. None of the normality tests

were passed (**Table 3**) and the data was right-skewed. Since the data was right-skewed and not normal, the data was unfit for any two-way ANOVA or repeated measures ANOVA analysis. Consequently, the data was transformed using the natural log function (*ln*) to create new log values to compare with the repeated measures ANOVA. This transformation resulted in the data having a normal distribution in all stages of development (**Table 4, Figures 23-30)**. All of the normality tests demonstrated the result of transforming values (**Table 5**). Using the transformed values, a repeated measures ANOVA was used to test for differences in infection across stage and variety.

Table 4. Tests of Normality.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Stage 1	.289	36	.000	.718	36	.000
Stage 2	.252	36	.000	.678	36	.000
Stage 3	.242	36	.000	.698	36	.000
Stage 4	.342	36	.000	.534	36	.000

a. Lilliefors Significance Correction

Table 5. Descriptive Statistics of the Mangiferin Content of All Mango Peels Analyzed During Each Stage of Development.

			Statistic	Std. Error
Stage 1	Mean		6995.10559	1589.379463
	95% Confidence Interval for Mean	Lower Bound	3768.49374	
		Upper Bound	10221.71744	

	Variance		90940574.780	
	Std. Deviation		9536.276778	
Stage 2	Mean		3733.79569	929.718694
	95% Confidence Interval for Mean	Lower Bound	1846.36640	
		Upper Bound	5621.22498	
	Variance		31117566.568	
	Std. Deviation		5578.312161	
Stage 3	Mean		2842.99487	670.523430
	95% Confidence Interval for Mean	Lower Bound	1481.75994	
		Upper Bound	4204.22980	
	Variance		16185660.108	
	Std. Deviation		4023.140578	
Stage 4	Mean		2490.61067	784.547596
	95% Confidence Interval for Mean	Lower Bound	897.89438	
		Upper Bound	4083.32697	
	Variance		22158537.477	
	Std. Deviation		4707.285574	

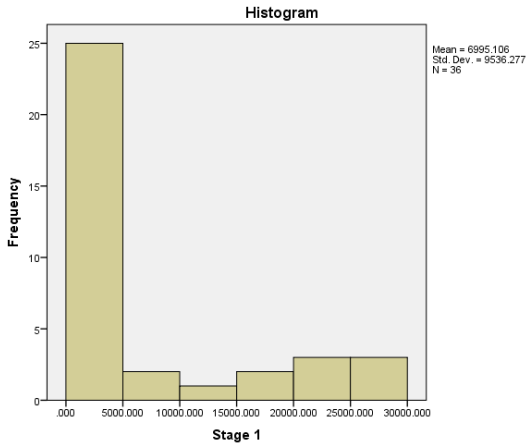


Figure 20. Stage 1: Distribution of all cultivars.

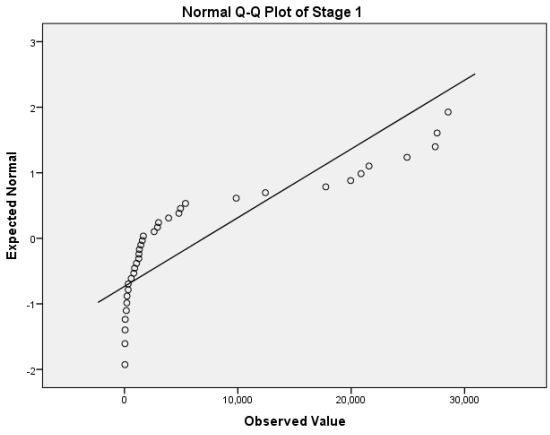


Figure 21. Stage 1: Normal Q-Q plot of all cultivars.

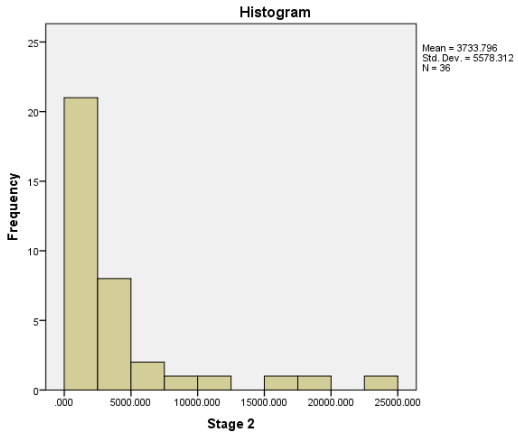


Figure 22. Stage 2: Distribution of all cultivars.

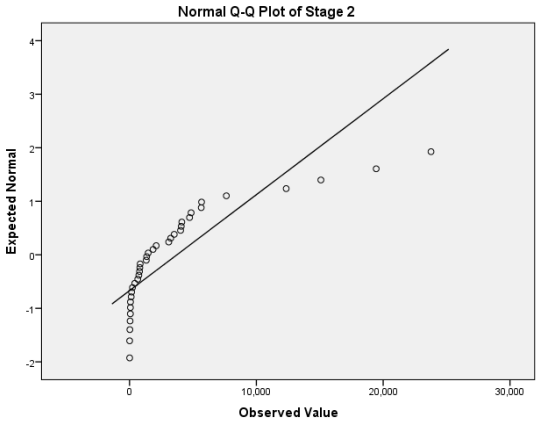


Figure 23. Stage 2: Normal Q-Q plot of all cultivars.

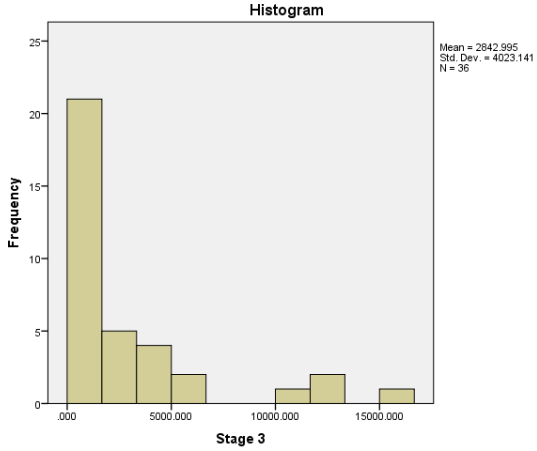


Figure 24. Stage 3: Distribution of all cultivars.

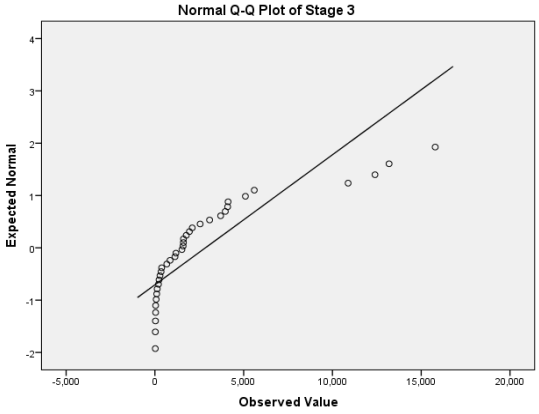


Figure 25. Stage 3: Normal Q-Q plot of all cultivars.

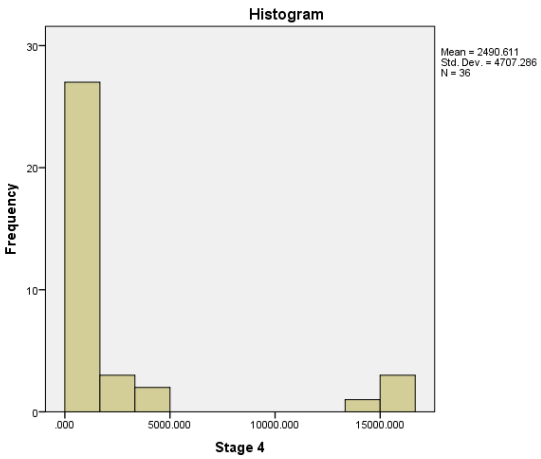


Figure 26. Stage 4: Distribution of all cultivars.

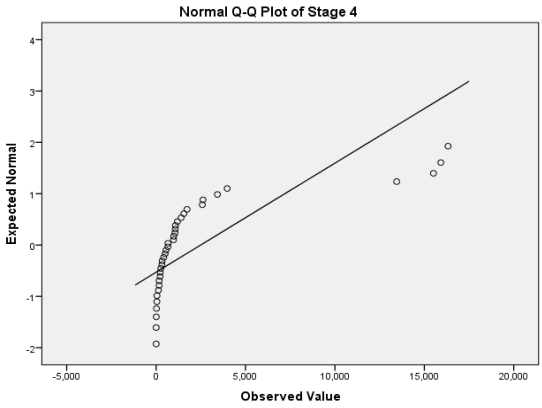


Figure 27. Stage 4: Normal Q-Q plot of all cultivars.

Table 6. Descriptive statistics for the Transformed Values of Mangiferin per Stage of Development.

			Statistic	Std. Error
ln_stage1	Mean		7.5112	.33057
	95% Confidence Interval for Mean	Lower Bound	6.8401	
		Upper Bound	8.1823	
	Variance		3.934	
	Std. Deviation		1.98343	
ln_stage2	Mean		6.8500	.35794
	95% Confidence Interval for Mean	Lower Bound	6.1233	
		Upper Bound	7.5766	
	Variance		4.612	
	Std. Deviation		2.14762	
ln_stage3	Mean		6.7389	.32017
	95% Confidence Interval for Mean	Lower Bound	6.0889	
		Upper Bound	7.3889	
	Variance		3.690	
	Std. Deviation		1.92105	
ln_stage4	Mean		6.2762	.34395

	95% Confidence Interval for Mean	Lower Bound	5.5779	
		Upper Bound	6.9744	
	Variance		4.259	
	Std. Deviation		2.06372	

Table 7. Tests of Normality for the transformed values of Mangiferin.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
ln_stage1	.096	36	.200*	.943	36	.063
ln_stage2	.126	36	.158	.939	36	.048
ln_stage3	.148	36	.044	.940	36	.050
ln_stage4	.098	36	.200*	.954	36	.136

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

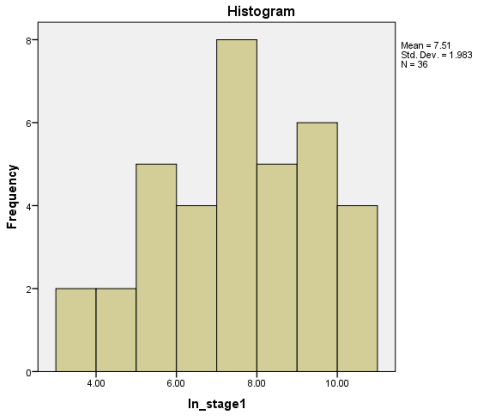


Figure 28. Stage 1: Distribution of transformed values for of all cultivars.

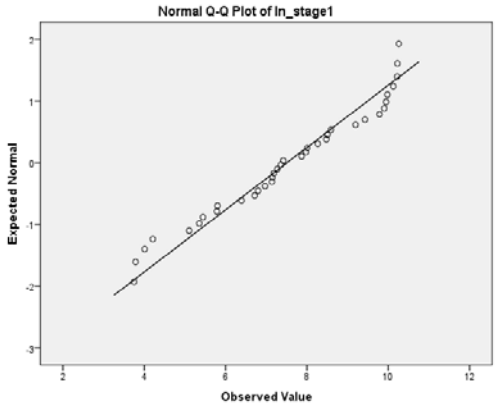


Figure 29. Stage 1: Normal Q-Q plot of transformed values for of all cultivars.

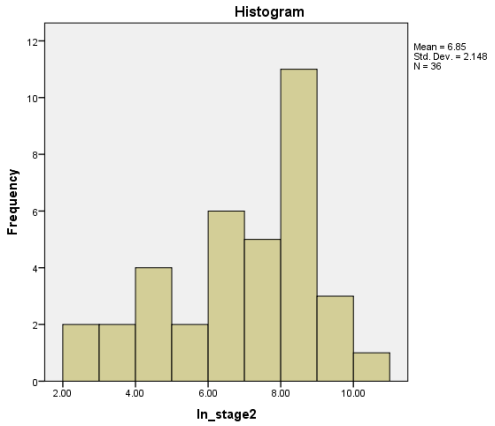


Figure 30. Stage 2: Distribution of transformed values for of all cultivars.

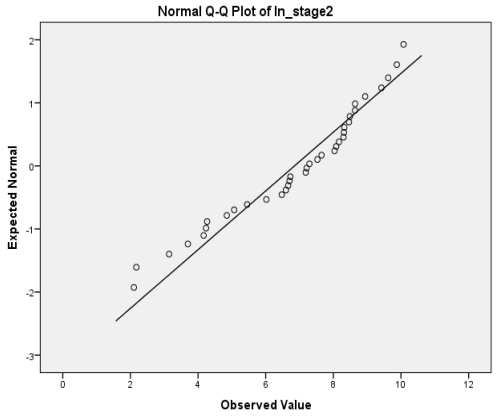


Figure 31. Stage 2: Normal Q-Q plot of transformed values for of all cultivars.

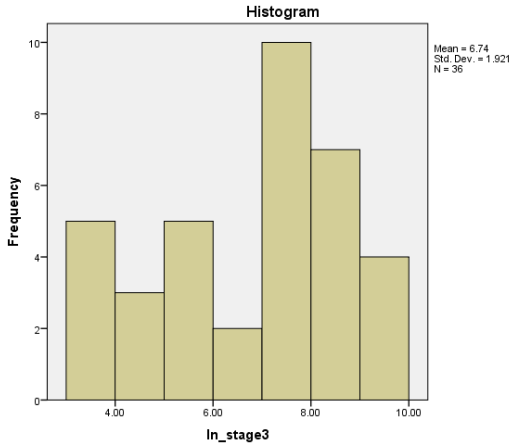


Figure 32. Stage 3: Distribution of transformed values for of all cultivars.

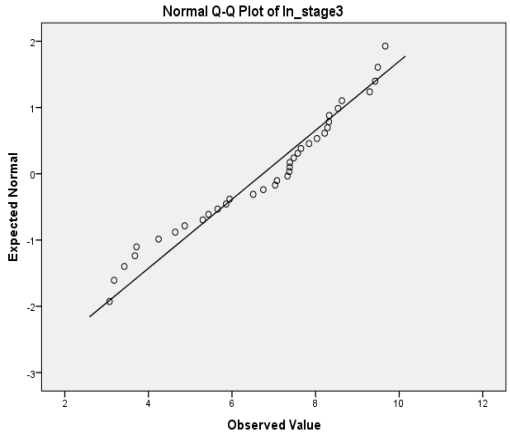


Figure 33. Stage 3: Normal Q-Q plot of transformed values for of all cultivars.

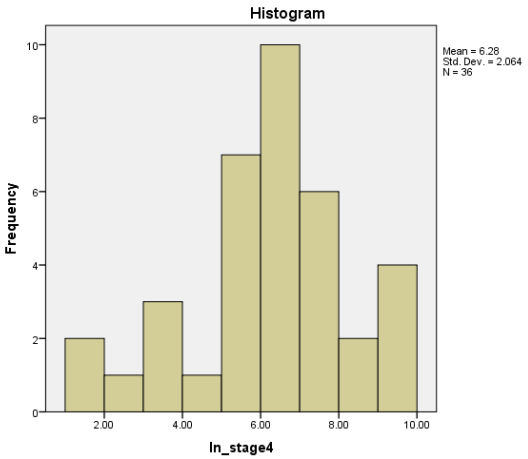


Figure 34. Stage 4: Distribution of transformed values for of all cultivars.

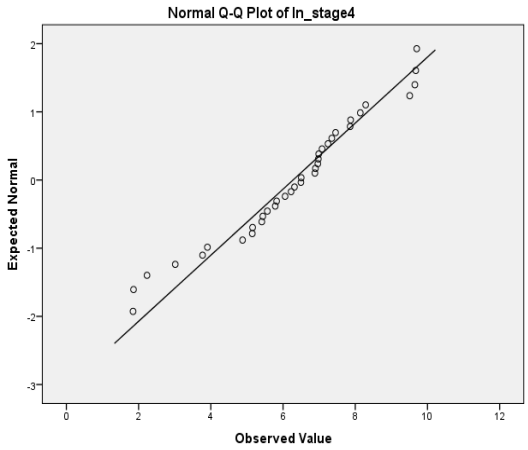


Figure 35. Stage 4: Normal Q-Q plot of transformed values for of all cultivars.

The descriptive statistics for the general linear model of the repeated measures ANOVA can be found in **Table 8** and the descriptive statistics for the region-stage interaction can be found in **Table 9**. Multivariate tests *via* the repeated measures ANOVA were implemented to determine the significance of the mangiferin content in each stage of fruit development and the interaction of the stage of fruit development and the region of origin. The tests showed that the stage of fruit development was significant but the interaction was not significant (**Table 10**). Tests of within-subjects contrasts were implemented to further confirm the significance of the mangiferin content in each stage of fruit development and the region of origin; and to determine the significance of the stage of fruit development and the interaction of the stage of fruit development and the region of origin in different trends (linear, quadratic, and cubic). The tests showed that the stage of fruit development had a significant linear trend (**Table 11**). The test of between-subject effects determined that the region of mango origin was significant (**Table 12**).

Table 8. Descriptive Statistics for the General Linear Model.

	Region	Mean	Std. Deviation	N
ln_stage1	0	10.2065	.05812	4
	1	8.0642	1.61572	8
	2	7.0641	1.08588	8
	3	6.7570	3.04673	8
	4	6.8118	.81105	8
	Total	7.5112	1.98343	36
ln_stage2	0	9.7487	.28676	4
	1	7.1287	1.50141	8
	2	6.7417	1.54902	8
	3	5.5952	3.05839	8
	4	6.4848	1.47077	8
	Total	6.8500	2.14762	36
ln_stage3	0	9.4686	.15470	4
	1	7.1459	1.34649	8
	2	6.2503	1.30806	8
	3	5.7152	2.56570	8
	4	6.4795	1.52986	8
	Total	6.7389	1.92105	36
ln_stage4	0	9.6330	.08667	4
	1	7.0010	.75156	8
	2	5.6783	1.43200	8
	3	4.9277	2.93300	8
	4	5.8192	.55574	8
	Total	6.2762	2.06372	36

Table 9. Region * Stage Descriptive Statistics.

Measure: Measure_

Region	Stage	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	10.207	.880	8.411	12.002
	2	9.749	.956	7.798	11.699
	3	9.469	.839	7.758	11.179
	4	9.633	.807	7.988	11.278
1	1	8.064	.623	6.795	9.334
	2	7.129	.676	5.749	8.508
	3	7.146	.593	5.937	8.355
	4	7.001	.570	5.837	8.164
2	1	7.064	.623	5.794	8.334
	2	6.742	.676	5.362	8.121
	3	6.250	.593	5.041	7.460
	4	5.678	.570	4.515	6.842
3	1	6.757	.623	5.487	8.027
	2	5.595	.676	4.216	6.975
	3	5.715	.593	4.506	6.925
	4	4.928	.570	3.764	6.091
4	1	6.812	.623	5.542	8.082
	2	6.485	.676	5.105	7.864
	3	6.480	.593	5.270	7.689
	4	5.819	.570	4.656	6.983

Table 10. Stage *vs* Stage- Region Interaction: Multivariate Tests[a].

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[d]
Stage	Pillai's Trace	.617	15.594[b]	3.000	29.000	.000	.617	46.782	1.000
	Wilks' Lambda	.383	15.594[b]	3.000	29.000	.000	.617	46.782	1.000
	Hotelling's Trace	1.613	15.594[b]	3.000	29.000	.000	.617	46.782	1.000
	Roy's Largest Root	1.613	15.594[b]	3.000	29.000	.000	.617	46.782	1.000
Stage * Region	Pillai's Trace	.322	.933	12.000	93.000	.518	.107	11.194	.507
	Wilks' Lambda	.704	.909	12.000	77.018	.542	.110	9.545	.422
	Hotelling's Trace	.383	.882	12.000	83.000	.568	.113	10.583	.474
	Roy's Largest Root	.240	1.861[c]	4.000	31.000	.142	.194	7.445	.499

a. Design: Intercept + Region
Within Subjects Design: Stage
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Computed using alpha = .05

Table 11. Stage *vs* Stage- Region Interaction: Tests of Within-Subjects Contrasts.

Measure: Measure_

Source	Stage	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Stage	Linear	22.017	1	22.017	41.073	.000	.570	41.073	1.000
	Quadratic	.483	1	.483	1.014	.322	.032	1.014	.164
	Cubic	1.027	1	1.027	1.049	.314	.033	1.049	.168
Stage * Region	Linear	2.343	4	.586	1.093	.377	.124	4.370	.302
	Quadratic	1.910	4	.477	1.001	.422	.114	4.004	.278
	Cubic	1.349	4	.337	.344	.846	.043	1.378	.117
Error(Stage)	Linear	16.617	31	.536					
	Quadratic	14.785	31	.477					
	Cubic	30.349	31	.979					

a. Computed using alpha = .05

Table 12. Region: Tests of Between-Subjects Effects.

Measure: Measure_
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	6789.798	1	6789.798	666.671	.000	.956	666.671	1.000
Region	194.270	4	48.568	4.769	.004	.381	19.075	.918
Error	315.724	31	10.185					

a. Computed using alpha = .05

Pairwise comparisons were implemented to determine if there were any significant differences in mangiferin content between the stages of mango fruit development (**Table 13**). Estimated marginal means were used to make these comparisons (**Figure 36**). Stage 1 had a significantly higher mean mangiferin content than stages 2-4. Stage 2 had a significantly lower mangiferin content than stage 1 and a significantly mean higher mangiferin content than stage 4. Stage 3 had a significantly lower mean mangiferin content than stage 1. Stage 4 had a significantly lower mean mangiferin content than stages 1 and 2.

Table 13. Stage: Pairwise Comparisons.

Measure: Measure_

(I) Stage	(J) Stage	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.641*	.198	.017	.084	1.198
	3	.769*	.212	.006	.173	1.365
	4	1.169*	.166	.000	.700	1.638
2	1	-.641*	.198	.017	-1.198	-.084
	3	.128	.251	1.000	-.581	.837
	4	.528*	.176	.031	.033	1.023
3	1	-.769*	.212	.006	-1.365	-.173
	2	-.128	.251	1.000	-.837	.581
	4	.400	.183	.218	-.115	.915
4	1	-1.169*	.166	.000	-1.638	-.700
	2	-.528*	.176	.031	-1.023	-.033
	3	-.400	.183	.218	-.915	.115

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

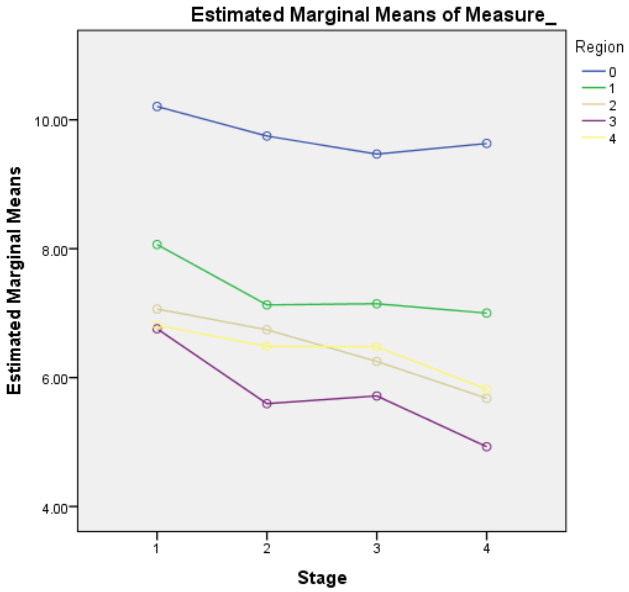


Figure 36. Region: Estimated Marginal Means.

Pairwise comparisons were implemented to determine if there were any significant differences in mangiferin content between the different regions of origin (**Table 14**). Region 0 (the controls) had a significantly higher mean level of mangiferin than all regions with the exception of region 1. Region 1 had no significant difference with any of the other regions. Regions 2, 3, and 4 had significantly lower mean mangiferin concentrations than region 0.

Table 14. Region: Pairwise Comparisons.

Measure: Measure_

(I) Region	(J) Region	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
0	1	2.429	.977	.185	-.524	5.382
	2	3.331*	.977	.018	.378	6.284
	3	4.015*	.977	.003	1.062	6.968
	4	3.365*	.977	.017	.412	6.318
1	0	-2.429	.977	.185	-5.382	.524
	2	.901	.798	1.000	-1.510	3.312
	3	1.586	.798	.557	-.825	3.997
	4	.936	.798	1.000	-1.475	3.347
2	0	-3.331*	.977	.018	-6.284	-.378
	1	-.901	.798	1.000	-3.312	1.510
	3	.685	.798	1.000	-1.726	3.096
	4	.035	.798	1.000	-2.376	2.446
3	0	-4.015*	.977	.003	-6.968	-1.062
	1	-1.586	.798	.557	-3.997	.825
	2	-.685	.798	1.000	-3.096	1.726
	4	-.650	.798	1.000	-3.061	1.761
4	0	-3.365*	.977	.017	-6.318	-.412
	1	-.936	.798	1.000	-3.347	1.475
	2	-.035	.798	1.000	-2.446	2.376
	3	.650	.798	1.000	-1.761	3.061

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Univariate tests were implemented to further confirm the presence of significantly different regions (**Table 15**). The tests were based on linearly independent pairwise comparisons among the estimated marginal means. The tests showed that there was at least one significantly different region of origin.

Significant differences in mangiferin content between the different regions of origin were further confirmed by implementing multiple comparisons tests using Tukey's HSD (**Table 16**). The multiple comparisons tests showed that region 0 was significantly higher than regions 2-4, region 1 had no significant differences with any other region or origin, and regions 2-4 were significantly lower than 0.

Table 15. Region: Univariate Tests.

Measure: Measure_

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Contrast	48.568	4	12.142	4.769	.004	.381	19.075	.918
Error	78.931	31	2.546					

The F tests the effect of Region. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Table 16. Region: Multiple Comparisons *via* Tukey's HSD.

Measure: Measure_
Tukey HSD

(I) Region	(J) Region	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	2.4292	.97714	.120	-.3994	5.2579
	2	3.3306*	.97714	.015	.5019	6.1592
	3	4.0154*	.97714	.002	1.1868	6.8441
	4	3.3654*	.97714	.013	.5367	6.1940
1	0	-2.4292	.97714	.120	-5.2579	.3994
	2	.9013	.79783	.790	-1.4082	3.2109
	3	1.5862	.79783	.295	-.7234	3.8958
	4	.9361	.79783	.766	-1.3734	3.2457
2	0	-3.3306*	.97714	.015	-6.1592	-.5019
	1	-.9013	.79783	.790	-3.2109	1.4082
	3	.6848	.79783	.910	-1.6247	2.9944
	4	.0348	.79783	1.000	-2.2748	2.3444
3	0	-4.0154*	.97714	.002	-6.8441	-1.1868
	1	-1.5862	.79783	.295	-3.8958	.7234
	2	-.6848	.79783	.910	-2.9944	1.6247
	4	-.6501	.79783	.924	-2.9596	1.6595
4	0	-3.3654*	.97714	.013	-6.1940	-.5367
	1	-.9361	.79783	.766	-3.2457	1.3734
	2	-.0348	.79783	1.000	-2.3444	2.2748
	3	.6501	.79783	.924	-1.6595	2.9596

Based on observed means.
The error term is Mean Square(Error) = 2.546.
*. The mean difference is significant at the .05 level.

The repeated measures ANOVA showed that the control cultivars were not significantly different from each other. To determine if there were any differences in mangiferin content per stage of development, a repeated measures ANOVA was implemented on the control cultivars alone. The biological replicates were analyzed individually to identify any differences in mangiferin content (**Table 17**). Both biological replicates for the cultivar 'Neelum' showed no significant difference in mangiferin concentrations per stage of development. One biological replicate for the cultivar 'Zebda' showed that stage 1 had a significantly higher amount of mangiferin than stage 3 and that stage 2 had a significantly higher amount of mangiferin than stages 3 and 4.

Table 17. Pairwise Comparisons: Cultivar *vs* Stage of Development.

Measure: MEASURE_1

Mango	(I) Stage	(J) Stage	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Zebda_1	1	2	-.860	.653	1.000	-2.795	1.074
		3	2.442*	.715	.019	.324	4.560
		4	1.304	.493	.099	-.157	2.764
	2	1	.860	.653	1.000	-1.074	2.795
		3	3.302*	.720	.001	1.169	5.436
		4	2.164*	.588	.010	.423	3.905
	3	1	-2.442*	.715	.019	-4.560	-.324
		2	-3.302*	.720	.001	-5.436	-1.169
		4	-1.138	.569	.364	-2.824	.547
	4	1	-1.304	.493	.099	-2.764	.157
		2	-2.164*	.588	.010	-3.905	-.423
		3	1.138	.569	.364	-.547	2.824
Zebda_2	1	2	-1.028	.653	.796	-2.963	.906
		3	-.015	.715	1.000	-2.133	2.103
		4	-.195	.493	1.000	-1.656	1.266
	2	1	1.028	.653	.796	-.906	2.963
		3	1.013	.720	1.000	-1.120	3.147
		4	.833	.588	1.000	-.908	2.574
	3	1	.015	.715	1.000	-2.103	2.133
		2	-1.013	.720	1.000	-3.147	1.120
		4	-.180	.569	1.000	-1.865	1.505
	4	1	.195	.493	1.000	-1.266	1.656
		2	-.833	.588	1.000	-2.574	.908
		3	.180	.569	1.000	-1.505	1.865
Neelum_1	1	2	.216	.653	1.000	-1.719	2.150
		3	.831	.715	1.000	-1.287	2.950
		4	.613	.493	1.000	-.847	2.074
	2	1	-.216	.653	1.000	-2.150	1.719

		3	.616	.720	1.000	-1.518	2.749
		4	.398	.588	1.000	-1.343	2.139
	3	1	-.831	.715	1.000	-2.950	1.287
		2	-.616	.720	1.000	-2.749	1.518
		4	-.218	.569	1.000	-1.903	1.467
	4	1	-.613	.493	1.000	-2.074	.847
		2	-.398	.588	1.000	-2.139	1.343
		3	.218	.569	1.000	-1.467	1.903
Neelum_2	1	2	.700	.653	1.000	-1.234	2.634
		3	.645	.715	1.000	-1.474	2.763
		4	.534	.493	1.000	-.927	1.994
	2	1	-.700	.653	1.000	-2.634	1.234
		3	-.056	.720	1.000	-2.189	2.078
		4	-.166	.588	1.000	-1.908	1.575
	3	1	-.645	.715	1.000	-2.763	1.474
		2	.056	.720	1.000	-2.078	2.189
		4	-.111	.569	1.000	-1.796	1.574
	4	1	-.534	.493	1.000	-1.994	.927
		2	.166	.588	1.000	-1.575	1.908
		3	.111	.569	1.000	-1.574	1.796

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Anti-fungal Assay Results

No anti-fungal activity was exhibited when the extracts and mangiferin standards were tested against four strains of *Colletotrichum gloeosporioides* Penz. and Sacc. In Penz. Consequently, no statistical analyses were performed.

VII. DISCUSSION AND CONCLUSION

Accuracy Determination

With the exception of samples spiked at the LOQ level, the error (represented by the coefficient of variance or CV) of the other samples were acceptable. The low coefficient of variance led to the conclusion that the extraction method had high reproducibility. The coefficient of variance of the LOQ may have been a consequence of the quantified amount of mangiferin recovered being below the limit of quantification.

The percent recoveries followed a Gaussian pattern. The lower levels had low mangiferin concentrations, the middle level (5LOQ) had the highest amount of mangiferin, and the higher levels had low amounts of mangiferin. The low percent recovery of the samples spiked at the LOQ level was a result of the mangiferin remaining in the sample composite. The percent recoveries that were greater than 100% was caused by the extraction of the mangiferin spiked onto the samples as well as mangiferin that naturally occurred in the samples.

Mangiferin Quantitation and Statistical Analysis

It was hypothesized that the mangiferin content would be the highest in mangoes originating from Region 4 (Pacific/South America/West Indies) and the lowest in originating Region 2 (Florida/Hawaii/Israel) during all four stages of development. Since all of the mangoes were collected from the same location, any variability due to local environmental effects (*e.g.,* UV, precipitation, nutrient absorption, and soil) would essentially be the same in all of the mangos collected. Therefore, any significant

differences in mangiferin content would be the result of genetic differences. Those genetic differences were outlined in the dendrogram constructed by Schnell *et al.* (2006) and utilized to group the mangoes into their respective "regions". Mangoes from Region 4 were cultivated for a considerable period of time in and around the equator. These areas are consistently exposed to high amounts of sunlight thereby increasing the probability of oxidative damage to the trees. Mangiferin is a free-radical scavenger so over time, the trees could have increased their mangiferin production considerably to protect the tissues from damage. Mangoes from Region 2 are north of the equator. They are not exposed to as much ultraviolet radiation as mangoes from the other regions so there was no need for mangiferin production to increase. As such, the mangiferin content in this region was expected to be considerably lower than the other regions.

According to the various tests implemented *via* the repeated measures ANOVA, the control cultivars had significantly higher concentrations of mangiferin than all other regions except region 1. Amongst the test cultivars, there were no significant differences between the different regions in any of the stages of development. These results show that the region of origin of a mango may be insignificant. The standard error for each region was considerable because of the high variability in mangiferin content per cultivar. For instance, the test cultivar with the highest overall mangiferin content, 'Tommy Atkins', was from the same region as the test cultivars with the lowest overall mangiferin content, 'Fukuda'. No regional differences in mangiferin content may also be because the sample size (144), effect size (0.25), and power (0.8) were too small to detect any significant differences. If there are any significant differences, another analysis will need to be implemented with a larger sample size with a more diverse array of cultivars. By

increasing the sample size, the effect size will decrease and the power will increase. By implementing these changes, when the data are analyzed by the repeated measures ANOVA, significant differences that were undetectable before will be detectable.

According to the various tests implemented *via* the repeated measures ANOVA, there were statistical differences in mangiferin content between the stages of mango fruit development. Stage 1 had a significantly higher mean mangiferin content than stages 2-4; stage 2 had a significantly lower mangiferin content than stage 1 and a significantly mean higher mangiferin content than stage 4; stage 3 had a significantly lower mean mangiferin content than stage 1; and stage 4 had a significantly lower mean mangiferin content than stages 1 and 2. Mangiferin may play a pivotal role up to the first stage of development that collections took place (mangos were collected after fruit approximately 1-2 months after fruit budding occurred). Mangiferin concentrations during this stage of development could potentially be indicative of the mango tree's ability to resist anthracnose. During this stage, 'Tommy Atkins', a moderately resistant variety, had the highest amount of mangiferin amongst the test cultivars at 21216ppm. 'Fukuda', a susceptible variety, had the lowest amount of mangiferin at 48.813ppm.

The control cultivars 'Neelum' and 'Zebda' were reportedly highly susceptible and highly resistant to mango anthracnose, respectively. As such, it was hypothesized that the mangiferin content would be considerably low and considerably high, respectively. The analysis showed that (1) the mangiferin concentrations of the control cultivars were considerably higher than any other cultivar analyzed and (2) there were no significant differences between the control cultivars in any stage of development. The mangiferin content of each stage of development were not statistically different for either

cultivar. The only exception was that the one biological replicate for the cultivar 'Zebda' showed that stage 1 had a significantly higher amount of mangiferin than stage 3 and that stage 2 had a significantly higher amount of mangiferin than stages 3 and 4. In order to determine if 'Zebda' has significantly different amounts of mangiferin during different stages of development, more samples need to be collected and analyzed.

The mangiferin concentrations of 'Zebda' and 'Neelum' superseded the concentrations expected of these cultivars. Also, the mangiferin content of these accessions did not significantly decrease like the test cultivars did. According to literature, the higher the amount of mangiferin, the greater the resistance of the mango plant to biotic and abiotic stressors. Contrariwise, if mangiferin accumulates excessively at the site of infection, this results in the manifestation of disease symptoms *via* a wide range of biological and physiological aberrations in the host cells. 'Zebda' and 'Neelum' may have mangiferin contents that lie on the border of concentrations that defend the plant against biotic and abiotic threats and concentrations that cause damage to the plant.

Anti-fungal Assay

It was hypothesized that there would be a negative correlation between fungus growth and mangiferin concentrations. The extracts were not effective against the four different strains of *C. gloeosporioides* Penz. and Sacc. In Penz. in the assay. This does not mean that mangiferin is not effective against *C. gloeosporioides.* Mangiferin may have another mode of action that is effective against the *Colletotrichum spp.* That mode of action may be similar to the mode mangiferin utilizes to combat the fungus responsible fofr mango malformation. When *Fusarium mangiferae* Britz., M.J. Wingf. & Marasas,

the fungus responsible for mango malformation, is present, mangiferin removes the nutrition source of the fungus by oxidizing into polymeric quinones (along with gallic acid conjugates). These quinones cause the collapse of adjoining cells, thereby preventing the multiplication of the fungus. Mangiferin also stops the breakdown of host starch. This process removes the nutrition source the pathogen requires to grow and sporulate.

In summation, the validated method utilized to extract mangiferin from mango peels had a high, reproducible percent recovery. According to the repeated measures ANOVA, there were no statistical differences between mangiferin content and the regions of origin. There were, however, statistical differences between mangiferin content and stages of mango fruit development. Mangiferin concentrations were significantly higher in stage 1 than in stages 2-4 and significantly higher in stage 2 than in stage 4 of development. The control cultivars, 'Neelum' and 'Zebda', had no significant differences in any stage of development. Some significant differences in mangiferin content during each stage of development were observed for 'Zebda', but further analyses need to be implemented to determine if there are indeed any significant differences during the stages of development. The extracts exhibited no anti-fungal activity in the antifungal assay employed. If mangiferin's mode of action is similar to its mode of action against the fungus responsible for mango malformation then the absence of any anti-fungal properties exhibited in this assay should be expected.

REFERENCES

Ajaiyeoba E., Oladepo O., Fawole O., Bolaji O., Akinboye D., Ogundahunsi O., Falade C., Gbotosho G., Itiola O., Happi T., Ebong O., Ononiwu I., Osowole O., Oduola O., Ashidi J., Oduola A. 2003. Cultural categorization of febrile illnesses in correlation with herbal remedies used for treatment in Southwestern Nigeria. *Elsevier: Journal of Ethnopharmacology*. 85: 179-185.

Anaruma, N., Schmidt, F., Duarte, M., Figueira, G., Delarmelina, C., Benato, I., Sartoratto, A. 2010. Control of *Colletotrichum gloeosporioides* (penz.) Sacc. in yellow passion fruit using *Cymbopogon citratus* essential oils. *Brazilian Journal of Microbiology*. 41(1): 66-73.

Andreu, Gilberto Lázaro Pardo; Delgado, René; Velho, Jesus Antonio; Curti, Carlos; Vercesi, Anibal E. 2005. Mangiferin, a natural occurring glucosyl xanthone, increases susceptibility of rat liver mitochondria to calcium-induced permeability transition. *Elsevier: Archives of Biochemistry and Biophysics*. 439: 184-193.

Asase, Alex; Akwetey, George A.; Achel, Daniel G. 2010. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *Elsevier: Journal of Ethnopharmacology*. 129: 367-376.

Ballab, Basant; Chauransia, O.P.; Ahmed, Zakwan; Singh, Shashi Bala. 2008. Traditional medicines of cold desert Ladakh-Used against kidney and urinary disorders. *Elsevier: Journal of Ethnopharmacology*. 118: 331-339.

Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R., Schieber, A. 2005. Screening of Mango (*Mangifera indica* L.) Cultivars for Their Contents of Flavonol *O*- and Xanthone *C*-Glycosides, Anthocyanins, and Pectin. *Journal of Agricultural and Food Chemistry*. 53: 1563-1570.

Ceuterick, Melissa; Vandebroek, Ina; Torry, Bren; Pieroni, Andrea. 2008. Cross-cultural adaption in urban ethnobotany: The Columbian folk pharmacopoeia in London. *Elsevier: Journal of Ethnopharmacology*. 120: 342-359.

Chakrabarti, D.K. 2011. Mango Malformation. *Springer Sciences & Business Media*. 39-47

Chang, C., Lin, T., Yang, W. 2012. Invading Time of *Colletotrichum gloeosporioides* Affects Fruit Drop and Infection Rate in 'Irwin' Mango (*Mangifera indica* L.). *Hort. Environ. Biotechnol.* 53(6): 452-459.

da Cruz Jr., José W.; de Moraes, Lucas R.; dos Santos, Marcelo H.; da Silva, Geraldo

Alves; Brigagão, Maisa R.P.L.; Ellena, Javier; Doriguetto, Antonio C. 2008. Crystalline Structure of Mangiferin, a C-Glycosyl-Substituted 9H-Xanthen-9-one Isolated from the Stem Bark of Mangifera indica. *Helvetica Chimica Acta*. 91: 144-154.

Doughari, J. H.; Manzara, S. 2008. *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. *African Journal of Microbial Research.* 2: 67-72.

Enzyme. 2011. Bioinformatics resource portal. *Swiss Institute of Bioinformatics.* Retrieved from http://enzyme.expasy.org/EC/1.13.11.- 5 October 2011.

Gómez-Zaleta, Berenice; Ramírez-Silva, María Teresa; Gutiérrez, Atilano; González-Vergara, Enrique; Güizado-Rodríguez, Marisol; Rojas-Hernández, Alberto. 2006. UV-vis, ^{1}H, and ^{13}C NMR Spectroscopic Studies. *Elsevier: Spectrochimica Acta Part A*. 64: 1002-1009.

Litz, R.E. 2009. The Mango: Botany, Production and Uses. Second Edition: 63, 77

Luo, F., L., Q., Zhao, Y., Hu, G., Huang, G., Zhang, J., Sun, C., Li, X., Chen, K. 2012. Quantification and Purification of Mangiferin from Chinese Mango (*Mangifera indica* L.) Cultivars and Its Protective Effect on Human Umbilical Vein Endothelial Cells under H_2O_2-induced Stress. *International Journal of Molecular Sciences*. 13: 11260-11274.

Masibo, Martin; He, Qian. 2008. Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food Safety*. 7: 309-319.

Morton, Julia F. 1987. Mango. p. 221–239. *Fruits of warm climates*.

Nelson, S. 2008. Mango anthracnose (*Colletotrichum gloeosporioides*). *Plant Disease*. 48: 1-9.

Ososki, Andreana L.; Lohr, Patricia; Reiff, Marian; Balick, Michael J.; Kronenberg, Fredi; Fugh-Berman, Adriane; O'Connor, Bonnie. 2002. Ethnobotanical literature survey of medicinal plants in the Dominican Republic used for women's health conditions. *Elsevier: Journal of Ethnopharmacology.* 79: 285-298.

Rao, B.S. Satish; Sreedevi, M.V.; Rao, B. Nageshwar. 2008. Cytoprotective and antigenotoxic potential of Mangiferin, a Glucosylxanthone against cadmium chloride induced toxicity in HepG2 cells. *Elsevier: Food and Chemical Toxicology*. 47: 592-600.

Schieber A., Ullrich W., Carle R., 2000. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection.

Innovative Food Science and Emerging Technologies 1:161-166.

Schnell, R., Brown, J., Olano, C., Meerow, A., Campbell, R., Kuhn, D. 2006. Mango Genetic Diversity Analysis and Pedigree Inferences for Florida Cultivars Using Microsatellite Markers. *J. Amer. Soc. Hort. Sci.* 131(2): 214-224.

Singh, S., Tiwari, R., Sinha, S., Danta, C., Prasad, S. 2012. Antimicrobial evaluation of mangiferin and its synthesized analogues. *Asian Pacific Journal of Tropical Biomedicine*. S884-887.

Stoilova, Ivanka; Jirovetz, Leopold; Stoyanva, Albena; Krastanov, Albert; Gargova, Stoyanka; Ho, Lien. 2008. Antioxidant activity of the polyphenol mangiferin. *Electronic Journal of Environmental, Agricultural, and Food Chemistry*. 7(13): 2706-2716.

Sun, Y., Zhang, X., Xue, X., Zhang, Y., Xiao, H., Liang, X. 2009. Rapid Identification of Polyphenol *C*-Glycosides from *Swertia franchetiana* by HPLC-ESI-MS. *Journal of Chromatographic Science*. 47: 190-196.

Tabuti, J.R.S.; Lye, K.A.; Dhillion, S.S. 2003. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *Elsevier: Journal of Ethnopharmacology*. 88: 19-44.

Thomasek, Paul H.; Crawford, Ronald L. 1986. Initial Reactions of Xanthone Biodegradation by an Arthrobacter sp. *Journal of Bacteriology*. 167(3): 818-827.

Ticktin, Tamara; Dalle, Sarah Paule. 2004. Medicinal plant use in the practice of midwifery in rural Honduras. *Elsevier: Journal of Ethnopharmacology*. 96: 233-248.

Vandebroek, Ina; Balick, Michael J.; Ososki, Andreana; Kronenberg, Fredi; Yukes, Jolene; Wade, Christine; Jiménez, Francisco; Peguero, Brígido; Castillo, Daisy. 2010. The importance of botellas and other plant mixtures in Dominican traditional medicine. *Elsevier: Journal of Ethnopharmacology*. 128: 20-41.

Volpato, Gabriele; Godínez, Daimy; Beyra, Angela; Barreto, Adelaida. 2009. Use of medicinal plants by Haitian immigrants and their descendants in the Province of Camagüey, Cuba. *Journal of Ethnobotany and Ethnomedicine.* 5:16-24.

Wang, Rui-Rui; Gao, Yue-Dong; ;Ma, Chun-Hui; Zhang, Xing-Jie; Huang, Cheng-Gang; Huang, Jing-Fei; Zheng, Yong-Tang. 2011. Mangiferin, an Anti-HIV-1 Agent Targeting Protease and Effective against Resistant Strains. *Molecules*. 16: 4264-4277.

Wauthoz, Nathalie; Balde, Aliou; Balde, Elhadj Saïdou; Van Damme, Marc; Duez,

Pierre. 2007. Ethnopharmacology of *Mangifera indica* L. Bark and Pharmacological Studies of its Main C-Glucosylxanthone, Mangiferin. *International Journal of Biomedical and Pharmaceutical Science*. 1(2): 112-119.

Yoshima, Naoki; Matsunaga, Kengo; Katayama, Masaki; Yamada, Yasuhiro; Kuno, Toshiya; Qiao, Zheng; Hara, Akira; Yamahara, Johji; Mori, Hideki. 2001. The inhibitory effects of mangiferin, a naturally occurring Glucosylxanthone, in bowel carcinogenesis of male F344 rats. *Elsevier: Cancer Letters*. 163: 163-170.

Zumteg, Isabelle S.; Weckerle, Caroline S. 2007. *Bakera*, an herbal steam bath for postnatal care in Minahasa (Indonesia): Documentation of the plants used and assessment of the method. *Elsevier: Journal of Ethnopharmacology*. 111: 641-650.

Figures Sources

Figure 3

http://www.nature.com/nature/journal/v174/n4435/images/174830a0-i1a.gif

Figure 4

http://www.lktlabs.com/images/products/b1853.gif

Figure 5

https://patentimages.storage.googleapis.com/WO2006026504A2/imgf000012_0001.png

Figure 6

http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/Images/polyarom.gif

APPENDICES

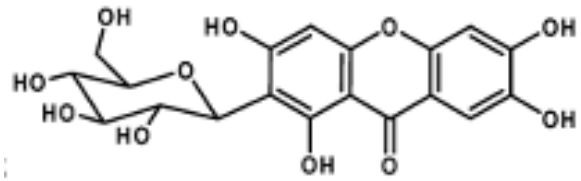
RAW DATA

Cultivar	Biological Replicate	Region	Stage 1	Stage 2	Stage 3	Stage 4
Peach	1	1	9855.444	2101.789	1587.328	667.770
	2	1	4947.635	3245.512	1521.856	1071.749
Pere Louis	1	1	597.473	69.067	103.676	324.705
	2	1	164.731	232.967	286.209	554.007
Turpentine #7	1	1	19946.808	4023.396	5596.370	2584.298
	2	1	12427.740	4854.113	4096.957	2620.439
Toledo	1	1	3003.331	1469.231	2548.207	1728.165
	2	1	2917.125	1859.931	1604.859	1399.058
Nam Tan Teen	1	2	4805.879	7634.840	852.918	988.089
	2	2	3892.465	158.429	380.705	660.953
Nam Doc Mai	1	2	325.233	64.432	230.045	43.378
	2	2	1442.028	648.832	41.033	49.674
Myatryaut	1	2	211.297	1350.047	1183.350	172.000
	2	2	1260.413	836.339	350.651	131.488
Katar Rum Rung	1	2	897.186	1317.258	1943.778	1554.864
	2	2	1666.400	3522.752	2102.132	1081.450
Tommy Atkins	1	3	20864.306	5649.948	5098.900	3429.206
	2	3	21567.834	5677.629	4123.126	3974.436
Fukuda	1	3	55.127	8.802	21.536	6.421
	2	3	42.499	8.192	24.048	6.336
Bombay	1	3	44.116	23.147	30.678	20.380
	2	3	67.365	40.369	39.510	9.316
Maya	1	3	17755.838	3090.348	1766.870	1055.951

	2	3	5379.928	4120.430	3081.829	1187.937
Sabre	1	4	825.171	71.477	669.937	173.458
	2	4	1274.903	127.749	1609.349	230.107
Diab	1	4	1571.916	783.774	3700.642	337.770
	2	4	1064.143	411.211	3963.821	426.462
Bullock's Heart	1	4	1319.082	4732.478	200.642	261.285
	2	4	2617.103	4076.706	130.192	974.048
Jehangir	1	4	330.640	734.235	1131.155	224.009
	2	4	231.034	813.679	69.589	503.870
Zebda	1	0	28545.899	23772.566	12397.64	13451.55
	2	0	24923.819	19448.142	10880.81	15510.26
Neelum	1	0	27572.860	15087.424	15787.58	15921.27
	2	0	27409.031	12349.405	13189.87	16325.83

I. Scope

The extraction method used is applicable to the quantitative determination of mangiferin in mango peels. The method has a validated limit of detection (LOD) of 13.32 ppm and a limit of quantitation (LOQ) of 39.99 ppm and has been validated over a concentration range of 39.99 ppm-399.9 ppm. This method generates approximately 4mL of organic waste per sample.



Mangiferin

II. Principle

A 1 gram sample is extracted with 50:50 Acetonitrile:Water. The extract is sonicated for 15 min, placed on a shaker overnight, vortexed, and centrifuged for 5 min (4500rpm). A 1 mL aliquot is transferred to an autosampler vial and analyzed by Ultra High-Performance Liquid Chromatography coupled with an Electrospray Ionizer-Mass Spectrometer with UV detection at 242nm, 367nm, and3D Field as well as MS-MS detection of the base peak 405*m/z*.

III. Process Description

a. Sample Preparation

i. Apparatus and Reagents

- Analytical Balance
- 50mL disposable centrifuge tube (Fisher Scientific cat#: 05-539-12)
- Liquid Nitrogen
- Dewar vessel
- Metal Mortar
- Ceramic Pestle
- Funnel
- Spatula
- Surgical Steel Blades

ii. Mixing and Preparation of Samples

- Using a surgical steel blade, remove viable peel (peel that is unbroken and does not have fungi growing on it)
- Adding bits at a time, grind the entire sample in liquid nitrogen using a metal mortar and ceramic pestle
- Transfer grinded tissue into a 50mL centrifuge tube
- Store sample in cryogenic freezer if extraction is not planned for the same day
  ***Samples must be thawed prior to extraction*
- Clean laboratory equipment between samples
  **Clean the metal mortar, ceramic pestle, funnel, and spatula with soap and water then solvent rinse

We have performed the operations stated in this section.

Initial_____ **Date**_____

iii. Weighing of Sub-Samples

- Verify balance suitability and record appropriate documentation in balance logbook.
- Place a 50mL disposable centrifuge tube into a beaker and tare the analytical balance
- Transfer 1 gram of sample into the 50mL centrifuge tube
- Record weight on sample worksheet.

We have performed the operations stated in this section.

Initial_____ Date_____

b. Sample Processing

i. Apparatus and Reagents

- 50:50 Acetonitrile:Water
- 15mL disposable centrifuge tubes
- 5mL pipette
- Vortex
- Sonicator
- Shaker
- Centrifuge

ii. Sample Extraction

- To the sample, add 5mL of 50:50 acetonitrile:water.
- Sonicate for 15min.
- Place on shaker overnight (setting: low)
- Vortex the sample.
- Centrifuge the sample for 5min (setting: 4500rpm).
- Transfer supernatant layer to a 15mL disposable centrifuge tube.

- Centrifuge the sample for 5min (setting: 4500rpm).
- Add 0.5mL of supernatant to HPLC vial with 1.5mL of 50:50 Acetonitrile:Water

We have performed the operations stated in this section.

Initial_____ Date_____

c. Sample Fortification

i. Apparatus and Reagents

- 2mL, 4mL Class A volumetric pipettes
- 50mL volumetric flasks
- Brown bottles
- 50:50 Acetonitrile:Water
- 1000µg/mL Mangiferin stock solution in 50:50 Acetonitrile:Water

ii. Preparation of Fortification Solution

- Using an eppendorf pipette, pipette 1.4mL of a 1000µg/mL Mangiferin stock solution into a 50mL volumetric flask and dilute to the mark with 50:50 Acetonitrile:Water. Concentration should be approximately 28µg/mL mangiferin
- Transfer solution to the appropriate size brown bottle and label
- Record activity on sample worksheet

iii. Preparation of Standard Check

- Using an eppendorf pipette, pipette 1.4mL of a 1000 µg/mL Mangiferin stock solution into a 50mL volumetric flask and dilute to the mark with 50:50 Acetonitrile:Water. Concentration should be approximately 28µg/mL mangiferin
- Transfer solution to the appropriate size brown bottle and label
- Record activity on sample worksheet

iv. Determination of Percent Recovery of Mangiferin

- To the blank composite, spike 5mL of the fortification solution
- Record activity on the sample worksheet

Amount Spiked of Fortification Standard (ppm) $= \frac{(5mL)(\frac{28\mu g}{mL})}{1g\ sample}$

Amount Spiked of Fortification Standard (ppm) = 140ppm Mangiferin

We have performed the operations stated in this section.

Initial_____ Date_____

d. Instrumental Analysis

i. Apparatus and Reagents

- Thermo Scientific UltiMate™ 3000 UPLC equipped with a photodiode array detector
- Thermo Scientific LTQ Heated Electrospray Ionizer (HESI) coupled with a Line Ion Trap Mass Spectrometer
- Analytical Column, Zorbax 300SB-C18 250 x 4.6mm, 5µm
- 0.1% Formic Acid in HPLC Water
- 0.1% Formic Acid in Acetonitrile
- 15mL disposable centrifuge tubes
- Autosampler vials
- 50:50 Acetonitrile:Water
- 0.5, 1, 2, 3, 8, and 10mL Class A volumetric pipettes

ii. Typical Operating Conditions

Parameter			
Instrumentation	Thermo Scientific UltiMate™ 3000 UPLC equipped with a photodiode array detector Thermo Scientific LTQ Heated Electrospray Ionizer (HESI) coupled with a Line Ion Trap Mass Spectrometer		
Column	Zorbax 300SB-C18 250 x 4.6mm, 5μm		
Column Temperature	25°C		
Mobile Phase	Solvent A: 0.1% Formic Acid in Water		
	Solvent B: 0.1% Formic Acid in Acetonitrile		
Gradient	**Time**	**%Solvent A**	**%Solvent B**
	0.00	95.0	5.00
	1.00	95.0	5.00
	15.00	0.00	100.0
	9.00	0.00	100.0
	16.00	95.0	5.00
	19.00	95.0	5.00
Flow Rate	0.500 mL/min		
Injection Volume	1μL		
MS Base Peak	423*m/z*		
MS/MS Base Peak	405*m/z*		
Normalized Collision Energy	35.0 V		
MS Polarity	Positive		
UV Wavelengths	242nm, 367nm, 3D Field		
Run Time	19 min		

iii. Preparation of Bench Standards

Using a Class A volumetric pipette, pipette 2mL of a 5000ppm stock of mangiferin into a 15mL disposable centrifuge tube and add 8mL of 50:50 acetonitrile:water. Label appropriately. Concentration should be approximately 1000ppm mangiferin. Shelf life is to be 6 months. This solution is the bench intermediate. Prep Date:_____

Working Standard	**Initial Concentration**	**Amount Added**	**Solvent Amount**	**Solvent**	**Final Concentration**	**Target Concentration**	**Prep Date**
LOQ Std Mangiferin	_____ (ppm)	**0.5mL Bench Intermediate**	**12.0mL**	**50:50 ACN:H_2O**	_____ (ppm)	**40ppm**	
5LOQ Std Mangiferin	_____ (ppm)	**1.0mL Bench Intermediate**	**4.0mL**	**50:50 ACN:H_2O**	_____ (ppm)	**200ppm**	
10LOQ Std Mangiferin	_____ (ppm)	**2.0mL Bench Intermediate**	**3.0mL**	**50:50 ACN:H_2O**	_____ (ppm)	**400ppm**	

$$ppm = \frac{\frac{\mu g}{mL}(Bench\ Standard)}{\frac{0.2g}{mL}(Final\ Sample\ Size)}$$

We have performed the operations stated in this section.
Initial_____ Date_____

iv. System Suitability

Criteria	Expected	Analyst Review
Standard check	5% RSD ≥	□ Y □ N
Peak Area at LOQ of first 3 standards	5% RSD ≥	□ Y □ N
Standard curve at 1, 5, and 10 LOQ	$R^2 \geq 0.98$	□ Y □ N

- Suitability is not met unless all criteria have been checked "Yes"
- If suitability is not met, perform maintenance and run again
- Do not proceed to *Analysis* until suitability has been achieved

We have performed the operations stated in this section.
Initial_____ Date_____

v. Analysis

- The 5LOQ standard is to be placed after every 5 samples and at the end of analysis
- Samples found to be outside of the calibration curve are to be diluted appropriately and the dilution factor applied to the final amount

vi. Calculations

- To determine the percent recovery of the Fortified Sample, use the following calculation:

  Percent Recovered = $\frac{Amount\ found\ (ppm)}{Amount\ spiked\ (ppm)} x100\%$

- To determine the amount of mangiferin, use the following calculation:

Amount of mangiferin = (Amount found (ppm) x Dilution factor)/ Recovery Factor

vii. System Clean Up

- In order to prevent matrix build up on the column, the system must be cleaned after each group. Below is a table with the operating conditions:

Parameter	
Column Temperature	25°C
Mobile Phase	Solvent A: 0.1% Formic Acid in Acetonitrile
Flow Rate	1.000 mL/min
Run Time	45 min

viii. Data Package Assembly

- A copy of the sample worksheet and accompanying field forms
- A copy of the appropriate technical procedure
- A printout of the sequence file used for analysis
- A printout of the calibration table for the reporting column
- A printout of the extended statistics of the peak height for mangiferin
- A printout of chromatograms supporting system suitability (3 LOQ standard injections, linearity curve, and standard check)
- A printout of chromatograms supporting sample analysis
- A printout of calculation spreadsheet if applicable
- A printout of report

We have performed the operations stated in this section.
Initial_____ **Date**_____

IV. Records

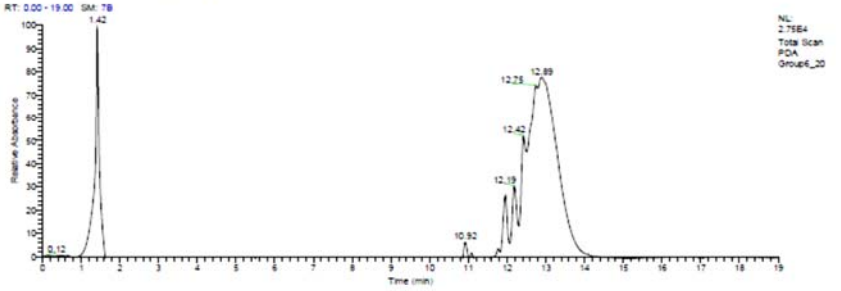
Data Package

SAMPLE REPORT GENERATED BY SOFTWARE

Quan Component's Peak Report

Sample ID:	Group6_RC4	Sample Type:	Unknown	Sample Name:	62
Operator:	Velos Pro			Vial:	RC4
Acquisition Date:	04/14/15 11:15:43 PM			Sample Vol (µl):	0.00
Low Mass(m/z):	115.00000			Inj Volume(µl):	1.00
High Mass(m/z):	455.00000			Sample Weight:	0.00
Scans:	7322			Dilution Factor:	1.00
Run Time(min):	19.00	Calibration Level:		Revision:	2.7.0 SP1
Comments:					

Instrument Method:	C:\Xcalibur\methods\Determination of Mangiferin-071614 isocratic ACN.meth
Original Processing Method:	C:\Xcalibur\methods\Mangiferin ppm
Current Processing Method:	N/A
Calibration File:	N/A

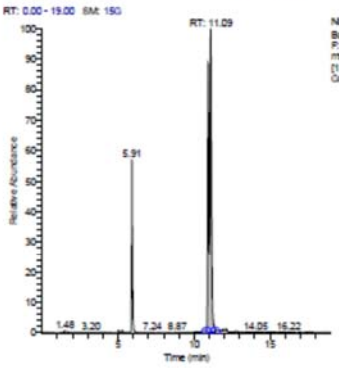


RT	Component Name	Area	Calculated Amount	Units
11.09	Mangiferin	614058.48	154.45	ppm

There is no signature data to report.

Quan Component's Peak Report

Component Name: Mangiferin

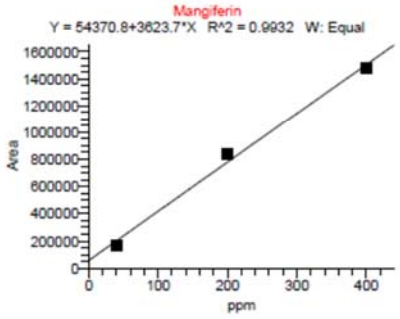


Details

Component Name:	N/A
Actual RT(min):	11.1
Specified Amount:	N/A
Calculated Amount:	154.45205
%Diff:	N/A
Response:	614058.47515
Response Ratio:	614058.475
Base Line:	SS
Signal To Noise:	556.19
Saturated:	No
Peak Status:	
System Suitability:	Not Tested: S R W T O B N C D
Data Flags:	
Calibration Flags:	Q

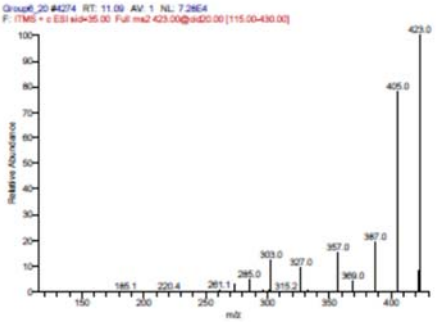
Calibration Curve

Component Name: Mangiferin



Component Spectra

Component Name: Mangiferin



There is no signature data to report.